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Michael A. Lang and **Martin D.J. Sayer**

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FOREWORD

***Michael A. Lang*[1]**
***Martin D.J. Sayer*[2]**
Co-Chairs
2013 AAUS/ESDP Curaçao Joint International Scientific Diving Symposium

[1]The Ocean Foundation
1990 M Street NW, Suite 250
Washington, DISTRICT OF COLUMBIA 20036 U.S.A.
mlang@oceanfdn.org

[2]NERC National Facility for Scientific Diving
Scottish Association for Marine Science
Dunstaffnage Marine Laboratories
Dunbeg, Oban, Argyll PA37 1QA, SCOTLAND
mdjs@sams.ac.uk

The American Academy of Underwater Sciences (AAUS) and European Scientific Diving Panel (ESDP) Joint International Scientific Diving Symposium in Curaçao, October 24-27 2013, marks the third time that the AAUS has held its annual meeting in partnership with other international scientific diving organisations. Jointly sponsored AAUS symposia occurred in 1985 with CMAS (Confédération Mondiale des Activités Subaquatiques; Mitchell, 1985) and in 1998 with the Canadian Association for Underwater Science (CAUS; Hartwick et al., 1998).

The AAUS and the ESDP share commonalities in their mission and objectives. They both represent a considerable number of scientific divers performing diving-based research covering the full range of scientific disciplines. Underwater scientific research is often conducted on an international scale in regions that frequently face growing pressures on resources and increased threats of pollution, urbanization of habitats, invasion by alien species, extreme weather events and sea-level change. The relatively shallow and multifaceted nature of many coastal areas can restrict the types of platforms that can be deployed in support of relevant research. Scientific diving is a cost-effective high-quality research tool that can sustain a wide range of scientific disciplines within operationally expedient timeframes. It has particular use in complex environments such as subtidal rocky substrates or urbanized habitats (marinas, wrecks, offshore wind farms, etc.) that are routinely inaccessible for study by other methods. Scientific diving has also provided unique multidisciplinary datasets that add value to other ocean observation platforms (Lang et al., 2013).

This symposium convenes scientists of multiple nationalities in an attempt to reduce insularity, encourage exchange of ideas and operational protocols, and expand the worldwide network of scientific divers. This joint initiative provides a forum that will highlight some of the research findings from within the international research communities. We are experiencing a developmental period of large-scale multi-national research programs established to observe, record and monitor change over various geographical scales. Engendering a research framework for scientific divers that facilitates multi-national collaboration is of immediate and long-term benefit.

Irrespective of the symposium venue selected, the diving scientist increasingly experiences cutbacks in research funding, extreme pressure in travel budgets and intense competition for proposal-dependent funding, not to mention government shutdowns. The island of Curaçao was identified for this symposium because of its historical ties to Europe, its proximity to the Americas and as a desirable venue for workshops such as coral reef ecology, performance freediving and a post-symposium field trip to Bonaire. The presence of Substation Curaçao's CURASUB provides delegates the opportunity for deep-reef observation dives and CARMABI and CIEE are local research organizations in Curaçao and Bonaire providing perspective on long-term coral reef research in the Caribbean. We are hopeful that the connections created through this joint international symposium will advance scientific diving as a research platform on a global scale.

We wish to acknowledge the Divers Alert Network for major symposium support. We also thank the following: Dutch Grier and Laureen Schenk for sponsorship of the CURAÇAO SEAQUARIUM reception and making CURASUB dives available to symposium participants; Nolo Ambrosi of OCEAN ENCOUNTERS for diving support; the Curaçao Tourism Board; AAUS scholarship-sponsoring organizations and individuals, in particular Kathy Johnston English for her signature artwork. Thanks are also due the presenters and authors for their contributions to the symposium program and proceedings. Finally, a special thanks to Heather Fletcher for outstanding symposium support and organizational efficiency.

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THE ROLE OF VOLUNTEER DIVERS IN LIONFISH RESEARCH AND CONTROL IN THE CARIBBEAN

Fadilah Ali [1,2]
Ken Collins[1]
Rita Peachey [2]

[1] Ocean and Earth Science
University of Southampton, National Oceanography Centre
Southampton SO14 3ZH, UNITED KINGDOM
fadilah.ali@soton.ac.uk

[2] CIEE Research Station Bonaire
Kaya Gobernador Debrot 26
Kralendijk, Bonaire, DUTCH CARIBBEAN

The Indo-Pacific lionfish (Pterois volitans) is a venomous, voracious predator that is currently causing ecological and economical harm throughout the Caribbean. Their generalist diet and habitat preference coupled with their rapid growth rate and lack of natural predators has allowed their population to explode throughout the Caribbean. As a means to control lionfish populations, countries have designed lionfish removal programs which, in some instances, depend primarily on volunteer divers. Activities such as lionfish tournaments or specified lionfish removal trips and events are another platform whereby volunteer divers help to remove substantial quantities of lionfish. These removal events are important for lionfish control, and they contribute greatly to research. In Bonaire, since October 2009, almost 5,000 lionfish have been submitted to CIEE Research Station Bonaire by volunteer divers for research on lionfish morphometrics, sexual maturity and feeding ecology. This submission of specimens has contributed to one of the most in-depth and long-term studies of lionfish feeding ecology in the Caribbean. Staff from CIEE have also attended lionfish hunting tournaments in Curaçao to collect data on lionfish ecology and make comparisons. During the first tournament in 2012, 317 fish of the 1,069 caught were analysed, whereas in 2013, 1,500 fish out of 2,403 caught were dissected. Thus, within two days an extensive sample size was attained from various depths throughout various locations in Curaçao, an achievement that would have taken a small group of researchers many weeks or even months. Thus, volunteer divers have the ability to play an instrumental role in lionfish research and control and should be implemented into further lionfish management strategies throughout the Caribbean.

Introduction

Lionfish signify the first marine fish invader from the Western Pacific to the Atlantic and are believed to have been introduced via intentional and/or unintentional aquaria releases (Morris, 2009). They are usually benthically associated and in their native range they occur over coral, sand and hard-bottom substrates from the surface to 50 m (Whitfield et al., 2002; 2007; Vasquez-Yeomans et al., 2011). In their invaded range, they have occupied all major seafloor and substrate types and occupy a range of depths, from the shoreline to more than 300 m deep. During the day, lionfish linger under ledges and crevices, but may hunt small fish, shrimps and crabs in the open water at night (Ruiz-Carus et al., 2006). Lionfish are adaptable to many habitats and have colonised areas ranging from 1 to 140 m on reef walls, patch reefs, rocky areas, hard bottoms, ledges, crevices, mangrove creeks, isolated coral heads, blue holes, ship wrecks, and man-made structures (NOAA's Coris, 2009). Lionfish tend to live in small groups as juveniles and during reproduction but disperse and hide in reef shadows when they are adults (Fishelson, 1997).

Based on captured specimens in the Caribbean, lionfish are reproducing throughout the year (REEF, 2012). Their reproduction shows no apparent timing relative to moon and tidal regimes, and a continuous supply of propagules is generated (Department of Marine Resources, 2008; Morris, 2009). Lionfish are prolific breeders with one female being able to eject up to 15,000 eggs during a single mating event, of which she can have at least three per month (Bervoets, 2009). The eggs are bound in an adhesive mucus that disintegrates a few days later, allowing the embryo and/or larvae to become free-floating (Hare and Whitfield, 2003; Morris et al., 2009). As they are not thermally limited in their native range, they can continuously reproduce throughout the year. The actual larval distribution of lionfish still remains unknown but has been estimated to be between 25-40 days (Morris, 2009). Laidig and Sakuma (1998) reported a larval growth rate of 0.33 mm day^{-1} for *Scorpaena*, a genus in the Scorpionfish family. Applying this estimate to the estimated 25-40 day larval duration, it can be suggested that larvae are in the water column and consequently susceptible to transport by ocean currents for approximately one month (Hare and Whitfield, 2003). Settling from the water column to the benthic habitat is proposed to occur at a total length of about 12 mm (Hare and Whitfield, 2003). The juveniles develop rapidly and begin to actively hunt at approximately 7 cm total length and have been observed to consume prey up to two-thirds their body length (Bervoets, 2009).

Lionfish are principally piscivorous but are known to feed on invertebrates (Morris, 2009). In their native range they occupy the higher levels of the food chain (Hare and Whitfield, 2003; Bervoets, 2009). In the Bahamas, the occurrence of teleosts in lionfish diets is size-dependent with larger lionfish feeding on teleosts and smaller lionfish feeding more heavily on crustaceans (Morris, 2009; Morris and Akins, 2009). Bervoets (2009) suggested that lionfish consume 10% of their body weight every day, whilst Morris (2009) proposed that lionfish have the ability to consume 2.5 to 6.0% of their body weight per day at 25-26^{o}C. Lionfish stomachs can expand to more than 30 times in volume (Freshwater et al., 2009). Lionfish are adept in undergoing periods of starvation of over 12 weeks without mortality because of their capability of long-term fasting (Fishelson, 1997; Morris, 2009). They can employ a diverse range of feeding strategies making them well suited for feeding on benthic and cryptic prey. Prey species in the Atlantic region are naïve to lionfish's novel predation strategies, resulting in lionfish having higher predation efficiencies in the invaded range compared with its native range.

Since their invasion of the North Atlantic/Caribbean region, various management measures have been instilled to quell lionfish populations (Morris et al., 2010). Lionfish management has been an evolutionary process in terms of the schemes established, the tools used, and the means to increase removal efforts (De Leon et al., 2011). At the beginning of the lionfish invasion, scientists and government or state department officials were the primary persons involved in lionfish removal. However, because of the invasion characteristics of lionfish, there is need for a larger, community effort to enhance the chances of more successful removal (Morris et al., 2012). Removal activities were introduced to dive operators and professionals and further extended to volunteer divers, no matter what their dive certification level (BNMP, 2010); this has been instrumental in the successful control and management of lionfish in some islands in the Caribbean.

Case Studies

Bonaire

Bonaire is a prime example of how the use of volunteer dives can be a successful means to control lionfish populations. The control, management and monitoring of lionfish in Bonaire is achieved through an intricate partnership between STINAPA (nature enforcement agency on the island), CIEE Research Station Bonaire, dive operators and volunteer divers. Not only do volunteer divers remove considerable quantities of lionfish, but they also submit a large proportion of their catch so that research can be conducted. Since the first lionfish sighting on 26 October 2009, more than 5,000 lionfish have been submitted to CIEE Research Station Bonaire. This represents one of the largest and most long-term lionfish datasets in the Caribbean; this would not have been possible without the commitment of volunteers. Time and especially monetary restrictions mean that scientists themselves are unable to achieve such a large sample size. However, for research purposes, large sample sizes are desired especially for dietary studies because a large proportion of the stomach content already tends to be too digested for analysis (Cocheret de la Moriniere et al., 2003). A larger sample size is also able to reveal the full spectrum of prey items in lionfish diets and any temporal changes (Hyslop, 1980; Pierce et al., 2004). Research has focused on lionfish ecology, primarily their feeding preferences, their growth rates, their reproductive status and their habitat and depth preferences. Conducting research on lionfish and monitoring any changes in their growth rate, diet, habitat and depth preferences helps to predict and quantify how lionfish may impact on the ecosystem, especially when coupled with prey density surveys (Morris et al., 2012).

Conducting research also acts as an important education and training tool. In Bonaire, all findings are communicated to the public via lectures, newsletters and postings on social media. There is also high involvement with the local schools with educational workshops whereby students get educated on the lionfish invasion and are then given the opportunity to view live lionfish and conduct research through an interactive dissection workshop. Getting the locals, especially the youth, involved is imperative as they can act as ambassadors and educate others. In Bonaire there is also a fantastic partnership between CIEE and the STINAPA Junior Rangers. This program targets the young people of Bonaire in the 12-21 age group. All Junior Rangers have received a lionfish education whilst those over the age of 18 have received training on lionfish removal. These Junior Rangers diligently attend lionfish removal trips offered by CIEE and GOOODIVE and have improved tremendously; they are now the top hunters on these trips. These Junior Rangers act as ambassadors to other youngsters who are aspiring to learn more about lionfish and become lionfish hunters themselves.

Klein Bonaire

Bonaire is renowned as being a shore diving heaven, which makes lionfish removal in Bonaire so convenient. However, Klein Bonaire is only accessible via boat, which accounts for a lower hunting pressure. As a result, a research and removal program was initiated in early 2012 whereby the entire island of Klein Bonaire would be covered. On each trip, a group of 8-10 hunters would remove lionfish from a particular dive site but also keep track of data such as the depth of capture, how many lionfish were missed, lionfish aggregation and behavior. Following the dives all lionfish were measured, weighed, dissected and their reproductive status and stomach contents analysed. Following analysis, lionfish were either sold to restaurants or used personally by hunters.

Figure 1 reveals a reduction in the number of lionfish seen at Klein Bonaire, from 2,240 in 2012 to 854 in 2013. The lionfish hunters in Bonaire have become more efficient at removing lionfish. Improving from a 40% successful capture rate in 2012 to 63.5% in 2013 is especially commendable because lionfish themselves have become ‘smarter’ and have learnt to associate divers with danger, thus making removal activities more difficult.

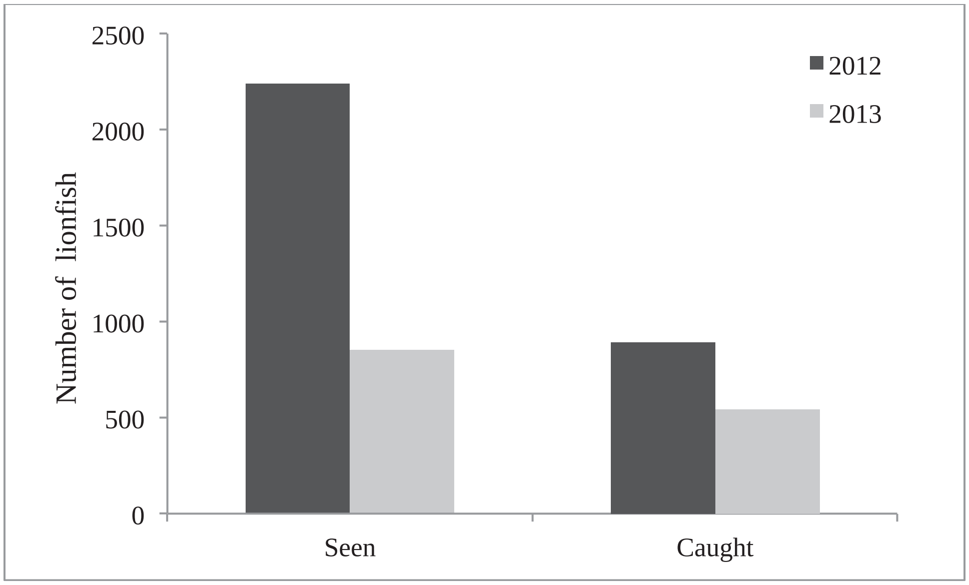

Figure 1. Comparison of the number of lionfish seen and caught at Klein Bonaire in 2012 and 2013

Curaçao

Lionfish were first confirmed in Curaçao on 27 October 2009 (NACRI, 2009). Since 2012, three lionfish tournaments have been hosted in Curaçao (Table 1). Personnel from CIEE Research Station Bonaire were present at the 1st and 3rd tournament, whilst scientists from CARMABI were on-hand to conduct research during the 2nd tournament. During the 1st tournament, 317 lionfish were analysed whereas during the 3rd tournament, an impressive 1,497 lionfish were analysed by CIEE staff.

Table 1. Diver participation and number of lionfish caught during tournaments in Curacao.

Tournament	No. Scuba divers	No. Freedivers	No. Lionfish Caught
1) March 2012	20	4	1084
2) August 2012	11	0	1591
3) April 2013	44	0	2403

Lionfish collected during the tournaments in Curaçao were used for an analytic comparison with fish collected at Klein Bonaire and Bonaire. Despite the close proximity of these islands to one another, there were some interesting differences with respect to lionfish feeding preferences, differences that may not have been discovered with a smaller sample size. Similarities of the top prey species in lionfish's diet were also observed amongst the three islands, which helped to confirm and also understand how lionfish feed which could provide a basis for predicting what their future impacts may be.

Importance and benefits of volunteer divers and lionfish tournaments

Lionfish tournaments allow for large numbers of lionfish to be eradicated in quite a short space of time. The fish caught from these activities provide valuable research specimens for scientists to examine lionfish growth characteristics and their feeding and reproductive ecology, among other aspects. The large quantities of fish caught can also be used as a means to encourage the locals and the general public to eat lionfish whilst also acting as an important vehicle for public education and raising awareness (Morris et al., 2012).

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DEEP REEF OBSERVATION PROJECT (DROP):
A SMITHSONIAN-SUBSTATION CURAÇAO COLLABORATION

Carole C. Baldwin
Smithsonian Institution
National Museum of Natural History
Washington, DISTRICT OF COLUMBIA 20560 U.S.A.
baldwinc@si.edu

Scientists have studied shallow Caribbean reefs for more than a century. In contrast tropical mesophotic and deep reefs, which are below the depths accessible by traditional scuba gear and above the depths typically visited by deep-diving submersibles, are underexplored ocean ecosystems that science has largely missed. Little is known about the diversity of life on deep reefs, how it changes over space and time, and what role deep reefs may play in the survival of shallow reefs above. Shallow reefs are in peril circumtropically but what is the status of deep reefs? Are water temperatures on deep reefs rising and are these ecosystems exhibiting degradation and loss from major agents of environmental impact? Are shallow reef inhabitants moving deeper in response to warming surface waters? Are deep-reef populations re-seeding shallow populations that have been impacted by reef demise?

In 2011 the Smithsonian initiated Deep Reef Observation Project (*DROP*), a research effort that utilizes Substation Curaçao's *Curasub* submersible (http://www.substation-curacao.com/, Fig. 1) to address questions related to biodiversity and conservation of deep reefs. *DROP's* three main research objectives are to (1) document local biodiversity to a depth of 300 m in a small, 0.2 km^2 "plot" of water directly off the Curaçao Seaquarium shoreline (the "Seaquarium plot") and create specimen and DNA libraries for diversity and monitoring studies; (2) implement standardized sampling methods for monitoring long-term changes in environmental and biological conditions on deep and shallow reefs off Curaçao; and (3) transport the *Curasub* to other Caribbean deep reefs to obtain comparative samples to broaden the evolutionary and biogeographic research questions that can be addressed.

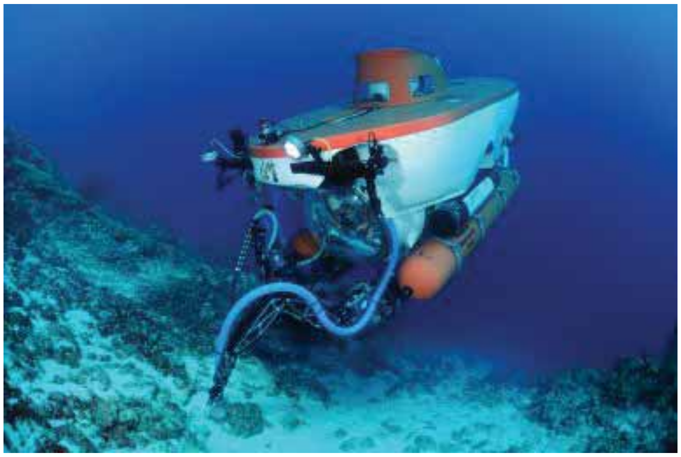

Figure 1. The *Curasub* submersible. Photo by Barry Brown.

In 2011-2013 Smithsonian marine scientists made 32 exploratory submersible dives in the Seaquarium plot and collected approximately 600 deep-reef specimens. Samples were photographed, processed for genetic analysis, and exported for archiving into Smithsonian collections. This material contains at least 30 new species of fishes and invertebrates (e.g., Baldwin and Robertson, 2013) and, for fishes, each submersible dive has yielded species new to the *DROP* project. This is remarkable considering the small size of the study area and it demonstrates the need for additional sampling. The increasingly comprehensive collection of specimens and DNA sequences resulting from specimens collected during submersible diving is invaluable for ongoing and future diversity and monitoring studies.

Progress also has been made in implementing standardized sampling methods for monitoring deep and shallow reefs in the Seaquarium Plot. In 2012, *DROP* broke new ground by deploying nine autonomous reef monitoring systems (ARMS) at depths up to 224 m using the hydraulic arms of the *Curasub* (Fig. 2). Three additional ARMS were placed on the shallow reef for comparative purposes and 11 oceanographic temperature loggers (set to record hourly temperatures) were deployed along a transect at regular intervals from 15 to 250 m. All of this equipment will remain in place for one year and then be retrieved, processed and redeployed. Successful acquisition of annual data on temperatures and invertebrate/algal diversity on a reef slope from 15 to 250 m will be a unique scientific accomplishment: there is no comparable dataset from anywhere else in the world. Repeated over time, these standardized sampling methods will provide information on long-term changes in temperatures and biodiversity on shallow and deep reefs off Curaçao.

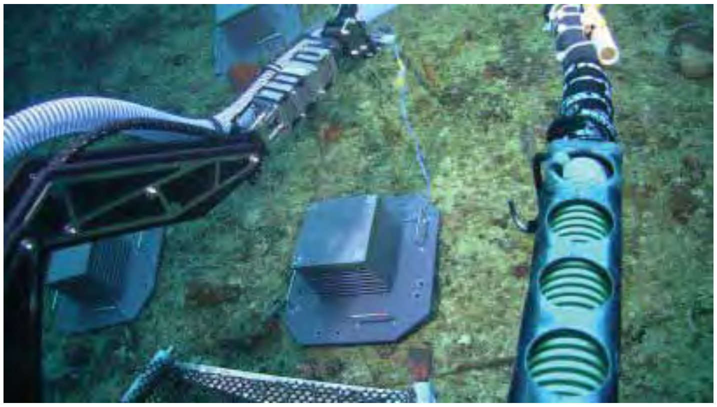

Figure 2. Deploying autonomous reef monitoring structure (ARMS) with the hydraulic arms of the *Curasub* submersible off Curaçao. Photo courtesy of Substation Curaçao.

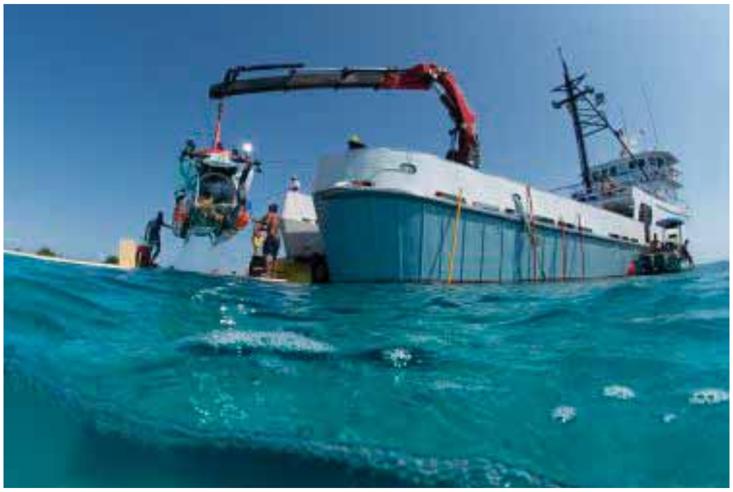

Figure 3. Deploying the *Curasub* at Klein Curaçao from the R/V *Chapman.* Photo by Barry Brown.

Regarding transporting the *Curasub* away from its home port at the Curaçao Seaquarium to investigate other southern Caribbean deep reefs, renovations to a former NOAA ship, the R/V *Chapman*, were completed in 2012 and sub dives are now being made from the shipboard platform (Fig. 3). Smithsonian marine scientists have participated in *Curasub* dives at Klein Curaçao, a 1.7 km^2 uninhabited island southeast of Curaçao, and off Bonaire. Putative new species were collected at both sites and additional dives at Klein Curaçao are planned. Genetic data from dives at the three localities will enable preliminary assessment of genetic connectivity among certain taxa inhabiting geographically distinct reefs. Ultimately, given sufficient submersible collections from numerous Caribbean deep reefs, biogeographic patterns of shallow and deep taxa can be compared.

In conclusion, facilities and staff at Substation Curaçao are enabling exploration and monitoring of deep reefs off Curaçao. Deep and shallow reefs are not isolated ecosystems and their interconnectedness may be significant. Like shallow coral reefs Caribbean deep reefs should be candidates for designation as marine-protected areas but such management decisions require scientific data on a multi-year basis. Results of *DROP* research will be used as a foundation for establishing Curaçao as a permanent site in the Smithsonian’s Marine Global Earth Observatory (SI MarineGEO) and to inform international marine policy related to the establishment of deep reefs as marine protected areas

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RESEARCH DIVING VERSUS RESEARCH DIVING: A MODEL FOR TRAINING TECHNICIANS AND NON-SPECIALISTS

Alex Brylske
Florida Keys Community College
5901 College Rd.
Key West, FLORIDA 33040 U.S.A.
alex.brylske@fkcc.edu

Many scientific diving programs are geared to training undergraduates in the latter stages of their degree, graduate students and practicing scientists. The primary objective is to train them to perform safely in an underwater environment. In this case, given the students' technical background, it is often perceived as unnecessary, perhaps even condescending, to emphasize or even include topics such as the nature of science, the scientific method, data collection, experimental design and other basic concepts/techniques covered in the early education of a scientist. Programs with this perspective might be referred to as Research DIVING courses where diving, rather than research, is the priority. However, other programs have a very different task of taking a relatively competent diver who has no prior scientific education, and training them to perform effectively underwater as a scientific technician. Students in this case are normally first or second year undergraduates, or perhaps not college students at all. In this case, if students are to fully understand their role in a scientific context, they must be given a grounding in basic scientific theory and practice. Because science, rather than diving, is the priority in this case, these programs might be referred to as RESEARCH Diving courses. This latter approach is used in the Marine Environmental Technology degree program at Florida Keys Community College for a required sophomore-level interdisciplinary science course entitled ISC 2132: Basic Research Diving. This paper provides an overview of the FKCC program as a model for others who have a similar goal of training students very early in their careers. The model may be useful as well for programs training "citizen scientists" in a non-academic setting.

Introduction

Scientific diving in the United States developed in the early 1950s to enable and support underwater research operations and the first "scientific divers" were scientists and graduate students. Even today many, if not most, of those who enroll in scientific diving courses are scientists, graduate students or upper-level undergraduates. This population typically has a strong background in their respective scientific disciplines, as well as subjects such as research design and statistical analysis. On the other hand, they often lack a similar depth of understanding and skill in diving. Facing the scenario of "strong scientific, but weaker diving background," scientific diver curricula often emphasize the diving aspect while deemphasizing the science side of the equation. For many scientific diver programs, particularly those at research universities, this approach is valid and appropriate. However, this is not the case in all instances.

Another characteristic of the early days of scientific diving is that programs were based almost exclusively at large research universities, making the profile of the typical scientific diver relatively homogenous. Yet, in the intervening years both scientific diving and scientific divers have become much more diverse. As evidence one merely needs to review the organizational membership of the American Academy of Underwater Sciences (AAUS). While large research universities still represent a major portion of AAUS Organization Members (OM), the Academy membership is now much more inclusive than it was at its inception. Today, entities as diverse as public aquaria, consulting practices, government agencies and community colleges fill the AAUS membership rolls. There are even several OMs that deal exclusively with high school-age students.

Students enrolling in scientific diving programs from these more non-traditional organizations represent very different constituency and program needs. In addition, some students now come into scientific diving courses with extensive diving credentials and experience. However, these students often have little, if any, formal scientific training. This is especially true in the case of the "citizen science" diving programs at public aquaria and other organizations that deal largely with non-specialists. Thus, rather than "researchers," students in this category are probably better viewed as research "technicians."

Unlike traditional scientific diver programs, the needs and goals of this technician group require that the training experience focus on science education as much as, if not more than, diving. Such is the case at Florida Keys Community College (FKCC), which may provide a useful model for other scientific diving programs with similar goals and diver characteristics.

The Marine Environmental Technology Program at FKCC

Given its location in Key West, FKCC has long had a strong commitment to marine science and underwater technology education. The College's Department of Marine Science offers two degrees. One is a traditional Associate of Arts degree in marine and aquatic biology, with a curriculum not unlike that of many other institutions. It also offers a unique Associate of Science degree in Marine Environmental Technology (MET).

The MET degree is designed to prepare students for diverse employment opportunities in marine-oriented careers. During the program students acquire the skills and knowledge necessary to enter the work force in a variety of ocean-based occupations including technicians at research laboratories, environmental consulting companies, public aquaria, marine tourism venues, aquaculture/mariculture facilities and marine restoration projects. A strong foundation in theoretical knowledge is applied through field study in the various marine habitats throughout the Florida Keys. At the conclusion of the program graduates are able to: (1) collect marine related data above and below the water (i.e. on scuba), (2) write technical reports, (3) navigate and operate marine vessels, and (4) understand basic business and management concepts. A steering committee comprised of employers in the marine sciences and environmental industries, as well as state and federal resource management agencies, meets regularly to provide input to FKCC faculty and administrators ensuring that the MET curriculum matches workplace demands.

An overview of the MET curriculum is provided here:

General Education Requirements (15 credits)

- ENC 1101 English Composition (3)
- MAC 1105 College Algebra (3) **OR**
  MGF 1106 Math for Liberal Arts I (3) **OR**
  STA 2023 Introduction to Probabilities & Statistics (3)
- SPC 1608 Introduction to Speech Communication (3)
- Any course from Humanities/Fine Arts (3)
- Any course from Social/Behavioral Science (3)

Core Requirements (36 credits)

- BSC 1010 Principles of Biology I (3)
- BSC 1010L Principles of Biology I Lab (1)
- GEB 2112 Introduction to Entrepreneurship (3)
- ISC 2132 Basic Research Diving (4) *
- MTE 1053C 2 & 4 Cycle Outboard Engine Repair and Maintenance (3)
- MTE 1811 Basic Seamanship (2)
- OCB 1000 Introduction to Marine Biology (3)
- OCB 2102C Marine Data Collection (4)
- OCB 2721C Survey of Mariculture (4)
- OCB 2263C Coral Reef Biology and Management (4) *
- OCE 1001 Introduction to Oceanography (3)
- PCB 2030 Environmental Biology (3)

Area of Specialization (9 credits)

Monitoring & Restoration Track:

- OCB 2107C Monitoring of Caribbean Reef Fish (3) *
- OCB 2132C Restoration of Coral Reefs (3) *
- OCB 2133C Restoration of Seagrass (3)
- OCB 2262C Assessment of Coral Reef Habitats (3) *

Marine Aquaculture Track:

- FAS 1419 Aquaculture Best Management Practices (3)
- OCB 2722C Mariculture Systems and Design (3)
- OCB 2723C Nutrition of Mariculture Species (3)
- OCB 2724C Tropical Ornamental Mariculture (3)
- OCB 2725 Disease and Parasites of Mariculture Species (3)
- OCB 2725L Disease and Parasites of Mariculture Species Lab (1)

Marine Mammals Track:

- OCB 1301C Dolphin Laboratory (3)
- OCB 1311 Marine Mammal Care and Basic Training I (3)
- OCB 2310 Cognitive and Behavioral Research with Marine Mammals (3)
- OCB 2313 Marine Mammal Training and Enrichment II (3)
- OCB 1315 Marine Mammal Rescue (3)
- OCB 2316 Cetacean Anatomy, Physiology and Pathology (3)

Total Credits Required: 62

* Denotes courses that have labs requiring scuba diving activities falling under the FKCC Dive Control Board. Students completing all five of these courses typically have between 50 and 70 logged dives as part of their required lab hours.

Scientific Diver Training at FKCC

At FKCC full Scientific Diver certification requires completion of two courses: 1) EMS 2081C: Scuba Rescue and Emergency Medicine; and 2) ISC 2132: Basic Research Diving. This two-phased approach enables the learning experience to concentrate on diving and emergency management skills in EMS 2081, while allowing ISC 2132 to emphasize the research aspect of scientific diving. This strategy also allows time in ISC 2132 to concentrate on other topics not addressed, or not emphasized, in traditional research diving courses.

As described in the course description, ISC 2132 is an interdisciplinary science course designed to expose students to the tools and techniques for collecting scientific data under water. This course includes a review of the theory and practical application of scientific diving through the use of scuba diving skills and research methodology as applied to the fields of marine biology, physical oceanography and marine archaeology. It carries four semester credit hours. The course objectives include:

1. demonstrate the theoretical and practical understanding of: the nature of science and scientific investigation, what data is and how it's collected, the role of the research diver and various methods used in conducting underwater research;
2. demonstrate the skills necessary to collect data as part of an underwater research team;
3. demonstrate how to plan and organize dives for underwater scientific research; and
4. identify the problems and hazards of research diving, and demonstrate the techniques and procedures needed to minimize their occurrence or impact.

The topics explored during the 60-hour theory and field lab of ISC 2132 include the following:

- The Role of the Research Diver
- The Nature of Science and Scientific Investigation
- Understanding Data and Research Design
- Research Methods
- Underwater Survey Methods
- Advanced Diving Theory
- Introduction to Underwater Photography & Photo-Documentation
- Submerged Cultural Resources & Marine Archeology
- Introduction to Coral Reef Ecology
- Protocols for Coral Reef Monitoring & Assessment (Reef Check)

One advantage to offering a program such as the MET degree at FKCC is that it houses the James E. Lockwood School of Diving and Underwater Technology. The campus contains facilities specifically designed for diver education, such as an Olympic-size pool, a 3,000 square-foot dedicated dive locker capable of outfitting 35 scuba divers and six surface-air-supplied divers and a 1,500 square-foot hyperbaric facility with two recompression chambers.

Open water training at FKCC is facilitated by an on-campus, five-acre 45-foot deep Underwater Training Area (UTA). This facility was originally a quarry dug for construction fill before the campus was built in 1966, and offers complete protection from adverse weather and sea conditions enabling open water training 365 days per year.

For ISC 2132, the UTA allows effective skill diving skill assessment and remediation before entering the more challenging offshore environment. Navigation training and a rescue workshop are held, as well, in the UTA before any offshore diving activities. About 20 years ago the treasure hunter Mel Fisher placed 24 timbers excavated from the wreck of the *Nuestra Senora de Atocha* in the UTA for use in training FKCC students in underwater archeology. The Florida Keys National Marine Sanctuary (FKNMS) has also placed in the UTA several historic artifacts confiscated from illegal salvors. These are all used during ISC 2132 to teach underwater surveying, trilateration and photo-documentation. The UTA is also used to provide training to research diving students in more advanced diving technologies such as full-face mask systems, and in showing how diving operations can augment remote sensing technologies such as sector-scan sonar.

The offshore portion of ISC 2132 is facilitated by an agreement between FKCC, NOAA and the Florida Department of Environmental Protection for access to the 55-foot Research Vessel *Dante Fascell*. In this portion of training students dive several sites within the FKNMS. Also, since 2010, as part of the maritime archeology component of ISC 2132, students have been working in cooperation with the Mel Fisher Maritime Museum documenting an historical shipwreck (the largest vessel ever built in the

Bahamas) in Key West Harbor, the schooner *Marie J. Thompson*. In addition, students have the option of interning at the Museum's conservation laboratory.

Students are introduced to the biological aspects of research diving through the coral reef monitoring protocol, Reef Check®. They are also taught quadrat use and rugosity calculation. Each semester students complete a team research project entitled, *A Comparison of the Community Structure and Condition of a No-Take Reserve and Unprotected Site Within the Florida Keys National Marine*. The culminating activity of the course is an oral presentation of research results, and submission of a report using appropriate scientific structure and formatting. The project is graded using both a rubric- and peer-evaluation. Depending on weather conditions, ISC 2132 students make between 12 and 18 open water dives during the term.

Conclusion

Although some of the ISC 2132 curriculum is specific to FKCC's program goals, location and facilities, the course content and approach do have broader applications for other scientific diving programs. While programs at research universities may have no need for a course with such extensive treatment of basic science education, this template may have great utility for programs geared to lower-division undergraduates who have not yet completed much formal course work in a scientific discipline. Programs training citizen scientists at non-academic institutions may also find ISC 2132 a useful training template.

Note: Copies of all ISC 2132 course materials are available by contacting the author.

FUNGAL DIVERSITY IN MARINE SPONGES FROM HIGHLY DIVERSE AREAS IN THE ISTHMUS OF PANAMA

***Catherina Caballero-George*[1]**
***Jessica Bolaños*[1]**
***Luis Fernando De León*[1]**
***Edgardo Ochoa*[2]**
***José Darias*[3]**
***Luis D´Croz*[4]**
***Huzefa Raja*[5]**
***Andrea Porras-Alfaro*[6]**

[1]Institute for Scientific Research and High Technology Services
Apartado 0843-01103
Panama, REPUBLIC OF PANAMA
c.caballerogeorge@gmail.com

[2]Smithsonian Tropical Research Institute
Apartado Postal 0843–03092
Panama, REPUBLIC OF PANAMA

[3]Instituto de Productos Naturales y Agrobiología
Tenerife, ESPAÑA

[4]University of Panama
Panama, REPUBLIC OF PANAMA

[5]Department of Chemistry and Biochemistry
The University of North Carolina
Greensboro, NORTH CAROLINA 27402 U.S.A.

[6]Department of Biological Sciences
Western Illinois University
Macomb, ILLINOIS 61455 U.S.A.

Fungal diversity studies using cultured-based and isolation-independent methods have shown that fungi occupy an important ecological niche in the marine environment. Although it has been suggested that the culture-based approach overlooks a large diversity of fungi, this method is still favored by research programs aimed at studying the biotechnological potential of fungi. Interactions between fungi and marine sponges are far less studied when compared with interactions between sponges and bacteria in terms of host specificity and microbial diversity. Living, apparently healthy, marine sponges were collected by scuba from 12 sites along the Caribbean and Pacific coasts of the Isthmus of Panama, at depths between 3 and 30 m. Sponges were collected in an area of ca. 3 m^2 at each site. Measures were taken to prevent cross contamination among samples during collection and isolation of the fungi. Total genomic DNA was extracted from 570 representative fungal strains and the ITS and LSU regions of the nuclear *ribosomal DNA were amplified and sequenced bidirectionally. Sequences were compared against the NCBI GenBank database using BLAST to estimate taxonomic placement. A total of 198 Operational Taxonomic Units (OTU) was found with 58% of the OTUs represented by singletons. Marine sponges were highly dominated by fungi within the Ascomycota (80.9%), with a minority within Basidiomycota (2.8%). Ascomycota fungi were represented by several major lineages, including Sordariomycetes, Dothideomycetes and Eurotiomycetes. Species accumulation curves for both geographical region and sponge orders indicated no obvious saturation. Several unique clades were found during phylogenetic analysis. Our results indicated that marine sponges from Panama could be a "hot spot" of fungal diversity as well as a rich resource for capturing, cataloguing, and assessing the pharmacological potential of previously undiscovered fungi associated with marine sponges.*

Introduction

Fungi occupy an important ecological niche in the marine environment. This is revealed by phylogenetic analysis of increasingly available molecular data which promise to unravel the yet to be described marine fungal diversity (Manohar and Raghukumar, 2013). Sponges can harbor microorganisms that are either acquired by the surrounding water or by parental sponges through reproductive stages (Taylor, 2007). Interactions between sponges and bacteria are well studied and characterized in terms of host specificity and bacterial diversity (Taylor, 2007; Simister et al., 2012; Taylor et al., 2012),; studies regarding archaea and fungi interacting with sponges are far less numerous. Although, it has been suggested that culture-based methodology overlooks a large diversity of fungi, this method is still favored by research programs aimed at studying the biotechnological potential of fungi (Blunt et al., 2009; Caballero-George et al., 2010; Kjer et al., 2010; Liu et al., 2010).

The Pacific shelf of Panama is wide and is divided by the southward projecting Azuero Peninsula, into two large areas: the Gulf of Panama (east-side) subject to strong seasonal wind-driven upwelling, and the Gulf of Chiriquí (west) where high mountains block the wind and prevent wind-induced upwelling (D'Croz and O'Dea, 2007). In contrast, the Caribbean coast of Panama is relatively straight with a narrow continental shelf exposed to ocean water except for the Bocas del Toro Archipelago on the west side, where rainfall and river discharge are higher (D'Croz et al., 2005; Collin et al., 2009). Thus, Panama´s "four oceanic zones" provide unique opportunities for understanding how and why low-latitude marine ecosystems vary and function as they do (Robertson et al., 2009). We used these widely differentiated oceanographic zones to compare fungal communities associated with 39 species of sponges collected by scuba in areas of high biodiversity.

Methods

Living, apparently healthy, marine sponges were collected by scuba at depths between 3 and 30 m from 12 primary sites locations along the Caribbean and Pacific coasts of the Isthmus of Panama (Figure 1). Three individuals of each sponge species were collected in an area of ca. 3 m^2 at each site. To prevent cross contamination among samples, each sponge was cut and sealed *in situ* (Figure 2) in an individual plastic bag after removing excessive water; they were processed for fungal isolation within 2 h. Sponges were identified following Zea (1987); Hooper and Van Soest (2002); and Collin et al. (2005). Collected sponges represented the following orders: Chondrosida, Dendroceratida, Dictyoceratida, Hadromerida, Halichondrida, Haplosclerida, Homosclerophorida, Lithistida, Poecilosclerida, Spirophorida, and Verongida.

Isolation of sponge-associated fungi was carried out under sterile conditions in a laminar flow cabinet (Portable Clean Air Unit, Liberty Industries). Whole fresh sponges were placed on a sterilized strainer and washed thoroughly with sterile artificial seawater (ASW, adjusted to 36 g/l for Caribbean sites and 32 g/l for Pacific sites). Samples were pressed on sterile, absorbent paper and cut with a sterile scalpel into ca. 1 x 0.5 cm pieces, making a cross section from the osculum to the holdfast. Pieces were washed three times with sterile ASW and dried as above. From the cleaned mesophyll of each sample, 50 cubes of 2-3 mm^3 were cut. Ten cubes were placed onto each of five solid isolation media (P15, P30, EM, M1D, SNA) prepared prior to sterilization in 1 l volumes of distilled water (Caballero-George et al., 2010).

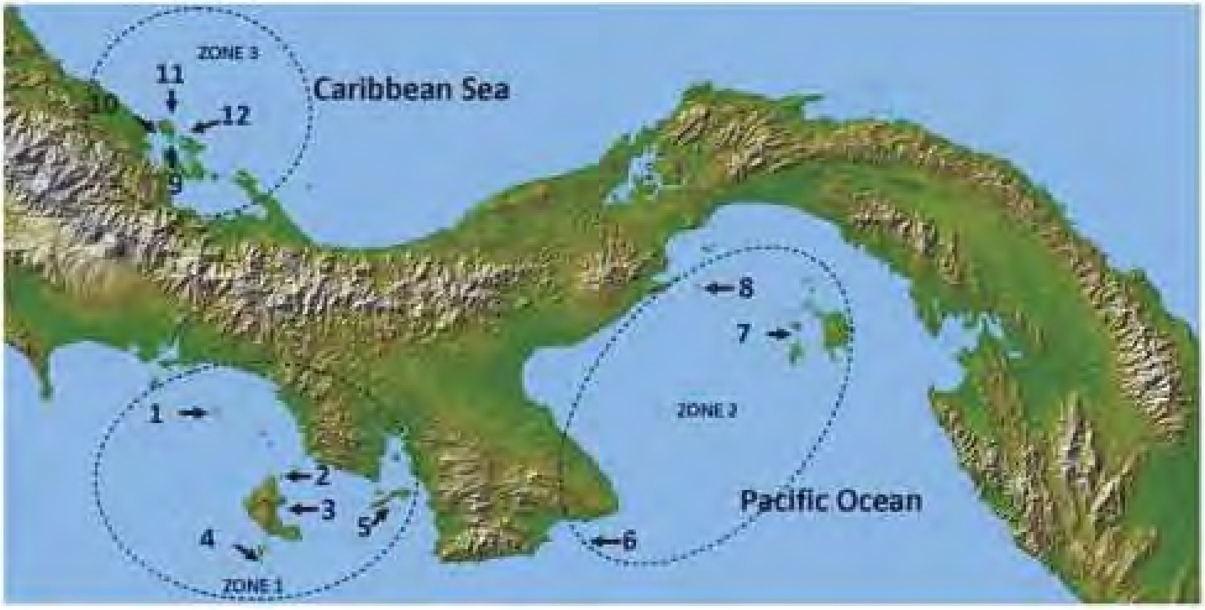

FIGURE 1 Distribution of sites for collection of sponges in the Republic of Panama. 1) Secas Islands, Gulf of Chiriquí; 2) Rancheria Island, Coiba National Park; 3) Bahia Damas, Coiba National Park; 4) Jicarón & Jicarita Islands, Coiba National Park; 5) West of Cébaco Island, Veraguas; 6) Frailes & Monjas Islands, Los Santos; 7) Las Perlas, Gulf of Panama; 8) Otoque & Boná, Gulf of Panama; 9) Airport, Bocas del Toro; 10) STRI's Point, Bocas del Toro; 11) Punta Caracol, Bocas del Toro; 12) Wild Cane Key, Bocas del Toro.

Figure 2. Diver cutting and sealing sponge individually in situ.

Total genomic DNA was extracted from 570 representative fungal strains; the Internal Transcribed Spacers (ITS) and Large Subunit (LSU) of the nuclear ribosomal DNA were amplified in a single reaction using ITS5 (or ITS1) and LR3 (or ITS4) primers (White et al., 1990) and sequenced bidirectionally. Sequences were grouped at 97% similarity, 40% overlap in sequencher as a proxy for species definition. OTUs were confirmed comparing the sequences against the NCBI GenBank database using BLAST analysis. The following criteria were used to interpret the sequences of the GenBank database: for sequence identities ≥97%, the genus and species were accepted; for sequence identities between 91% and 96%, only the genus was accepted; and for sequence identities ≥90%, isolates were labeled as the order or family name. All three criteria were defined on the basis of sequence similarity over shared sequence lengths with at least 80% overlap. We used the Shannon-Weaver diversity index in order to estimate the diversity of isolated fungal species from different regions (Caribbean, Pacific) and from different sponge orders. We used analysis of variance (ANOVA) to compare relative abundance of fungal species as a function of regions and sponge orders. Finally, we generated accumulation curves to test for the cumulative number of fungal species in relation to our sampling effort. These analyses were performed using the R (R Development Core Team, 2008).

Results

A total of 198 different species were found as defined by the Operational Taxonomic Units (OTU) with 58% of the OTUs found only once (singletons). The majority of the isolated fungi were Ascomycota (80.9%), with a minority within Basidiomycota (2.8%). Ascomycota fungi are represented by 15 orders dominated by Hypocreales, Xylareales and Pleosporales (Figure 3a) while Basidiomycota was represented by Agaricales, Polyporales and Russulales (Figure 3b).

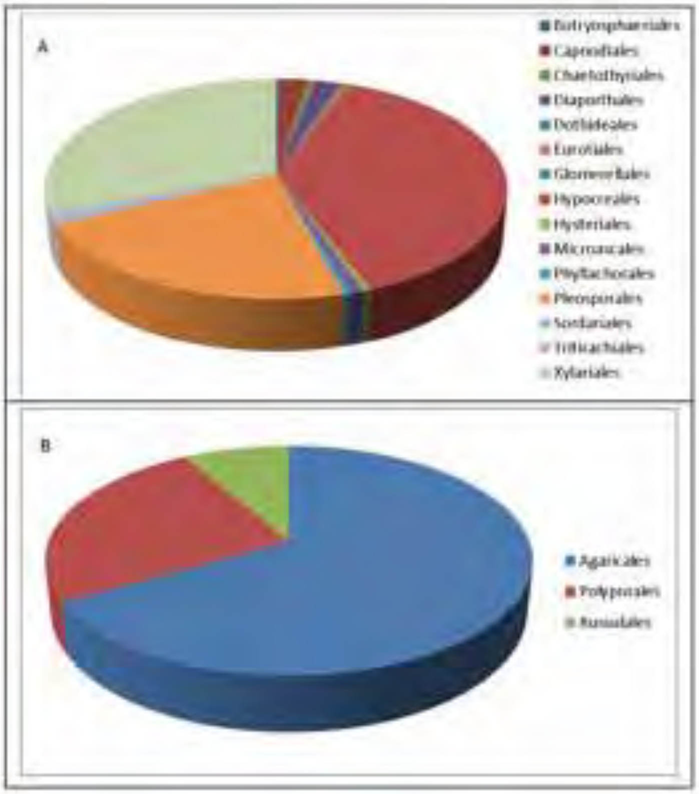

Figure 3. Graphical representation of isolated sponge-associated fungi from Ascomycota (A) and Basidiomycota (B) grouped by orders.

Shannon-Weaver diversity index revealed a high diversity of cultivable fungi, and this diversity was comparable between the two geographical regions (3.51 for Caribbean, 3.52 for the Pacific). The following diversity indices were found for fungi isolated from different taxonomic orders of sponges: Dictyoceratida (1.20), Chondrosida (1.68), Hadromerida (1.68), Haplosclerida (2.74), Haplosclerida Verongida (2.55), non-identified species from the Caribbean (N.I.C) (3.40), Spirophorida (1.09), Homosclerophorida (2.45), Halichondrida (2.10), Poecilosclerida (3.02), non-identified species from the Pacific (N.I.P) (1.53). The analyses of variance indicated significant differences in the relative abundance of fungal species between geographical regions ($F_{(2)}$= 31.2, $P<0.001$) and among sponge orders ($F_{(10)}$= 7.55, $P<0.001$). Finally, our species accumulation curves for both geographical region and sponge orders indicate an increasing trend of species accumulation with no obvious saturation (Figure 4).

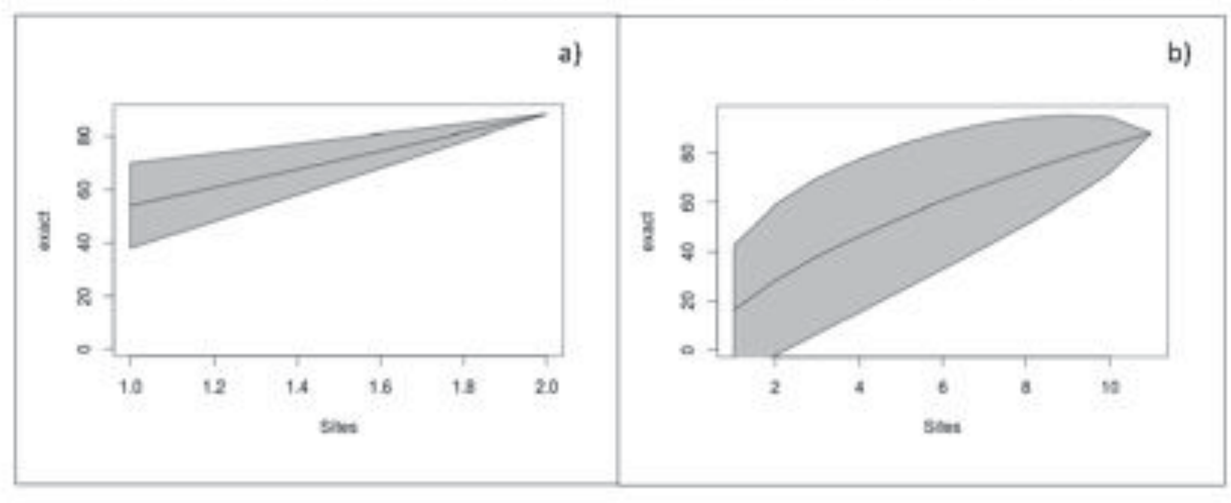

Figure 4. a) Species-accumulation curves per site of collection; b) Species-accumulation curves per taxonomic order of host sponge.

Effect of isolation media on isolation of sponge associated-fungi

In general, isolation media based on malt extract (Baker et al., 2009; Caballero-George et al., 2010) like EM appeared as a good culture media to obtain a high number of fungal isolates. Nevertheless, the question remains whether these high numbers also involve a high diversity. Figure 5 shows that EM media allowed isolation of the highest number of different fungal species (25%) followed by P15 and P30 (20%), which are based on glucose, peptone and yeast extract (Caballero-George et al., 2010). However, fungal species like *Armillaria tabescens* and *Microdiplodia hawaiiensis* were only found in M1D culture media, and *Colletotrichum gloeosporioides*, *Microdiplodia miyakei*, *Corynespora cassiicola* and *Eutypella kochiana* were only found in PD culture media. In the present study, fungi from the order Hypocreales, particularly the genera *Acremonium* and *Hypocrea* would grow more readily on EM than the other five media. Interestingly, the genus *Fusarium* showed equal preference for media P15, SNA and EM, while *Lecanicillium kalimantanense* showed no preferences. The order Dothideales and unidentified strains of Ascomycota had equal preference for media EM as well as P30. Fungi from the order Xylariales had equal preference to grow in EM and P15. This was also the case for *Eutypa consobrina*. However, *Eutypella sp*. was isolated mainly from P15. Interestingly, *Annulohypoxylon stygium*, *Anthostomella conorum*, *Arthrinium phaeospermum* and *Cryptosphaeria eunomia var. eunomia* were isolated from medium EM and *Daldinia cf. loculatoides* was isolated only from PD and SNA. Fungi from the order Pleosporales were isolated mainly from media P30. The order Diaporthales was isolated in media EM, P30 and PD and the order Eurotiales was only isolated with PD and EM. The order Agaricales showed no preference for any media.

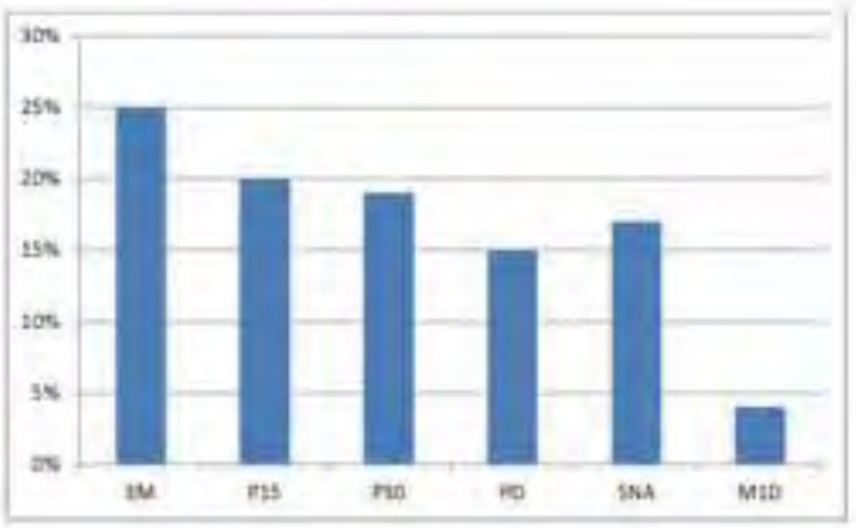

Figure 5. Number of different species of fungi isolated with each type of culture media.

Discussion

Very little is still known about diversity, nature of association and ecological significance of sponge-associated fungi. It is crucial to improve this knowledge in order to estimate a more precise fungal diversity (Richards et al., 2012) and to maximize their biotechnological application (Suryanarayanan, 2012).

The majority of the fungi isolated from this study belong to the Phylum Ascomycota and many branched close to clades of known terrestrial fungi. Marine ascomycetes are described as primary or secondary inhabitants of marine environments (Kohlmeyer, 1986). Primary marine species are hypothesized to be derived from ancestral lineages that originated in marine environments, while secondary marine species represent the reintroduction of fungi into the marine environment and are suggested to share a more recent common ancestry with terrestrial lineages (Spatafora et al., 1998).

Most of the current knowledge about the diversity of sponge-associated fungi comes from culture-based studies that aim to discover pharmacologically active metabolites within individual strains. Although these studies are conservative and might not be truly representative of natural diversity (Richards et al., 2012) they remain the goal of research programs aimed at isolating bioactive compounds from fungi. This is why special efforts have been made to optimize culture media in order to isolate the largest possible diversity. In the present study, media based on malt extract was suitable to isolate a large number of fungi with the highest number of different species. Nevertheless, isolates like M2034-2-M1D (*Microdiplodia hawaiiensis* DQ885897 ID 90%) and M188I-1-M1D (*Armillaria tabescens* AY213589 ID 83%) that were found only once came from isolating media that yielded a low number of isolates such as M1D. This suggests that the use of diverse cultivation media increases the efforts to capture a large diversity of fungi. Our results suggest that fungi from different taxonomical orders may show preferences regarding culture media. This knowledge might facilitate the isolation of specific groups of fungi.

Together, these results suggest that there is a high diversity of sponge-associated fungal species in neotropical waters and that this diversity varies both between geographical regions and among host-sponge species. Interestingly, our results also indicate that our sampling design was rather limited and that a more systematic effort could help in a better understanding of the species richness and diversity of sponge-associated fungi.

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SCIENTIFIC DIVING FOR GEOLOGICAL RESEARCH: EXAMPLES FROM ITALY

Giorgio Caramanna[1,2]
[1]Italian Association of Scientific Divers (AIOSS)
c/o Centro Interdipartimentale di Ricerca per le Scienze Ambientali
Alma Mater Studiorum
Universitá di Bologna
Via S. Alberto 163
48123 Ravenna, ITALY
giorgiocaramanna@yahoo.it

[2]Heriot-Watt University
School of Engineering and Physical Sciences
Edinburgh, SCOTLAND EH14 4AS UNITED KINGDOM
G.Caramanna@hw.ac.uk

The Italian landscape is characterized by a widespread presence of limestone and karst phenomena with the formation of caves and sinkholes often hosting springs or small lakes. Scientific diving is a powerful tool to study the geological features of these cavities as it allows for data collection on geomorphology, palaeontology, palaeoclimatology and hydrogeology. Case studies are illustrated of geological investigations in karst springs, sinkholes and submarine caves. Owing to the potentially hazardous environments, sound procedures, training and specific methodologies for data collection have been developed and applied. In all of these situations, direct exploration by scientific diving permitted the collection of information which otherwise would not have been possible to attain.

Introduction

The geology of Italy is characterized by a large presence of both limestone outcrops and bedrock. The climatic and pluvial regime, temperate with an abundance of rain mostly on higher grounds, is favorable for the development of karst phenomena. Caves and sinkholes are widespread throughout the country and often host small lakes or springs (Societá Speleologica Italiana, 2013). The genesis of sinkholes in Italy is related not only to the presence of calcareous rocks, which originate dissolution-sinkholes classifiable as dolines (Delle Rose and Parise, 2002), but also to specific hydrogeological and geochemical conditions where rapid groundwater flows. This is eventually associated with chemically-aggressive fluids rising through fractures in the bedrock that may destabilize sedimentary covers of poor physical-mechanical characteristics thereby generating sudden collapses of these materials. Other triggering mechanisms are associated with human-induced vibration, earthquakes and abrupt variation of the groundwater level such as those following floods or droughts (Bono, 1995; Salvati and Sasowsky, 2002; Nisio et al., 2007; Caramanna et al., 2008). The presence of travertine deposits inside or surrounding flooded sinkholes is a relatively common feature, which highlights the presence of mineralized fluids carrying high-concentrations of carbonates originated by the dissolution of calcareous bedrock (Tuccimei et al., 1886; Caramanna and Bono, 2001; Caramanna et al., 2012).

When studying flooded caves and sinkholes, it is possible to collect data on a variety of subjects including hydrology, geomorphology and the potential impact of pollutants that may enter the groundwater. These environments are also isolated niches where endemic life forms may develop along gradients of light and, in cases such as marine caves hosting fresh-water springs, gradients of salinity. The speleothems inside the caves often retain important geological records of paleoclimatic events.

Scientific diving is a useful method for researchers to reach environments that otherwise would not be possible to study. Ambient conditions such as overhead restrictions, reduced visibility, cold water and potentially toxic fluids, mean that dedicated training and robust procedures must be applied, not only for the safety of the divers but also for the reliability of the gathered data.

Examples of scientific diving activities for geological research in flooded environments are illustrated in this paper. The sinkhole Lake Paterno (in northern Latium) was studied to achieve information on its stratigraphic setting and on its connection with local and regional groundwater. The karst spring of La Foce (in southern Latium) was explored for the first time, and the identification of the source of volcanic sands inside the main conduit gives important clues for the potential extension of the cave. A speleothem was retrieved from the marine cave Argentarola (in southern Tuscany) through a complex operation leading to important paleoclimatic observations. Lastly, a marine cave along the peninsula of Maddalena (in Sicily) was surveyed.

Methods

Because of potential hazards in the studied areas, specific diving techniques were used. Scientific diving in environments presenting overhead obstructions is a demanding activity requiring knowledge of techniques and procedures commonly used in cave diving. The main rules, in summary, are: use a line as a continuous connection with the exterior of the cave, which should also indicate the distance from the nearest exit; use multiple light sources; use independent breathing-gas equipment and apply conservative rules for breathing-gas reserve management; be confident in emergency procedures including de-tangling, searching for lost line, and removal of diving gear (Palmer, 1986; Iliffe and Bowen, 2001; Ward et al., 2008; Caramanna et al., 2012a).

The geomorphological survey of the studied environments required both surface-orientated and submerged methods. The bathymetric map of the sinkhole Lake Paterno was obtained by sounding the lake from a small inflatable boat along a series of transects aligned on four main directions (N-S, W-E, NW-SE, NE-SW). Each transect was performed by fixing a mooring point along the shore and deploying a metered line from the boat until the opposite point on the shore was reached. Several buoys were used to float the line above the lake’s surface. Once the line was fixed, the depth was measured every 10 meters along the transect or, where sharp changes of the bathymetry were observed, more often using a weighted metered line. A section of the lake was surveyed by scuba diving in order to identify the stratigraphy and composition of the sinkhole’s walls. In this case, a metered line was deployed along the wall as a horizontal reference while a digital depth gauge was used to track vertical dimensions of the observed features. Plastic corers were used to collect samples of the sediments for further analysis.

The submerged cave of the Maddalena peninsula was surveyed using a metered line deployed on the floor of the cave along its axial line. The cave was divided in twelve subsections; the azimuth of each section was measured using a compass. Lateral dimensions were measured using secondary metered lines; vertical dimensions were measured using a digital depth gauge. A map and a vertical section were produced.

A complex sampling procedure was necessary for the collection of a large stalagmite from inside the Argentarola cave. The main issue was related to the presence of large quantities of extremely mobile silt accumulated on the floor of the cave that was a serious problem potentially leading to reduced visibility and, therefore, enhanced hazardous conditions for the divers. Before removal of the stalagmite, which was tightly encased inside the sedimentary cover, a safety line to be used as reference guide for the divers was deployed along the walls from outside the cave entrance to the sampling area. An airbag was used to lift and free the stalagmite from the sediments; a second thicker line was fixed close to the ceiling to be used as cable-rail for the movement of the airbag carrying the stalagmite's weight. Despite the highly skilled divers employed for the operation, the complexity of the required procedures (which included wrapping the stalagmite in a harness, connecting the airbag, linking and pulling the system along the line) caused the sediments to be stirred up. However, the divers managed to safely reach the exit of the cave in almost zero visibility. This confirms the importance of having guidelines in place and the correct management of breathing-gas reserves. Breathing gas management needed to take into account the extended time required for performing any kind of operation under difficult environmental conditions, such as the extremely poor visibility.

For hydro-chemical measures in many of the studied areas, samples of water were collected under water by divers using 0.5 l plastic sampling bottles. The bottles were carried underwater capped and filled with de-ionized water to avoid their implosion. Once at the desired sampling depth, the bottle was opened, placed upside-down, purged using an air-source, filled with the water sample and then capped. A simple rack was designed and used to carry up to eight bottles underwater at the same time.

Case studies

In the following case studies, researchers extensively used scientific diving as a valuable tool for achieving their goals. The case studies include a sinkhole, freshwater and marine caves. In each environment, a variety of operations were conducted ranging from geomorphologic survey to specimen sampling.

The sinkhole of Lake Paterno

The sinkhole of Lake Paterno is the largest of a series of sinkholes in the S. Vittorino Plain (northeast Latium, central Italy). The plain has a sub-triangular shape and is bordered by direct and transtensive faults generating a pseudo-graben structure. Limestone outcrops surround the area and the calcareous bedrock is buried below the sedimentary cover. The sedimentary cover is composed of fluvial and lacustrine deposits (sand, gravel, clay and local travertine) with thickness up to 170 m in the center of the plain (Centamore and Nisio, 2003; Nisio, 2003).

A series of springs are aligned along the northern and southern borders of the plain, which acts as drainage basin. The main recharging area is the karst limestone ridge of the Nuria-Velino mounts covering an area of more than 1,100 km^2 with annual average rainfall of 1,250 mm and average infiltration of 800-1,000 mm. The main springs are the Peschiera springs with a flux of 18 m^3/s, of which 9.5 m^3/s are used for the main aqueduct of the city of Rome (Bono, 1993). Other smaller springs feed small ponds and lakes along the plain; several springs emit mineralized fluids (CO_2 and H_2S) and migrated over time in correlation with seismic events (Nolasco, 1998; Solenghi et al., 1999).

Lake Paterno has been well known since ancient times. Diogenes of Halicarnassus (1st century B.C.) describes the lake in his “Historiae” with the name of “Lacus Cutiliae” (from the name of the nearby thermal springs). The presence of travertine deposits along its shore indicates that highly mineralized waters were once flowing in the lake. The lake has a sub-circular shape with main diameter of 180 m, minor diameter of 165 m and maximum depth of 55 m (Fig. 1). The bottom of the lake is covered by fine unconsolidated silt. The thickness of the silt deposits has been measured up to 0.8-1.0 m using a steel probe. Below the silt, solid bedrock is encountered. A vertical section along the southern shore of the lake highlighted the presence of travertine layers, clay deposits, sand and clay, and marl (Fig. 2).

The lake is drained by a small artificial channel and has no inlet; therefore, the source of the water should be a submerged spring. It is reasonable to assume that the travertine acts as permeable barrier allowing the local water table to filter inside the lake. The limited water circulation leads to high levels of evaporation, mostly during the warmer summer season, as highlighted from the $\delta^{18}O$ values (-6.45) that shows an enrichment in the heaviest oxygen isotope. The lake has thermal stratification in summer while during the winter the water column is more homogenized with a very limited inverse thermal stratification below 25 m (Table 1). The water chemistry is typical of low mineralized carbonate aquifer (Table 2).

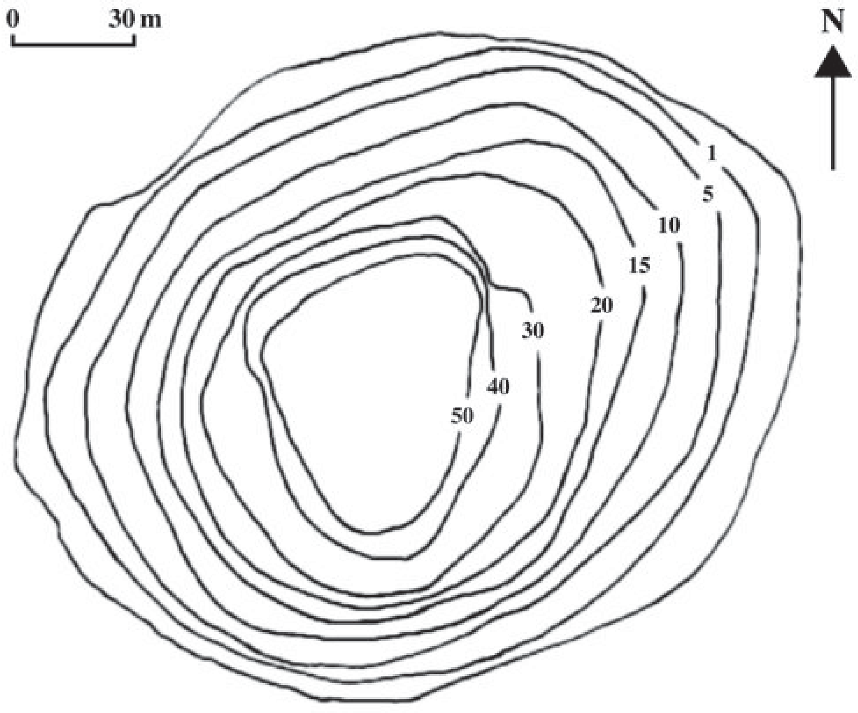

Figure 1. Bathymetric map of the sinkhole Lake Paterno.

Table 1. Temperature along the water column in Lake Paterno, winter versus summer.

Depth (m)	Winter (°C)	Summer (°C)
0	6,0	23,7
0.50	6,0	23,6
1	6,0	23,4
2	6,0	23,1
3	6,0	22,9
4	6,0	18,1
5	6,0	13,6
6	6,0	10,9
7	6,0	8,7
8	6,0	7,6
9	6,0	7,0
10	6,0	6,8
15	6,0	6,7
20	6,0	7,1
25	7,1	7,1
30	7,1	7,1
35	7,0	7,0
40	7,0	7,0
45	7,0	7,0
50	7.1	7.1
55	7.0	7.1

Table 2. Main ions composition, electric conductivity (E.C.) and total dissolved solids concentration (TDS) of water in the sinkhole of Lake Paterno.

Depth (m)	**T (°C)**	**pH**	**E. C. (µs/cm)**	**TDS (mg/l)**	**Ca^{++} (mg/l)**	**Mg^{++} (mg/l)**	**Na^{+} (mg/l)**	**K^{+} (mg/l)**	**HCO_3^{-} (mg/l)**	**SO_4^{--} (mg/l)**	**Cl^{-} (mg/l)**
50	5.0	6.96	688	392	62	8	5	2	292	6	7
30	5.3	6.93	684	390	52	8	5	2	231	6	9
15	5.5	7.16	646	368	62	8	5	2	265	7	8

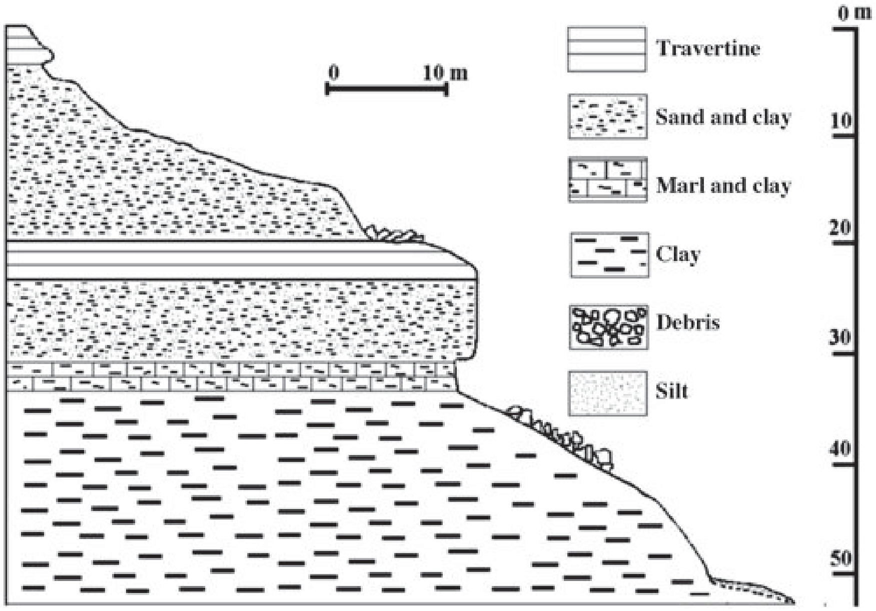

Figure 2. Lithological section of Lake Paterno.

The origin of this sinkhole may be related to dissolution of travertine lenses and/or deep carbonate bedrock followed by the collapse of the overlying sedimentary cover. The observation of levels of travertine inside the lake supports this genetic hypothesis.

La Foce cave

A few km south of the village of Trevi nel Lazio in the calcareous outcrops of the Ernici Mounts lies the cave of La Foce. From the mouth of the cave, a semi-perennial spring feeds a creek that merges in the high section of the Aniene River. The area is characterized by the presence of a large number of karst springs and caves often aligned along regional structural lineaments.

The cave has been known since the 1800s, and was explored during a dry period in 1969 from the entrance to an inner lake. In 2001, a cave diving exploration revealed the presence of abandoned guide lines likely from former explorers in the late 1990s (Mecchia et al., 2003). In 2004-2006, a new explorative phase, the use of semi-closed rebreathers allowed a depth of 105 m to be reached at about 900 m from the entrance. This exploration is still in progress.

La Foce cave can be divided into two sections. The first 600 m is a sub-horizontal conduit with the presence of speleothems. During the summer season, this section is partially dry (Fig. 3). The second section is in the phreatic zone showing clean walls and no presence of speleothems (Fig. 4). A large accumulation of black sand (Fig. 5), which was identified by XRD analysis to be of volcanic origin, is present inside the phreatic conduit. The origin of the sand is likely from volcanic deposits along the

Apennine ridge, the closest of which is about 5 km from the cave, which suggests a large extension of the cave.

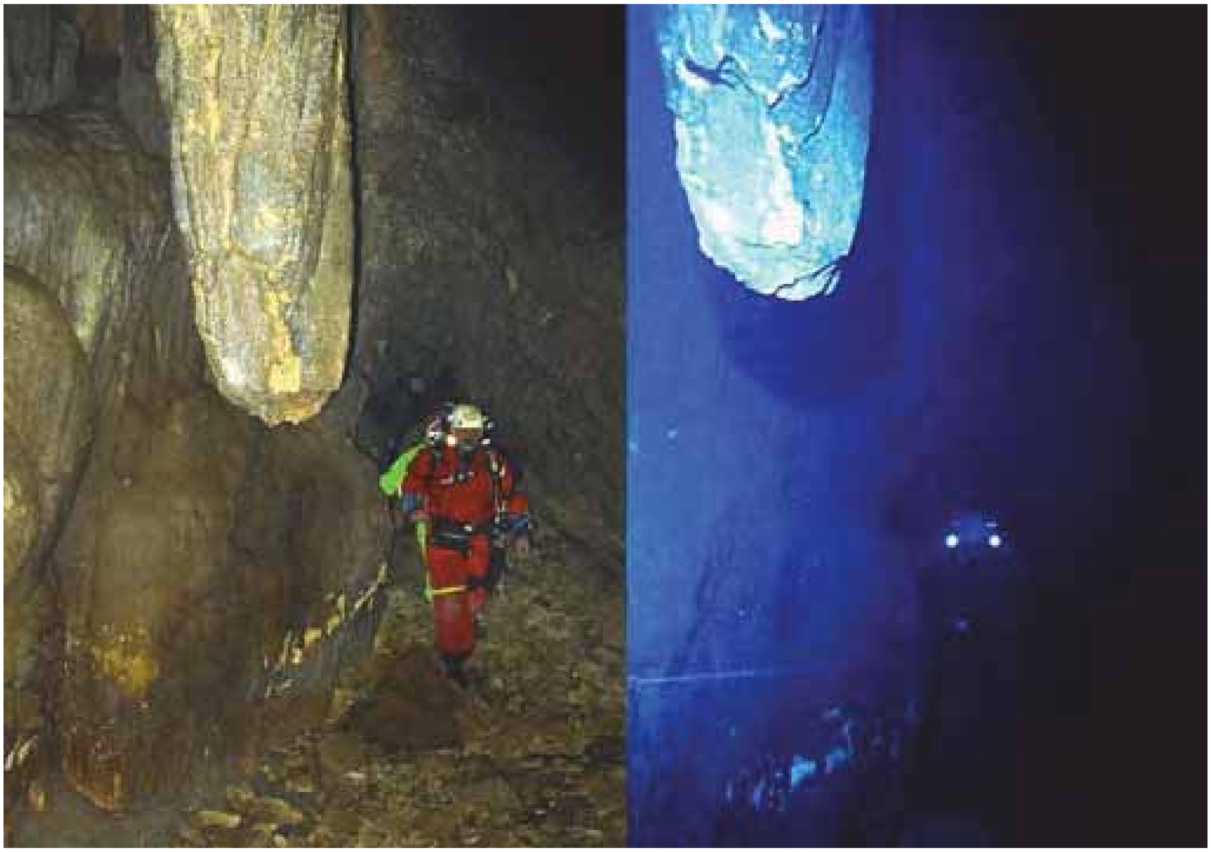

Figure 3. La Foce cave, dry versus flooded condition of the vadose sector.

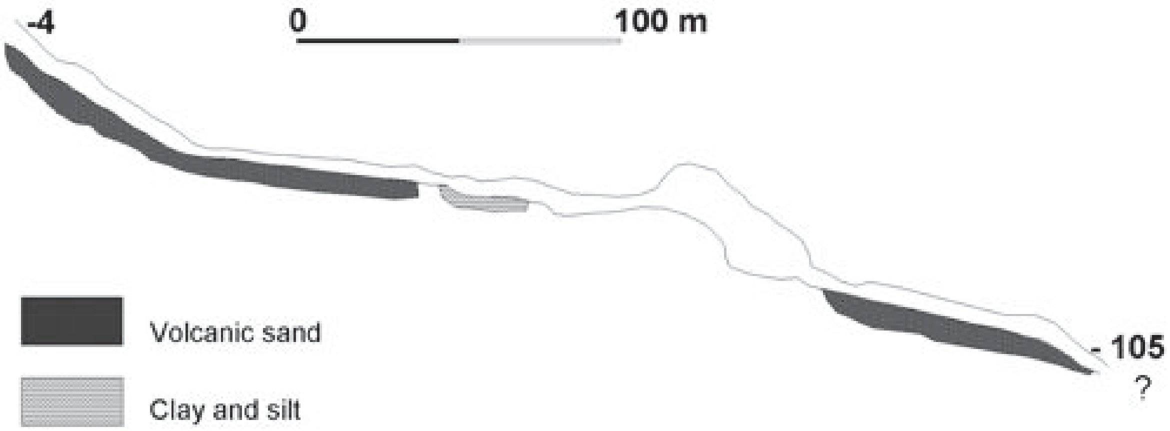

Figure 4. La Foce cave, simplified section of the deeper vadose sector

Water samples collected inside the cave have medium mineralized values with pH 7.25, electric conductivity of 351 µs/cm and total alkalinity (bicarbonates) of 177 mg/l. The water temperature is between 4 and 6^{o}C.

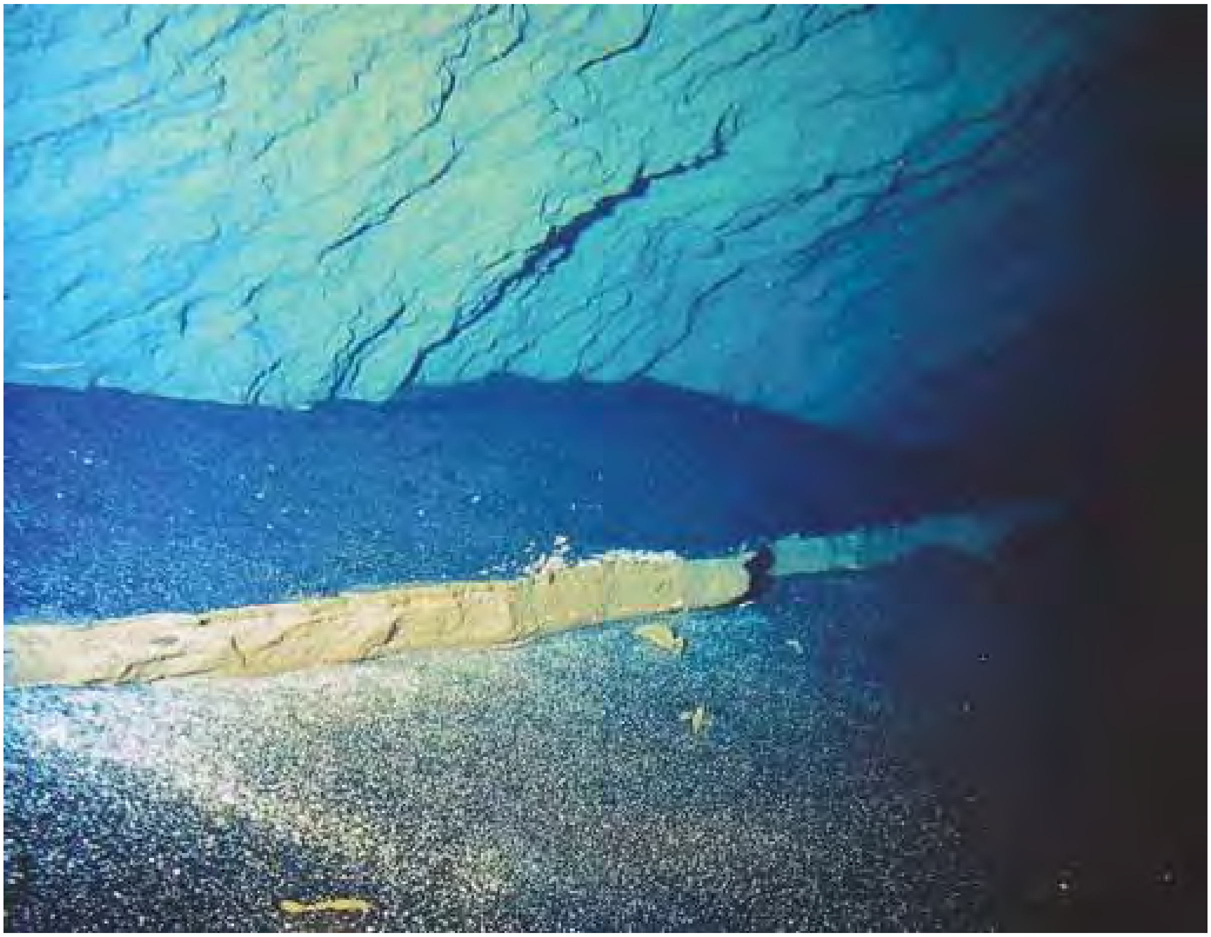

Figure 5. La Foce cave, accumulation of black volcanic sand inside the phreatic sector.

The hydrologic regime of the cave shows a correlation with the melting of the snow leading to high-fluxes during late spring early summer. In late summer, and sometimes during dry winters, the first section of the cave is partially dry. These hydrologic conditions allow only a few weeks per year for diving in the cave when the flux is not too high or too low; the latter case makes it impossible to proceed with diving gear in the semi-dry section because of a large accumulation of mud.

Argentarola cave

This cave derives its name from a small islet close to the shore of the Argentario promontory (Tuscany, Italy). The islet is composed of dolomitic limestone in monoclonal setting with NNW 45° dip (Carta Geologica d'Italia, 1968).

The entrance to the cave is a narrow fracture on the southeast cliff of the island at about 6 m of depth (Fig. 6). The main cave is a large room reaching a maximum depth of about 20 m. A secondary tunnel connects the cave with a deeper entrance at approximately 30 m of depth but it is now completely filled with sediments and, therefore, the only available entrance is the shallow one. Because of the low-energy environment, a large accumulation of fine silt covers the floor of the cave and any stirring of the deposits makes the visibility almost zero. Once the deposits have been stirred, several days are needed for the silt to redeposit and for the water to clear.

The geological importance of the cave is that it is in a very stable region and was cyclically flooded during the marine high stands in Middle and Late Pleistocene. This, coupled with the relative isolation of the cave caused by the small dimensions of the entrance, allowed the preservation of a large number of speleothems (Fig. 7) where continental layers mark the emersion phases and marine serpulids grow during the submerged phases.

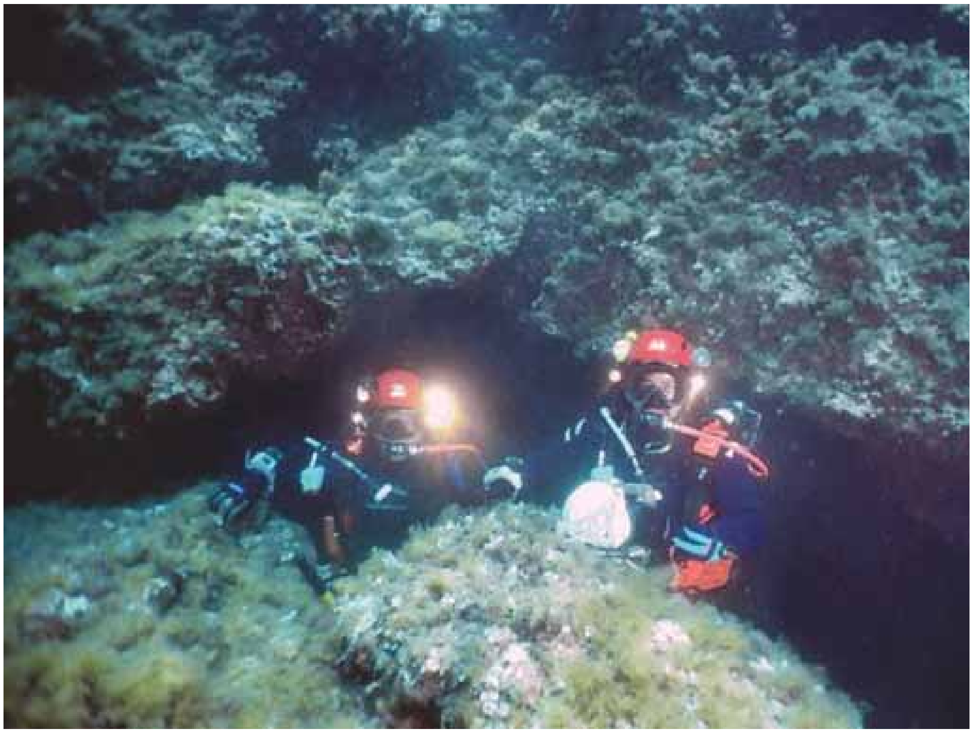

Figure 6. Entrance of Argentarola cave.

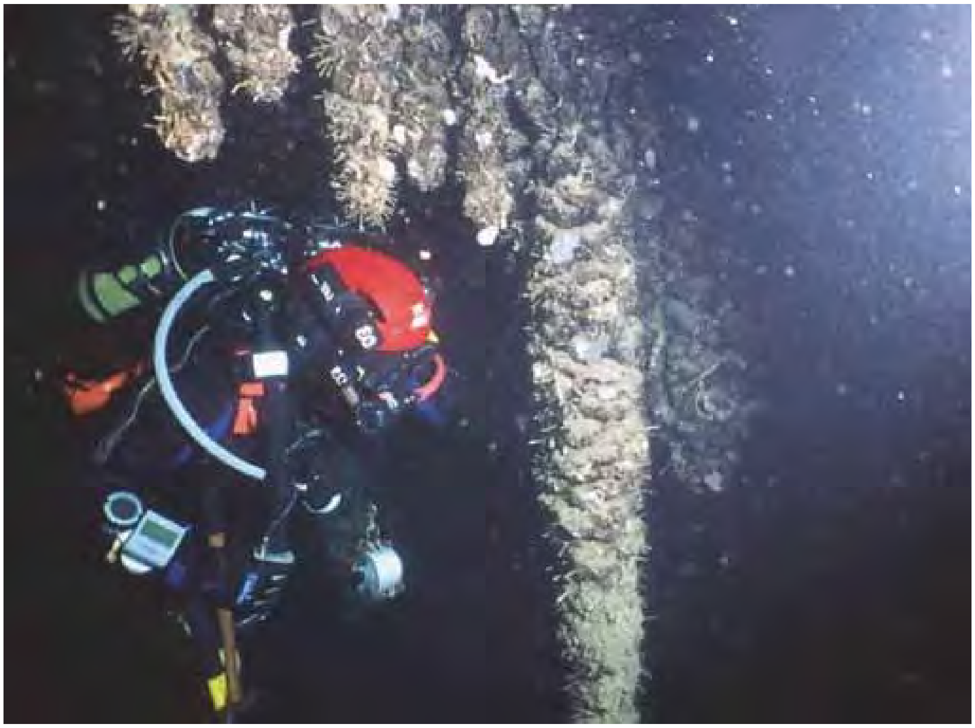

Figure 7. Stalactites inside Argentarola cave.

Since 1990, a series of speleothems have been sampled inside the cave and their cyclical layers have been studied in order to identify the timescale and magnitude of the relative sea-level oscillations and to compare this information with that from other sites. In 2009, a further, larger, stalactite was retrieved from the cave as described above, completing an impressive record of high-quality specimens. Through their study it was possible to identify sea-level oscillations during the last 215,000 years with extreme accuracy (Antonioli et al., 2004; Dutton et al., 2009).

The cave of the Maddalena peninsula

The peninsula of Maddalena is in the southern part of the bay of Porto Grande di Siracusa (southern Sicily, Italy). The main lithology is calcareous sandstone of Miocene age overlying volcanic deposits of Cretaceous age and a sedimentary hiatus divides the two depositions. Six series of marine terraces are visible and can be correlated to alternating high- and low-standing phases of the sea level (Di Grande and Raimondo, 1983). The submerged morphology is characterized by landslides and fluvial erosion patterns developed during emersion periods. Fractures and faults enhance the circulation of water and the dissolution of the calcareous components of deposits leading to the formation of voids and, where the erosion was stronger, of caves.

The studied cave, Grotta del Faro, is a shallow sub-horizontal conduit with an average depth of 3 m and a total length of 20 m (Fig. 8); the direction is along the local fault system. The lithology is calcareous sandstone with deposition of several speleothems. At the end of the cave, a small spring feeds freshwater originating a halocline and a mixing zone. The water collected inside the cave in the mixing zone has electrical conductivity of 49 ms/cm and pH 8.20 compared with the values of 59 ms/cm and pH 8.53 of the seawater outside the cave. The mixing of freshwater and seawater enhance the chemical dissolution of the calcareous deposits and causes a sharp biological gradient with marine organisms disappearing in vicinity of the freshwater emission. Mechanical erosion is visible on the floor of the cave and is likely caused by the effect of the water flowing from the spring during the past low standing of the sea level and emersion phases of the cave.

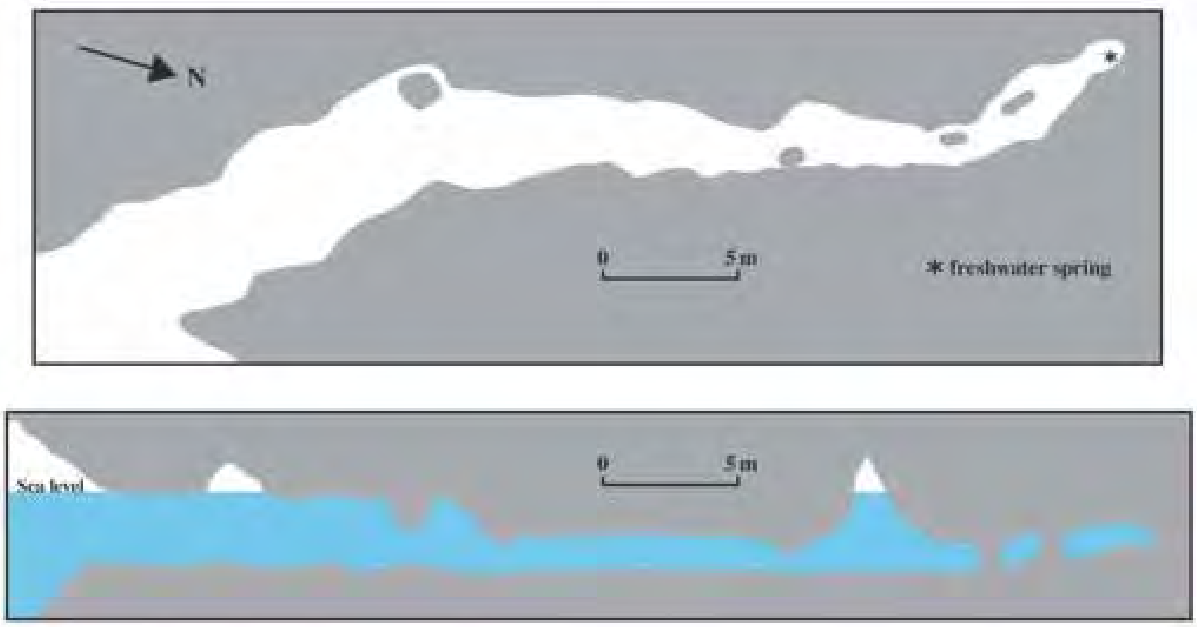

Figure 8. Map and section of Grotta del Faro.

Conclusions

The case studies presented cover only some of the geological researches that can benefit from scientific diving. Through diving activity it was possible to identify key factors that otherwise would not have been possible to study, such as underwater morphology and stratigraphy, and to collect samples in complex environments as in the case of Argentarola cave.

Even if automated vehicles are available for underwater investigations and sampling, they cannot replace the presence of researchers or the quality of collected data. Many environments, such as caves, cannot be studied by commercially available underwater vehicles as they would require the development of custom-made equipment (Gary et al., 2008).

The progress towards new and more reliable scuba diving equipment, such as rebreather technology, will make the range of underwater activity even more extensive, allowing deep or extremely prolonged dives which can be safely conducted by scientists performing more complex tasks. Although scientific diving is as common a tool as any of the other methods used by researchers for their field-work activity, the associated potential risks of underwater activity and especially those in challenging environments, require adequate training and solid procedures both for the safety of the operators and for the reliability of the results.

Acknowledgments

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RESTORATION OF CRITICALLY ENDANGERED ELKHORN CORAL (*ACROPORA PALMATA*) USING SEXUALLY PRODUCED RECRUITS

***Valérie F. Chamberland*[1, 2]**
***Dirk Petersen*[2]**
***Mark J.A. Vermeij*[1]**

[1]CARMABI Foundation
Piscaderabaai z/n
Willemstad, CURAÇAO
chamberland_valerie@live.ca

[2]SECORE Foundation
Parkstraße 34
65189 Wiesbaden, GERMANY

Introduction

Prior to the 1980s, the Caribbean Acroporids (i.e., *Acropora palmata* and *Acropora cervicornis*) were dominant shallow-water reef building species that provided shelter for a large variety of other reef organisms and significantly contributed to coastal protection during storms and hurricanes. In the early 1980s their abundance declined by >95% caused by a white band disease outbreak and has remained at low densities without noticeable recovery since then (*Acropora* Biological Review Team, 2005). As a result, both species were listed as "threatened" under the U.S. Endangered Species Act (Hogarth, 2006) and "critically endangered" under the IUCN Red List (Aronson et al., 2008). To facilitate these species' recovery, Caribbean-wide restoration efforts were started.

In 2010, the SECORE Foundation, in collaboration with CARMABI and the Curaçao Sea-Aquarium, launched a restoration program in Curaçao aimed at developing the techniques required to assist the recovery of depauperate *Acropora palmata* populations throughout the Caribbean. In contrast to more commonly used methods that depend on the production of offspring by fragmenting existing colonies (reviewed in Young et al., 2012), SECORE uses sexually produced offspring (i.e., more genetically diverse offspring) that are reared in nursery conditions at the Sea-Aquarium prior to their reintroduction to the reef. Since the beginning of this project, SECORE has succeeded in developing methods to culture coral larvae in a land-based nursery and has reintroduced several hundred recruits to the reef to date.

The present study was aimed at evaluating the effectiveness of the restoration techniques developed by the SECORE Foundation. The main objectives were i) to compare naturally-settled versus laboratory-reared recruits, and ii) to determine the optimal age for introduction of recruits to the reef. Coral settlers reared from gametes were introduced to the reef at various time points and their survival and growth were monitored over 6 to 11 months and compared with that of recruits kept within a land-based nursery. Since spawn quality and environmental conditions may significantly differ between spawning seasons, these objectives were investigated over a period of two consecutive spawning seasons, from August 2011 until August 2012.

Methods

Study location and facilities

The study was carried out in Curaçao (12°N69°W), a relatively small island in the Southern Caribbean that still harbors healthy and dense thickets of *Acropora palmata* that spawn annually in the fall. In 2010, SECORE started a coral rearing facility at the Curaçao Sea-Aquarium consisting of five aquariums (each 2150 x 685.8 x 635 cm; 936 L volume), to which fresh seawater was continuously supplied from the nearby ocean through a 100-m pipeline. The system was specifically designed to maintain corals during their earliest life stages and to provide settled corals with a controlled environment aimed at increasing their survival compared to natural conditions.

Gamete collection and larvae rearing

Acropora palmata is a broadcast spawning species that releases gametes once or twice a year (Szmant, 1986) and for which spawning timing is well known. Larvae from this species were reared from gamete bundles collected in the field on predicted spawning nights (Fig. 1a, b, c). Fertilized eggs were reared in specially designed flow-through rearing devices (called 'kreisels'; see Hagedorn et al. 2009) in the nursery system at the Curaçao Sea-Aquarium where they completed embryogenesis (Fig 1d). After approximately 24-72 hours, larvae began to swim downward in the water column and after 4 to 7 days, they were transferred to plastic containers lined with artificial settlement substratum (i.e., tripods made of clay) and allowed to settle (Fig 1e). Young corals were then grown within the nursery (Fig 1f) and introduced to the reef.

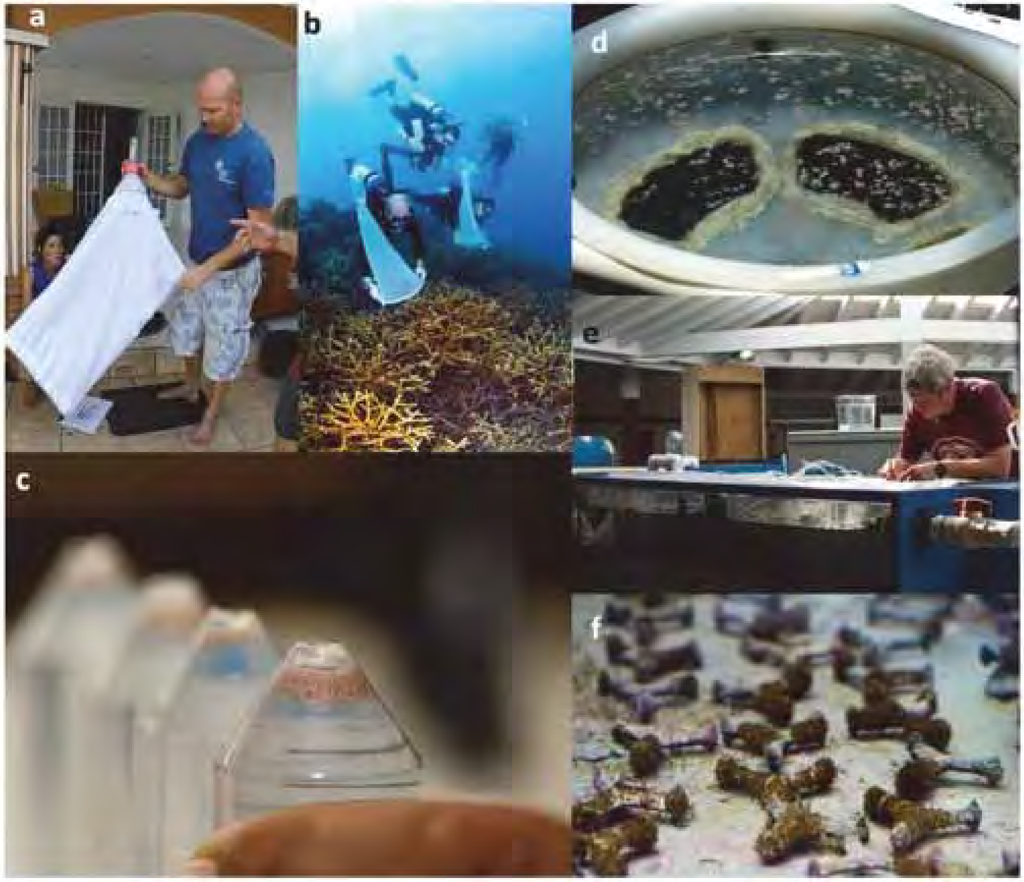

Figure 1. Collection of spawn and coral rearing: a. Coral spawn is collected with specially designed nets into which positively buoyant sperm/egg bundles are trapped; b. At the predicted

spawning time, divers set the nets on coral colonies; c. The spawn is transported in collecting tubes and brought back to the laboratory for fertilization; d. Once fertilized, the eggs are transferred to kreisels where they will complete development; e. After a few days, the coral planulae are placed into settlement bins in which they are provided with suitable settlement substrate and water flow; and, f. Coral settlers are reared within the Curaçao Sea-Aquarium nursery. Photo credits: Paul Selvaggio and Barry Brown.

Introduction of settlers to the reef

To compare naturally-settled recruits with nursery-reared recruits, we first attempted to locate naturally-settled recruits on the reef using belt transects and photo quadrats. However, since no natural *Acropora palmata* settlers were found *in situ*, artificially-settled juveniles were introduced to the reef as primary polyps (i.e., 2 weeks old) in 2012. Their survivorship was monitored after 1 month, 6 months and 11 months as a proxy for this species' potential natural post-settlement success, which was compared with that of recruits from the same generation kept within the Sea-Aquarium nursery (n=30 settlement tiles, with an average of 11.4 ± 4.2 SD recruits per tile). In order to determine the optimal recruit age for introduction to the reef, coral settlers collected in August 2011 were introduced to the reef at the age of 1 week , 2 months, and 1 year old. In 2012, settlers were again introduced at an age of 1 week. Because of limited spawning in 2012, the time points of 1 month and 2 months were excluded and additional recruits from that spawning season will be out planted only at the age of 1 year (August 2013). Table 1 summarizes the planting and monitoring time points and includes all sample sizes from 2011 and 2012. All the settlement tiles seeded with recruits were transported from the coral nursery to the reef and tied to a previously secured nylon rope on the reef flat near the Sea-Aquarium, with the exception of the 1 year old recruits from the 2011 spawning which were stabilized to a wave breaker near the Sea-Aquarium using marine epoxy. The survivorship of each recruit was assessed regularly for a period of 11 months and compared with that of controls kept in the nursery.

Table 1. Summary of all planting and monitoring time points of *Acropora palmata* recruits and their respective sample sizes from 2011 and 2012. Survival rates are included for each time point and are expressed as the average percent survival and their standard deviations.

Generation	Planting treatment	*N* (no. of tiles)	Monitoring time points (months)	Survival (Mean % ± SD)
2011	Nursery	10	6	16.0 ± 10.0
	1 week	40	6	6.0 ± 6.0
	2 months old	22	6	9.0 ± 8.0
	1 year old	8	6	100.0 ± 0.0
			11	100.0 ± 0.0
2012	Nursery	30	1	80.9 ± 28.7
			6	9.6 ± 10.1
			11	3.7 ± 8.3
	1 week	30	1	65.8 ± 23.2
			6	13.8 ± 10.3
			11	11.1 ± 8.6

Data analysis

Since our data did not meet the criteria of homoscedasticity (Levene, p<0.001) and of normality of distribution (Shapiro-Wilk, p<0.0001), one-way NPMANOVA was performed to test for differences in survival rates between the corals reared *in situ* and those kept within the nursery. The analysis was performed with Bray-Curtis distances as measure of dissimilarity among untransformed data and significance was based on *F* tests obtained with 9999 permutations. All analyses were performed with PAST 1.97 (Hammer et al., 2001).

Results and Discussion

Objective i) comparison of naturally-settled versus laboratory-reared recruits

In 2012, the survivorship of recruits introduced on the reef as primary polyps (65.8% ± 23.2 SD) after 1 month was significantly lower than that of recruits within the nursery (83.7% ± 28.7 SD), (F = 2,941 $p<0.05$) (Fig. 2). In contrast, after 11 months, the recruits raised within the nursery had suffered higher mortality rates than recruits placed on the reef immediately after settlement (survival rate: 3.7 % ± 8.3 SD and 11.1 % ± 8.6SD, respectively, F = 20.1, $p<0.001$) indicating that, overall, the nursery conditions were suboptimal for young *Acropora palmata* corals. The high mortality rate of the artificially-reared recruits could be the result of stressful conditions occasionally experienced by the latter within the land-based nursery. For instance, between October and November 2012, the sea surface temperature (SST) reached 29.5°C on the reef and often exceeded 31.0°C in the aquaria. Previous data showed that the variability of daily mean temperature within the coral nursery is greater than on the reef adjacent to the Sea-Aquarium (SECORE, unpublished data). Thus, coral recruits kept in aquaria at the Sea-Aquarium were exposed to higher ranges of thermal differences while the reef provided them with more constant thermal conditions. Furthermore, these results show that the first 6 months after settlement of young *A. palmata* represent a crucial life stage during which mortality is highest (i.e., > 85%), both within the nursery and on the reef. Newly settled corals experience severe mortality because their small size makes them extremely vulnerable to stressors such as competition, predation, diseases, runoff and bleaching (Ritson-Williams et al., 2009). Thus, this half-year time window is critical for coral restoration programs such as SECORE.

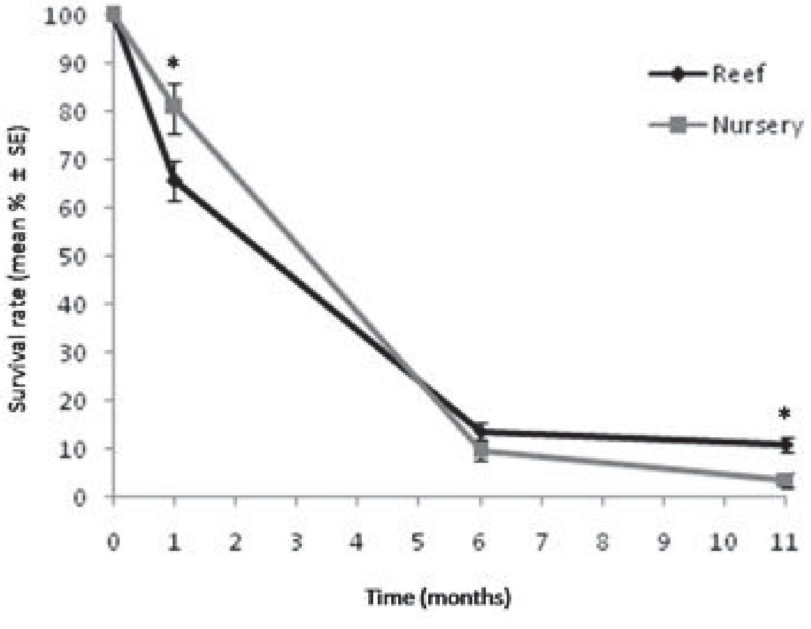

Figure 2. Average survival rates of *Acropora palmata* settlers introduced to the reef as primary polyps (black line) compared to settlers kept within the land-based nursery (grey line). Their survival was monitored after 1, 6, and 11 months. Error bars indicate standard errors (SE) and asterisks (*) indicate significant differences between the two coral populations (NPMANOVA, $p < 0.001$).

Objective ii) determination of the optimal age/size for planting of recruits

When planted as primary polyps, the survival rates of *Acropora palmata* settlers after 6 months in 2011 and 2012 were 5.8 % ± 6.0 SD and 13.8% ± 10.3 SD, respectively. Survival rates did not significantly increase when recruits were planted out at the age of 2 months. However, coral recruits that had spent a full year within the nursery before being introduced to the reef had a survival rate of 100% ± 0.0 SD after 11 months. Additionally, after 6 months spent on the reef, they had started growing vertically and their size was about 2 fold larger than the size of their counterparts kept within the nursery. This indicated that growth of juveniles kept for longer than a year within the nursery was somehow limited compared with those introduced to the reef.

Conclusions

The sudden and unexpected die-off of Caribbean Acroporids in the early 1980s alongside with their dramatic decline in sexual recruitment (Vermeij et al., 2011) has prompted restoration efforts across the region to promote their recovery. The present study demonstrates that using sexually produced recruits has potential in enhancing restoration strategies for critically endangered Elkhorn coral. Indeed, the survival rates achieved after one year in the field are promising. Our results show that the first 6 months after settlement of young *Acropora palmata* represents a crucial life stage during which mortality is highest both within the nursery and on the reef, and that increased nursing periods yield higher survival rates after planting out. However, keeping recruits within a nursery for an entire year represents excessive labour and cost investments, is suboptimal for the coral recruits and is, therefore, not ideal. Alternatively, we suggest placing the settlers as primary polyps within a mid-water nursery for half a year to provide recruits with stable and natural reef conditions and allow them to achieve a bigger size class before being introduced directly to the reef substrate.

Acknowledgments

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EXPLORATION OF DEEP HOLE, MYAKKA RIVER STATE PARK, FLORIDA

***James K. Culter*[1]**
***Curt Bowen*[2]**
***John Ryan*[3]**
***Jon Perry*[3]**
***Rene Janneman*[3]**
***Weiqi Lin*[3]**

[1]Mote Marine Laboratory
1600 Ken Thompson Parkway
Sarasota, FLORIDA 34236 U.S.A.
jculter@mote.org

[2]ADM Exploration Foundation
327 Snapdragon Loop
Bradenton, FLORIDA 34212 U.S.A.

[3]Sarasota County Water Resources
1001 Sarasota Center Blvd
Sarasota, FLORIDA 34240 U.S.A.

Deep Hole is a karst sink located in Myakka River State Park, Sarasota County, Florida. The park is popular for hiking, fishing, camping, and wildlife observation. Deep Hole is located on the northwest bank of the Myakka River just outside the southern terminus of the Lower Myakka Lake in the Wilderness Preserve section of the park. Lake levels exhibit pronounced depth variation from wet to dry season and Deep Hole is a refuge for wildlife during the dry season. Prior studies of river rocks estimated the age of the Myakka River basin at several million years although the formation period for Deep Hole is unknown. Notably, evidence for one of the earliest documented human settlements in the area now defined as the United States occurs within the Myakka River basin from two sinkhole features, Warm Mineral Springs (WMS) and Little Salt Spring (LSS) which yielded artifacts that have established human habitation in the area to 10,000 - 12,000 years before present. The authors collaborated to compile existing information and conduct preliminary investigations of water quality and underwater topography. Water quality data were collected in August 2011 and in April 2012 a technical dive team explored the sink to determine the overall depth and document similarities to WMS and LSS, and offshore submarine springs. Unique characteristics of the site necessitated planning for unknown depth, potential caves, poor visibility and a large number of adult alligators. Findings showed the maximum depth to be 41 m for ambient dry season lake levels, no significant ledges or caves were found. There was a strong thermocline at ~9 m in August 2011and 6 m in April 2012 below which the water was anoxic. For the August visit water temperature was uniformly 14.2°C below 12 m, colder than the typical ~22.5°C for Florida spring water indicating the sink does not have underlying spring flow. Long-term temperature records indicated there is some seasonal warming near the thermocline. Water quality parameters varied with the temperature stratification and

were dissimilar to Warm Mineral Springs and Little Salt Spring where warm, mineralized water rises from a deep aquifer.

Introduction and Background

Study Site

Deep Hole is a sinkhole located in Myakka River State Park, one of Florida's oldest and largest state parks. The "Florida Wild and Scenic Myakka River" flows through 57 square miles of wetlands, prairies and woodlands. The Park is popular for hiking, fishing, camping, and wildlife observation. The main entrance of the park is located off State Road 72 in eastern Sarasota County. Deep Hole is a sinkhole located on the southwest shoreline of the Lower Myakka Lake in the Wilderness Preserve section of the park, which requires a permit for access. Prior to creation of the State Park the area surrounding Deep Hole was used for grazing ranchlands.

Deep Hole is located just southwest of the shoreline of the Lower Myakka Lake where the lake empties into the lower Myakka River. The sink is approximately 90 m in diameter (Fig. 1). The sink perimeter is well defined in the dry season as shown in Figure 1, but is mostly obscured during a strong wet season when lake levels may rise by +2 m (Fig. 2).

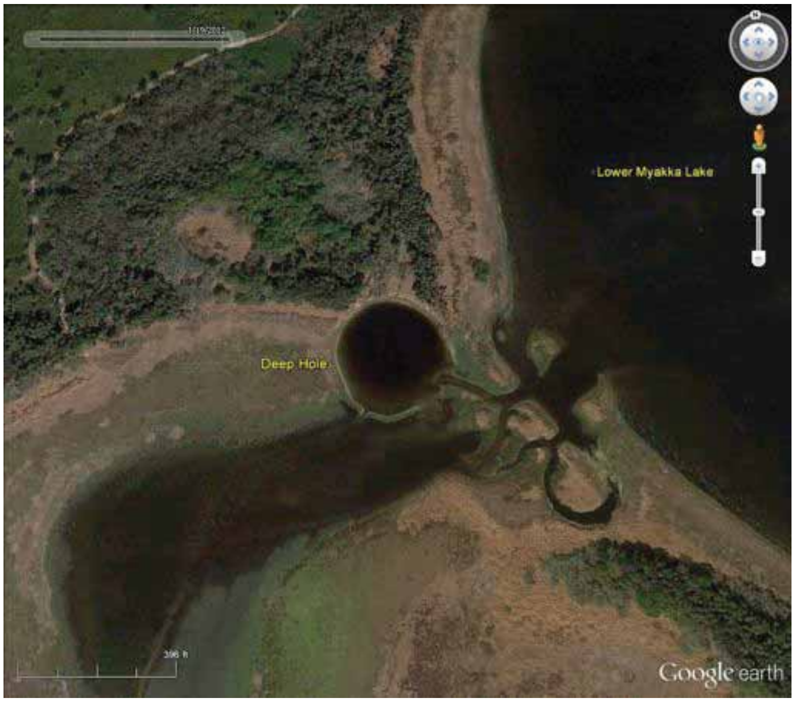

Figure 1. Image of Deep Hole and Lower Myakka Lake taken during the dry season low lake level.

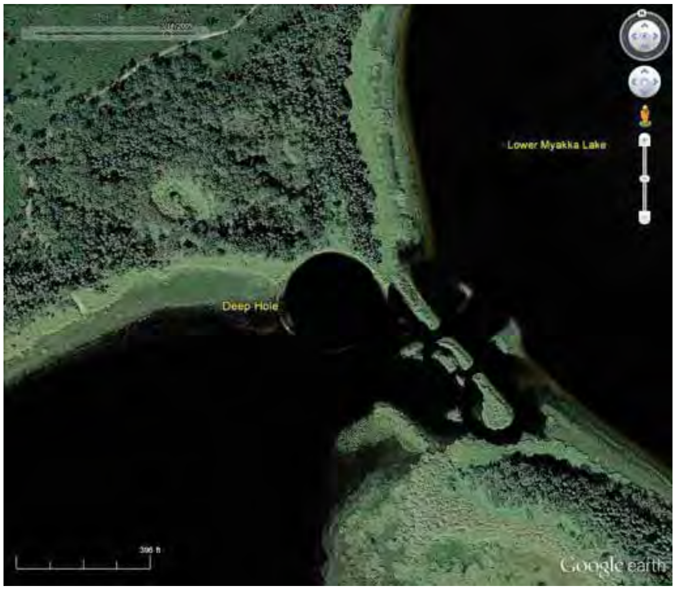

Figure 2. Image of Deep Hole and Lower Myakka Lake taken during wet season with high lake water.

During severe dry seasons the depth and area of the Lower Myakka Lake decline greatly. Deep Hole subsequently becomes a refuge for fishes and other fauna as the water levels drop and the tremendous diversity of the park becomes evident as many species aggregate near water sources. At least 40 species of fishes are known to occur in the Myakka River State Park. In addition, according to Park records, there are 48 species of reptiles, 22 species of amphibians, 32 species of mammals, and the Park checklist includes 255 species of resident and migratory birds. The dry season lake water drop and the resultant high numbers of fish contained within Deep Hole results in the aggregation of the American alligator (*Alligator mississippiensis*). During the period of the April 2012 survey in excess of 150 alligators were counted within Deep Hole from still images of the site.

The report "A History of the Myakka River Sarasota County, Florida" (McCarthy and Dame, 1983) relates that the estimated age of the river basin is several million years old based on rock samples from the river. It is notable that evidence for the earliest documented human settlements in the United States occurs within the Myakka basin from two sinkhole features, Warm Mineral Springs and Little Salt Spring. The proximity of these sites to Deep Hole was one aspect of the motivation to conduct this study.

There are relatively few sinkholes in Sarasota County compared with other areas of west Florida. However, Little Salt Spring and Warm Mineral Springs have proved to be of great archaeological significance for documentation of early human settlement in North America. Deep Hole (27.214928°N, 82.335214°W) is located approximately 11.5 miles from Little Salt Spring (27.074678°N, 82.233111°W) and 11.6 miles from Warm Mineral Spring (27.059900°N, 82.260327°W). Warm Mineral Springs is located 1.97 miles from Little Salt Spring at a bearing of 238 degrees from north. The scientific relevance of these nearby sites was the impetus for the investigation of Deep Hole for which there are no known records of scientific investigation and only undocumented reports of scuba divers having investigated the sink. The overall dimensions and the depth of the site were unknown prior to this survey.

Little Salt Spring (LSS), located within the City of North Port, owned by the University of Miami since 1982, has been a scientific study site since the 1970s and has provided human remains and artifacts dating to ~12,000-13,450 years before present (Clausen et al., 1979; Purdy, 2008; Gifford and Koski, 2011). Only recently have the sources of the water from the spring vents been investigated (Van Ee and Riera-Gomez, 2009) with results suggesting multiple sources.

Warm Mineral Springs (WMS) is also within the City of North Port in Sarasota County. WMS has a primary deep source spring of highly mineralized warm water (~30°C) with the vent located ~63 meters below the spring surface within a cave and also has additional lesser flow freshwater seeps closer to the surface. WMS was a privately owned tourist attraction until 2010 when it was jointly purchased by Sarasota County and the City of North Port. In July 2013, WMS was closed to the public and the future fate is dependent upon development of a plan for the future use and development.

In the 1950s, a retired Air Force Colonel William Royal began scuba diving in WMS and discovered stalactites, human remains and artifacts. In 1959, Colonel Royal invited Dr. Eugenie Clark, then the Director of Mote Marine Laboratory in Placida, to examine the site. During these dives additional artifacts were collected including an intact human skull with remains of brain tissue. A charred log beneath the human remains subsequently was carbon dated to 8,000 B.C. ± 200 years, the earliest known habitation for man in Florida at that time (Royal and Clark, 1960). Like LSS, this site proved to be a valuable archaeological site but was largely ignored by the archaeological community until 1972 when an archaeologist from Florida State University began working in WMS (Cockrell, 1987).

Studies of LSS and WMS provided conclusive evidence of human habitation. WMS, LSS and Deep Hole would have served as important sources of fresh water for human settlements when lower sea level may have resulted in a lack of available surface waters during a period that was generally more arid with a lower water table (Clausen et al., 1975, 1979). Stream and river flows may have been seasonal features. The distance between these sites is relatively short and would have increased the available foraging range of the early communities. These potential links and the possibility of discoveries of archaeological significance were the impetus for the exploration of Deep Hole.

Methods and Materials

Physical water parameters and water quality samples were collected in August 2011 by Sarasota County Water Resources with personnel accessing the site in a small Jon boat. Water levels were higher than for the following spring when the bathymetry and diving surveys were conducted. In situ measurements of temperature, salinity, conductivity, dissolved oxygen and pH were measured in the field with a multiparameter meter. Water quality samples were obtained at depths of 3, 15, 30, 45, 60, 90, and 105 ft for analysis of nutrients, minerals and trace metals according to National Environmental Laboratory Accreditation Conference (NELAC) standards by an independent contract laboratory certified by the Florida Department of Health Environmental Laboratory Certification Program.

The collaborative research team consulted with the Myakka State Park personnel to arrange for a research permit and vehicle access permission to conduct an investigative survey and scuba dive in Deep Hole within the wilderness preserve. Abnormally dry conditions allowed the team to transport equipment to the edge of the hole by vehicle. In early 2012, the site was visited several times to map the subsurface contours using an aluminum Jon boat equipped with a WAAS enabled Global Positioning System coupled with a digital fathometer. A surface deployed drop camera was also deployed to provide the team with estimates of the expected visibility and the composition of the bottom. On February 12, 2012 two Onset Computer HOBO® thermographs were placed in the hole, one near the surface and the second below the thermocline at ~9 m (30 ft) to obtain a long-term record of temperature variation. The thermographs were recovered on March 11, 2013. The surface thermograph had been damaged from what appeared to be an alligator bite. The thermocline thermograph was recovered and data are considered reliable through November 28, 2012 at which time there was a sudden spike in temperature. This anomaly was believed to be a result of the thermograph being pulled closer to the surface possibly by entanglement of the line with an alligator.

In April 2012, a technical dive team explored the sink to determine the overall depth and document similarities to WMS, LSS, and offshore submarine springs and sinks. Diving safety was focused on accounting for unknown depth and water conditions and the presence of unusually large number of adult alligators. The overall depth and subsurface configuration of the hole was unknown to the dive team. The team planned for the possibility of depths as great as 76 m (~250 ft), the presence of caves or caverns and low visibility. There were two deep divers, one diving a side-mount configuration and the second a traditional double tank back mount cave diving configuration. Bottom gas consisted of trimix and separate stage tanks for travel gas (Nitrox) and oxygen decompression were also carried by each diver. Both divers wore drysuits since the water quality survey in 2011 indicated a large temperature decrease at 6-9 m. A weighted downline buoyed to the surface was placed as a descent and ascent guide for the divers. Surface support consisted of fully outfitted stand-by divers and alligator proximity observers. Surface team members were also briefed in first aid and evacuation protocols in the event of a medical emergency.

After consultation with several alligator behavior research scientists it was deemed prudent to arrange for a shark cage enclosure to use at the 6-9 meter (20-30 ft.) decompression stops. The shark cage was made available for the project and transported to the site by Think Out Loud Productions®. The cage was suspended with surface buoys so the bottom of the cage was located at ~9 m. The bottom panel of the cave was removed to allow easy entry by the deep dive team. Divers conducted their final decompression stop at approximately 7 m.

The divers descended the down line and upon reaching the bottom attached a cave reel line to the dropline weight. The divers then swam out perpendicular to the down line until encountering the rock wall and proceeded to circumnavigate the cavern.

Results

Water Parameters, Minerals, Nutrients and Trace Metals

It should be noted that a one-time sampling event is insufficient for definitive conclusions regarding water quality in natural systems. For that reason the results of the water quality analysis are presented with the caveat that they should be used only to provide insight on the general conditions of the hole at the time of the survey. It is possible that Deep Hole may exhibit significant seasonal variation.

Data for the August 2011 field water measurements and water quality analyses are found in Tables 1 - 4. The physical water parameters were generally stable but stratified by two depth zones; a shallow warm zone, 0 to ~10 m (0-30 ft.) and a cold deep zone greater than ~10 m (Table 1). Water temperature dropped from 28.5ºC at the surface to 14.2ºC at 12 m ($\Delta t = 14.3$ºC). The low water temperature at depth indicates a lack of spring flow into the system. During the April 2012 diver survey it was noted that the thermocline was close to the 6-m (20 ft.) depth corresponding with the sharp drop past the lip of the collapse rim. The data from the thermograph located near the thermocline (~10 m) are shown in Figure 3. The water temperature at this depth illustrated a gradual warming as a result of the combination of summer warming and continued lake level drop during the dry winter months. The anomaly caused by the presumed alligator bite is observed on 11/28/2012.

Total dissolved solids, turbidity and biochemical oxygen demand were all higher below 10 m (30 ft.) but total suspended solids (TSS) and total organic carbon were greater near the surface. The temperature gradient of the system most likely keeps TSS in suspension over the more dense cold water. The pH of the system was only slightly lower than a typical Florida stream.

Table 1. Physical water measurements for Deep Hole, August 31, 2013.

Depth (ft)	3	15	30	45	60	75	90	105	Shallow Avg. (3-15 ft)	Deep Avg. (30+ ft)	All Avg	Typical Florida Stream
Depth (~m)	1	4.6	9.1	13.7	18.2	22.9	27.4	32.0				
Temp C	28.5	28.2	21.6	14.2	14.2	14.2	14.2	14.2	26.1	14.2	18.7	--
Temp F	83.3	82.8	70.8	57.6	57.6	57.6	57.6	57.6	83.1	57.6	65.6	--
Salinity (PSU)	0.2	0.2	0.4	0.4	0.4	0.4	0.4	0.4	.2	0.4	0.3	0.5
Conductivity (umhos/cm)	375	375	638	795	801	803	798	795	375	772	673	475
Total Dissolved Solids (mg/l)	268	292	440	532	528	520	548	536	333	533	458	214
Color (PCU)	250	250	250	100	100	100	100	100	250	100	156	350
Dissolved Oxygen[1] (mg/l)		0.2	0.9								0.5	5.5
Dissolved Oxygen %		1.9	0.8								1.4	62
Biochemical Oxygen Demand (mg/l)	2	2	7	14	14	14	15	15	4	14	10	1.3
Turbidity (NTUI)	2	2	60	50	52	40	50	50	2	50	38	2.5
Total Suspended Solids (mg/l)	2.8	2.0	1.8	1.0	0.8	0.8	1.0	0.8	2.2	0.9	1.4	4.0
Total Organic Carbon (mg/l)	26	26	21	18	19	18	18	18	24	18	20	17
Fecal Coliform (cfu/100ml)	400								400		400	60
pH	6.7	6.8	6.9	6.9	6.9	6.9	6.9	6.9	6.7	6.9	6.9	7.2

Footnotes: 1, Dissolved oxygen probe malfunction in the field make data for this parameter unreliable.

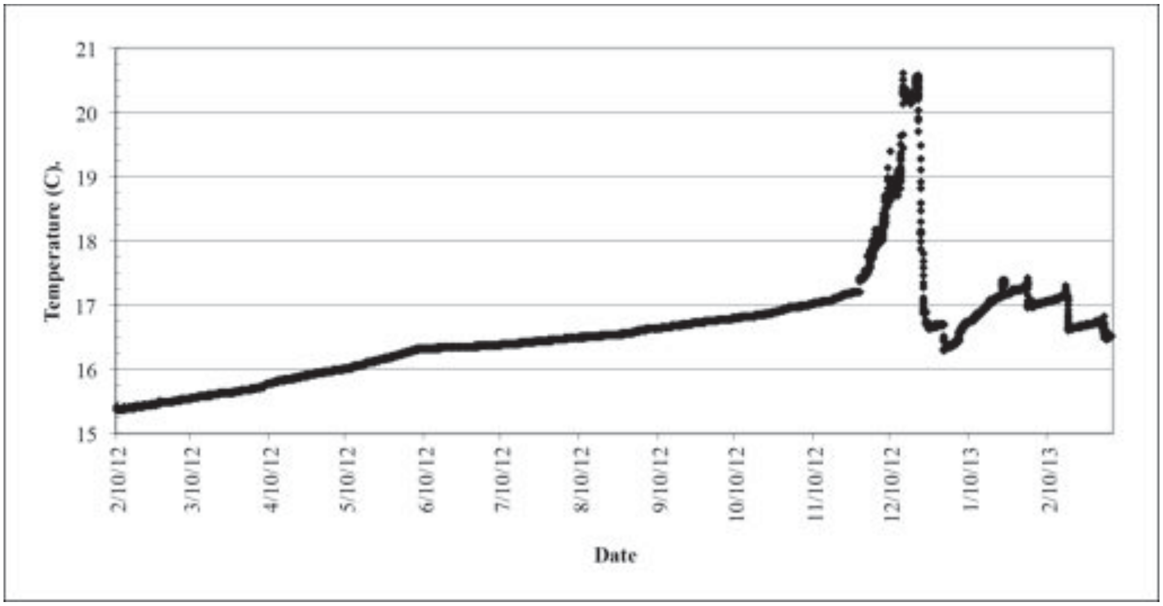

Figure 3. Deep Hole temperature record at a depth of ~10 m for the period of Feb. 2012 to March 2013.

Dissolved oxygen levels were not reliably recorded during the August site visit. The dissolved oxygen membrane probe failed during the visit to the site. Hydrogen sulfide levels were high below the temperature thermocline (see Table 2) and this may have poisoned the oxygen electrode.

Table 2. Mineral concentrations for water samples obtained from Deep Hole, August 31, 2011. Values in mg/l except where noted.

Depth (ft)	3	15	30	45	60	75	90	105	Shallow Avg. (3-15 ft)	Deep Avg. (30+ ft)	All Avg.
Depth (~m)	1	4.6	9.1	13.7	18.2	22.9	27.4	32.0			
Hardness total	152	146	336	359	363	342	384	360	149	357	305
Sulfate	104	105	217	225	226	226	226	227	105	225	195
Iron	377	353	338	119	84	83	110	82	365	136	193
Alkalinity, bicarbonate	39.0	39.0	70.0	110.0	114.0	112.0	112.0	112.0	39.0	105.0	88.5
Alkalinity, total	39.0	39.0	70.0	110.0	114.0	112.0	112.0	112.0	39.0	105.0	88.5
Calcium	34.6	32.8	77.1	82.9	85.5	82.2	91.6	84.4	33.7	84.0	71.4
Manganese (ug/l)	23.5	22.9	108.0	63.6	48.4	47.2	49.8	48.7	23.2	61.0	51.5
Magnesium	15.9	15.5	34.9	36.8	36.2	33.1	37.8	36.3	15.7	35.9	30.8
Chloride	17.4	17.4	25.3	28.9	29.0	28.9	29.0	29.1	17.4	28.4	25.6
Sodium	11.4	11.1	15.9	17.3	18.3	17.6	17.7	18.0	11.3	17.5	15.9
Sulfide	0.054	0.264	11.000	22.900	23.500	19.800	21.400	21.500	0.159	20.017	15.052
Silica, dissolved	15.9	15.8	11.2	8.2	8.2	8.3	8.4	8.5	15.9	8.8	10.6
Hydrogen Sulfide, unionized	0.032	0.156	5.750	13.900	13.800	12.200	12.800	13.000	0.094	11.908	8.955
Potassium	8.0	7.7	11.3	8.8	8.5	8.0	8.9	8.5	7.9	9.0	8.7
Strontium	1.9	1.9	2.9	3.4	3.6	3.4	3.4	3.5	1.9	3.3	3.0
Alkalinity, carbonate	0.594	0.594	0.594	0.594	0.594	0.594	0.594	0.594	0.594	0.594	0.594
Fluoride	0.224	0.223	0.259	0.308	0.295	0.293	0.301	0.297	0.224	0.292	0.275

Fecal coliform levels were somewhat elevated, 400 cfu/100ml, and likely caused by the presence of high densities of waterfowl, feral pigs and deer in the area and the low flow conditions at the site. Statewide numeric criteria whereby counts of colonies do not exceed a monthly average of <200 colonies/100 ml, a maximum of 400 colonies/100 ml in 10% of the samples, or a maximum of 800 colonies/100 ml on any one day.

Dissolved mineral data for Deep Hole water are presented in Table 2, which illustrates the site is fresh water without substantial input of deep mineralized spring water. There was some stratification by depth with mineral concentrations at depth usually different than the shallow water values. Iron and silica were the only minerals with higher concentrations near the surface. All others were higher at depth, except strontium, which did not vary by depth. There was no carbonate alkalinity.

Water nutrient data are presented in Table 3. Ammonia levels were high a deeper depths as a result of the anoxic conditions and consequently nitrate and nitrite levels were very low, and are typical of low oxygen environments. TKN also illustrated high values at depth. Total nitrogen was high at depth and was dominated by the ammonia and organic constituents. Total and ortho-phosphorus were high reflecting the phosphorous rich soils of the Myakka River basin. Chlorophyll levels near the surface were typical for a Florida lake as was phaeophytin.

Table 3. Nutrient parameters for Deep Hole water, August 31, 2013. Values in mg/l except as noted.

Depth (ft.)	3	15	30	45	60	75	90	105	Shallow	Deep		Typical Florida	
Depth (~m)	1	4.6	9.1	13.7	18.2	22.9	27.4	32.0	Avg.	Avg.	Average	Stream	MDL
Ammonia	0.121	0.111	2.660	3.100	3.420	3.210	3.550	3.160	0.12	3.18	2.417	0.04	0.008
Nitrate + Nitrate	0.008	0.012	0.005	0.006	0.008	0.006	0.004	0.004	0.01	0.01	0.007	0.07	0.004
Total Kjeldahl Nitrogen	1.62	1.55	3.25	4.81	4.93	4.94	4.88	4.96	2.14	4.90	3.868	1.06	0.05
Total Nitrogen	1.63	1.56	3.25	4.82	4.94	4.95	4.88	4.96	2.15	4.91	3.874	1.05	0.05
Ortho Phosphorus	0.555	0.559	0.861	0.512	0.483	0.448	0.436	0.498	0.56	0.54	0.544	0.06	0.002
Total Phosphorus	0.712	0.725	1.03	0.918	0.906	0.894	0.898	0.891	0.72	0.92	0.872	0.08	0.008
Chlorophyll a (mg/m^3)	17.2	13.5	7.47	3.33	1.78	2.51	2.09	2.13	12.72	2.37	6.251	4.22	0.25
Pheophytin (mg/m^3)	2.17	0.25	4.33	3.20	1.82	2.31	2.07	2.20	1.21	2.66	2.294	1.66	0.25

Results for trace metal analysis are presented in Table 4. All concentrations were very low. Cadmium, mercury, molybdenum, nickel, selenium and zinc were not detected at all and are shown as minimum detection levels (MDL). The practical quantification limit (PQL) is shown on the far right of Table 4. Arsenic, chromium, copper and lead were at very low levels.

Table 4. Results of trace metal analysis for Deep Hole water, August 31, 2013. Values µg/l.

Depth (ft.)	3	15	30	45	60	75	90	105	Average	MDL	PQL
Arsenic	0.951	0.853	1.270	0.855	1.350	1.120	1.550	1.400	1.169	0.689	2.756
Cadmium	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.9	3.6
Chromium	3.10	3.40	3.00	3.30	3.00	3.00	3.20	2.70	3.09	2	8
Copper	4.30	4.30	4.00	5.80	4.30	4.40	3.20	4.60	4.36	4	16
Lead	0.670	0.670	0.670	0.670	0.758	0.670	0.670	0.670	0.681	0.670	2.680
Mercury	0.198	0.198	0.198	0.198	0.198	0.198	0.198	0.198	0.198	0.198	0.792
Molybdenum	2.0	2.0	2.0	2.0	2.1	2.0	2.0	2.0	2.0	2	8
Nickel	1.18	1.18	1.18	1.18	1.18	1.18	1.18	1.18	1.18	1.18	4.72
Selenium	1.57	1.57	1.57	1.57	1.57	1.57	1.57	1.57	1.57	1.57	6.28
Zinc	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.4	5.6

Sink Topography and Diver Observations

The circular basin of Deep Hole has a substratum that consists primarily of medium to coarse quartz sands typical of the Myakka River soils. In the shallows the surface of the sand exhibited a greenish layer of periphyton. Shells and live individuals of the Florida Shiny Spike, *Elliptio buckleyi* (Lea, 1843), a Unionidae bivalve, were very abundant in the shallows of the bowl. During several site visits it appeared that both alligators and hogs feed on this mussel. The location of the collapse feature within the bowl of Deep Hole is shown in Figure 4. The collapse portion of the sink is located toward the northern side of the bowl and is irregular in shape. Figure 5 presents an oblique view of the hole. Figure 6 illustrates a cross section of the collapse section that is irregular in shape but approximately 33 m (110 ft.) across and drops to a depth of ~34 m (~112 ft.) from the water surface for conditions at the time of the survey in February 2013. For the time of the depth survey the Myakka River level gauge was 1.5 ft. as recorded at a flow control structure near Laurel (USGS gage 02298880). The top of the debris cone was approximately 25 m (82 ft.) below the edge of the collapse opening. The vertical section of wall along the collapse area was small generally <3 m (10 ft.). Divers noted a shallow sand covered ledge around a portion of the vertical wall section but the ledge was not examined in detail for the entire circumference because of the extremely poor visibility.

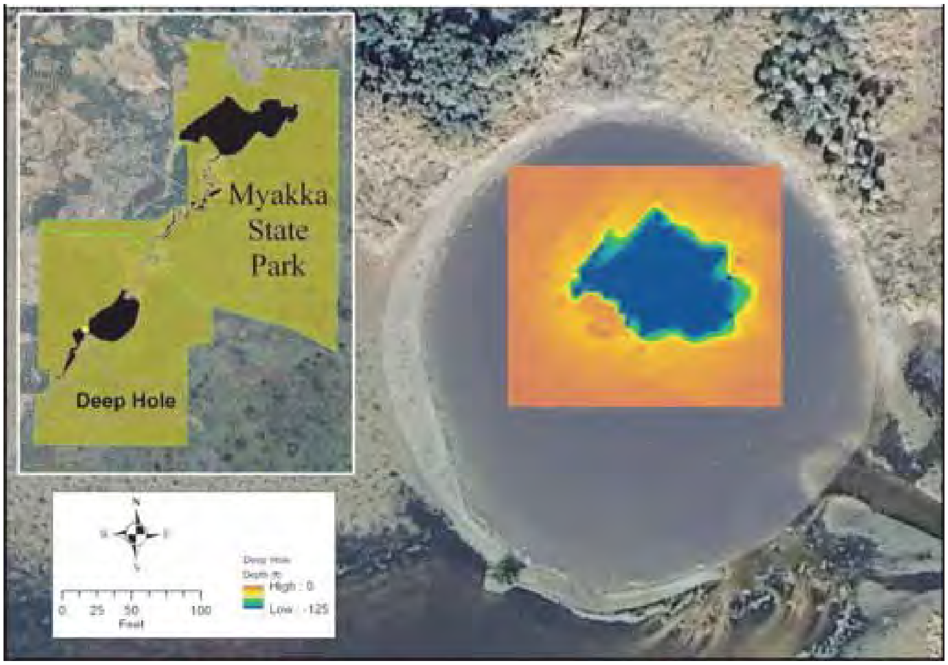

Figure 4. Image of Deep Hole illustrating the location of the collapse feature.

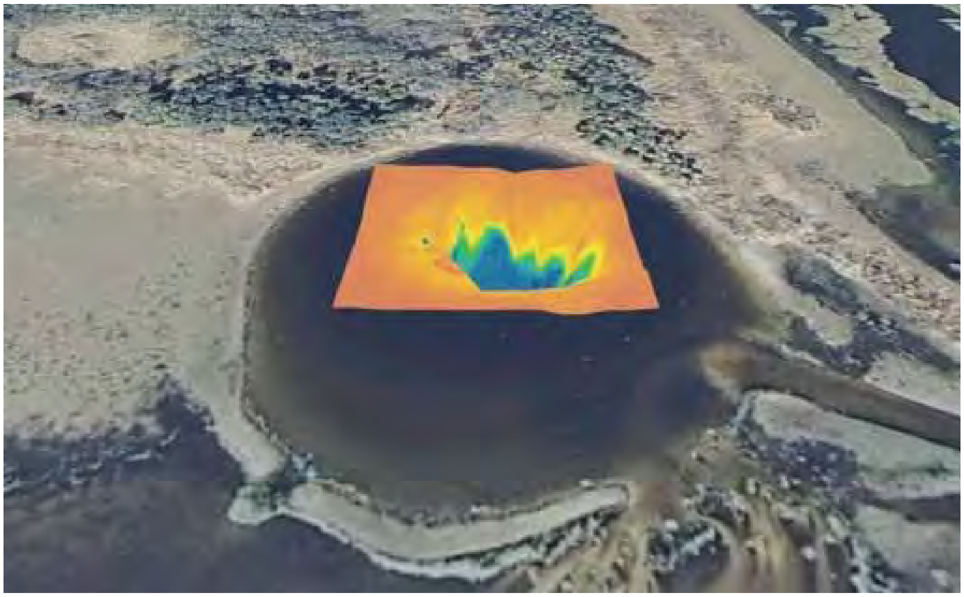

Figure 5. Oblique image of Deep Hole illustrating the location of the collapse feature.

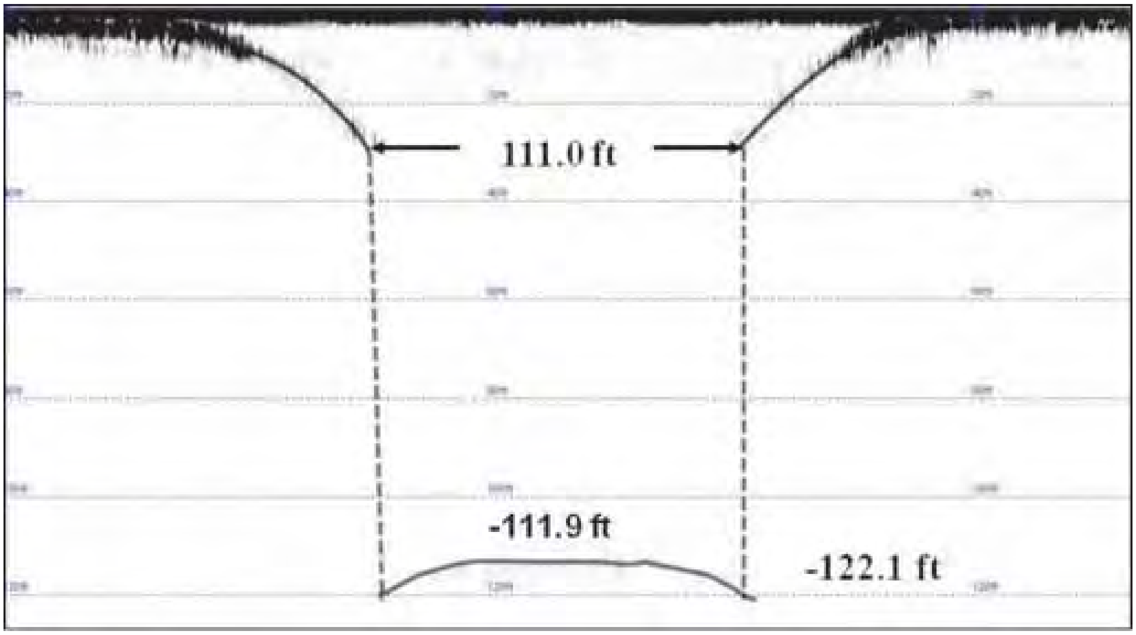

Figure 6. Cross section of the collapse feature of Deep Hole illustrating the overall dimensions.

The divers reported that underwater visibility was very poor ranging from 30 - 60 cm (1-2 ft.). Hydrogen sulfide was present below the thermocline. The bottom of the sink consisted of very soft brownish-gray sediment pocked with numerous circular holes ~4-5 cm in diameter. The sediment was not probed to determine the thickness. Dead fish were common across the bottom. There was relatively little identifiable plant material or other debris. From the down line near the apex of the debris pile the bottom sloped gently down toward the periphery and a maximum recorded depth of 40 m (131 ft.). The diameter of the sink at the bottom was estimated to be in excess of 46 m (150 ft.) although exact measurements were not obtained. Divers made their ascent along the sloping wall and with the exception of the small ledge at approximately 9 m (30 ft.) no caves or significant structures were noted although such structures could be present.

Discussion

The primary objectives of this project were accomplished. The relative size, depth and shape of the feature have been described and basic water chemistry determined. The results of this project show that for the present day Deep Hole is a very different sinkhole habitat than Warm Mineral Springs and Little Salt Spring. Although the chemistry and most of the physical data collected by this project represent only one summer period the information illustrates that Deep Hole is a static sink and does not have obvious spring activity. The sharp thermocline with a much lower temperature than Florida spring water indicates that groundwater influence is minimal since temperature would be moderated if there were significant spring flow into the sink. During winter cold periods the surface waters sink into the basin and remain trapped. The hole is likely a net nutrient sink with plant debris and animal carcasses, such as the observed fish, being trapped under the thermocline.

We speculate that the concentration of fishes during the dry months may exceed the carrying capacity of the basin. Fish may succumb as a result of starvation, cold shock or the depletion of oxygen caused by the hydrogen sulfide layer being relatively close to the surface. A moderate winter turnover associated with the sinking of cold water could result in some release of nutrients to the Myakka River but the volume of water displaced would likely be relatively small and in years of extreme low lake level the sink does not have much direct interaction with the river.

Much remains to be learned about the site although the utility of scientific diving will be limited because of the uniformly poor visibility. A geologic determination of the age of the sinkhole would be valuable to determine if the feature was present during the same time period as WMS and LSS. If Deep Hole formed from a collapse of the same rock strata as WMS and LSS then the overall depth of the original collapse hole could be much deeper. If Deep Hole was present during the period of human occupation of WMS and LSS it may have been utilized as a seasonal hunting area because of the accumulation of fish and wildlife during dry periods. Additional relevant archaeological information could possibly be obtained by a close examination of the shallow ledge observed along a portion of the break. However, the small size of the ledge would likely preclude significant use as human habitat.

Perhaps the most intriguing possibility for Deep Hole is that it may serve as a biological record of the flora and fauna of the recent geologic past. The bottom sediment is likely mostly undisturbed and may be quite thick. Observations during this study indicate that potentially large numbers of fish and possibly other organisms may be entombed in the bottom sediments such as those found in a sinkhole known as "Sawmill Sink" on Abaco, the Bahamas where numerous well preserved skeletal remains were recovered including extinct tortoises (Steadman et al., 2007). The direct accessibility of the hole to a variety of wildlife during low water periods provides for a high probability that remains of organisms other than fish and alligators may be present in the bottom sediment.

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BIOLOGICAL ACTIVITY INVESTIGATIONS OF SPONGES COLLECTED FROM HAWAII AND MAUI

***Jaaziel E. Garcia Hernandez*[1,2]**
***Kehau A. Hagiwara*[3]**
***Nicole Tabanera*[3]**
***Nicole de Voogd*[4]**
***Dovi Kelman*[23]**
***Anthony D. Wright*[3]**

[1]Department of Marine Sciences,
University of Hawaii
Hilo, HAWAII 96720 U.S.A

[2]Department of Biology,
University of Puerto Rico – Mayagüez
Mayagüez, PUERTO RICO 00681 U.S.A
jaaziel.garcia@upr.edu

[3]College of Pharmacy
University of Hawaii at Hilo
Hilo, HAWAII 96720 U.S.A.

[4]National Museum of Natural History Naturalis
PO Box 9517
2300 RA Leiden, THE NETHERLANDS

Introduction

Marine sponges were once thought to be some of the simplest multicellular organisms in the animal kingdom. In reality, these organisms are relatively complex life forms that are important members of reef ecosystems. It has been highlighted (Diaz and Rützler, 2001) that on coral reefs sponges play a variety of important roles: primary production and nitrification through complex symbiosis; chemical and physical adaptation for successful space competition; capability to impact the carbonate framework through calcification, cementation and bio-erosion; and the potential to alter the water column and its processes through their ability to filter large volumes of water. The majority of marine sponges are sessile filter feeders. Consequently, most marine sponges need to reside where there is strong water movement and surge in order to survive; the water movement provides oxygen and nutritional intake that sponges need. This water is actively pumped through hundreds of pores found on a sponge's surface (Riisgård et al., 1993). Furthermore, they lack a "conventional" organ system for digestion (Hentschel et al., 2002) that is present in higher animals. This results in an unconventional distribution of microbes within sponge tissue, regardless of the nature of the transient association between microbe and sponge, whether for nutritional or alternative benefit. It is known, however, that marine sponges utilize their ability to filter large amounts of sea water to trap microbes for food and also to benefit the sponge in other ways (Radjasa and Sabdono, 2009).

Sponge metabolites

In the search for novel bioactive compounds for the development of pharmaceuticals many species of marine sponges have been collected (El Sayed et al., 2000; Baker et al., 2007; Zhu et al., 2008; MarinLit 2013). Typically, sponges produce secondary metabolites as do their associates, including bacteria and fungi. These secondary metabolites often play an important role in the life cycle of a given marine sponge where they serve ecological functions such as competition for space, protection from predation, prevention of fouling, and from ultraviolet light (Rohwer et al., 2002). The microbes in turn use their secondary metabolites to protect themselves from the sponge and other organisms and conditions within the sponge's tissue. In doing so, they often also impart positive protective properties to the sponge, for example, against predators, parasites, pathogenic bacteria, reactive oxygen species and ultraviolet light.

Bergmann and Feeny (1950) in one of the earliest reported marine natural products research efforts discovered two novel nucleosides, spongothymidine and spongouridine, in the marine sponge *Cryptotethia crypta*. This discovery led to the synthesis of two compounds known as ara-A and ara-C that were the first marine natural-product inspired pharmaceuticals. Today, ara-A is employed as both an anticancer and an antiviral agent (Field et al., 2004) while ara-C is used in the treatment of leukemia and lymphoma (Wang et al., 1997; Sipkema et al., 2005). Since that early finding, many thousands of natural products have been discovered from the marine environment (Marinlit, 2013). Of the compounds discovered, many have been shown to have potent pharmacological activities, including anti-tumor (Erba et al., 2001), antifungal (Lee et al., 2007), anti-viral (Field et al., 2004), antimalarial (Wright et al., 1996), antitubercular (König et al., 2000), anti-HIV (Matthée et al., 1999), anti-inflammatory (De Silva and Scheuer, 1980), as well as anti-bacterial (McCarthur, et al., 2008).

Hawaiian sponge biological activity

The current study investigated the biological activity of organic solvent extracts derived from shallow water marine sponges collected along the eastern coastline of Hilo, Hawaii and from mesophotic sponges collected from the Au'au channel, Maui. Three shallow-water sites were surveyed during this project at which 13 sponges were observed and collected *in situ* using either scuba or snorkel diving. Five mesophotic sponges collected from the Au'au Channel off Maui using the NOAA HURL *PISCES IV* and *PISCES V* submersibles from February 26 - March 10, 2011 were also analyzed. Samples averaging 1-6 cm^3 in size were taken from each sponge in order to perform tissue and spicule analysis for preliminary taxonomic identification. Of the 13 shallow-water sponges, six were identified to genus level based on scanning electron microscope (SEM) images of their spicules. Organic extracts from each sponge were tested for their antioxidant and antibacterial properties. Total antioxidant activities of individual organic solvent extracts of all 18 sponge samples were determined employing the ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996, 1999). Of the sponges tested, extracts of the mesophotic sponges showed the highest total antioxidant activity. Mesophotic sponge D0016 showed the highest antioxidant activity with a FRAP value of 1780 ± 239 µM/µg extract. Antimicrobial properties of the extracts were determined using the Kirby-Bauer antibiotic sensitivity test (Schultz et al., 1995). Again, extracts of the mesophotic sponges showed the highest levels of inhibition against the panel of Gram-positive bacteria: *Staphylococcus aureus, Bacillus cereus,* and *Enterococcus faecalis*, with sponge D0015 demonstrating the highest activity.

Conclusion

These results show that numerous Hawaiian sponges, from both shallow and deep waters, exhibit significant antioxidant and antibiotic activities that may lead to discovery of novel compounds for potential use by the pharmaceutical and related industries.

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SCIENTIFIC DIVING IN COASTAL GEORGIA: 8000 YEAR-OLD TREES, PREHISTORIC SHELL MIDDENS AND SEA LEVEL CHANGE

***Ervan G. Garrison*[1]**
***Jessica W. Cook Hale*[1]**
***Michael K. Faught*[2]**

[1]University of Georgia
Department of Anthropology
Baldwin Hall, Room 265A
Athens, GEORGIA 30602 U.S.A.
egarriso@uga.edu

[2]Department of Anthropology
Florida State University
Tallahassee, FLORIDA 32306 U.S.A.

We describe scientific diving during an initial study of prehistoric landscapes characterized by drowned trees and shell middens discovered by a remote sensing and diving reconnaissance in 2012. Subsequent work in the summer of 2013 has focused on better characterizing these sites and their archaeological research potential. Modern sea levels are only the present day stage of the last glacial cycle that ended 15,000 years ago with the retreat of the Laurentide Ice Sheet (LIS). The study sites are well below modern low tide cycles suggesting the presence of preserved stream or estuarine paleoenvironments for the Georgia coast before modern sea levels were reached in the Mid-Holocene. Their continual inundation since submergence has preserved many organic features such as trees and the shell deposits adjacent to those trees. The aim of this research was to go beyond the reconnaissance level of study and to recover additional scientific information to determine their relationship or lack thereof with prehistoric human groups who occupied the Georgia coastal plain before its modern submergence. We present remote sensing data as well as the analysis of materials recovered from the submerged landforms.

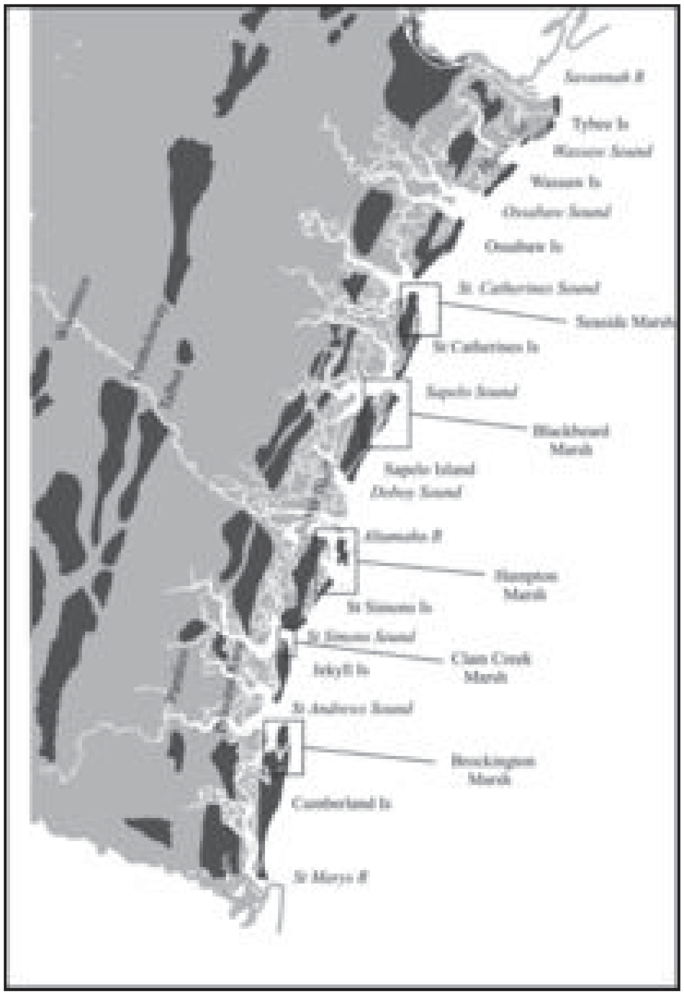
Introduction

More North American archaeologists are investigating tidal and submerged sites for evidence of later Quaternary or early Holocene settlement of southeastern U.S. coasts (Garrison, 1992; Faught, 2004; Thompson and Turck, 2009). These types of archaeological sites were subaerial during long stretches of the late Quaternary and early Holocene, having only recently been inundated by sea level rise after the end of Last Glacial Maximum (LGM) at 18 ka. Present-day, or near present-day, sea level was only reached by the 7th millennium BP (before present), leaving potential sites from earlier periods underwater and invisible to terrestrial surveys.

One particular facet of the Holocene that has puzzled archaeologists in the Southeast is minimal evidence for cultural materials on the coastline during the Middle Archaic Period. This period elsewhere dates to ca. 8000-5000 BP. In Georgia, archaeologists have noted the low number and density of Middle Archaic Period sites on the Coastal Plain for some time (Williams, 1994; Elliott and Sassaman, 1995;

Kowalewski, 1995; Williams, 2000; Williams et al., 2013). Within the present day coastal zone (which extends from the seaward shores of the barrier islands to about 30 km inland), only three Middle Archaic sites have been found (Turck, 2012). Offshore and inshore studies of submerged archaeological locales have found little or no definitive evidence for this period either (Faught, 2004).

Material remains left behind by Middle Archaic populations are also not currently very well characterized for the tidewater zone (Turck, 2012). Middens made up of marine and/or estuarine shell and dating to this time are not present in the Georgia coastal area, while pottery, the other main diagnostic artifact type for archaeological sites in coastal Georgia, had not yet been invented. The main evidence for sites from this time period, therefore, will be lithic material. Even more frustratingly there is a strong possibility that perishable materials such may have composed a large part of the Middle Archaic toolkit (Elliott and Sassaman, 1995). This would fit the pattern noted elsewhere in the Southeast, where there was a notably increased reliance on local raw materials at that time. Coupled with this factor, there is a dearth of lithic material on the coast of Georgia. It seems highly likely then that traces of Middle Archaic activity will be more difficult to locate than later cultural material remains.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Sapelo Island
Doboy Sound
Altamaha R
Hampton Marsh
St Simons Is
St Simons Sound
Clam Creek Marsh
Jekyll Is
St Andrews Sound
Brockington Marsh
Cumberland Is
St Marys R

Figure 1. Georgia coast showing the principal modern and ancient barrier island systems. The AIWW locations mentioned in this article are referenced to this figure. Detailed locations for the sites discussed herein cannot be given because of the danger of unauthorized personnel exploiting explicit geodetic information.

In this paper, we describe the initial phases of study of submerged locales, along sections of the Atlantic Intracoastal Waterway (AIWW; Fig. 1) using scuba and air helmet diving techniques, to attempt to locate prehistoric sites, particularly those dating to the Middle Archaic Period.

Objectives and Rationale

The primary objective of our study was the location and identification of submerged prehistoric archaeological/paleontological sites in estuarine/tidal waters that are difficult to explore because of low or no visibility and other detrimental environmental factors such as currents, tides and sedimentation.

The rationale for doing such a study resides in the fact that underwater environments such as these are under-investigated by archaeologists. Additionally, because of the height of the present-day sea level, many previously habitable paleolandscapes are now continuously submerged. With modern rates of sea-level rise, any potential prehistoric locales will only become more deeply submerged and even less accessible for study.

The potential for the discovery of previously unidentified archaeological/paleontological locations, seems to be significant enough to justify this exploratory research. As described in our introduction, one of the present-day lacunae in the archaeological knowledge of the Southeastern U.S. coast is that of the lack of Middle Archaic Period sites. In justifying our study we would invoke the well-known maxim, "an absence of evidence is not evidence for absence." A large part of the reason for a lack of evidence for Middle Archaic Period archaeological sites may simply be the lack of systematic exploration by archaeologists who have skill sets and technology to investigate submerged environments where these sites may be found.

Methods

Scientific Diving

In 2012, co-author Faught, during a reconnaissance level archaeological study of areas along the Atlantic Intracoastal Waterway (AIWW), used air helmet diving techniques (Fig. 2). Subsequent diving work in the summer of 2013, by co-authors Cook Hale and Garrison used scuba diving techniques. Both diving methods were found to be efficient for the shallow, tidal-stream depths encountered in the study area.

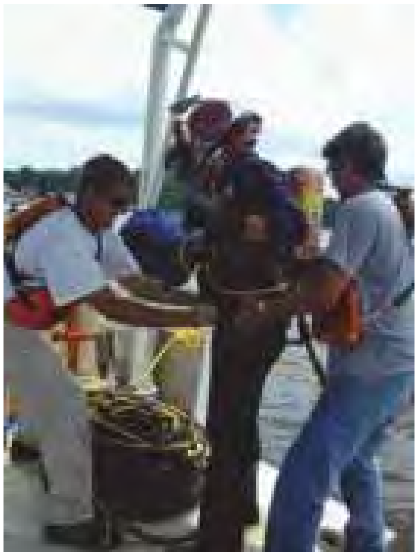

Figure 2. Diver with Superlite 17 air helmet, AIWW location.

Diving reconnaissance followed up on the identification of suspected prehistoric features and landforms using side-scan sonar and subbottom profiler surveys. In low-to-no visibility conditions, which are typical for estuarine and tidal streams in Georgia, diving for initial reconnaissance was an unproductive method because it is very difficult, if not impossible, to identify submerged sites. However, diving used as a means to identify and characterize suspected submerged prehistoric locations that were previously located using remote-sensing methods is a very useful tool.

Geophysical

To provide locations for diving assessments to be used, we utilized shallow-water geophysical survey methods such as side-scan sonar, sub-bottom acoustic profiling and magnetometry. In the summer of 2012, working on parallel but separate research projects, co-authors Faught and Garrison investigated differing locales along the AIWW in Georgia. Faught's research focused on locating potential submerged archaeological sites along extended reaches of the AIWW as part of a project for the U.S. Army Corps of Engineers (USCOE) and utilized a full suite of sonar, sub-bottom profiling and magnetometry. The research done by Garrison's team was part of a graduate research study and utilized sonar and magnetometry (Fig. 3). Faught's research utilized photogrammetric (Fig. 4) and reconnaissance data from a much earlier (1979) survey of the AIWW in a project conducted by Garrison and Tribble (1981).

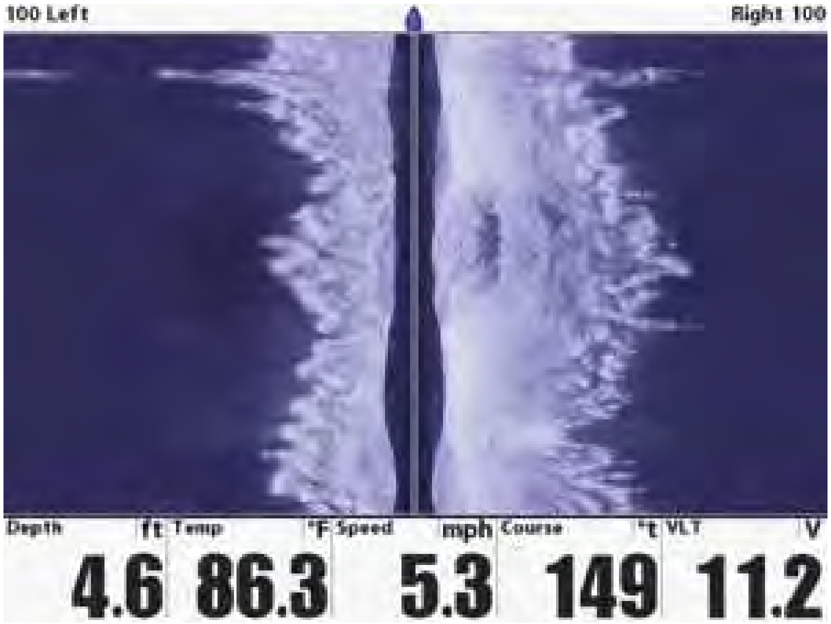

Figure 3. Side-scan sonar of Grove's Creek thalweg, Skidaway Island area.

Videography

The *R/V GEORGIA BULLDOG*, used in the summer of 2013, had underwater video capabilities. This equipment was used to examine a suspected prehistoric site's bottom conditions in low-to-zero visibility conditions. As has been observed in other "telepresence" studies, the camera is superior to the human eye in these low visibility conditions. This quality enabled us to discern bottom type and/or sediment type as well as biota (Fig. 5) before carrying out additional investigations in the estuarine/tidal streams of the AIWW.

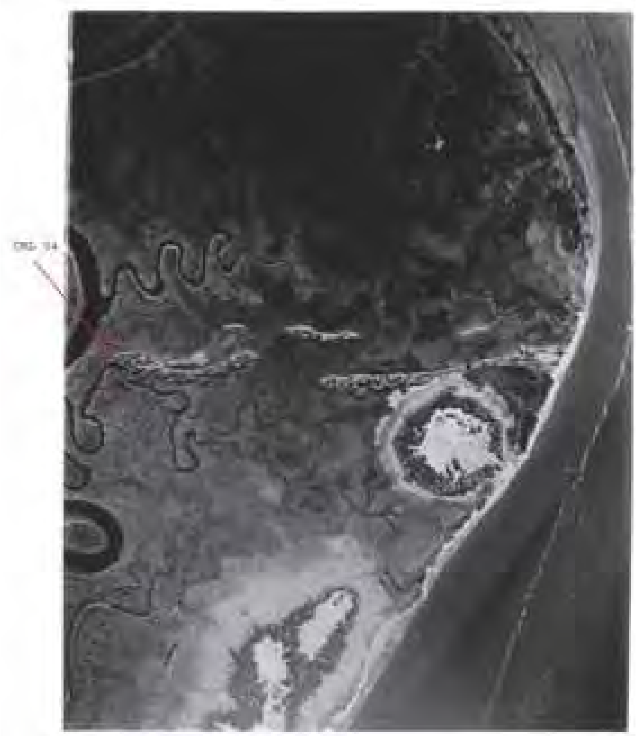

Figure 4. Aerial photograph of prehistoric archaeological site (CRL 04) located in the 1979 AIWW survey (Garrison and Tribble, 1981).

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Figure 5. Video image of coralline algae "fronds" in Jekyll Creek, AIWW.

Sediment Grabs and Shallow Cores

The collection of bottom and subbottom sediment samples was a primary objective of both diving and shallow sediment core/grab sampling. The latter techniques were deployed alone or in concert with diving collection activities. The core/grab samples were taken using easily deployed over-the-side apparatus (Fig. 6 a, b).

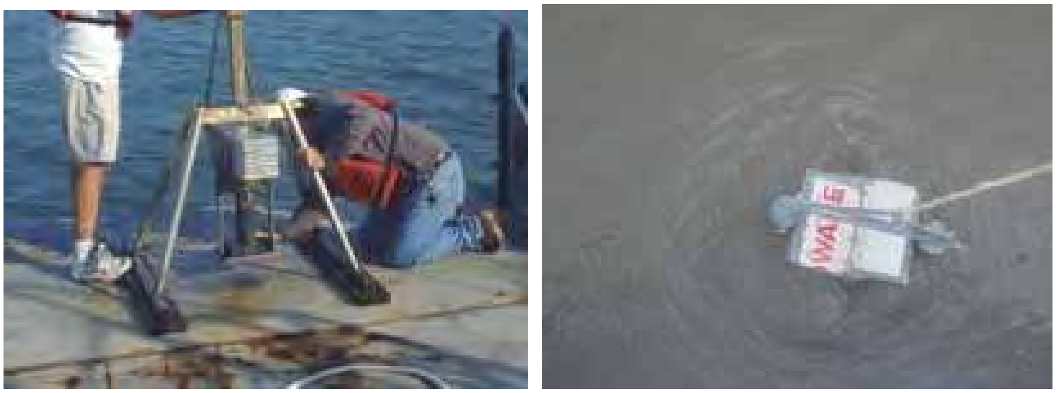

Fig. 6. (a) above, shallow sediment corer, (b) right, a sediment grab deployed at Jekyll Creek, AIWW.

Results

Divers investigated numerous potential prehistoric targets during the study led by Faught (Panamerican Consultants, Inc., 2012). Some of these locales had been previously identified in the 1981 report (Garrison and Tribble, 1981) as noted above. Those locations were shared with Faught and several were subsequently investigated in the course of the 2012 research. The 2013 research revisited three of the potential sites identified and investigated in 2012. A fourth location was investigated in 2012 and was not studied in 2013 but future research is planned for that site. The three sites, jointly studied in 2012-2013, will be addressed in order.

1. C 41, Jekyll Creek
2. SB feature 7/20, Rockdedundy, South & Little Mud River
3. SB feature 49, Rockdedundy, South & Little Mud River
4. C282, St. Augustine Creek

The shorthand used, above, is “SB” for sub-bottom profiler contact; “SS” for side-scan sonar contact; and “C” for “contact”.

1. C 41, Jekyll Creek

This site was a high backscatter sonar contact and recommended for avoidance or additional study by the 2012 study. Diver investigation confirmed the deposit as made up of whole and fragments of oysters. The sample collected and studied showed all specimens were disarticulated, with evidence of oxidation (rust color) on many. The deposit was considered as a secondary dump, most probably from dredging operations. The oyster shells were large and there were equal numbers of left and right valves suggesting a natural deposit (i.e., not prehistoric).

The 2013 study returned to the C 41 site to carry out additional study. Sonar was carried out primarily to relocate the features described in the 2012 study. Once the location was satisfactorily identified videography and sediment sampling was carried out. Shown in figure 7 the sediment grab and one such grab's contents are displayed. Two transects were sampled roughly 30 m apart with 14 grabs taken. The transects provided adequate sampling of the bottom sediments from shallow to mid-channel depths in Jekyll Creek.

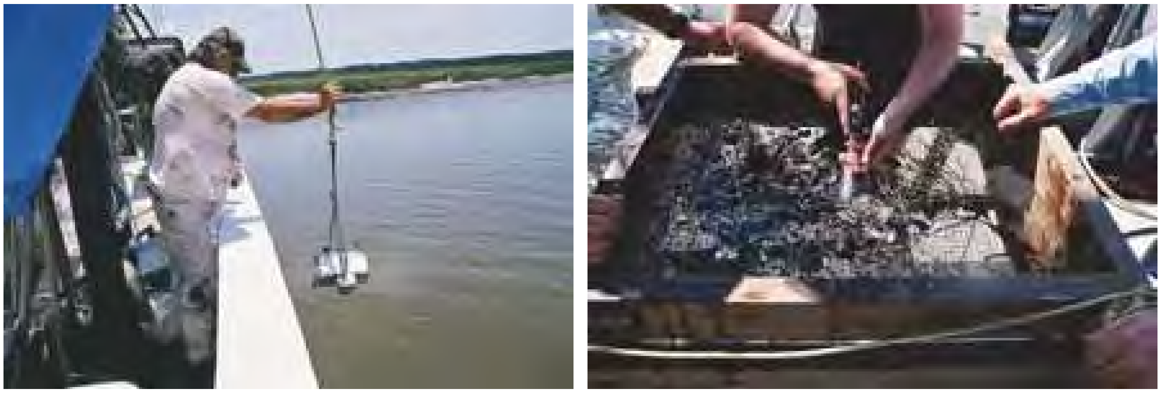

Figure 7. 2013 studies of C 41 site, Jekyll Creek. Left, sediment grab deployed; right, washing a sample grab through wire mesh screens (note algae seen in Fig. 5).

As illustrated in figure 7, all sediments recovered were washed through wire mesh screens to recover small inclusions such as shell, bone, lithic material, etc. No diving was conducted at the site in 2013. No archaeological materials were recovered in the sediment samples thereby confirming the deposits were of a "natural" origin as surmised by the 2012 study.

2. SB features 7 and 20, Rockdedundy, South and Little Mud River

The feature is labeled as 7/20 because it was mapped on one sub-bottom line file as SB Feature 7 and another as SB Feature 20. SB Feature 7/20 was recognized in 2012 as anomalous by its layered and mounded character in contrast to the surrounding returns. The layering is the result of bands of light and dark reflections in the sub-bottom record (Fig. 7). The top of the feature is at 5 feet (1.6 m) under the water, its base at 14 feet (4 m) and its overall length is 700 feet (200 m). Figure 8 shows numbers on three possible sets of couplets that make up the structure, each with three to five sets. Diver excavations showed that the couplets were intercalated oyster shell and gray clayey-silt or silty clay layers. Oyster shell was dominant with few specimens of other species; there were also small fragments of reeds. All specimens were disarticulated.

SB Features 20/7 and nearby SB 49 described below are essentially the same kind of seismic reflector (the boundary between beds with different properties) but SB Feature 49 is somewhat deeper and, therefore, possibly older. The formation process is unknown but includes alternating beds of clay, shell, clay, and shell, situated on the edge of the channel which suggests a migrating channel bed facies scenario (Farrell et al., 1993). Additional sampling was done in 2013 to help answer these questions. Likewise, a study of the oyster shells was conducted to ascertain whether the deposit was caused by natural or anthropogenic processes.

Irv Quitmyer (Florida Museum of Natural History; pers. comm. 28 August 2012) commented that the normal distribution of valves in a natural setting should have a preponderance of left (cupped/bottom) valves. This is because the right (flat/top) valves are more susceptible to reworking and fractionation than the left (cupped) valves. The 2012 report suggested that it was possible that these numbers are the result of human intervention (e.g., collecting). The 2013 study results indicated an obvious preponderance of top to bottom valves in the assemblage. Those data are: top/ right valve, n = 43; bottom/left valve, n = 16. If Professor Quitmyer's opinion is correct, then this result suggests: (1) the assemblage is not the result of natural processes and (2) there may have been selective sorting of valves by predators, human or otherwise.

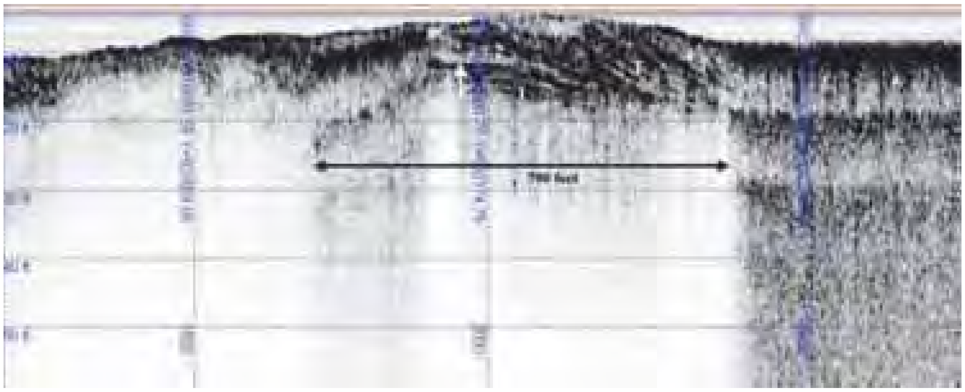

Figure 8. Feature 7/20 as shown in sub-bottom profile. White arrow points at location of induction dredge excavation carried out in 2012.

3. SB feature 49, Rockdedundy, South and Little Mud River

SB Features 7/20 and 49 are essentially the same kind of seismic reflector, but SB Feature 49 is somewhat deeper and therefore possibly older. The formation process is unknown but includes alternating beds of clay, shell, clay, and shell, the same as seen at SB 7/20 (Fig. 9). The overall length, 775 feet (220 m) is quite similar as well. Futhermore, sedimentologically and geomorphically the two locations are similar.

The shell deposits at both show a similar distribution in shell types and the sorting of their valves. The 2012 study identified 81 “top” valves in relation to 14 “bottom” valves. Further study results, in 2013, based on a larger assemblage from SB 49 agree with the results of 2012, i.e., top valves, n = 73; bottom valves, n =11. As previously noted, these results suggest selective sorting of the shell assemblage. Based on the analyses of the shell assemblage alone, these results imply that the deposits are anthropogenic in nature and may represent prehistoric activities at both sites. Figure 10 illustrates an interesting contrast in the shell species diversity at SB 49 in comparison with those of C 41 and SB 7/20 assemblages which were predominantly oyster.

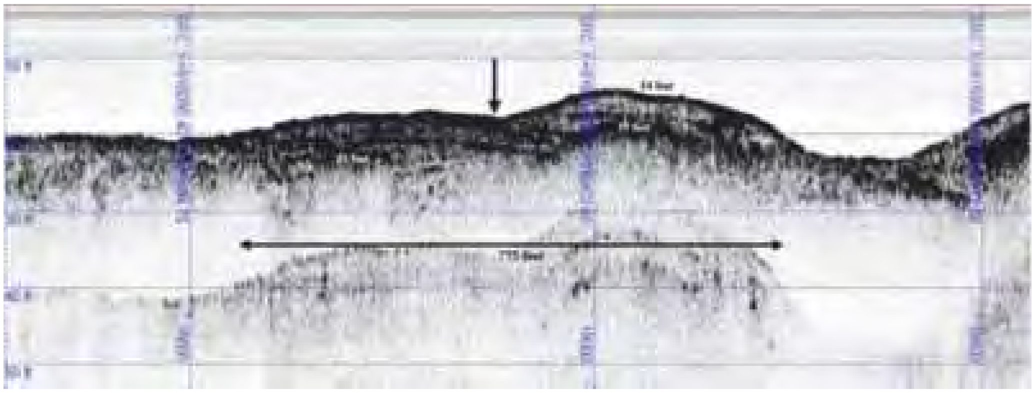

Figure 9. SB 49 as shown in sub-bottom profile.

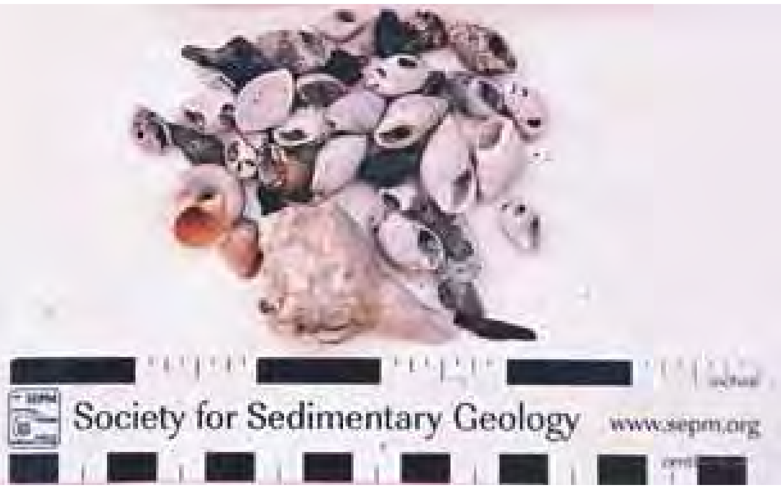

Figure 10. Mixed shell assemblage from SB 49 site.

As seen in Figure 10, gastropod shells are the predominant component. One whelk (*Busycon*) and one clam (unidentified) are shown but the remainders are *Littorina littorea* (common periwinkle) and one Naticidae species ("moon snail"). Gastropods, marine and estuarine, in the oyster midden suggests human agency for their presence. Marsh periwinkle (*Littorina littorata*) would have been collected on *Spartina* grass stems throughout the year (Fierstien and Rollins, 1987) and whelks (*Busycon* sp.) were likely picked up while collecting bivalves, as they are commonly found feeding in *Tagelus* beds (Purchon, 1977).

4. C 282, St Augustine Creek

In Figure 11, Contact C 282 remains an area of potentially significant submerged cultural resources. In the sonar data this area included larger (> 1 foot (20 cm) diameter) and smaller (< 3 inch (7.25 cm)) protuberances that were confirmed as tree stumps and smaller "posts" or smaller trees, sticks, branches or bushes. Some of these smaller specimens appeared to be in possible alignments in the 2012 sonar record, suggesting the possibility of fish weirs or other features constructed near-shore.

During investigation of these targets water visibility was near zero but divers could determine the limits of the exposed materials by feel (smooth bottom downstream *versus* abundant biomass upstream and upslope, away from the thalweg). Four tree stumps were measured by the diver wrapping his arms around the stump; their diameters were noted to be greater than 1 foot with one almost 3 feet (~ 1 m) in diameter. Samples of this stump (fig. 12) were collected, including a broken off rootlet from one main root trunk into the sediment bed. A portion of this specimen was radiocarbon dated to 7300 +/- 40 YBP (Beta 36234). This is a significant age, indicating the age of a tree preserved in a surface that was probably flooded during and after MIS 1, after 7,300 YBP. The surface was at 28 feet (9 m) at the time of the 2012 dive operations; the root was from about 1 foot (20 cm) below the surface exposure.

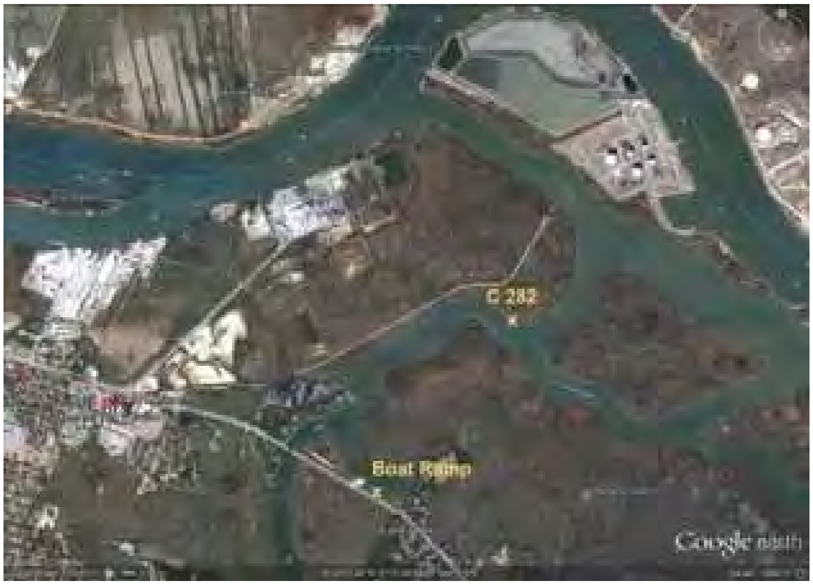

Figure 11. Google Earth image of location of C 282 in St. Augustine Creek, AIWW.

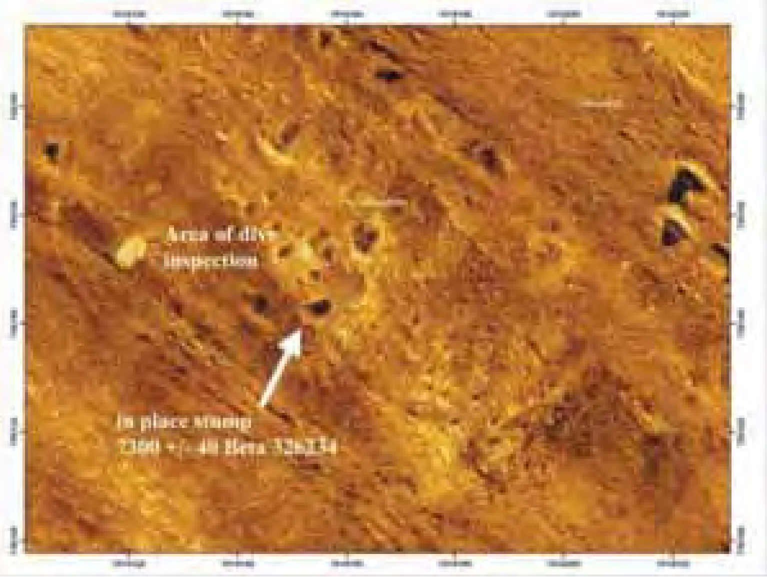

Figure 12. High backscatter area inspected by geoarchaeologists showing location of submerged forest stumps.

C 282 will not be investigated in 2013 but future studies are planned. Samples will be collected by divers to establish the various arboreal taxa present as well as their respective ages. If possible, dendroclimatology studies will be conducted on cross-sections of selected samples.

Summary and Conclusions

A multi-method approach to the studies of these four example sites has stressed the use of scientific diving as a key element. Combined with geophysical and sediment sampling methods, diving made possible the controlled and systematic recovery of materials found on these sites. Only one of these four sites has been eliminated as a potential prehistoric archaeological site, C 41, but the other three remain active study locations because of the nature of the invertebrate assemblages coupled with the discovery of drowned prehistoric trees (C-282). The key defining attribute of all prehistoric archeological sites – artifacts - have not yet been found. Continued study by divers and sediment sampling techniques will focus on recovering definitive prehistoric cultural remains that may include lithic material, shell artifacts, bone/horn items, pottery and organic items such as wood and/or woven materials. To date the results may be only indicative of prehistoric garbage. That being said, however, further collection may find evidence for the folk who produced the garbage.

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AN IMPROVED NEW CLASS OF DECOMPRESSION MODELS THAT ARE RELEVANT TO SCIENTIFIC AND RECREATIONAL DIVING

Saul Goldman
Department of Chemistry
University of Guelph
Guelph, Ontario, N1G 2W1, CANADA
sgoldman@uoguelph.ca

A new class of interconnected compartmental models is projected as the 'gold standard' for decompression modeling. These models are consistent with empirically based physiological data on gas washout rates from mammalian tissue. In these models a central risk-bearing compartment exchanges dissolved gas both with the circulatory system and with contiguous connected non-risk-bearing compartments. These models fit calibration data more accurately and with fewer fitted parameters than do current parallel compartment models. These models also extrapolate beyond their calibration regime much more accurately than do parallel models. This is highly relevant both to scientific and recreational diving where the hit rate is orders-of-magnitude less than that of the calibration dataset. This discussion provides a general overview of the properties of these models in relation to standard parallel models, illustrates their superior capability of accurately predicting hit rates both for very high-risk and very low-risk diving and describes the practical implications of these models for effective decompression in low-risk diving. Using a large subset of the DAN PDE dataset (approximately 70,000 air dives) for which the hit rate (0.015%) is comparable to that found with scientific diving, these models are shown to accurately predict the observed hit rate while the older parallel models do not.

Introduction

About sixty-five years ago, Morales and Smith proposed that compartmental models of the kind shown in the lower left quadrant of Figure 1, which they called "a competitive parallel arrangement," were a more realistic representation of tissues in the body than was the simpler Haldanean compartmental structure shown in the upper left quadrant of this figure (Morales et al., 1944, 1945, 1948). However, because their work preceded the computer era, and because their mathematical solution for the kinetics of these models was cumbersome (albeit correct), their suggestion was not pursued by the decompression modeling community. Models of this kind were, nonetheless, used quite extensively for the last several decades in pharmacokinetic studies in medicine and physiology (Jacquez, 1985). The interest was to predict the distribution and washout times from the body for both non-volatile and volatile solutes such as drugs, metals, anesthetics, toxins, etc. It was established in these studies that the independent parallel (i.e., Haldanean) compartmental structure could not account for the observed washout kinetics but the interconnected parallel arrangement, suggested by Morales and Smith, could.

More recently, Doolette et al. (2003; 2005) found inconsistencies with the Haldanean structure in their studies of gas washout from both sheep and humans. They found that interconnected models were consistent with their gas washout results. In addition to these studies, the general idea that DCS-prone tissue is unlikely to be isolated from less susceptible tissues, and may be indirectly affected by them, has been around for some time. For example, Vann (1990) pointed out the possibility that unsusceptible tissue (such as lipid) may act as a reservoir of dissolved inert gas relative to contiguous, more susceptible tissue, and may thereby indirectly affect DCS risk by diffusive exchange of inert gas with the susceptible tissue. Depending on the dive profile, these contiguous gas reservoirs may either reduce or increase the risk by either respectively drawing off or adding to the quantity of excess gas in the central risk-bearing tissue (Goldman, 2007).

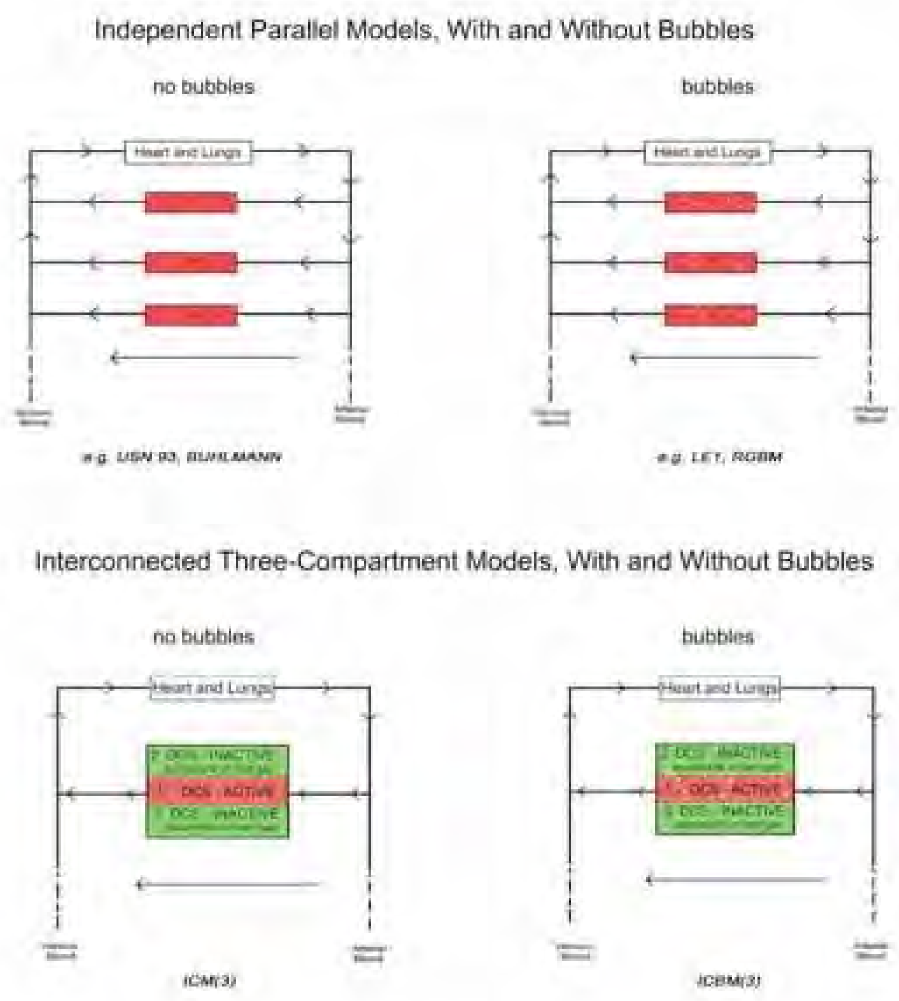
Independent Parallel Models, With and Without Bubbles
no bubbles
bubbles
Heart and Lungs
Heart and Lungs
e.g. USN 93, BUHLMANN
e.g. LET, RGBM
Interconnected Three-Compartment Models, With and Without Bubbles
no bubbles
bubbles
Heart and Lungs
Heart and Lungs
2 DCS INACTIVE
1 DCS ACTIVE
3 DCS INACTIVE
2 DCS INACTIVE
1 DCS ACTIVE
3 DCS INACTIVE
ICM(3)
ICBM(3)

Figure 1. Independent and interconnected three-compartment models, each with and without bubbles in their risk-bearing compartments. The red compartments bear risk; the green compartments do not bear risk, but affect risk indirectly.

Theory

There are two basic components to a decompression theory or model: 1) the kinetics, and 2) the risk.

Kinetics

The detailed kinetics of the interconnected models have been described previously (Goldman, 2007). The most relevant result for the present purpose is:

$$rate\ = C_1 \exp(-\lambda_1 t) + C_2 \exp(-\lambda_2 t) + C_3 \exp(-\lambda_3 t)\ .\ (1)$$

In Equation 1, the Cs and λs are constants (the latter are effective rate constants) and "rate" is the rate at which excess dissolved gas is removed (or "washed out") from the central risk-bearing compartment for the diver at the surface after a dive for any specific dive profile. The sum of three exponentials is a consequence of all three compartments collectively contributing to the washout rate of the central compartment. Equivalently, this can be thought of as the peripheral non-risk bearing compartments acting as sources of or sinks for the excess gas relative to the central compartment.

In contrast to this, for an independent (e.g., Haldanean) compartmental structure, one "controlling" compartment almost invariably dominates the washout rate for any given profile, so that here:

$$rate\ \cong C \exp(-kt)\ (2)$$

Here there are no sources of, or sinks for, the excess gas. All the relevant excess gas is in the 'controlling' risk-bearing compartment that off-gases directly to the circulatory system. As will be shown below, Equation 1 results in a relatively fast washout rate at small “*t*” (i.e., short times), and a relatively slow washout rate at large “*t*” (i.e., long times), as opposed to the more uniform washout rate at all times produced by Equation 2.

Risk

This is handled differently in different models. A number of so-called 'risk functions' have been developed, two examples of which are given below (Thalmann et al., 1997; Goldman, 2007;).

One-phase (no bubbles). This empirically-based risk function has been used both for independent and interconnected compartmental models. Here:

$$risk = K_1 \Delta P / P_0;\ \Delta P = P_t - (P_0 + B)\ (3)$$

B is a constant and (P_t, P_0) is the gas pressure in the tissue and the ambient pressure, respectively.

Two-phase (bubbles). This risk function has been used for the interconnected models and is based on the author's theoretical description of Strauss et al.'s Ruffini type-2 pain-sensing organelle model (Strauss et al., 2008; Goldman, 2009, 2010) and is given by:

$$risk = K_2 (\Delta P)^2\ ;\ \Delta P = P_t - (P_0 + B)\ (4)$$

Results

Table 1 provides the approximate % DCS for various types of diving (Vann, 2004) and shows an overlap between scientific and recreational diving. Scientific diving entails a risk level that is comparable to low-risk recreational diving (e.g., diving from 'liveaboards' with a dive computer). Consequently, this subset of the recreational diving regime is used as a substitute for a scientific diving dataset. In the figures color-coding has been used to distinguish the results obtained for the different models.

Table 1. Perspective on DCS Risk (Source: Vann, R.D. 2004. In: Bove and Davis' Diving Medicine", 4th ed.)

Type of diving	% DCS
Scientific	0-0.03
Recreational	0.01-0.08
Commercial	0.04-0.3
US Navy	0.03-1.3
Military dive trials	4-5

Table 2 shows the quality of the fits of one parallel and two interconnected models to a large calibration dataset. These models all have the same number (four) of fitted parameters. Akaika's Information Criterion (AIC), which is lowest for the best-fitting model, shows that the two interconnected models [ICBM(3) and ICM (3)] fit the data better than the parallel model, and that the bubble-based interconnected model [ICBM(3)] provides a better fit than the one-phase interconnected model [ICM(3)].

Table 2. Model parameters.

Model (# compartments)	**# Fitted Parameters**	**Akaike's Information Criterion (AIC)**
Two-Compartment Parallel PCM(2) Not bubble-based	4	500* (worst)
Three-Compartment Interconnected ICM (3) Not bubble-based	4	469* (middle)
Three-Compartment Interconnected ICBM (3) Bubble-based	4	450* (best)

[a] AIC = 2 [# Fitted Parameters- Ln(L)], L= "Maximized Likelihood Function"

[b] Calibration dataset: Approx. 2200 exposures, all air, consisting of a mix of recreational and military profiles. Average hit rate was about 4%.

[*] Lowest AIC gives the best model

A good fit to a calibration dataset is a necessary, but not sufficient, basis for deciding on the quality and/or value of a model. A more stringent and meaningful criterion is how well a calibrated model 'projects' (or extrapolates) to dive profiles that are outside of, or not well-represented by, the calibration dataset. Clearly, if a model, simplified though it may be, captures enough of the underlying physiology to extrapolate correctly, that model is obviously much better than one that does not, even if they both fitted some given calibration dataset equally well. Table 3 and Figure 2 illustrate how well or otherwise a number of models extrapolate to profiles not well-represented by the calibration dataset.

Table 3 compares the observed versus predicted % DCS for a very large, very low-risk dataset. This is a subset of about 68,000 dives, which is about half of the full PDE dataset being collected and smoothed for computational use by DAN. The observed % DCS for these dives is 0.015 % for which the 95% binomial confidence interval is 0.007-0.027%. It is seen from this table that the two interconnected models [ICBM(3) and ICM(3)] each correctly predict the observed % DCS, while both parallel models in this table significantly overestimate it. The weakness here of the LE1 model (a three-compartment, independent parallel, bubble-based model, a form of which is used by the USN) is particularly significant. It has 12 fitted parameters while the two interconnected models that correctly predict the observed % DCS have only four.

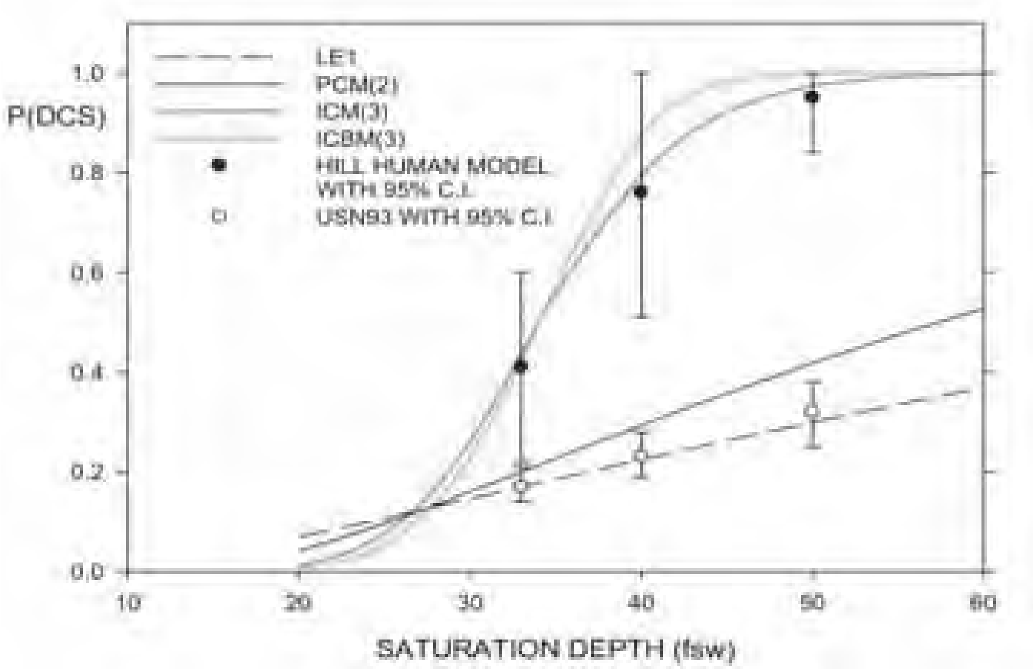

Figure 2. The probability of decompression sickness [P_{DCS}] predicted by different models for very high-risk direct ascents from saturation using air.

The quality of extrapolations in the opposite direction, to the very high-risk regime, is illustrated in Figure 2. The P_{DCS} and observed decompression sickness for direct-ascent from saturation, for a range of saturation depths for which the P(DCS) is extremely high. This graph clearly shows that both interconnected models (bubble-based and non-bubble based) correctly predict the rapid rise in P_{DCS} with increasing saturation depth, while all the parallel models (bubble-based and non-bubble-based) do not.

The reason that the interconnected models extrapolate accurately while the independent parallel compartmental models do not can be understood physically from the role of the contiguous non-risk-bearing compartments that only the interconnected models have. For extrapolations to very low-risk profiles the contiguous compartments act as receptacles for excess inert gas. They thus reduce the amount of excess gas in the risk-bearing compartment resulting in a modulation of the risk. On the other hand, for extrapolations to very high-risk direct ascents from saturation the contiguous compartments, which are saturated, add to the excess inert gas burden of the central risk-bearing compartment causing a rapid increase in risk with increasing saturation depth.

Simply putting bubbles, or a bubble-based risk function, into an independent parallel (or Haldanean) compartmental structure, does not result in a model that extrapolates accurately. This is evident from the poor results for the LE1 model exposed in both Table 3 and Figure 2.

Table 3. Observed and predicted hit rates for recreational diving.

Model [# Parameters]	**% DCS**	
	Predicted	Observed*
		Mean, 95% BCI
LE1 [12] (Calibrated elsewhere)	0.062	0.015 (.007-.027)
PCM(2) [4]	0.20	0.015 (.007-.027)
ICM(3) [4]	0.013	0.015 (.007-.027)
ICBM(3) [4]	0 .014	0.015 (.007-.027)

* Based on 67,679 air-only dives, with 10 hits. This is a preliminary sub-set of the approximately 140,000 PDE dataset provided courtesy of Drs. Petar Denoble and Richard Vann (DAN). This subset is mostly from live-aboard diving with smaller numbers of day-boat diving, cold-water wreck diving and recreational dive professionals in the Caribbean and Brazil, diving mostly from day-boats.

As indicated previously, interconnected models predict a relatively fast initial washout rate and a relatively slow washout rate at long times, relative to independent compartmental models for which the washout rate is more uniform over time. This is true whether the model is bubble-based or not and it has important manifest consequences on how to decompress effectively. As shown in Figs. 3 and 4, this results in a much more rapid rate of risk-abatement relative to parallel models at early times in a decompression stop.

On the other hand, at very long times the rate of risk abatement of the interconnected models is very slow (relative to parallel models). Consequently, according to these models, a great deal of benefit accrues from doing a relatively short stop but very little benefit is gained by a very prolonged decompression stop.

A similar effect is illustrated in Figure 5 that shows the P_{DCS} predictions of the different models for different rates of ascent in the absence of a stop. The interconnected models, whether bubble-based or not, predict much greater benefit from slowed rates of ascent than the independent parallel compartment model. The origin of this is the faster washout time at short times manifested by the interconnected models.

Finally, Figure 6 shows that while the gross or overall P_{DCS} predictions for the 17 standard USN no-stop square profiles are rather similar for parallel and interconnected models (all are within (1.5-3.0)%), there are differences as to which particular square profiles are the riskiest. The LE1 model predicts that the risk is greatest for the very longest shallowest profile (35 fsw for 310 min) while the two interconnected models have the risk greatest in the center (100 fsw for 25 min). It should be borne in mind, however, that this last result may in part be a consequence of different calibration datasets used for the LE1 and the interconnected compartment model calibrations.

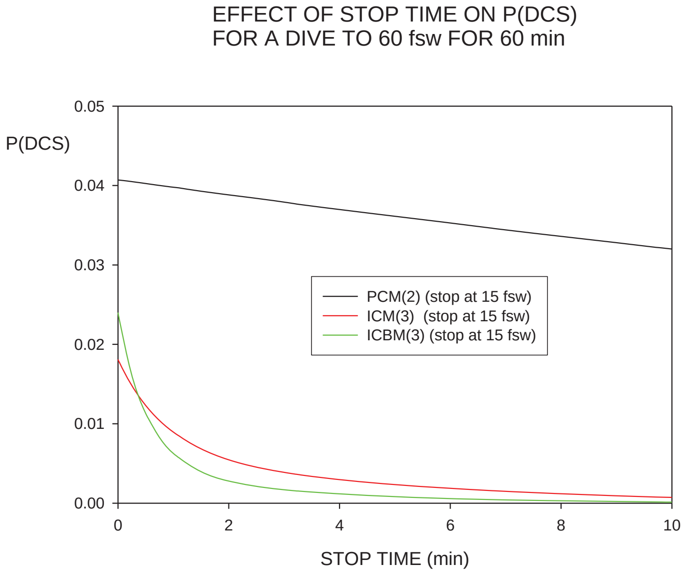

Figure 3. The effect of stop time on P_{DCS} at a stop depth of 15 fsw (5 msw), following a dive to 60 fsw (20 msw) for 60 min for parallel and interconnected models.

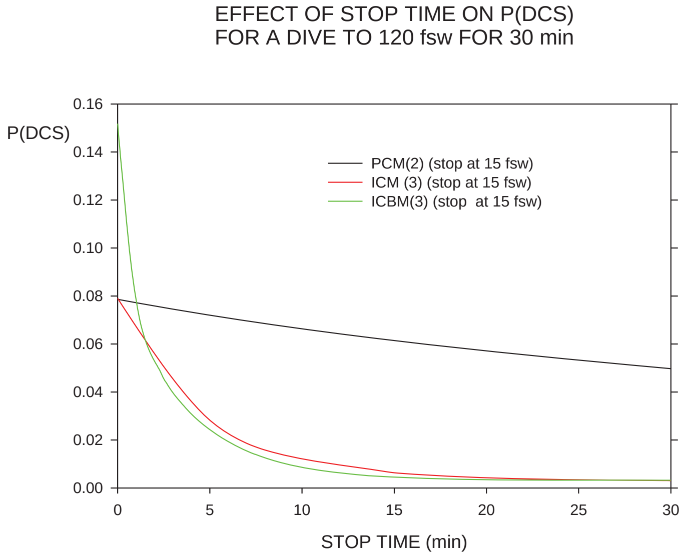

Figure 4. The effect of stop time on P_{DCS} at a stop depth of 15 fsw, following a dive to 120 fsw for 30 min for parallel and interconnected models.

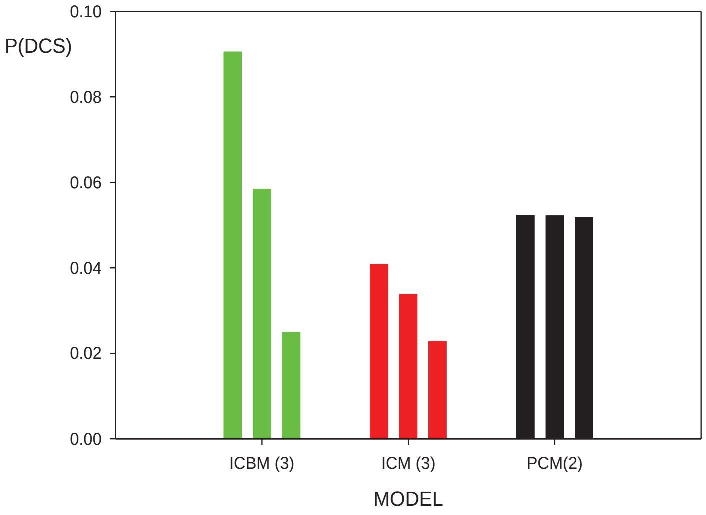

Figure 5. The effect of ascent rate on P_{DCS} following a no-stop dive to 100 fsw (30 msw) for 25 min for parallel and interconnected models. Ascent rates from left to right: 100 ft·min^{-1}, 60 ft·min^{-1}, 30 ft·min^{-1}. (30 m·min^{-1}, 20 m·min^{-1}, 10 m·min^{-1})

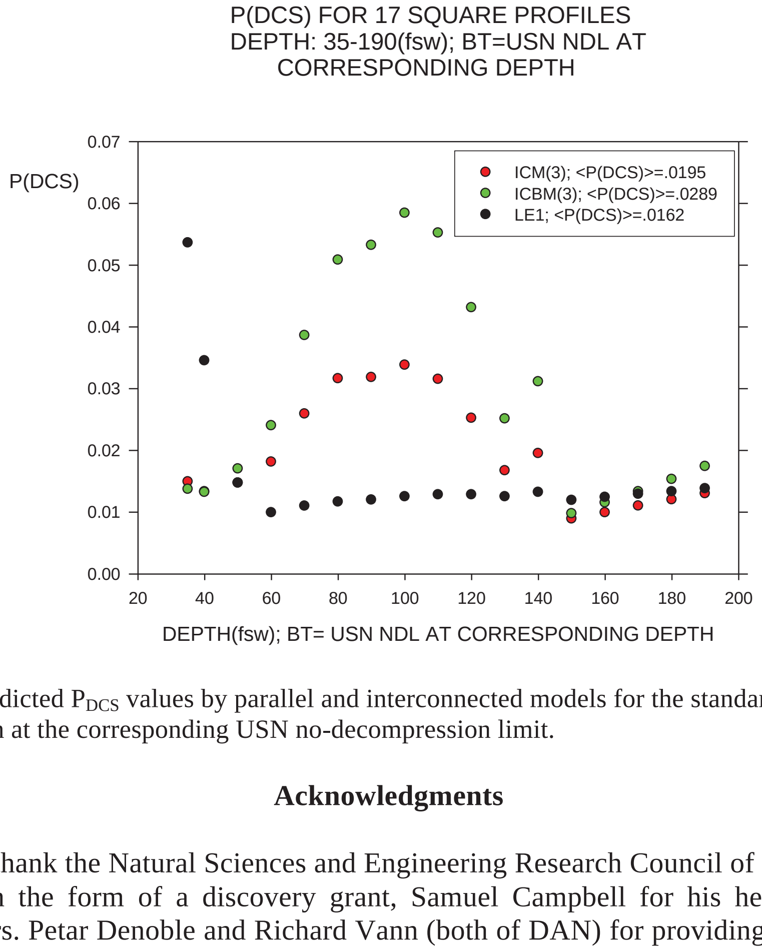

Figure 6. Predicted P_{DCS} values by parallel and interconnected models for the standard 17 square profiles, each at the corresponding USN no-decompression limit.

Acknowledgments

I would like to thank the Natural Sciences and Engineering Research Council of Canada (NSERC) for financial support in the form of a discovery grant, Samuel Campbell for his help with some of the calculations, and Drs. Petar Denoble and Richard Vann (both of DAN) for providing a preliminary subset of the full PDE dataset.

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SCIENTIFIC DIVING WITH COASTAL AND OCEANOGRAPHIC ENGINEERING: A STUDENT'S PERSPECTIVE

Uriah Gravois
University of Florida
Environmental School of Sustainable Infrastructure and the Environment
Coastal & Oceanographic Engineering Program
PO Box 116580, 365 Weil Hall,
Gainesville, FLORIDA 32611 U.S.A.
uriah@coastal.ufl.edu

Introduction

This paper details my involvement with scientific diving as an undergraduate and graduate student at the University of Florida (UF). Throughout my 6 years of involvement, I have surpassed 100 logged dives, earned a divemaster certificate, reached full American Academy of Underwater Sciences Scientific Diver certification and am currently a member of the UF Diving Safety Board. Participation in the science diving program has been a huge asset to my class work and research. Documenting and sharing my science diving experiences might encourage future generations of scientists to take the extra step to engage in scientific diving, particularly in the field of Coastal and Oceanographic Engineering (COE).

A project on chasing hurricanes led to my initial involvement in science diving and on to pursue a Master's thesis. In an undergraduate hydrodynamics class, students were recruited with an interest in a research project to study hurricane waves within the COE graduate program. From the perspective of an avid Florida surfer who is fascinated with science, this was a perfect opportunity. My knowledge and abilities as a so-called “waterman” did not earn me this research position but my previous experience with electronics and good grades did. This summer research project changed the course of my college career and my initiation to scuba diving.

Objectives

The basic premise for this research was to develop a rapid-response method using helicopters to install temporary wave and surge gauges ahead of hurricane landfall. Gauges are then recovered post-storm by scuba divers. Although not a project requirement, I volunteered to start scuba dive training. The University of Florida COE department has a long and rich history of field work and a fabulous academic diving program. Unfortunately, because of budget cuts, regulations and general shift in research towards computer modeling, it is not hard to imagine a future without scientific diving in the COE department.

Methods

In 2006, during the first year of the project, open water scuba and science diver-in-training were completed. Alongside the dive training, by the end of summer the project had successfully developed pressure-sensor wave gauges and a method to deploy them from helicopter (Fig. 1). Gauges were deployed by helicopter several days prior to a storm in water depths between 30-50 ft by lowering the approximately 50-lb gauge to the water surface and releasing it. After 3 to 4 weeks, divers returned by boat *via* GPS coordinates to retrieve the gauges after a hurricane. Once in the proximity, the instruments were located by scuba divers carrying sonar receivers that pick up the signal from acoustic pinger locators. Recovery was aided by a small line of marker floats attached to the pressure gauge. Once located some minor digging was usually required to free the instruments. In a few cases a jet pump was needed to dig out the pressure sensors. This has proven to be an effective method with recovery of 45 out of a total of 49 sensors after 6 storms.

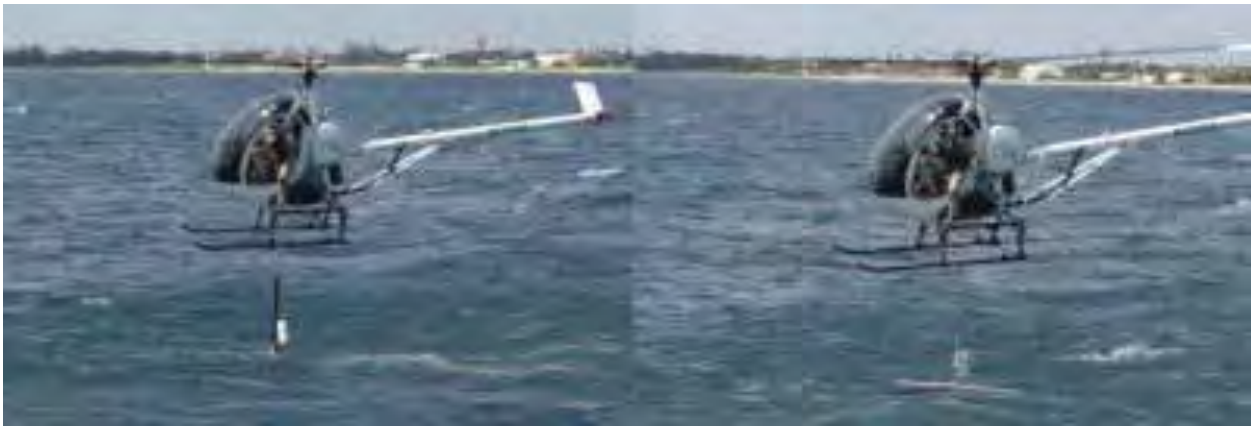

Figure 1. Sensor deployment via helicopter.

The project was still in the funding stages during the notoriously bad 2005 hurricane season and once off the ground the hurricane activity waned. In 2006 we completed a test run deploying only 4 sensors off the South East coast of Florida for Tropical Storm Ernesto.

Results

The proof of concept was achieved and we had a whole season to improve our pressure sensor (Fig. 2) and methods of helicopter deployment.

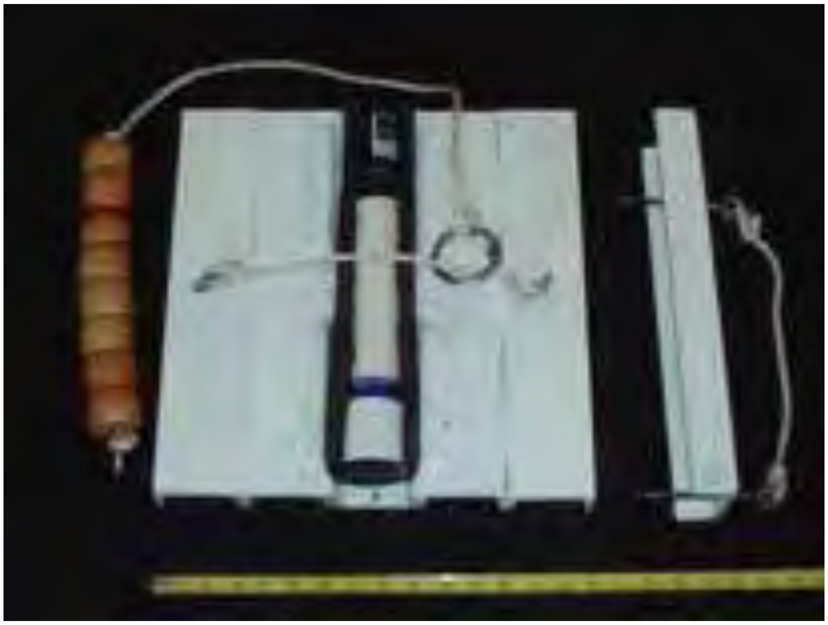

Figure 2. Pressure sensor assembly.

For the 2007 hurricane season, we had refined our methods and were ready to apply our methodology to a big storm but the hurricane season was quiet. A small sensor deployment was made near the same location as the previous year for Tropical Storm Noel, resulting in 10 for 10 with deployment/retrievals. The new sensor design was equally successful and much more efficient.

In 2008, the hurricanes we had been expecting materialized. Over the course of a few weeks we had deployed 39 gauges from the outer banks of North Carolina to southern Texas for storms Fay, Gustav, Hanna, and Ike. That year highlighted that deployment of gauges was a lot easier than retrieval. The North Carolina gauge deployments and retrievals for Hanna were handled by project collaborators at University of North Carolina. The United States Geological Survey assisted in the recovery of Fay gauges off the West coast of Florida. This left 29 Gauges deployed for Gustav and Ike from the Florida panhandle to south Texas. On average, we were able to recover 2-3 gauges per day, weather permitting, using an array of boats: a small UF vessel, boats from Louisiana University Marine Consortium (LUMCON) and University of Texas Marine Science Institute and a private boat. The gauge recovery (26 of 29) was completed with next to zero visibility. This was a true proof of project concept with both storms being major hurricanes and causing substantial damage. The data from these measurements led to 4 scientific journal publications (Kennedy et al., 2010; 2011a; 2011b; 2011c).

Conclusion

Although the project of chasing hurricanes with helicopters is over, I have had involvement with several other COE projects involving scientific diving, each with its own peculiarities for training, preparation, safety precautions and logistics. Through scientific diving I have developed a better practical understanding of the natural environment involving coastal and oceanographic engineering. I continue to inform, influence and encourage others to utilize scientific diving as a research tool.

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USING RISK MANAGEMENT PRINCIPLES TO OPERATE A SAFE SCIENTIFIC DIVING PROGRAM

Vallorie Hodges
Oregon Coast Aquarium
2820 SE Ferry Slip Road
Newport, OREGON 97365 U.S.A.
vallorie.hodges@aquarium.org

Dennis Nixon
University of Rhode Island
Graduate School of Oceanography
215 South Ferry Road
Narragansett, RHODE ISLAND 02882 U.S.A.

All organizations are exposed to risk. How these risks are identified and addressed range from a proactive to a reactive approach. Despite the enviable diving safety record enjoyed by the scientific diving community, accidents still happen. The potential costs of a crisis include people, property, income and goodwill. Organizations want to avoid these costs but often devote inadequate time and attention toward developing a strategy to address them throughout all levels of their organization. This paper describes the principles of risk management and their importance in dive program management; outlines the considerations and tools of risk management; applies risk management principles to scientific diving programs; and describes how to develop a strategy for coping with incident management.

Introduction

In 2009, the authors were tasked by the AAUS (American Academy of Underwater Sciences) BoD (Board of Directors) to create a PowerPoint presentation on risk management. The intent was to create a product that could be used in two ways. First, by AAUS to train new DSOs (Dive Safety Officers) and second, as a training resource to provide to AAUS organizational members as a member benefit for use within their diving safety programs.

The resulting Risk Management PowerPoint was presented to the BoD and to a group of Diving Officers at the 2009 AAUS annual symposium and was approved by the BoD. This paper serves as a resource document for the PowerPoint, providing context and background so that it may be more effectively used by DSOs and the AAUS. The PowerPoint consists of three modules, the Principles of Risk Management, Applying Risk Management to Scientific Diving Programs and Incident Management.

Why should RM (risk management) be taught? Risk in scientific diving programs is not a theoretical concept, it is an operational occurrence. Egstrom (2006) stated “We will never be without risk in diving but we must use reasonable care in determining the degree of risk we are prepared to accept.” RM must be taught and practiced with due diligence. It is an important management tool that must be updated and used as part of the daily diving operation.

How RM is taught will vary and each module can be adjusted to the needs of the target audience. For example, higher level administrators with demonstrated knowledge of RM principles might be presented with the first module as a review. Non-diving personnel may have a rudimentary knowledge of diving, so more thorough grounding in modules two and three may be appropriate. Personnel with duties to assist in emergencies and crises will likely benefit from an in-depth briefing on module three, combined with an overview of site-specific emergency protocols. Diving Control Board members, DSOs, and dive program staff are good candidates to receive all three modules in some depth and detail. The PowerPoint session is deliberately divided into these three distinct modules to allow for such flexibility in delivery and to build on prior learning.

Principles of Risk Management - Module One

When the term risk management is used, many people immediately think of finance and insurance. It is certainly an application of RM but financial risk is only part of the picture. Broadly speaking, risk is anything that threatens the organization’s mission and RM can be described as a discipline or process for dealing with uncertainty. In simple terms, RM is about identifying risks and deciding what to do about them. Can all risks really be identified? That is unlikely given that all risks are not created equal. RM is not a static assessment. Weather, sea conditions and the financial marketplace are all dynamic environments so RM must be a management process used on a daily basis. The most important thing about an organization is its mission and its most important resource its people. The most important decisions organizations make are how it uses its resources to achieve its mission. RM helps us protect our mission and resources by guiding the decision making process.

Risk Management is a Process

What are we trying to accomplish; what or who are we trying to protect; what are the priorities; and what will our strategy be to implement the plan and monitor and update it? Typically organizations will want to reduce or prevent injuries and incidents, avoid costly legal claims, preserve the reputation of the organization, free up resources for mission-critical activities and ensure adequate risk financing. The first step is thus to identify these answers and set goals.

Next, risks need to be categorized and prioritized. What and/or who are we trying to protect? Commonly this includes people, property, income and goodwill. There is a need to keep things in perspective so that action items can be put in priority order.

The next step in the process is to evaluate the risk. This is done by identifying what the hazard or risk is and providing a description of the risk event. Then the risk position can be evaluated by examining both the probability and the severity of an occurrence. Finally, a plan is formulated to mitigate or mediate the risk.

It is important to emphasize the two-dimensional nature of evaluating the total risk position (i.e., examining both the probability and the severity of a risk event). An excellent example of this two-dimensional aspect can be seen in Figure 1, which provides a qualitative approach of using colored quadrants to represent low, medium and high levels of the risk position. This graphically demonstrates that even when the likelihood of an incident is low, if the consequences of such an incident are high, the risk position is in the yellow “caution” area and steps should be taken to mitigate or mediate that risk. For example, even though it is very rare for a high pressure cylinder to catastrophically fail (low likelihood) the consequences of such an event are high and can result in great risk to life and property, so the resulting risk position is one that requires we take preventive action.

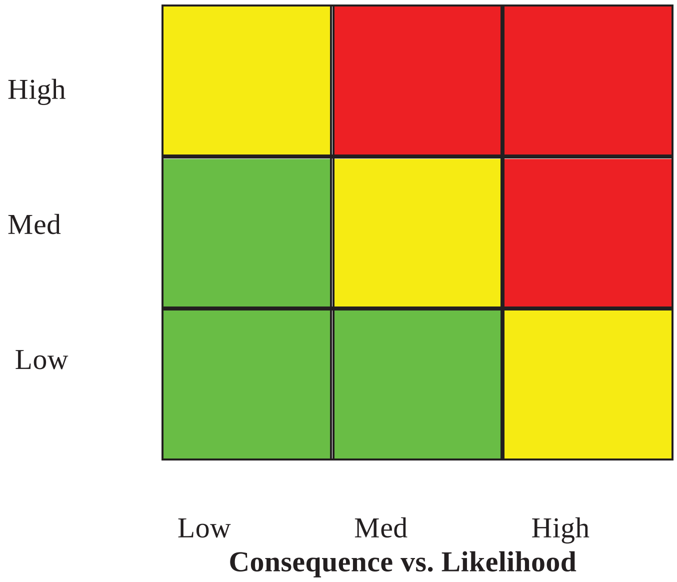

Figure 1. Total Risk Position – Qualitative (source: NASA)

Noel Hinners (pers. comm., 2010) opined that “One must also recognize that [these types of] diagrams are simply a means of visually summarizing the risk assessments; in and of themselves they do nothing else. Behind them is the whole process of risk management. The figures are a useful way to sum up the prime risks and stimulate discussion. Experience shows that there is a lot of room for disagreement as to whether a particular risk belongs in a particular 'box.' That aside, it sure does focus one on critical risks. If a risk is in the high consequence (e.g., death, loss of mission) and low probability, one better pay attention to it and implement risk reduction approaches. The NASA shuttle accidents demonstrate that in a very forceful way. The reports of the accident investigations are sobering and demonstrate the consequence of ignoring risks that are staring you in the face.”

The next step in the process is to develop a strategy and then monitor and update the plan. Recall that RM is a dynamic process and change is challenging, i.e., this process must be ongoing.

There are four techniques that are generally used in a RM strategy, including avoidance, modification, retention and sharing. Avoidance is for when the risk is too high, such as when an organization has insufficient resources, training, equipment, supervision and/or safety measures to complete a mission. Retention is when the organization assumes the risk (e.g., $1,000 deductible on an insurance policy). Modification is a technique that is used frequently in diving programs and is effective when there is a way to do something safely by simply changing the way it is done. Policies and procedures are developed just for this purpose. Sharing is another technique and is commonly done through a contract, such as a caterer that has insurance policy coverage working at a venue, which also holds insurance coverage.

Before applying this process to AAUS organizations, the categories of risk must be examined. People are clearly a high priority for any ethical organization and, unlike property, they cannot always be “made whole” again. The long-term impact of a serious injury often extends well beyond the person injured and can have lasting negative effects on other members of the organization. Another category of risk is goodwill or the reputation of an organization. Crises can tarnish the image of an organization, impacting their customer base, bottom line, ability to secure funding or simply the degree to which they have influence in their community or industry. These two categories of risk alone can have a profound effect on an organization.

The following activity can be merely discussed theoretically, actually accomplished during the PowerPoint session or alternatively set as a homework assignment. First, the participants are asked to identify the "people assets" in their organization. Next, some of the risks to these people are listed. The organization's reputation is described and then some of the risks to the organization's goodwill assets identified. What events or crises could shatter the reputation that has been built over the years? Participants then put this all together by listing the priority risks they have decided to focus on from the lists previously compiled, leaving several lines of space between priorities. Then below each risk are listed some of the steps or actions the organization could take to minimize the likelihood of the risk materializing or reduce its negative effect on the organization should the risk materialize. The next steps in this process should be to involve others and keep in mind that additional expenditures may be necessary. The process requires common sense and sound planning. By keeping things simple there will be a better chance of success and with time this process should become more inclusive. A visual example of how this process is implemented in an organization is provided in Figure 2, which shows NOAA's (National Oceanic and Atmospheric Administration) six-step process for risk management.

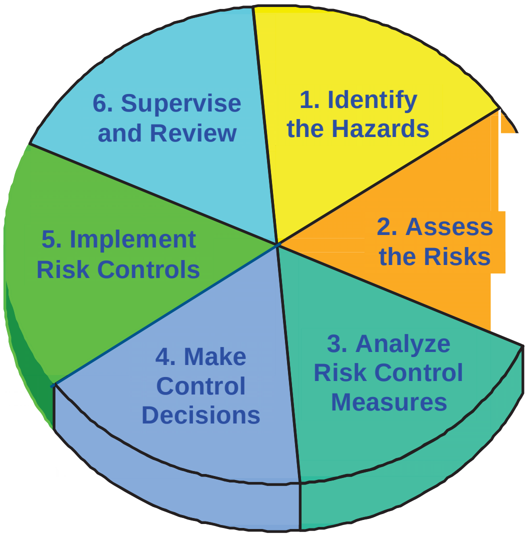
1. Identify the Hazards
2. Assess the Risks
3. Analyze Risk Control Measures
4. Make Control Decisions
5. Implement Risk Controls
6. Supervise and Review

Figure 2. NOAA Risk Management Six-Step Process

Applying Risk Management to Scientific Diving – Module Two

Negligence is a legal construct that incorporates four components; the duty of care, a breach of that duty, causation, and damages. The duty of care is commonly referred to as the community or industry standard of care. In the scientific diving community an example would be the AAUS Standards. A breach of that duty would be a failure to comply with the standard. Causation relates to whether the acts or omissions were the cause of the loss or damage sustained. Damages relates to the harm caused (injury, death or property damage).

Negligence is not the same as carelessness. A person might exercise as much care as they are capable of yet still fall below the level of competence expected of them. It is the failure to use reasonable care, or conduct, that falls short of what a reasonably prudent person in our community would do to protect another person from foreseeable risks of harm. Note that this is not simply what a reasonably prudent person would do but rather, what a reasonably prudent person in our community would do (http://dictionary.law.com/Default.aspx?selected=1314).

How do Diving Officers avoid negligence? Follow the standards, exercise sound judgment, follow local practices, have appropriate safety equipment available, be prepared to respond to emergencies and certify only divers that meet requirements (Richardson, 2004).

The Costs

There are two types of costs of RM failures; direct and hidden. The direct costs include the obvious financial burdens of multi-million dollar insurance policies, legal representation and settlements. The hidden costs include the incurred personal toll. This can include the humiliation and stigma of being involved in an incident and the resulting impact on a person's professional reputation. Media reports are frequently inaccurate and worded in layman's terms. There is often an impact on both personal and professional relationships. A colleague that was previously considered a friend may testify against you. If a case is settled it is usually confidential or often goes unreported which means there is no opportunity for you to speak out. The time lost to the legal process (questions, depositions, reviewing documents) can take a considerable toll. The cost of replaying and reliving an incident for those involved can be horrifying as they continue to ask the "what if" questions of themselves. And finally, if you are found to not be culpable, who will remember or even be interested? The hidden costs of incidents can be onerous.

Tools and Practices

To assist in avoiding such costs, there are a number of available tools and practices. Written releases and waivers are one such tool, though some dive programs that are dealing with only employees will not need them because an employee cannot release an employer. Programs that have students, volunteers, interns or other non-employees that are not covered by worker's compensation of the DSO's employer may need to use such forms, as well as non-employee divers that are under any level of supervision or control by the DSO or employer. If a program hosts visiting divers under reciprocity, third party liability may apply, so the application of coverage must be established (e.g., which organization will provide medical coverage to the diver in the event of an incident?). If a release is used it must be written and administered correctly, which means that it must be written to apply to the unique activity and in accordance with state law so it should be drafted by an attorney in that state. To be administered correctly, the release must be based on informed consent. "Informed' means that the signer has been provided an overview of the risks. "Consent" requires questions to be answered adequately, with time to think and decide provided in the absence of undue pressure or duress. Filling out a release in a dark vessel at five o'clock in the morning on the way to the dive site as the instructor tells you it is "just paperwork you need to sign" does not constitute consent and would not be enforceable.

Another tool is the AAUS medical release. Who is qualified and authorized to make decisions? The correct answer is both the medical practitioner and the DSO. While clearly the decision of a person's medical fitness to dive is the realm of a medical practitioner, the DSO has a role in identifying potential medical issues to refer to a medical practitioner. If the DSO is aware of a recent illness or injury that might impact a diver's medical fitness, the diver may be counseled to seek medical evaluation. In the event of a major illness or injury or a condition requiring hospital care, the diver is required to obtain medical evaluation (AAUS standards section 6.10). Another role of the DSO is to examine the medical release paperwork for errors and omissions. Is the form complete? Were required tests conducted and initialed? A very important element of this tool is that to comply with scientific diving standards, it must be signed by an MD (Medical Doctor) or a DO (Doctor of Osteopathy). The DSO must check the medical signatures before accepting the medical release as valid.

Waivers and medical releases are examples of legal forms that may be used in dive programs but there are a host of other documents that are commonly used to help organize, plan and document activities. Written schedules or databases that track equipment maintenance, training and frequency of medical clearance are powerful management tools. Written checklists for briefings and pre-dive equipment checks are RM tools that not only provide a quick reminder to divers of the things they need to do. When actually used to check off tasks, they also document that these checks are being done. Dive plans are documents that guide divers through the planning process but are also designed to be used in an emergency by providing the information needed. It is important to have them present at the dive location.

Contingency planning is a practice that is a cornerstone of safe diving operations even if the contingency plan itself is never used. It is a discipline that is preventive by its nature. When contingencies are planned for the more likely it is to recognize hazards and plan to avoid them. Pre- and post-dive safety briefings and the protocol used for their conduct are some of the most critical preventive practices. It is also a practice that tends to erode over time as divers become comfortable and complacent. Pilots conduct preflight checklists before every flight regardless of how many times they fly the plane. Diving should be no different. Another practice common to dive operations is to reduce ratios or numbers of divers depending on conditions that can vary tremendously.

There are also tools and practices for equipment inspection and maintenance. Scheduling and performing “annual” servicing requires accurate and complete logs and records. Who performs the servicing and repairs and how often is it done? Diving equipment should be serviced by an authorized technician. How frequently equipment is tested and/or serviced varies with the equipment type, federal laws, state laws, the AAUS standards, frequency and type of use and other considerations. For example, some cylinders in heavy use may need visual inspections monthly rather than every 12 months (High and Gresham, 2009).

Fill station operations require a specific area of expertise and should be restricted to only current certified/authorized fill station operators. Air test results, a list of prohibited cylinders and fill station instructions should be posted at the fill station. Training for fill station operators must be provided every three years or whenever new regulations emerge. Maintaining records is also an important RM practice and should include compressor and fill station inspections and servicing as well as training and content (High and Gresham, 2009). Worthy of consideration is inviting an independent professional to inspect the fill station operations and provide a report to the organization.

A valuable tool available to dive programs is the practice of conducting program audits. There are several ways this can be done from using a standard self-audit form to inviting third party audits of the program or parts of the program (e.g. fill station operations). AAUS provides a Scientific Diving Program Compliance Review Checklist for its members to use that conforms to the minimal standards of AAUS as a simple self-audit tool. AAUS Organizational Member Program accreditation policy and procedures are now in place. The National Oceanic and Atmospheric Administration (NOAA) has some useful tools and documents that can be used in this fashion as well, including the NOAA Diving Program’s Unit Diving Inspection Checklist (www.ndc.noaa.gov).

Another critical RM practice is access to continuing education and professional development. While it is important to provide continuing education to all of the divers in an organization, it is critical that the DSO, as the person responsible for administration of the program, is current in all aspects. The law presumes that you are proficient. Do you have the knowledge, skills and abilities to continue to serve as a DSO, a dive leader and teacher? Are you comfortable in all areas needed to do your job? Standards, regulations and equipment are not static so these areas must be updated regularly. Skills are also not static and must be continuously upgraded and maintained. Attending conferences and symposia is certainly one option for professional development. This might include attending the annual AAUS symposium and Diving Officer meeting, Regional Diving Officer meetings, diving trade shows, commercial diving conferences or discipline-specific conferences (e.g., Benthic Ecology Meetings, Western Society of Naturalists, or the Association of Zoo and Aquaria annual conference). Reading publications and literature, participating in forums and news groups or simply picking up the phone and speaking with colleagues are additional options. Another valuable educational technique is debriefing minor incidents or studying accident reports and reviewing these with the Dive Control Board (DCB). Taking or auditing a course or more specifically attending a DSO orientation or accreditation offered by AAUS are excellent professional development options. Publishing and presenting papers or teaching a course or assisting another instructor with a course can provide growth opportunities. A myriad of options exist for continuing education and professional development but most dive programs will require a broad set of skills so a multidisciplinary approach to maintaining DSO proficiency is recommended. In other words, sending a DSO to one workshop every few years will likely be inadequate. There are also required proficiencies that must be met, such as instructional requirements for currency, depth certifications, gas (mode), special environments, fill station operator training, equipment technician training and compressor maintenance certification to name a few. Again, the emphasis should be placed on assuring that the DSO is proficient and competent.

Finally, in terms of tools and practices, there is probably no more profound RM tool as developing a culture of safety. The diver's attitude toward safety may have the most significant impact on safety. What is your attitude? What is the attitude of your divers, your supervisor and your organization? Safety may be the middle word in the title DSO but it is not the sole job of the DSO; it must be shared. Personal responsibility, responsibility toward the dive buddy, toward the dive team and toward the organization must be nurtured. The primary tool is communication. How safety issues and needs are communicated is part of this. The use of active listening skills and the responsiveness to safety communications are paramount.

Incident Management - Module Three

An incident is a terrible time to discover lack of preparedness to effectively handle a crisis. Should a serious diving incident occur right now what is the confidence level that all will respond correctly? Is effective response tested with drills and practice sessions? Is there an incident response plan?

The first step to dealing with an incident is to proactively develop an incident response plan. This should include written instructions on who must be contacted (chain of communications), how to organize and treat participants in an incident, rescue and recovery procedures, the preservation of equipment and counseling in preparation of an incident report. A spokesperson for the organization should be identified, as well as an attorney. There should be a prior clear understanding of post-incident roles, especially among the spokesperson, the attorney, the DSO, witnesses, responders and divers. The early interviews of witnesses should also be established.

In today's lightning fast multi-media world the news of a diving incident will spread rapidly. It is not uncommon in metropolitan areas to have the media at the gate before the ambulance arrives. The incident response plan should include an information packet about scuba diving for use during the incident. A cover sheet should be included that lists newspaper and electronic media contacts with names and telephone numbers to send the information packet to. A similar electronic version that can be posted to the internet should also be available. Chat rooms, news groups and other social networks are hotbeds of misinformation. The organization should have a clear policy on how this will be managed.

The second step to dealing with an incident is to have a clear understanding of how to use a legal advisor during incident management. Communications are privileged so the details, process and questions for management of the incident can be freely communicated. However, the DSO should be clear that the attorney is the organization's attorney, not the DSO's attorney. The legal advisor can assist in gathering evidence and interviews and act as an analyst from a legal perspective.

The third step is to understand the role of the insurance company in an incident. First things first: it is critical to notify the insurance company within the required minimum time provided on the policy or take the risk that there will be no coverage. With respect to insurance companies, do not expect early participation or assistance and be aware that your agent/broker is not the person who will investigate the claim. Understand that the relationship often becomes adversarial and that the insurance company calls the shots on settlements. This means they may settle the claim even if no wrong was done. Just like the attorney, the insurance company is under contract with the organization not the DSO. This raises the question of who protects the DSO. Recreational instructor coverage will only apply to the direct teaching of courses, not other DSO duties. There currently exists no custom product available to the DSO. And finally, some institutions are "self insured" so the process may be even more difficult to navigate. Consider these issues in advance of an incident and be proactive in establishing an incident response plan. This is the same concept as "plan the dive and dive the plan."

Even with a rigorous, relevant and updated incident management plan there may be many difficulties encountered managing an incident but there are a number of available resources including the AAUS, NOAA, UNOLS, AZA, the US Navy and others, including Diving Officers from other institutions that maybe able to act as advisors, investigators or simply as a second opinion or a sounding board. Tap into these resources as may be appropriate, or even list them in your response plan. A list of names, e-mail addresses and phone numbers of trusted advisors or colleagues that is placed in a convenient location can be a time saving and stress relieving feature of a well-managed crisis.

These are a few of the most common tools and practices that are available to organize, plan and document diving activities and many of them are required by the AAUS standards.

Summary and Recommendations

Three modules have been provided on the subject of RM in scientific diving programs that can be used as training or review tools at all levels of an organization. Depending on the audience and need, any one module or combination may be used and any may be tailored to the audience in terms of scope and delivery. Each module may also be used as a project template for developing an organization's RM plan, or as an evaluation template to scrutinize an existing RM plan.

In a training application it is recommended that the modules be delivered in the order presented here to take advantage of the sequential learning approach that is built into the program. It is also recommended that the program be required reading for DSOs, DCB members and lead divers, though there are likely others in the organization that would benefit from this information.

It is worth repeating the mantra that following the standard reduces the risk. There are different environments, missions and priorities but the requirement of adhering to the standards is shared. How do we assure we are following the standards? The obvious answer is to become well educated on what the standards are and rigorously and frequently compare activities with them. Staying actively involved in AAUS is helpful to this end. Perhaps less obvious is the contribution to the RM position that is made by becoming or remaining an organizational member of AAUS. How does one know if the diving safety manual, diving control board and the Diving Safety Officer's credentials are all compliant with AAUS standards? To become an organizational member of AAUS these elements of a diving safety program must be submitted to the standards committee for review and approval. Scrutiny of the program by the organization that sets the standards for scientific diving is quite simply the highest level of RM provided for those elements. We strongly recommend that all organizations claiming the scientific diving exemption become AAUS organizational members, attend annual meetings, and become actively involved in AAUS.

RM is a dynamic and daily process, comprised of a vast network of often simple decisions and activities fraught with the potential for complacency. A current, well-articulated RM plan super-imposed over this network provides a safety net for the diver, the dive program and the organization, protecting human life, property, goodwill and the missions of our valuable institutions.

Acknowledgments

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DEVELOPING A CULTURE OF SAFETY IN SCIENTIFIC DIVING PROGRAMS

Vallorie Hodges
Jenna M. Walker

Oregon Coast Aquarium
2820 SE Ferry Slip Road
Newport OREGON 97365 U.S.A.
vallorie.hodges@aquarium.org

A set of defined and time-tested safety standards is an obvious asset towards maximizing the protection of scientific divers from accidental injury or illness. What may not be as well understood is the impact that culture plays on the safety of divers. Diving Safety Officers can implement safety standards, train in rescue techniques and provide dive plans and checklists but if divers fail to use them, fail to take responsibility for safety or become complacent, accidents can occur. This paper explores how a culture of diving safety can be a potent accident prevention mechanism, how such a culture can be developed in a scientific diving program and some of the tools and techniques that may be useful for dive program managers.

Introduction

A primary purpose of the scientific diving standards promulgated by the American Academy of Underwater Sciences is to maximize protection of scientific divers from accidental injury and/or illness (AAUS, 2011). The standards provide specific requirements for training, dive procedures, certification, equipment, and medical approval, and additionally require a Diving Safety Manual, Diving Control Board (DCB) and a Diving Safety Officer (DSO) who is responsible for routine operational authority of the local dive program. While these standards provide the foundation for the strong safety record enjoyed by AAUS divers, the role of culture in dive safety management has not been investigated in the daily operations of dive programs, though its role in other industries has been widely studied. The Occupational Safety and Health Administration (OSHA) has observed at OSHA VPP (Voluntary Protection Programs) sites and confirmed through independent research that the single greatest impact on accident reduction is the development of a strong safety culture (OSHA, 2005).

A variety of ‘safety culture’ definitions are provided in the literature though it generally is defined as the ways in which safety is managed in the workplace and often reflects the attitudes, beliefs, perceptions and values that employees share in relation to safety (Cox and Cox, 1991). Safety culture can also be described as a product of the vertical distribution of attitudes and behaviors throughout the organization, from upper management to groups and individuals at every level, and the extent to which each commits to personal responsibility for safety (Von Thaden and Gibbons, 2008). As observed by Clarke (1999), an equal perception of intergroup safety culture may best encourage the development of trust and understanding between individuals and regulation enforcers.

Developing a culture of diving safety is an ongoing process and takes time, patience, commitment at all levels of the organization and a focus on the big picture or mission. Some of the steps toward developing a safety culture include assessing the current culture, setting goals, identifying the key personnel that can effect positive changes, providing training, building commitment, defining specific roles, developing a system of accountability and establishing a company safety vision that includes key policies, and strategic and operational plans (Thompson, 2012). In scientific diving programs the Diving Safety Officer and the Diving Control Board play pivotal roles in this process but will be less successful if the entire organization is not engaged in the development of a positive safety culture, from the individual employee to top level management. However, it has been the experience of the authors that the dive program can be the engine that drives the system, improving not just the culture of the dive program but also of the entire organization.

Building the Safety Culture "Engine"

Reason (1998) considers an ideal safety culture to be the "engine" that drives the system toward the goal of sustaining maximum resistance towards its operational hazards. The role of the Diving Safety Officer in managing day-to-day operations and identifying hazards is a critical component to any scientific diving program but one person does not constitute a culture. A DSO cannot be everywhere at once, nor can they be "in the skin" of the diver and know that a diver is experiencing sinus congestion, family concerns or slept poorly the night before. Many of the scientific diving programs that make up our community are large with hundreds divers and conduct thousands of dives annually. Even the most talented DSO cannot attend every dive or monitor each diver. Our programs depend on every diver taking personal responsibility for the safety of the team. Every diver must be willing to assure that the group is following safety protocol, which means speaking up and speaking out. It means calling out another diver who is not properly equipped or redressing a diver who is failing to maintain buddy contact. A Diving Safety Officer can train, drill and coach divers to be prepared for emergencies but in the face of a potential incident will they follow their training? Clearly, a strong safety culture can make a big impact on the safety of a scientific dive program by reinforcing these desired behaviors.

Perhaps the best place to start is with the Diving Control Board. While the DSO is the most suited to serve as the daily vanguard for navigating the scientific diving program toward a positive safety culture, the DCB should serve as a directorate and reservoir of political will. A strategic plan should be developed that embraces specific steps toward a safety culture but it should be understood that this is a dynamic and ongoing process. It is more about the journey than the destination. Strategic and operational plans should be reviewed and updated regularly. There are a number of tools and techniques that can help in this process and, while we discuss several here, they are not necessarily meant to be prescriptive nor is this an exhaustive list. Researching how other industries manage organizational and cultural change can be instructive and discussions among professional diving officers could help drive this process forward within our organizations and within the scientific diving community.

A core tool in developing a safety culture is the provision of the scientific diving standards and the organization's dive safety manual. These should provide the content of how a program should operate but the mere provision of policies and procedures does not integrate them into diver behavior. Divers must be informed not only of the policies but how they are implemented in daily operations with relevant examples provided *in situ*. Training divers to read, interpret and implement the standards and policies should be a key component of scientific diving training programs. When divers are aware of the standards and regulations it also helps with upward accountability providing a necessary check and balance.

The second core tool in developing a safety culture is effective training. AAUS Scientific Diving Standards provide a list of required and optional training. However, the range and diversity of research projects, tools and techniques used within our community is considerable. It is probably unreasonable to expect all scientific divers to be trained on all of them or indeed all training programs to be homogenous. What is more important is to establish a priority for the skills, knowledge and abilities that are required of a particular diver (typically based on the project they will be involved with) and to focus on developing a high level of competence and proficiency with these. Diver behavior should be observed, measured against desired behavioral outcomes and reinforced. "Train, train, train" instructs Borbidge (2008) who asserts that changing behaviors is a key element in changing the culture and that training must be ongoing to adapt to physical and personnel changes. This embraces the notion that changing behaviors or breaking bad habits is typically more difficult than training a completely novel behavior. Most organizations will need to do both because of the often transient nature of divers in their programs. The need to provide ongoing training is one of the reasons that the AAUS standards require the DSO to be a certified instructor. Training is not just teaching accredited and curriculum-based certification courses (e.g., entry level scuba, Scientific Diver Courses, First Aid). Training also includes daily observation and positive reinforcement or correction of specific behaviors. It is frequently in the small details that diver safety goes astray. Perhaps the most critical impact on developing a culture of safety is developing good habits among divers. Divers with ingrained habits of conducting buddy checks will do them every time because it is habit. This same principle applies to dive planning, gear set-up, dive briefings, air management and post-dive incident reports. No detail is too small to overlook in the development of habits. Good habits save lives and are the best inoculation against complacency.

Safety culture can be enhanced by addressing perceived risk among divers. Biased perception of risk can lead to misjudgments or inappropriate decisions regarding safety measures (Rundmo, 1996). For example, divers may become complacent regarding certain risks after low incident rates from routine dives. Without an established habit of safety checks, divers could potentially overlook important details in their dive plans over time. A similar phenomenon has been observed in commercial divers within the UK Continental Shelf Oil and Gas Industry where divers give lesser emphasis on safety culture in controlling accident risk, possibly caused by the nature of short-term contract work where culture may not have enough time for development (Adie et al., 2005).

Training should also include conducting drills. We would like to emphasize how instrumental emergency drills can be in both testing competency and in changing the safety culture of a dive program. Many dive programs employ a regular schedule of emergency drills such as rescue scenarios for this very reason. Over the past six years at the Oregon Coast Aquarium we have slowly shifted from running annually scheduled training-based rescue drills to adding unscheduled drills where most of the participants are not aware they are responding to a drill. This has required a higher level of preparation and consideration for how to prevent undue stress and injury for the participants but has also had a strong impact on our safety culture. Divers are not only better trained in dive rescue techniques but the attitude and emotional response has been far more realistic allowing a more "real world" experience that the participating divers have reported they value highly. The outcome has been not only skills, abilities and knowledge but a palpable shift towards a safety culture.

A third core tool in developing a safety culture is the availability of adequate resources. This includes equipment, personnel and other budgetary resources. For example, it is difficult to build a safety culture in the absence of safety equipment that is considered standard of care in the industry. If the organization does not provide needed safety resources, employees will see this as a lack of commitment and be more likely to mirror that with an equal level of apathy. This demonstrates the importance of a commitment to a safety culture by all levels of the organization, including financial managers.

This "equipment" category not only embraces emergency gear like first aid and oxygen kits, or vessel safety gear but also includes the quality, fit and standard of scuba gear, servicing/repair schedules and the standard to which equipment is serviced and by whom (e.g., factory authorized technicians). A related issue here is that standards and equipment change over time and dive program managers must stay informed of these changes and work to incorporate improvements in their programs to maintain the community standard of care. An example of this is the recent changes in the Divers Alert Network (DAN) oxygen course. The availability of bag-valve masks and oxygen regulators that ventilate a non-breathing diver have improved the available level of care and these skills are now incorporated in the DAN oxygen course. Equipment also includes structural needs such as the design and provision of safe storage of dive gear, air fill stations, vessels appropriate for the local conditions and safe entry/exit facilities in an aquarium.

The availability of personnel or human resources includes not only the number of personnel but the required level of skills and qualifications. If an organization is committed to building a safety culture this element must be carefully considered, particularly since it is often one of the more expensive items in the annual budget. The cost of providing adequate staffing should be weighed against the benefits of a safe, legally compliant dive program. Failure to provide adequate staffing may cost the organization financially, legally, and in public opinion and good will. Some indicators against which staffing levels may be measured include: the extent of deferred maintenance and quality/promptness of maintenance provided; the range, availability and frequency of offered training programs; the number of divers/dives and projects undertaken; the accuracy and completeness of records; the number and complexity of programs managed by the dive office; and the availability/coverage by DSO staff for needed projects or events. A DSO is a professional title that is defined by AAUS standards and scientific diving operations need to meet that standard, but there also needs to be a continued investment in the ongoing development of dive program managers to assure that they maintain proficiency and stay abreast of industry standards.

Effective communication is another core tool and often one that is diluted by time, distance and the medium used. For example, the Oregon Coast Aquarium’s dive program includes over 150 divers that live throughout Oregon and Washington and dive at different locations and different times. A primary communication medium for the group is electronic mail which has its limitations. Pre-shift briefings, lead diver meetings, scheduled training, monthly publications, a web-based forum and programmatic events that provide opportunities for divers to meet and interact are some methods the Aquarium has used to bolster the communications cycle. Effective communications in such an environment continues to be a challenge and one that modern social media (e.g., Facebook, Twitter, etc.) has offered as many obstacles as it has solutions. Communication is a tool that must continuously be fostered, improved and reviewed.

Another core tool is an emphasis on reinforcement of policies and regulations and de-emphasis on enforcement as the primary response to errors and failures. There is no doubt that there are times that a punitive response to a behavior is necessary. But when this is the singular response to policy violations the impact on culture can be damaging and sometimes severely negative. A diver’s willingness to self-report an injury or policy infringement will likely be dampened or even extinguished. On the other hand, encouraging information-sharing with all divers and allowing for an environment where honest errors and mistakes are discussed without penalty can have a potent impact on safety culture. This principle was recently examined in a model that defines progressive culture growth (Lock, 2012). The idea is that safety culture begins with an informed group, develops to an environment in which both accidents and incidents are reported and shared within a community, and eventually becomes a culture where both the organization and individuals are capable of adapting safety protocols based upon the needs of dive demands (Lock, 2012). Additionally, Skinner (1948) argued that positive reinforcement results in lasting behavioral modification (long term), whereas punishment changes behavior only temporarily (short term) and has many detrimental side effects. Another advantage to positive reinforcement is the cost-benefit ratio. Reinforcement techniques are frequently inexpensive, providing higher performance for lower costs.

Perhaps the most compelling tool for developing a safety culture is leadership, which Chemers (1997) described as a process of social influence in which one person can enlist the aid and support of others in the accomplishment of a common task. According to Leonard and Frankel (2012), great leaders know how to wield attitudinal and behavioral norms to protect against risks, such as psychological safety, where no one is hesitant to voice a concern and will be treated with respect when they do. But a single, even strong, leader is not likely to impact culture in a significant way unless it is multiplied by handing down leadership skills to others so they can become part of the process. When several layers of leaders are developed the effect can be orders of magnitude greater. Leadership is a skill that can be learned and mentoring leaders is the true essence of building a safety culture.

Summary

It is important to remember that a safety culture is developed over time, not created overnight. Organizations, like organisms, adapt and a safety culture develops as a result of history, work environment, the workforce, health and safety practices, and management leadership (Reason, 1998). This paper argues that a culture of diving safety can be a potent accident prevention mechanism and presents a template for how such a culture can be developed in a scientific dive program. Also described are some of the core tools and techniques that may be useful toward reaching this goal.

AAUS standards for safe diving and an organization's diving safety manual provide the essential content and foundation for building a safe diving program. An informed and committed Diving Control Board can be the prime mover and heavy lifter with respect to direction and political will, while the Diving Safety Officer provides leadership as the vanguard for implementation of this process into the daily lives of divers.

A culture of diving safety can not only save lives, money and good will, but should contribute significantly to the organization's mission and to the richness of the workplace and those in it.

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ENVIRONMENTAL RESPONSE TEAM STANDARD OPERATING PROCEDURES FOR CONTAMINATED WATER DIVING OPERATIONS

***Alan Humphrey*[1]**
***Scott C. Grossman*[2]**
***Jonathan McBurney*[2]**

[1]U.S. Environmental Protection Agency
Environmental Response Team
2890 Woodbridge Avenue
Edison, NEW JERSEY 08837 U.S.A.
humphrey.alan@epa.gov

[2]Lockheed Martin SERAS
2890 Woodbridge Avenue
Edison, NEW JERSEY 08837 U.S.A.

Recreational, government, military, law enforcement and commercial diving can all potentially occur in contaminated harbors and enclosed bodies of water. Contaminants can be found in sediments and the water column and may include biological hazards or persistent chemicals. In the absence of real-time analysis for water or sediment borne pollutants, EPA divers often use an enclosed outfit, such as drysuit, drygloves, and full-face mask. This overview of standard US EPA Environmental Response Team (ERT) polluted water diving protocols can serve as a template and be adapted for other dive team operations. Types of water pollution, considerations for diver dress and diver decontamination are examined and available testing data on dry suit permeability to pollutants discussed. The extent of decontamination required, from a potable water rinse on the stern of a vessel to a full multi-zone system, is reviewed. Portable decontamination kits, possible decontamination solutions, full-face mask cleaning procedures and equipment handling procedures are illustrated based on real-world projects conducted by ERT.

Introduction

The mission of EPA is to protect human health and to safeguard the natural environment upon which life depends (air, water and land). As part of EPA’s mission, the Superfund Program was initiated in 1980 to address abandoned hazardous waste sites and spills of oil and hazardous materials. As a Special Team under the National Contingency Plan, the EPA Environmental Response Team (ERT) provides a wide array of technical experts to all EPA regional offices in the Superfund program. Over the past 30 years, the ERT has investigated, analyzed and assisted in the remediation of thousands of oil and hazardous chemical spills and waste sites and helped to protect public health and the environment from toxic chemicals.

All work activities at Superfund sites are regulated by the OSHA regulation 1910.120, Hazardous Waste Operations and Emergency Response Standard (HAZWOPER). HAZWOPER contains specific requirements for Superfund clean-up, assessment, removal and remedial activities to protect worker health and safety, but it does not apply to diving operations. OSHA regulation 1910 Subpart T does establish mandatory occupational safety and health requirements for commercial diving operations but it also specifies an exemption for scientific diving operations. All EPA dive operations are conducted under this scientific dive exemption. Subpart T also covers conventional safe diving practices but does not address any potential mechanisms of chemical exposure to commercial divers.

Following the OSHA scientific dive exemption, the EPA Dive Safety Board oversees all EPA dive activities and requires adherence to the EPA Diving Safety Manual by all dive units. Individual EPA dive teams may develop specific Dive Safety Plans or Standard Operating Procedures (SOPs) as necessary. Because no regulations exist to protect divers from potential chemical exposure, the ERT provided EPA dive units in 2006 with guidelines for drysuit diving, surface-supplied diving operations and dive operation safety. The 2010 version of the EPA Diving Safety Manual included more comprehensive policies for conducting contaminated water dive operations and diver decontamination procedures.

Members of the US EPA dive team have historically become HazMat experts by working on land-based oil and chemical spills and abandoned chemical sites in the Superfund program. To operate safely in potentially hazardous environments, several levels of personal protective equipment (PPE) were instituted. Chemical resistant suits and various types of respiratory protection, including self-contained breathing apparatus (SCBA), are still used widely. Primarily used for protection against cold water, drysuits keep the diver dry and minimize the diver's contact with potentially contaminated water. Although these outfits were not designed as chemical resistant dive suits, they do provide a diver with the best available protection for diving in contaminated water. To provide specific performance data, several manufacturers have tested their suits against a variety of chemicals in the laboratory. To date, industry has not developed a drysuit that is chemically impervious but under typical conditions the suits provide enough protection to minimize the diver's contact with water or sediment-borne contaminants for most chemical hazards. The greatest risk for divers is not chemical permeation of the suit material; it is from leaks in suit material, seams or seals that should be repaired or replaced during regular maintenance. In general, if high chemical concentrations are known or suspected using divers should be the last option.

Operations

Dive Planning

Prior to conducting any dive operations a site specific Dive Safety Plan should be developed. The Dive Safety Plan should outline all aspects of the dive to ensure safe and efficient diving operations. The Dive Safety Plan should include a hazard assessment (including any potential chemical and biological hazards), a list of required/recommended dive equipment (drysuit and/or full-face mask), emergency procedures and contacts, and a Decontamination Plan (U.S. EPA, 2006a).

Dive Equipment

When selecting equipment to protect the diver, a conservative approach is required. Unless a body of water is known to be clean, some degree of contamination should be assumed. This is especially true in harbors, small enclosed water bodies, near cities/industrial areas, near any outfalls or contaminated sites (e.g., Superfund sites). The use of a drysuit with drygloves and a mated dive helmet or full-face mask would typically provide the diver with adequate protection for most polluted water dive operations but may not be suitable for diving in the vicinity of chemical spills/releases or on highly contaminated dive sites. When selecting a full-face dive mask or a helmet (which provides a greater degree of protection but is logistically more demanding) the following properties should be considered. The mask or helmet should have positive pressure, be suitable for decontamination and contain a reliable second stage regulator. If using a helmet it should be mated with the drysuit as opposed to using a neoprene seal. Dry suits should be constructed out of materials such as vulcanized rubber or polyurethane that contain a smooth surface to minimize adherence of contaminants and provide improved decontamination efficiency. The material/suit should have permeation test data, be resistant to abrasion, be simple to repair in the field and be fitted with double exhaust valves (Trelleborg-Viking, 2001).

Hazards

Although general water quality conditions have improved persistent organic chemicals and metals, along with biological contaminants, remain a potential hazard to inadequately protected divers. Most persistent biological and chemical contaminants tend to concentrate in sediment rather than in the water column (Hendrick et al., 2000; Hoffman et al., 2003; US Navy, 2004). Water quality conditions may change frequently and the presence of biological contaminants may be highly variable, especially within 24-36 hours of a rainfall event. The water body type and shoreline activities dictate the potential for contamination. A river or a large body of water (e.g., a lake or ocean), which has flow or circulation allowing for removal or dilution of suspected contaminants is generally of less concern than diving in a closed body of water (e.g., a pond or a flooded quarry). Lists of the chemical substances most commonly spilled in inland and coastal waterways of the United States are available from a number of sources. The chemical spill lists were summarized for the U.S. Navy's Experimental Diving Unit by Southwest Research Institute (Henkener and Ehlers, 2000).

If specific chemical contaminants are known a brief hazard analysis should be included in the Dive Plan to address potential exposure pathways and identify specific equipment or procedures to minimize risk factors. Several online chemical data bases such as www.epa.gov/iris, cameochemicals.noaa.gov, www.cdc.gov/niosh/npg/, or www.atsdr.cdc.gov, contain useful information on the potential human health hazards posed by chemicals that may be encountered and potential routes of exposure (inhalation, ingestion and/or skin contact hazard). Understanding the physical properties of the potential contaminants is also important. The properties should include solubility in water (maximum concentration that may be encountered in the water column), density (sinkers/in sediments or floaters/on water surface), water reactivity and persistence in the environment. If available, drysuit compatibility testing data should also be consulted and included in the Dive Safety Plan.

Biological contaminants include harmful algal blooms (e.g., red tide), bacteria (e.g., fecal coliforms), viruses and parasites. The levels of these contaminants are often highly variable but would typically be found at the highest levels near sewage outfalls, after a rainfall or as water temperature and biological nutrient levels increase in the water column. The EPA's BEACHES Environmental Assessment and Coastal Health Program (BEACH) and NOAA's Mussel Watch Program are valuable dive planning tools and should be consulted when preparing Dive Safety Plans.

Site Control/Work Zones

When conducting dive operations in contaminated water, several zones are established in the site Dive Plan (U.S. EPA, 2006a). These zones are dictated by the location of the contaminants and activities that will be performed in each of the zones. The Exclusion Zone (EZ), also called the Hot Zone, is the area believed to be contaminated (U.S. EPA, 1992). This is the area in which site work will normally be performed. In some cases when divers are entering the water from the shoreline, performing their duties and returning to the shore, the body of water and a portion of the shoreline may be considered the EZ. In the case of boat operations, the body of water and that portion of the boat that a contaminated diver contacts may be considered the EZ. It is imperative that no personnel enter the EZ without the proper personal protective equipment (PPE). It is also imperative that no personnel, equipment or samples pass from the EZ to the Support Zone without going through the Contaminant Reduction Zone.

The Contamination Reduction Zone (CRZ), or Contamination Reduction Corridor, is defined as the area through which all personnel leaving the Exclusion Zone must pass to be decontaminated (U.S. EPA, 1992). This is the primary working zone for decontamination personnel. All personnel in the CRZ must wear proper PPE for the task they have been assigned. The SZ is defined as the clean area outside of both the EZ and the Contamination Reduction Zone. The CRZ is a straight line operation; divers enter from the EZ and go through the decontamination process until fully decontaminated and ready to enter the Support Zone (SZ) on the other end. All equipment must also be decontaminated before moving into the SZ. No one should be allowed to leave the EZ and enter the SZ without completing the decontamination procedure, except in the event of a diving accident.

Diver Decontamination

The required diver decontamination will depend on the level and hazard of the contaminants present at the dive site. For operations with low levels of contaminants and/or those with limited hazards posed to the diver, decontamination is typically performed on the dive ladder or swim platform of the dive vessel. Under such a scenario, the EZ is typically considered to be the water and, if necessary, a container on the boat used as the equipment drop. The dive ladder or swim platform become part of the entire CRZ. It is the responsibility of the Divemaster to determine the zone boundaries and whether the space available on the boat is sufficient for the level of decontamination required.

If the levels of contaminants or their hazards warrant a more extensive decontamination than can be performed on the dive vessel, a larger boat/barge or shore based operation may be necessary to stage an appropriate multi-stage decontamination line. In such an instance, the dive vessel may be considered part of the EZ while conducting the dive operations and transporting the diver to the decontamination area (CRZ). At the conclusion of the project, the dive vessel would then need to also be decontaminated as necessary.

Decontamination Solutions

There are numerous decontamination solutions to choose from but, unfortunately, many of the most effective decontamination solutions are very aggressive, corrosive and toxic (LBL, 2006). Many disinfectants and sterilants are well suited to cleaning hospital surfaces and equipment but are not safe to use on divers or some dive equipment. The objective of decontaminating the diver is to remove the contamination from the diver’s suit so that the suit can be safely removed. It is not necessary to use solutions that are potentially dangerous to the diver or the equipment when other less dangerous solutions yield satisfactory results. Removing the contaminants from the diver is more important than neutralizing chemical contaminants or killing biological contaminants. Killing biological contaminants on the divers suit/equipment will usually not be the goal of the initial stage of the decontamination process (while the diver is still dressed), because of the wet contact time required to achieve this. A secondary definitive decontamination of drysuits and equipment may be required after the drysuit/equipment has been removed. Since some of the contaminants at a site may be unknown it is necessary to use a decontamination solution that is effective for a variety of contaminants (EPA 1985).

It is recommended that prior to the start of site activities the contaminants of concern be identified and consideration be given to select the most appropriate decontamination solution(s). If contaminants are anticipated but not well documented a conservative approach should be used in selecting the most effective broad-based decontamination solution(s).

A plentiful supply of potable water from a low-pressure hose is the first and last step of all decontamination procedures. If a large tank is not available, smaller containers (e.g., 5-gallon buckets, collapsible plastic containers, Hudson sprayers) of potable water are also suitable. Water from a hose should not be under pressure any higher than typical municipal water pressure (40 to 70 psi). High pressure hoses (e.g., pressure washers) should not be used; they may damage the diver’s suit or force contaminants into seams or contaminate nearby surface support personnel. In some instances a thorough rinse with potable water is all the decontamination the diver needs.

Commercial soaps/cleaning solutions are readily available and produced by numerous companies using various synthetic and/or natural active ingredients. When selecting a soap/cleaning solution the following properties should be considered:

Surfactant effectiveness: The greater the surfactant effectiveness the easier the solution will remove contaminants and oil/grease during the decontamination process. A soap's surfactant action will remove most organic contamination and scrubbing with soapy water will remove sediment-associated inorganics (e.g., metals). Soap will also wash away biological contaminants (when biological contaminants are washed off, they are not killed but their physical removal can result in effective decontamination). When decontaminating oil and grease the surfactants effectiveness is usually a key consideration when selecting an appropriate decontamination solution.

Antimicrobial properties: Some soap/cleaning solutions include antimicrobial additives. The active ingredient used in most antimicrobial soaps is triclosan. Triclosan works, even at very low concentrations, by blocking enoyl-acyl carrier-protein reductase (ENR) preventing bacteria and fungi from producing fatty acids needed for cell membranes and other vital functions (Senese, 2005). Humans do not have the ENR enzyme, i.e., triclosan is harmless enough for use in a wide variety of consumer goods including cosmetics and toothpaste (Senese, 2005). Because of its effectiveness and safety, antimicrobial dish soap is often the solution of choice for decontaminating patients arriving at hospital emergency rooms (USVA, 2006; Jagminas, 2006). In hand-washing experiments, antimicrobial soap was shown to be more effective at removing biological agents than soap with no antimicrobial additive (CDC, 2002).

Biodegradability: Many biodegradable products are readily available. When decontamination solutions may be released into the environment during the decontamination process a biodegradable product should be used. When the decontamination solutions are controlled and contained this criterion is of less importance. The product's biodegradability is usually specified on the product's label or the associated Material Safety Data Sheet (MSDS).

Safety: When selecting an appropriate soap/cleaning solution the safety to the all personnel and equipment should be considered. To access the safety of a solution the Material Safety Data Sheet (MSDS) should be consulted. When possible non-hazardous solutions with a HMIS health rating of 1 or less should be utilized. The MSDS will identify any specific health hazards (eye, skin, ingestion, and inhalation) and the appropriate protective equipment that should be used if needed. The MSDS will also list any applicable first aid measures, accidental release measures, handling and storage requirements, exposure controls and the solutions stability and reactivity (which is important when using multiple decontamination solutions and/or compatibility with dive equipment materials). Biodegradable antimicrobial soap is a useful decontamination solution because it has wide applicability, is readily available, is safe for use on both the diver and the diver's suit, and requires no special PPE or disposal. The leftover soap solution can be used to clean the decontamination zone, the boat or other equipment.

In instances where the use of soap and water alone are not sufficient to adequately decontaminate the diver additional decontamination solutions may be considered. When the diver's suit is contaminated with oil and/or grease a decontamination solution with degreasing properties may be effective as a single decontamination solution or in conjunction with other decontamination solutions. Although an iodine based decontamination solution such as Betadine or alcohol may not be useful as a primary decontamination solution it may be most effective for use decontaminating various pieces of dive equipment such full-face masks (e.g., AGA masks). Harsher or more aggressive decontamination solutions such as tri-sodium phosphate (TSP) and quaternary-ammonium compounds (quats) may not be an ideal primary decontamination solution but may be useful in performing a secondary definitive decontamination on certain equipment after it has been removed from the diver. Certain commercially available decontamination solutions such as DF200 have been tested and shown to be effective on specific biological and chemical contaminants. Although this solution is more expensive and less available than most other decontamination solutions it may be the most effective and reliable decontamination solution available for specific contaminants at the dive site.

Case Histories

The ERT dive team has conducted thousands of dives on contaminated sites throughout the United States. For each site, prior to conducting dive operations and as outlined above, the contaminants were identified (if possible) and the appropriate equipment and decontamination procedures were determined. The information was then incorporated in a site specific dive safety plan. As summarized in Table 1, dry suits with mated dry gloves and full-face masks were used for most dive operations while the decontamination plan was more variable depending on contaminants and site logistics.

Table 1. Case Histories.

Site	Contaminants	Equipment	Decontamination	Comments
Pyramid Lake – Diver transects and sediment sampling for submerged oil in reservoir (drinking water supply).	Heavy Oils	• Drysuit with Drygloves • Full-Face Mask	Multi-stage decontamination line on support barge using soap and degreasers.	Divers tried to minimize contact with sediments to limit exposure to contaminants.
Manistique Harbor Superfund Site – Sediment core samples collected by diver for assessment and cleanup verification.	PCBs	• Drysuit with Drygloves • Full-Face Mask	Shore based soap and water decontamination.	Dedicated vessel remained part of EZ; diver transported to shore; decon after two hour dives (surface supplied gear).
Raritan Slag Superfund Site – Collection of sediment core samples for site assessment	Lead	• Drysuit with Drygloves or Wet Suit • Full-Face Mask	Diver was decontaminated with soap on platform at stern of dive vessel.	Diver equipment downgraded on portions of the site after review of analytical data by Dive Master and Health and Safety Officer. Heat stress hazard considered extremely high, lead chemical hazard was extremely low.
Athos Oil Spill - Diver operated suction dredge for submerged oil recovery.	Heavy Oils	• Drysuit with Drygloves • Dive Helmet	Multi-stage decontamination line on barge using soap and degreasers.	Scope of dive operations was considered outside definition of scientific diving so commercial divers were hired to conduct work.
16th Street Quarry – Search for drums in a flooded quarry in urban area.	Unknown	• Drysuit with Drygloves • Dive Helmet	Shore based soap and water decontamination.	Search for drums allegedly dumped in quarry. EPA divers located drums and commercial divers over-packed underwater for removal and disposal.

Conclusions

In many diving situations some degree of contamination should be assumed. Diving in contaminated water can be performed safely when conservative procedures are followed to keep the diver dry. Through the use of Dive Safety Plans (including a chemical/biological hazard assessment), proper protective gear and thorough decontamination, diving operations can be safely employed to investigate contaminated water bodies or perform other activities under hazardous conditions. The methodologies outlined in this paper are currently followed by the EPA ERT Dive Team to perform sampling, drum searches, determining oil spill impacts and other environmental tasks in contaminated water. It is important that all groups performing dive operations be informed of any potential exposure to water contaminants and the proper methods for protecting divers and tenders from any identified hazards. No one should dive in contaminated water without medical monitoring and the proper training and equipment.

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CAVERNS, COMPRESSED AIR AND CRUSTACEAN CONNECTIVITY: INSIGHTS INTO HAWAIIAN SPINY LOBSTER POPULATIONS

Matthew Iacchei[1,2]
Robert J. Toonen[1]

[1]Hawaiʻi Institute of Marine Biology
University of Hawaiʻi at Mānoa
Honolulu, HAWAII 96744 U.S.A.
iacchei@hawaii.edu

[2]Department of Zoology
University of Hawaiʻi at Mānoa
Honolulu, HAWAII 96817 U.S.A.

Since the arrival of the first Polynesian voyagers to the Hawaiian archipelago spiny lobsters have proved to be valuable fisheries species in Hawaiʻi. However, the combination of long-term commercial and recreational fisheries for this species, and changing environmental conditions, have led to declining catch rates since the 1950s. The Papahānaumokuākea Marine National Monument (PMNM), established in 2006, now provides an extensive harvest refuge area for lobster species in the Northwest Hawaiian Islands (NWHI). This study aims to investigate the potential for the PMNM to rejuvenate lobster populations and fisheries in the Main Hawaiian Islands by using mitochondrial DNA markers to examine how lobster populations are related throughout the Hawaiian archipelago. State and federal agencies, dive shops and recreational and commercial lobster fishermen all collaborated to obtain genetic samples across the ~2,500 km stretch of ocean spanned by the State of Hawaiʻi. Over 1,249 samples from 15 islands, atolls, reefs and banks throughout the archipelago were collected prior to 2011. In the process of collecting samples for genetic analysis, we observed a significant shift in species abundance between the MHI and the NWHI. Panulirus marginatus *is the most abundant spiny lobster (73%) in the NWHI catch while* P. penicillatus *(88%) dominates the MHI catch (Fisher's exact test, $P << 0.001$). We also observed* P. marginatus *living at shallower depths in the NWHI where they would not be found in the NWHI. This could be due to thermal regime, habitat availability, species competition, or fishing effects.*

Introduction

Both researchers and resource managers around the globe generally agree that efforts to establish effective marine protected areas require detailed information regarding connectivity among disjunct populations of species (e.g., Botsford et al., 2001; Halpern and Warner, 2003; Palumbi, 2003; Cowen et al., 2006). The size and number of connected populations and frequency of larval influxes can profoundly influence the density and persistence of local populations (MacArthur and Wilson, 1963; Stacey and Taper, 1992). This fact is particularly pertinent for benthic marine invertebrates living in isolated island chains where populations are more likely related through larval dispersal than adult movement. Unfortunately for managers this pelagic developmental period, coupled with a small size and the ability to swim against prevailing oceanographic currents, make larvae extremely difficult to track (reviewed by

Levin, 2006). The lack of knowledge of population connectivity in organisms with a pelagic larval stage (~85% of benthic marine organisms) has caused much of the difficulty in successfully managing marine species (Carr et al., 2003). For example, the Northwestern Hawaiian Island (NWHI) lobster fishery was closed in 2000 because of increasing uncertainty in population and stock assessment models particularly with regard to spatial heterogeneity and the assumption of synchronous dynamics among bank specific populations (Botsford et al., 2002). Quantitative estimates of population connectivity are required to develop models that more accurately represent island/bank specific population dynamics.

Spiny lobsters have one of the longest larval durations of any taxa (6 months to over 1 year) making dispersal patterns in these species difficult to predict. Intuitively, species with larval durations of this length should be panmictic across broad geographic ranges (e.g., Shanks et al., 2003; Siegel et al. 2003); however, more recent meta-analysis suggests there is little to no relationship between larval duration and the degree of population structure across a species' range (Shanks, 2009; Weersing and Toonen, 2009). Accordingly, previous *Panulirus sp.* genetic studies have found indications of localized recruitment despite an 8-12 month larval duration (Silberman and Walsh, 1994; Johnson and Wernham, 1999). Hypotheses of dispersal patterns of *Panulirus marginatus* in Hawaiʻi have also been controversial and results contradictory. Pollock proposed that phyllosoma mix together in the Pacific subtropical gyre and remain there for up to four years before recruiting to Hawaiian reefs (Pollock, 1992), while MacDonald contended that larvae are retained around the archipelago for shorter time periods before settlement (MacDonald, 1986). Polovina et al. (1999) concluded that ocean currents drive phyllosoma in a southeasterly direction until they reach Necker Island, at which time they travel southwest. Previous genetic studies have failed to resolve these issues. Shaklee and Samollow (1980) used allozymes to examine the structure of *P. marginatus* populations across a subsection of the archipelago and suggested panmixia. Seeb et al. (1990) subsequently studied *P. marginatus* populations at just Maro Reef and Necker Island and found significant differences at one of seven allozyme loci but no differences at the other six. No study to date has sampled the entire archipelago nor looked at connectivity patterns in *Panulirus penicillatus*, a co-distributed congener that is the primary focus of recreational and commercial fisheries in the Main Hawaiian Islands.

The overall goal of this study is to determine the scales of population connectivity in the two congeneric species of spiny lobsters in the Hawaiian Archipelago, *P. marginatus*, and *P. penicillatus*, using natural genetic variation in both mitochondrial and microsatellite DNA markers. These data will enable managers to determine whether the recently established Papahānaumokuākea Marine National Monument (PMNM) has the potential to rejuvenate lobster stocks in the Hawaiian Archipelago or if additional reserves will be needed to sustain populations in the MHI. This work is ongoing and results to date are preliminary. Here we discuss the initial stages of this work, the collection of samples throughout the species range and some of the additional, previously undocumented, non-genetic results we have discovered in the sampling process.

Methods

Sampling

Lobsters were collected using three distinct methods: by hand while scuba diving or free diving, with standard Fathoms Plus® commercial lobster traps onboard both large and small research vessels, and through collaborations with commercial and recreational fishers. To cover such a broad geographical area and depth gradient for sample collection the assistance of a number of organizations was employed: two National Oceanic and Atmospheric Association (NOAA) vessels (R/V Hiʻialakai and R/V Oscar Elton Sette), six dive shops located throughout the state of Hawaiʻi, five Hawaiʻi Division of Aquatic Resource (DAR) Offices, five commercial fishermen, and over 20 recreational divers/fishermen, in addition to University of Hawaiʻi scientific divers. Whenever possible carapace length, sex, and a GPS coordinate of sampling locations were recorded. However, with the large diversity of sampling personnel, it was not always possible to obtain all of this information for each specimen. All samples collected by scientists employed non-lethal sampling protocols as outlined in Skillings and Toonen (2013). In all sampling instances, a single leg or antenna segment was taken for a tissue sample and lobsters were returned to the site of capture (with the exception of commercial and recreational fishers, who retained all legal lobsters). Removal of a walking leg is a standard sampling technique for crustacean genetic surveys because these appendages typically grow back during the next molt (Mykles, 2001).

The tissue samples were taken in the field and stored in either 20% dimethyl sulfoxide salt-saturated buffer (Seutin et al., 1991; Gaither et al., 2011) or 95% ethanol at room temperature. At all locations in the Northwestern Hawaiian Islands where > 50 samples have been collected (particularly, Necker Island, Gardner Pinnacles, Maro Reef, and Laysan Island where sample sizes are >> 50) the samples were obtained from the NOAA National Marine Fisheries Service (NMFS) annual lobster tagging cruises, prior to the establishment of the Papahānaumokuākea Marine National Monument (PMNM) in 2006.

Laboratory procedures

Genomic DNA was extracted from each tissue sample using the DNeasy Animal Tissue kits (Qiagen Inc., Valencia, CA, USA) following the manufacturer's protocol. For *Panulirus marginatus* a 662 bp region of mtDNA cytochrome oxidase II (COII) was amplified for each sample using the polymerase chain reaction (PCR; Saiki et al., 1988) and a standard PCR protocol (Palumbi, 1996) on a Bio-Rad iCycler Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA). For *Panulirus penicillatus* a 460 bp region of the cytochrome oxidase I (COI) region of the mitochondrial DNA was amplified using the same procedures described above. Purified PCR products were sequenced in the forward direction with an ABI 3730XL or an ABI 3130XL capillary sequencer (Applied Biosystems, Foster City, CA). Sequences were edited, aligned, and trimmed to a uniform size using GENEIOUS PRO v. 4.8.5 (Biomatters Ltd., Aukland, New Zealand).

Sequence analysis

DNA sequences were edited using SEQUENCHER version 4.9b (GeneCodes Corporation). Edited sequences were evaluated using MODELTEST 3.06 (Posada and Crandall, 1998) to identify the most appropriate model of DNA evolution. Analyses are forthcoming. Briefly, we will investigate population subdivision between the MHI and the NWHI using a hierarchical analysis of molecular variance (AMOVA) (Weir and Cockerham, 1984; Excoffier et al., 1992). To assess individual population connectivity relationships we will compare pairwise population ϕ_{ST} values using ARLEQUIN 3.1 (Excoffier et al., 2005). ARLEQUIN will also be used to calculate nucleotide (π) and haplotype diversity (h) for each sampling site. Sequences will be used to create a statistical parsimony network in TCS 1.21 (Clement et al., 2000) to visually represent the relationship and distribution of haplotypes among populations. Migrate-N (Beerli, 2004) will be used to determine directionality in gene flow and quantify exchange between the MHI and the NWHI.

Results

Sampling

Prior to 2011 967 *P. marginatus* and 282 *P. penicillatus* have been collected from 15 islands/atolls throughout the Hawaiian Archipelago (Table 1). Subsequently 67 *P. marginatus* and 12 *P. penicillatus* have been collected and added to the dataset of sequences but these samples and the effort to collect them are not included in this analysis. In both the NWHI and MHI 49% of lobsters captured were female (Table 2), which was not significantly different than the expected 1:1 ratio (NWHI: $\chi^2 = 0.184$, df = 1, $P = 0.668$; MHI: $\chi^2 = 0.039$, df = 1, $P = 0.844$). Because there is a ban on taking female lobsters in the MHI the sex ratio for lobsters that can be legally harvested in the MHI (>3.25 inches carapace length (CL)) was examined. For individuals in this legal size class 45% of lobsters in the NWHI were female (Table 2), which was significantly different than the expected 1:1 ratio ($\chi^2 = 4.111$, df = 1, $P = 0.043$). In the MHI

39% of lobsters captured were female (Table 2), but this deviation was not significant due to lower sample size ($\chi^2 = 3.125$, df = 1, $P = 0.077$).

Table 1. Total DNA samples collected to date from each island, atoll, and bank in Hawai‘i.

		Panulirus marginatus	*Panulirus penicillatus*
Main Hawaiian Islands	Hawai‘i	1	47
	Maui Nui Complex	17	84
	O‘ahu	63	0
	Kaua‘i	2	47
Northwest Hawaiian Islands	Nihoa Island	0	0
	Necker Island	161	5
	French Frigate Shoals	13	37
	Gardner Pinnacles	125	0
	Maro Reef	232	5
	Laysan Island	126	1
	Lisianski Island	50	21
	Pearl and Hermes Reef	67	30
	Midway Island	53	4
	Kure Island	57	1

Table 2. *Panulirus marginatus* and *P. penicillatus* tissue collections by sampling method, stakeholder group, sex, and size category (sub-legal versus legal-sized). Numbers listed are totals collected prior to 2011 from the Main Hawaiian Islands (MHI) and the Northwest Hawaiian Islands (NWHI).

		Panulirus marginatus		*Panulirus penicillatus*	
		MHI	NWHI	MHI	NWHI
Catch methods	Snorkel/dive	24	275	175	102
	Trap	59	609	3	2
	Commercial Fishers	72	N/A	81	N/A
	Recreational Fishers	4	N/A	35	N/A
	Scientists	7	884	62	104
Males	Males	3	276	50	1
	Sub-legal males	0	72	9	0
	Legal-sized males	3	204	41	1
Females	Females	4	266	46	0
	Sub-legal females	4	101	18	0
	Legal-sized females	0	165	28	0

Specimens of at least one of the two lobster species were successfully collected while snorkeling or scuba diving at all islands/atolls in the Hawaiian Archipelago with the exception of Gardner Pinnacles and Nihoa Island. From 2006 through 2009 one of the authors (MI) was involved in dive collection trips resulting in 11,091 diver-hours searching for lobsters: 4,453 hours in the MHI, and 6,638 hours in the NWHI. A best-guess estimate places the total diver-hours spent from 2006 to 2010 searching for lobsters at close to 20,000. Diving depths ranged from 0 to 30 m, with a mean depth of 14 m (45 ft), and median of 13 m (43 ft).

In the Main Hawaiian Islands (MHI) snorkeling or scuba diving was used to collect 24 *P. marginatus* and 175 *P. penicillatus.* In the Northwest Hawaiian Islands (NWHI) 275 *P. marginatus* and 102 *P. penicillatus* were collected while snorkelling or scuba diving (Table 2, Fig. 1). This is a highly significant shift in the species abundances across the two regions, with roughly three-fold more *P. marginatus* than *P. penicillatus* collected in the NWHI, whereas roughly seven-fold more *P. penicillatus* than *P. marginatus* were sampled in the MHI (Fisher's exact test, $P << 0.001$).

Trapping was not as widely utilized as snorkeling and scuba diving for lobsters due to difficulty in obtaining scientific collecting permits to fish with the Fathoms Plus® commercial traps. Lobsters of at least one of the two species were succesfully collected with traps at six islands/atolls in the Hawaiian Archipelago and traps were fished unsuccesfully at four additional islands/atolls. Trapping depths ranged from 10 m (33 ft) to 117 m (384 ft), with a mean depth of 42 m (139 ft), and a median of 35 m (114 ft). The vast majority of lobster samples obtained through trapping were *P. marginatus* (59 samples in the MHI; 609 in the NWHI; Table 2, Fig. 1) obtained from the annual NOAA/NMFS lobster stock assessment and tagging cruises that visited the NWHI each summer. Trapping proved unsuccessful for collecting *P. penicillatus* samples (3 samples in the MHI; 2 in the NWHI; Table 2, Fig. 1).

All samples collected in the Northwestern Hawaiian Islands were collected by University of Hawai‘i or NOAA/NMFS biologists. In the MHI, though, both commercial and recreational fishers assisted in the sample collection. Figure 2 shows a distribution of samples collected in the MHI according to the stakeholder group that collected them.

From 2006 to 2010 261 lobster samples have been collected in the MHI: 83 *P. marginatus*, and 178 *P. penicillatus* (Table 1). Of these 59% (72 *P. marginatus*, 81 *P. penicillatus*) were collected by commercial fishers, 15% (4 *P. marginatus*, 35 *P. penicillatus*) by recreational fishers, and 26% (7 *P. marginatus*, 62 *P. penicillatus*) were collected by University of Hawai‘i scientists (Table 2, Fig. 2).

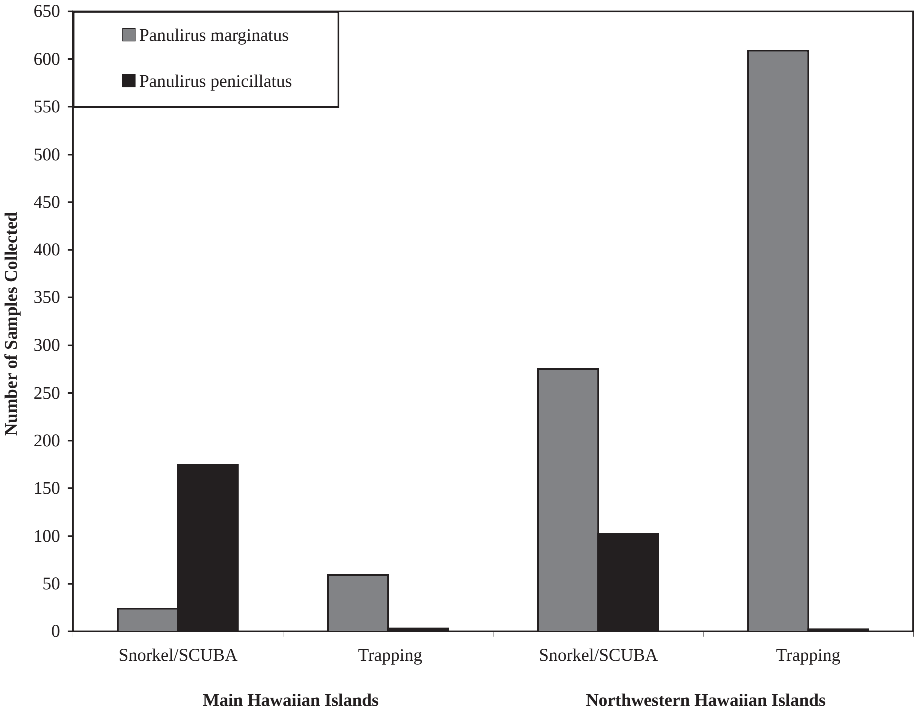

Figure 1. Number of lobster tissue samples collected per species in the Main Hawaiian Islands and the Northwest Hawaiian Islands by methodology.

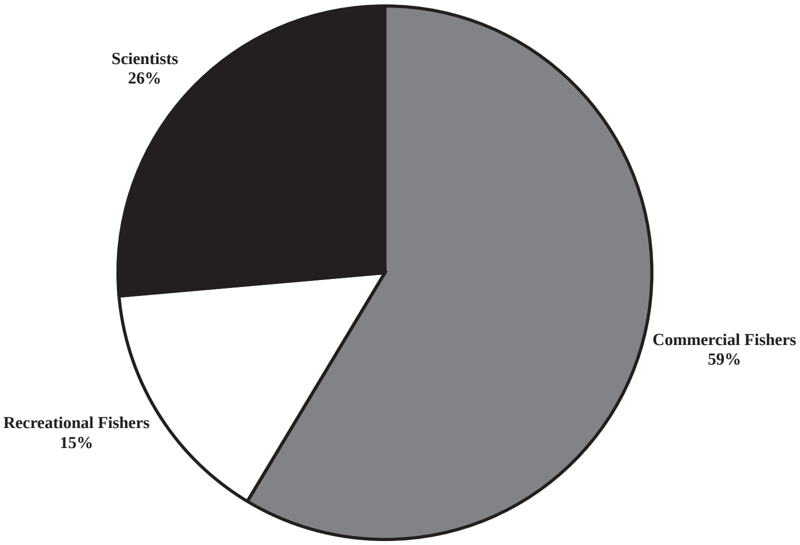

Figure 2. Percentage of lobster tissue samples collected in the Main Hawaiian Islands by stakeholder groups

Laboratory procedures

DNA was extracted from a total of 449 *P. marginatus* individuals from 14 islands/atolls and 227 *P. penicillatus* individuals from 8 islands/atolls. All of these individuals were sequenced at their respective gene (662 bp COII for *P. marginatus*; 460 bp COI for *P. penicillatus*).

Sequence analysis

Analysis of sequence data is currently underway and results will be the subject of future publications.

Discussion

Data on size, sex, and depth distributions are not usually recorded or published in marine phylogeographic or population genetic studies because these data are generally not available or relevant to the research questions being addressed. However, the collection of morphology, sex, fishery, and distribution data in addition to tissue samples for this genetic analysis has revealed interesting ecological patterns. For example, anecdotal evidence suggests that *Panulirus penicillatus* will not readily enter traps (Holthuis, 1991) but no study to date has documented this phenomenon with experimental trap catch reports. Here we report trap data (Fig. 1) that corroborates this hypothesis. Of the 673 spiny lobsters trapped in total for this study, only 5 (0.7%) were *P. penicillatus*. This result may be explained partially by the fact that the majority of lobsters (90.8%) were caught in the NWHI and at deeper depths where our data suggests *P. marginatus* is the more abundant species. However, even in traps that were fished at shallower depths in the MHI, where *P. penicillatus* makes up 68.2% of the total spiny lobster catch, *P. penicillatus* only comprised 1.6% of the trap catch. These data demonstrate that while *P. marginatus* will readily enter baited traps, this method of capture is relatively unsuccessful for *P. penicillatus*. Holthuis (1991) argues that the majority of tropical spiny lobster species will not enter traps with the exception of *P. marginatus* in Hawaiʻi and *P. pascuensis*, which is endemic to Easter Island. In contrast, temperate lobster species support lucrative commercial trap fisheries in the North Atlantic, East Pacific, Western and Central Pacific, and South Africa (Holthuis, 1991). Physical, ecological, or behavioral drivers of these differences in trapability are unclear.

The recording of the sex of each individual lobster when possible allowed a test of sex ratio differences in the MHI and the NWHI. The NWHI have been closed to lobster fishing since 2000 (Botsford et al., 2002) but recreational and commercial lobster fishing still occur throughout the MHI. In 2006 legislation was passed banning all take of female lobsters in the MHI (previously males and females >3.25in CL could be taken). All of the lobsters collected in the MHI for this study were obtained after the ban on female lobster take was implemented. While the sex-ratio in the MHI prior to this ban is undocumented, 49% of lobsters collected in the MHI in this study were female, with this percentage decreasing to 39% when only the lobsters of legal size (>3.25 in. CL) are considered (Table 2). This compares with 45% of lobsters >3.25 in. CL that were female in the NWHI, where there is currently no fishing (Table 2). This divergence from a 50:50 sex ratio for legal-sized lobsters in both the MHI and NWHI may be due to a number of factors including differences in species composition and growth rates, bank-specific growth-rate differences (O'Malley, 2009), or illegal and undocumented poaching.

The discrepancy in patterns seen in the MHI and NWHI could also be due to differences in sample size between legal-sized lobsters in the MHI and in the NWHI (Table 2). However, the consequences of the ban on taking of female lobsters in the MHI deserves further consideration. The continued monitoring of the ratios of female and male spiny lobsters in the MHI would provide valuable data on the effect of the State's regulation on population dynamics of spiny lobsters here. If these management regulations continue to skew the sex ratio of lobsters so that the the majority of males are small there is the potential that female fecundity will become sperm-limited, which in turn could limit overall egg production as has been demonstrated in the congeneric *P argus* as well as *Jasus edwardsii* (MacDiarmid and Butler, 1998). This in turn would stymy any hope for stock recovery of spiny lobsters in the MHI.

One of the most interesting and unexpected results obtained from this sampling effort is the significant shift in abundance of lobster species between the MHI and the NWHI. *Panulirus marginatus* is the most abundant spiny lobster (73%) in the NWHI catch, while *P. penicillatus* dominates the MHI catch (88%). *Panulirus marginatus* is known to inhabit shallow depths down to 143 m, and *P. penicillatus* inhabits 0 to 4 m depth (Holthuis, 1991) but in the MHI it is rare to find *P. marginatus* shallower than 15 m. The same pattern was expected in the NWHI; however, at multiple atolls *P. marginatus* were commonly found in 1- 3 m of water on patch reefs in calm areas inside of the atoll. *Panulirus penicillatus* in the MHI is commonly found in shallow depths but can be as deep as 12 m in areas of high wave action. In the NWHI *P. penicillatus* were much less common and where found were restricted to a much narrower depth range (5 m maximum). It is clear that *P. marginatus* is able to thrive at shallow depths; however, the species may have a low tolerance for turbidity and wave action.

In the NWHI the only *P. marginatus* found in very shallow (< 3 m) waters were found in calmer areas inside of atolls where they were protected from high wave energy. However, even on the forereef where no such protection is available they could be found as shallow as 7 or 8 m. Shallow areas with low wave energy may be relatively rare in the main eight Hawaiian Islands so the species depth range has shifted deeper in the MHI. Alternatively, they may prefer cooler water temperatures and therefore need to move deeper waters in the MHI as do a number of fish species (Chave and Mundy, 1994) because surface water temperature in the MHI is warmer. It is also remotely possible that heavy fishing pressure through time has removed most of the shallow-water individuals or shifted the species distribution as a whole. *Panulirus penicillatus* is morphologically adapted to survive in high energy environments with legs that are both longer and greater in diameter than individuals of the same CL in other spiny lobster species (Iacchei, pers. obs.) Areas with high wave action may provide a natural harvest refuge for this species in the MHI but are more rare in the NWHI (hence the lower numbers there) although there is unlikely to be such a large discrepancy in the amount of shallow water habitat between the NWHI and the MHI. Further experiments would be required to test the hypothesis that *P. marginatus* outcompetes *P. penicillatus* where they co-occur but is excluded from high energy environments where *P. penicillatus* is the only spiny lobster species present. Alternatively, the two species may have originally colonized the Hawaiian Archipelago from opposite ends of the chain and only had limited success expanding to the far opposite ends. This hypothesis will be tested to the extent possible using a phylogenetic framework with the genetic data obtained in this study. However, given the presence of both lobster species at all islands/atolls in the archipelago it is more likely that the differences in abundance and distribution are driven by ecological factors.

The genetic analyses of the *P. marginatus* and *P. penicillatus* mtDNA sequences obtained from these collected tissue samples contribute to the growing number of population genetic surveys of congeneric species in Hawaiʻi (i.e., Bird et al., 2007; Craig et al., 2010; DiBattista et al., 2011; Skillings et al., 2013), leading to a broader understanding of the factors driving meta-population dynamics in the Hawaiian Archipelago. Knowledge of spiny lobster connectivity patterns will provide valuable insight into whether the protection of the PMNM will allow the rejuvenation of lobster stocks in both the NWHI and the MHI, or if further actions will be required to protect MHI lobster stocks. This added ecological component illustrates the importance and the additional insight that can be gained by recording ecological data during the collection of tissue samples for phylogeographic and population genetic studies, rather than just blindly treating each organism as “simply a bag of DNA” (M.J. Donahue, pers. com.).

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MINING- AND STORM-INDUCED BENTHIC DISTURBANCES IN NORTON SOUND, ALASKA

Stephen C. Jewett
University of Alaska Fairbanks
Institute of Marine Science
128 O'Neill, P.O. Box 757220
Fairbanks, ALASKA 99775-7220 U.S.A.
scjewett@alaska.edu

The effects of offshore placer gold mining on benthic invertebrates were assessed on 'sand' and 'cobble' substrates in Norton Sound, Alaska, northeastern Bering Sea. Mining with a bucket-line dredge occurred nearshore in 9-20 m water depths during June to November 1986-1990. Sampling via scuba divers nearly a year subsequent to mining demonstrated that benthic macrofaunal community parameters (total abundance, biomass, diversity) and abundance of dominant families were significantly reduced at mined stations. Many of the dominant taxa are known prey of the locally important red king crab (Paralithodes camtschaticus). Following the termination of mining recovery of the biota was underway in both substrates within four years but this process was interrupted in the fall of the fourth year (1990 and after sampling) by several severe storms. In the fall of 2011 a "superstorm" battered the Norton Sound coast and in the following spring divers resampled many of the sites previously examined 20+ years earlier. This paper contrasts benthic effects from mining with local natural disturbances, primarily storms.

Introduction

Onshore placer gold mining has a rich history in the vicinity of Nome in western Alaska dating back to the early 1900s. Offshore marine mining has occurred in only one region along Alaska's extensive coastline, in the Nome Offshore Lease Area adjacent to the City of Nome in Norton Sound within the northeastern Bering Sea (Fig. 1). Offshore placer gold mining was accomplished there by Western Gold Exploration and Mining Company Limited Partnership [WestGold] in the ice-free months of 1986-1990 and was conducted with the world's largest bucket-line dredge, the BIMA. An associated monitoring program (1986-1993) assessed the influence of mining on the benthic soft and hard substrates with emphasis on the effect on red king crab (*Paralithodes camtschaticus*), a species harvested commercially and for subsistence from southeastern Alaska to Norton Sound (Jewett, 1999; Jewett et al., 1999; Jewett and Naidu, 2000). Scuba sampling of the benthos nearly a year subsequent to mining demonstrated that benthic macrofaunal community parameters (total abundance, biomass, diversity) and abundance of dominant families were significantly reduced at mined stations. Following the termination of mining, recovery of the biota was underway in both substrates within four years but this process was interrupted in the fall of the fourth year (1990) by several severe storms, thus resetting the recovery clock (Jewett et al., 1999).

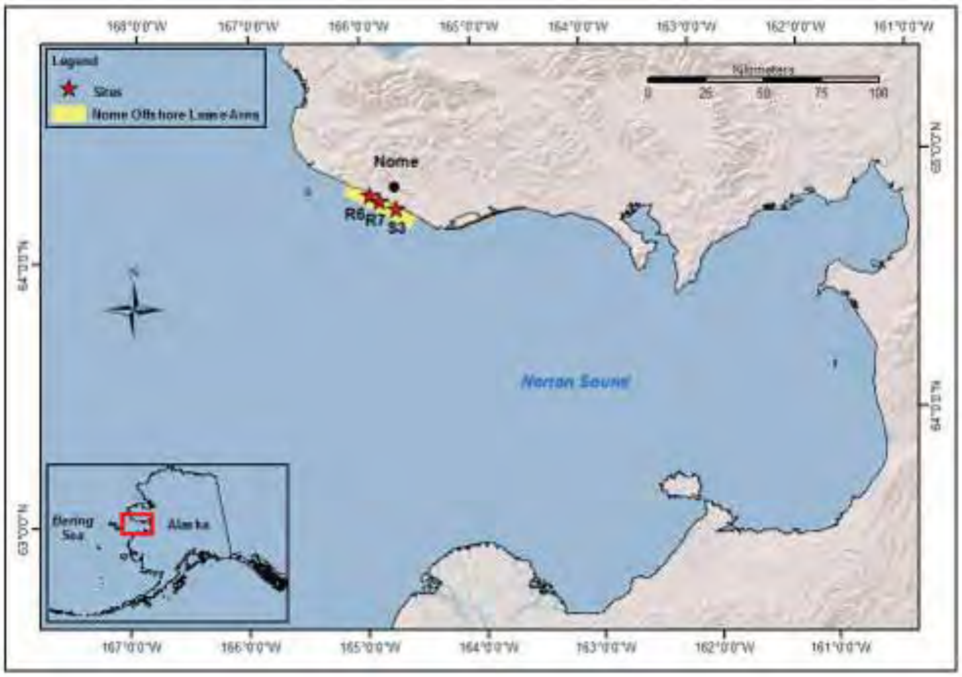

Figure 1. General study area and station locations in Norton Sound.

On 8-9 November 2011 a “superstorm” battered eastern and northern Norton Sound with winds to 106 km/h and seas surging to nearly 3 m in height (Nome Nugget, 2011). The destruction from this storm not only brought havoc to coastal communities like Nome but also caused severe harm to the nearshore marine community. Observations following the storm along a ~13 km-stretch of coast from Nome west to the Penny River revealed windrows of marine organisms in the surf zone including nudibranchs, sea raspberries (soft coral), cockles, crabs, sea cucumbers, sea stars, sea urchins, sea peaches (tunicate), sculpins, wolffish, greenlings and seals (Charles Lean, pers. comm., 17 November 2011).

Since the cessation of WestGold’s mining in the late 1990s, worldwide gold prices have steadily risen (from ~$408/oz in September 1990 to ~$1,402/oz in June 2013) creating resurgence in offshore gold mining in the coastal waters of Norton Sound. During the summer of 2012 divers returned to Norton Sound to resample some of the 1986-1993 stations for comparison in addition to establishing new baseline stations for a new potential mining operation. The objective of this paper is to contrast the effects of mining on the benthos with local natural disturbance effects, primarily storms.

Methods

Sampling/Field Methods

Sampling and field methods used for 2012 were similar to methods used in 1990 (Jewett et al., 1999). In June 2012, divers resampled soft (sand) and hard (cobble) substrates at three of the stations (R6, R7[R7A] and S3) that were sampled during 1986-1993. These three stations were within the Nome Offshore Lease Area (Fig. 1) in 11-13 m water depths adjacent to the City of Nome. Station R6 was dredged only during one season in 1986; station R7 was dredged in 1987 but also indirectly impacted by dredging in 1988, 1989, and 1990 because of mining activities occurring immediately adjacent to it. For 2012 Station R7 was renamed as R7A. Station S3, a reference station established at comparable water depths, was not previously dredged. A hand-held GPS unit was used to locate the station positions (WGS 84). An anchor with an attached surface buoy marked each station. All diving adhered to the University of Alaska (UA) Scientific Diving Program, an active member of the American Academy of Underwater Sciences. All diving was conducted from the Nome-based 9.75-m F/V *Kuzilvak*. Four divers on open-circuit scuba collected benthic samples using a suction dredge sampler. The suction sampler vacuum force was obtained with a 265 l/min water pump and a 3.8-cm diameter Venturi jet dredge nozzle. The influent side of the dredge nozzle was connected to a pump and the effluent side to a flexible discharge hose terminating in the 1.0-mm mesh collection container (cod end). The divers vacuumed the sediments within a 0.1-m^2 surface area-collecting frame and to a depth of ~10 cm. At that point, the collection container was removed, placed into a dive bag and a new sample container was attached. Five replicate samples were collected at each sampling site (six replicates were collected in 1990). Each successive replicate was collected upcurrent and approximately 2 m away from the previous replicate. For cobble substrates, rocks were removed from the quadrat frame before beginning suctioning. Any rocks that had organisms attached were placed into a 1.0-mm mesh sample bag and returned to the surface. All macrofaunal samples were returned to the workboat and then processed by the dive tender. The material in the mesh bag was removed and placed in a labeled polyethylene collection jar. Organisms attached to rocks were removed from the rocks prior to preservation. Sample preservation with 10% buffered formalin and 0.1% Rose Bengal occurred once onshore. Divers also collected surface sediment samples *ca.*5 cm deep for grain size analysis in conjunction with the macrofaunal sampling. Three replicate sediment samples were collected immediately adjacent to each macrofaunal sampling location within two substrate types: soft (mainly sand) hard (mainly cobble). At the completion of the field-sampling program macrofauna and sediment samples were air shipped to the UAF/IMS Laboratory.

Laboratory Methods

At the UAF/IMS lab samples were rinsed in fresh water through a 0.5-mm mesh screen and sorted according to major taxonomic groups. Each organism was identified to the lowest practicable taxonomic level and counts and blotted wet weights were obtained for each taxon by sample replicate. All processed material was preserved in 50% 2-propanol alcohol and labeled appropriately. Preserved samples were retained at the UAF taxonomic laboratory and some specimens were added to UAF's permanent taxonomic archive. Laboratory methods were similar to that used during the 1986-1993 studies. Sediment grain size was analyzed with sieve and hydrometer according to ASTM D422 at AmTest Laboratories, Inc. in Kirkland, WA.

Data Analyses and Statistical Methods

The benthic data included compilation of taxa numbers, abundance, biomass totals, as well as percent compositions of taxonomic groups for each station. The community metric most often used in benthic literature is abundance (e.g., Clark and Ainsworth, 1993; Olsgard and Hasle, 1993; Warwick and Clarke, 1993; Olsgard and Gray, 1995); thus, it is used here for temporal comparisons. Analysis of Variance (ANOVA) was applied to compare temporal abundance data. All data received 100 % QA/QC inspection. Archived 1990 WestGold Project data were retrieved and reviewed for taxonomic changes so the historic WestGold dataset would be compatible with the current 2012 dataset.

To standardize the datasets for between-year comparisons, the following modifications were made to the infaunal datasets prior to summarization and analyses: all meiofauna, pelagic invertebrates, highly-motile epifaunal invertebrates, large organisms weighing more than 5 grams, unidentified animal tissues and fishes were deleted. These exclusions eliminated the organisms that were not intended for collection by sampling device because of size or motility. Highly motile individuals were excluded from analyses to assess disturbance because their presence was most likely transient in a given area and their inclusion would obscure the responses of non-transient species. The assessment of disturbance relies on relatively sedentary species that are incapable of avoiding localized disturbance. Fragments of animals were not considered in abundance computations for any of the datasets.

Analysis of the benthic survey data was conducted using Primer v.6.1.15 with PERMANOVA add-on version 1.05 software. Benthic invertebrate taxa were summarized by station and substrate. Total number of taxa collected, average number of taxa collected and average density (individuals m^{-2}) were calculated.

Where historic data were available, the dominant invertebrate taxa by average abundance collected in 2012 were compared by station and substrate to the dominant taxa collected in 1990.

Multivariate statistical tools were also applied to determine patterns among sampling sites. The environmental data were standardized prior to analysis. Standardization transforms the data to have a mean of zero (0.0) and a standard deviation of one (1.0). A natural logarithm-transformation (*ln*-transformation) reduces the effect of variables having larger values and, overall, the values of numbers are similar but may still be a bit different. Thus, the data were analyzed on a common scale of mean 0.0 and standard deviation of 1.0.

Cluster analysis of the standardized environmental data was performed using the Euclidean distance coefficient. Cluster analysis is a method for grouping and classifying objects based on their similarity as determined through a distance matrix (McCune and Grace, 2002). A distance matrix represents the similarity of individual objects to each other and the goal of cluster analysis is to graphically represent object similarities as closely as possible to the distance matrix. Interpretation of the dendrogram is based on the closeness and linkage of branches within the tree diagram. Sites that share close branches are more similar than sites that are separated by more distant branches of the dendrogram. A subjective decision is made as to which sites are grouped together, i.e., meaning which sites are close enough in the dendrogram to infer that they should be considered as being similar. The Euclidean distance matrix is the accepted choice for biological data where few zero values are present. Group-average hierarchical cluster analysis was used to analyze the standardized environmental data selected.

Because interpretation of the dendrogram is subjective another multivariate tool, non-metric multidimensional scaling (MDS), was applied as used in Jewett et al. (1999). Taxa used in the analyses were at the family level or above. The Bray-Curtis similarity coefficient (Bray and Curtis, 1957) was used to calculate separate dissimilarity matrices for the sand and cobble substrates using *ln* (x+1) abundance data. The MDS ordination technique constructs a “map” of station similarities from the taxon abundance data (Bray-Curtis dissimilarity matrix) where the more similar stations are closer together in space (Gray et al., 1988). The extent to which the data are adequately represented by the two-dimensional map is summarized by the stress coefficient (square root of a normalized residual sum of squares: should be ≤ 0.15 [Clark and Ainsworth, 1993]). MDS ordination procedures have been applied widely to abundance data to assess disturbance effects upon marine communities (e.g., Field et al., 1982; Clark and Ainsworth, 1993; Olsgard and Hasle, 1993; Warwick and Clarke, 1993; Olsgard and Gray, 1995).

The two multivariate methods are presented to balance the weaknesses of the procedures. Cluster analysis tends to misclassify objects while ordination procedures have other weaknesses. If the patterns of separation among sites are similar in both methods then an inference about associations between sites can be made with greater confidence. Therefore, two methods are used as agreement in the groupings of stations that the cluster and MDS ordination provides. This agreement is evidence that the station groupings represent a good summary of the multidimensional relationships of the data. Cluster analysis and MDS were performed using PRIMER.

Results and Discussion

Mining disturbance

Based on a series of yearly assessments (1986-1993), following WestGold’s mining operations between 1986 and 1990, Jewett et al. (1999) concluded that mining impacts were local and apparent for the duration of the study period. The total area mined was only 1.5 km^2. Differences in sediment granulometry were not obvious for sand substrates but increases in coarse material were observed on cobble substrates. The benthos nearly a year subsequent to mining demonstrated benthic macrofaunal community parameters (total abundance, biomass, diversity) and abundance of dominant families were significantly reduced at mined stations. The recovery of the area mined once at Station R6 in 1986 and subsequently monitored for seven years by side-scan sonar imagery revealed a progressive smoothing of ocean bottom relief, decreasing size of tailing footprint and shoaling of depressions left by mining. The disturbance was best illustrated by the continued separation and slow convergence of mined with unmined stations in MDS ordinations.

Recovery of the biota was apparent within four years but this process was interrupted in the fall of the 4th year (1990) by severe storms. Opportunistic and/or early colonizing species dominated the nearshore (< 20 m) zone but were more prominent in mined areas. Opportunistic polychaetes included tube-dwelling polychaetes like oweniids and spionids, burrowing polychaetes like capitellids, and surface-dwelling cirratulids and goniadids. When opportunistic taxa show highly elevated abundance and dominate the community this often is indicative of a relatively recent disturbance. Jewett et al. (1999) noted recolonization of disturbed communities from dredging appeared to begin immediately after dredging stopped. Other less transient taxa such as bivalves can then begin to increase and account for more of the overall biomass. However, in areas with frequent disturbances such taxa do not reach a large adult size and are often replaced with opportunists on a regular basis. Following any major disturbance, man-made (dredging, crab fishing, ship anchoring) or natural (strong currents, ice grounding, feeding/excavating by animals), benthic recolonization and long-term community composition depended on numerous factors such as stability of disturbed areas, changes in local oceanographic regimes, tolerance of organisms to physical changes and availability of recruits (Pearson and Rosenberg, 1978; Boesch and Rosenberg, 1981; Thistle, 1981; Poiner and Kennedy 1984; Hall, 1994). In shallow benthic systems like Norton Sound, where natural disturbances are frequent, animals requiring a stable substrate probably never occur. Instead, such regions can be expected to comprise less diverse communities of resilient and resistant species (Boesch and Rosenberg, 1981).

In contrast to the Nome offshore area dredging studies conducted elsewhere demonstrate that recolonization can be a fairly rapid process. Assessment of the effects of repeated dredging elsewhere reveal the time for infaunal communities to return to conditions similar at control reference stations ranged from one month (McCauley et al., 1977; Hall et al., 1990) to six months (Lopez-Jamar and Mejuto, 1988), to approximately one year (Oliver et al., 1977, Swartz et al., 1980). Kenny and Rees (1996) did not observe a return to pre-dredge conditions in the North Sea two years after gravel extraction although the dominant species quickly recolonized. The longer recolonization process in the 1986-1993 study area (at least four years) compared with other shallow-water dredging operations, appears related to the interaction of the effects of BIMA dredge mining (i.e., initially unconsolidated sediments associated with the depressions and tailings piles that become more consolidated with time) with the frequent natural disturbances present in Norton Sound. Of the natural disturbance events, high dynamics in the study area (e.g., current scouring and associated bedload movement) are probably the most common factors influencing the overall success of particular species while severe storms tend to `reset' the successional clock within the study area. It was concluded that natural disturbances, some unique to the area, were major forces structuring the benthic community within Norton Sound.

The assessment of mining impact on red king crabs was judged to be negligible. This is based upon finding no differences between mined and unmined areas regarding crab catches, crab sex and size composition, prey quantity and few differences in crab stomach contents (prey composition) (Jewett, 1999). Also, mining activities did not result in elevated concentrations of any of eight heavy metals measured in crab tissues or sediments. Furthermore, the metal values observed in Norton Sound crabs were below or within the range of values of king crabs from other mined and unmined locations (Jewett and Naidu, 2000).

Storm disturbance

The Bering Sea "superstorm" of 8-9 November 2011 battered eastern and northern Norton Sound with winds to 106 km/h and seas surging to nearly 3 m in height. The destruction from this storm not only brought havoc to coastal communities like Nome but also caused severe harm to the nearshore marine community. Longtime Nome resident, Charlie Lean, made the following observations following the storm after driving ~13 km west along the coast from Nome to the Penny River (pers. comm., C. Lean, Norton Sound Economic Development Corporation, 17 November 2011): "There were deposits of large amounts of sea creatures every 1/2 mile (0.8 km) that would extend from about 100 to 200 yards (91-183 m) ranging from about 3 to 10 yards (3-9 m) wide; too dense to see the sand underneath. Most were starfish with huge numbers of small sea cucumbers. Black nudibranchs were common, as were sea strawberries (sea raspberry) and sea peaches. There was a king crab every 10 feet (3 m) with occasional snow crab and other crabs. The cucumbers were in densities up to100/lineal foot; nudibranchs were one per square yard; and small sculpins were there every 10 feet (3 m). Wolffish were found one every mile while large sculpins were eaten by gulls and ravens, probably every 50 feet (15 m). A ribbon seal and a spotted seal were in the 8-mile (~13 km) section. I saw one greenling (fish); I was just driving and getting out occasionally to kick things around. There were some things that were reddish animals on a holdfast that had broken off a rock, never saw one before. There were remnants of clams mostly without shells, probably cockles. The storm tore stuff off the bottom well beyond the ice scour zone. They said the waves were up to 30 feet (9 m) high offshore; that would take everything out to a mile offshore. King crab are not freshwater tolerant. We have not got ice cover yet so I was surprised to find so many king crabs since I thought they were still out in salt water several miles out."

It is probable that the effects of this storm disturbed the bottom much deeper and further offshore than Mr. Lean suspected. During ice-free months, red king crabs are known to occur in deeper (> 20 m) waters where salinities are higher than shallow, nearshore waters (Jewett, 1999).

Storms are responsible for altering substrates in the shallow depths (< 20 m) of Norton Sound. Nelson (1982) determined major storms increase the average 10-m water depth in southern Norton Sound by as much as 5 m. At one station (D8) at 18.6 m of water depth divers found only cobble in 1988, only sand in 1991 and only cobble in 2012 (unpublished). So clearly, fine surficial sediments are periodically mobilized by the dynamic conditions such as storms. Another possible line of evidence of destruction from the 2011 storm was observed in a core taken during geotechnical sampling in 2012 (pers. comm., G. van Eck, AuruMar (Pty) Ltd, March 2013). A decomposed Arctic armored cucumber, *Psolus fabricii,* was found 0.5 m deep in a core taken in 16-m water depth and 2.5 km offshore of Nome. This organism is a surface dweller and is not capable of burrowing. Thus, it is likely this sea cucumber was suddenly buried by the 2011 storm.

To look at changes through time, comparisons of selected benthic sites were made of a relatively non-disturbed community (1990) and a naturally disturbed community (2012). The 1990 WestGold dataset was selected for this temporal comparison for three reasons. First, this dataset was determined to be the least impacted of the 1986-1993 data collected during the WestGold environmental monitoring program because it existed without disturbance four years after being dredged in 1986. Second, these 1990 data were collected prior to a series of storm events that occurred at the end of the 1990 open-water season that greatly impacted the benthic communities in Norton Sound (i.e., the 1991 dataset showed decreases in abundance, biomass and number of taxa for all mined and unmined sites sampled; Jewett et al., 1999). Third, no known mining activity had occurred at these stations since 1990. The three stations sampled for both substrate types (i.e., sand and cobble) in both 2012 and in 1990 were selected for these analyses (i.e., BIMA-dredged Stations R6 and R7/R7A, and Station S3, a reference station established at comparable water depths that was not previously dredged). Station R6 was dredged only during one season in 1986. Station R7 was dredged in 1987 but also indirectly impacted by dredging in 1988, 1989, and 1990 because of mining activities occurring immediately adjacent to it.

Sediment grain size at the stations previously mined (R6 and R7) was highly variable between the two periods (Table 1). Stations R6 and R7 Cobble had significantly ($p < 0.05$) more sand in 2012 than in 1990. Likewise, Station R6 Sand had more ($p < 0.05$) mud in 2012. However, Station R7 Sand had less ($p < 0.05$) mud in 2012. By contrast, sediment composition at unmined Station S3 Cobble and Sand revealed little change between years ($p > 0.05$).

Table 1. Summary of surface sediment grain size at stations sampled offshore of Nome, AK in 1990 and 2012.

			Mean Percent Composition*		
Year	**Station**	**Substrate**	**Gravel**	**Sand**	**Mud**
1990	R6	Sand	0.0	98.4	1.6
2012	R6	Sand	0.0	76.2	16.7
1990	R6	Cobble	97.0	3.3	<0.1
2012	R6	Cobble	18.4	78.8	1.4
1990	R7	Sand	0.0	73.3	26.7
2012	R7A	Sand	0.0	93.7	2.4
1990	R7	Cobble	86.2	13.6	0.3
2012	R7A	Cobble	44.6	51.5	1.4
1990	S3	Sand	0.0	98.7	1.3
2012	S3	Sand	3.1	92.1	2.4
1990	S3	Cobble	61.2	37.6	1.1
2012	S3	Cobble	56.2	42.2	1.3

* = Gravel > 2.00 mm, Sand 2.00 – 0.0625 mm, Mud 0.0625 – 0.0039 mm

The benthic community observed at all of the shallow sites (11-13 m; R6, R7A and S3) in 2012 was indicative of a major disturbance by the storm of November 2011. The cluster analysis and MDS methods revealed clear separation of the benthic communities sampled in 1990 and 2012. Composition of the 2012 fauna is another indicator of the storm disturbance. The preponderance of opportunist or stress-tolerant taxa on sand and cobble coupled with the absence of slow-growing taxa like bivalves reflected a widespread disturbance, especially when compared to 1990 when the benthic community was less disturbed. Further evidence of substrate change, presumably from storms, was observed at an 18.6-m station (D8) that had cobble in 1988, sand in 1991, and only cobble in 2012 (unpublished). This most recent substrate change is indicative of storm-induced erosion, presumably from the storm of 2011.

Upon examination of total abundance at stations sampled in 2012 and 1990, there was no clear pattern, i.e., no increasing or decreasing trends (Table 2). For example, abundance was not higher or lower at all 2012 cobble sites than at 1990 cobble sites. The 2012 Sites R6 Sand, R7A Sand and Cobble had significantly greater abundance than the same 1990 sites (Fig. 2). However, for cobble Sites R6 and S3 there was no significant difference in abundance between years. Only one 2012 site, S3 Sand, had significantly lower abundance than in 1990. This interpretation of no clear pattern is presumably because substrate heterogeneity is too varied to yield consistent patterns among all stations, whether on cobble or sand, particularly when little information is known about forcing factors that may have occurred between the two periods, e.g., other storms, dredging, ice gouging, crab fishing activity.

Table 2. Summary of benthic abundance at stations sampled offshore of Nome (1990 & 2012).

Year	**Station**	**Substrate**	**Abundance (Individuals m^{-2})**
1990	R6	Sand	1,956
2012	R6	Sand	6,260
1990	R6	Cobble	741
2012	R6	Cobble	382
1990	R7	Sand	328
2012	R7A	Sand	1,980
1990	R7	Cobble	403
2012	R7A	Cobble	796
1990	S3	Sand	7,106
2012	S3	Sand	712
1990	S3	Cobble	1,496
2012	S3	Cobble	1,452

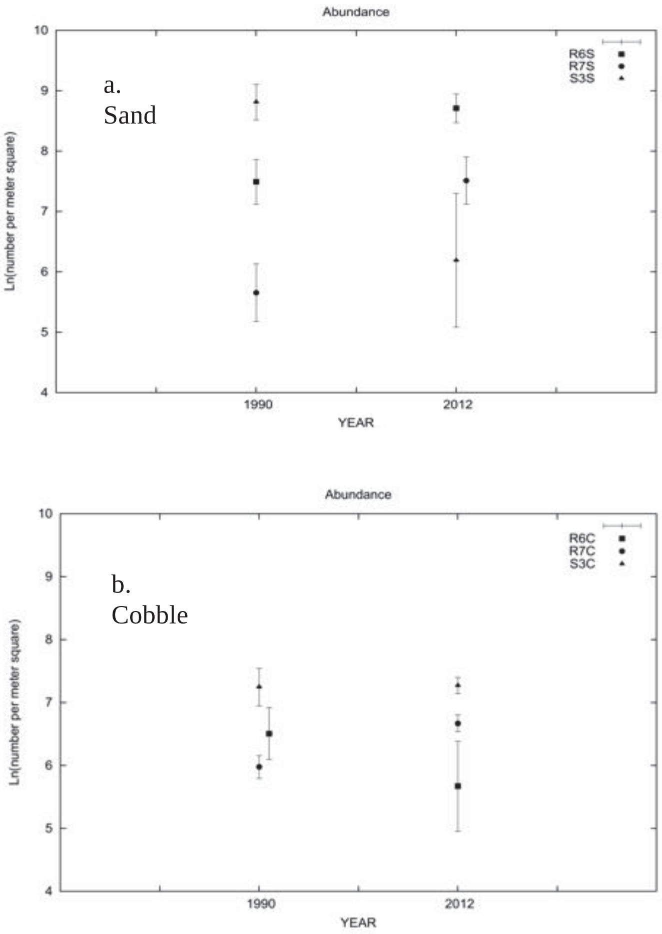

Figure 2. Plots of means and 95% confidence intervals of 1990 and 2012 benthic infauna/epifauna abundance in the sand (a; top) and cobble (b; bottom) substrates at mined Stations R6 and R7/R7A and unmined Station S3.

Comparisons between the dominant taxa in 1990 versus 2012 appear in Tables 3 and 4. These tables show the top-10 benthic organisms (by family or higher taxa) that dominated (individuals m^{-2}) Stations R6, R7/R7A, and S3 in 1990 and 2012, for both sand and cobble substrates. For the sand Stations R6 and R7/R7A, opportunistic or stress-tolerant polychaetes dominated more in 2012 than 1990. However, this was not the case with S3 Sand. For this site, not only was abundance substantially lower in 2012 than 1990 but there were low numbers of opportunistic polychaetes. Activities like ice gouging or subsistence fishing may have impacted this site prior to sampling. Predatory and scavenging nemertean worms (Nemertea) were the most abundant group at S3 Sand in 2012. For between-year comparisons on cobble substrates, there were fewer non-opportunistic species like brittle stars (Amphiuridae) and cockles (Cardiidae) in 2012 than in 1990.

Table 3. Dominant benthic higher taxa, in terms of abundance (individuals m^{-2}), at selected "Sand" substrate stations in 1990 and 2012. A = Amphipod, B = Bivalve, BS = Brittle star, C = Cumacean, G = Gastropod, N = Nemertean, P = Polychaete, SD = Sand dollar.

1990								
Sta	**Taxonomy**	**Ind/m^{-2}**	**Sta**	**Taxonomy**	**Ind/m^{-2}**	**Sta**	**Taxonomy**	**Ind/m^{-2}**
R6	Oweniidae P	1032	R7	Oedicerotidae A	110	S3	Oweniidae P	5378
	Oedicerotidae A	180		Amphiuridae BS	90		Spionidae P	225
	Magelonidae P	135		Goniadidae P	68		Oedicerotidae A	238
	Goniadidae P	128		Diastylidae C	43		Goniadidae P	143
	Amphiuridae BS	73		Magelonidae P	28		Tellinidae B	138
	Orbiniidae P	73		Oweniidae P	22		Amphiuridae BS	122
	Apistobranchidae P	53		Tellinidae B	10		Echinarachniidae SD	102
	Opheliidae P	50		Isaeidae A	10		Haustoriidae P	100
	Tellinidae B	47		Cardiidae B	8		Orbiniidae P	85
	Spionidae P	42		Capitellidae P	7		Magelonidae P	77
2012								
Sta	**Taxonomy**	**Ind/m^{-2}**	**Sta**	**Taxonomy**	**Ind/m^{-2}**	**Sta**	**Taxonomy**	**Ind/m^{-2}**
R6	Capitellidae P	2724	R7A	Oweniidae P	414	S3	Nemertea N	160
	Oweniidae P	908		Spionidae P	284		Cirratulidae P	110
	Cirratulidae P	590		Cirratulidae P	206		Oedicerotidae A	73
	Amphiuridae BS	456		Oedicerotidae A	134		Paraonidae P	70
	Nemertea N	410		Retusidae G	132		Capitellidae P	55
	Goniadidae P	236		Goniadidae P	120		Syllidae P	55
	Ampharetidae P	170		Echinarachniidae SD	112		Opheliidae P	28
	Sigalionidae P	142		Nemertea N	110		Spionidae P	28
	Oedicerotidae A	112		Orbiniidae P	106		Lampropidae C	23
	Orbiniidae P	66		Sigalionidae P	86		Sabellidae P	20

Table 4. Dominant benthic higher taxa, in terms of abundance (individuals m^{-2}), at selected "Cobble" substrate stations in 1990 and 2012. A = Amphipod, B = Bivalve, BS = Brittle star, C = Cumacean, G = Gastropod, N = Nemertean, P = Polychaete, SD = Sand dollar, SU = Sea urchin.

1990								
Sta	**Taxonomy**	**Ind/m^{-2}**	**Sta**	**Taxonomy**	**Ind/m^{-2}**	**Sta**	**Taxonomy**	**Ind/m^{-2}**
R6	Amphiuridae BS	272	R7	Cardiidae B	197	S3	Amphiuridae BS	353
	Oweniidae P	108		Amphiuridae BS	87		Oweniidae P	293
	Oedicerotidae A	72		Oedicerotidae A	32		Oedicerotidae A	267
	Cirratulidae P	38		Atylidae A	18		Goniadidae P	147
	Nemertea N	30		Ischyroceridae A	10		Nemertea N	80
	Cardiidae B	25		Oweniidae P	10		Cirratulidae P	63
	Strongylocentrotidae SU	22		Mytilidae B	8		Syllidae P	52
	Serpulidae P	22		Nemertea N	8		Cardiidae B	48
	Goniadidae P	20		Goniadidae P	7		Sigalionidae P	38
	Capitellidae P	17		Leuconidae C	5		Tellinidae B	38
2012								
Sta	**Taxonomy**	**Ind/m^{-2}**	**Sta**	**Taxonomy**	**Ind/m^{-2}**	**Sta**	**Taxonomy**	**Ind/m^{-2}**
R6	Cirratulidae P	66	R7A	Nemertea N	202	S3	Nemertea N	380
	Nemertea N	66		Cirratulidae P	144		Syllidae P	234
	Sigalionidae P	42		Syllidae P	142		Cirratulidae P	232
	Oweniidae P	40		Sabellidae P	54		Paraonidae P	132
	Capitellidae P	40		Sigalionidae P	42		Amphiuridae BS	68
	Syllidae P	22		Hiatellidae B	22		Spionidae P	44
	Bryozoa	16		Trochidae G	20		Capitellidae P	34
	Goniadidae P	12		Ampharetidae P	20		Cardiidae B	32
	Cardiidae B	8		Oweniidae P	20		Oligochaete	30
	Hydrozoa	8		Spionidae P	20		Sigalionidae P	28

To look at changes through time, the three stations sampled in both 2012 and 1990 during the WestGold environmental monitoring were selected for multivariate analyses (i.e., Stations R6, R7/R7A, and S3). Both cluster analysis and MDS multivariate methods were completed to look at groupings/aggregations of sites across sand and cobble substrates. Many analytical runs were done to investigate changes through time for the various multi-year datasets, as well as analyzing them with different levels of taxonomic differentiation (i.e., at the species level as well as at a higher taxonomic level such as family). These analyses were an iterative process and ultimately resulted in the following findings regarding temporal trends.

Ultimately, the 1990 WestGold data (“0” sites in Fig. 3) were used to compare to the 2012 data to look for temporal changes within mined and unmined stations. Figure 3 shows the results of cluster analyses for abundance at the species taxonomic level. This analysis suggested separation of sampling sites into two groups. These site groupings were:

- Group I: 2012 sites (R6 Cobble/Sand, R7A Cobble/Sand, S3 Cobble/Sand)
- Group II: 1990 sites (R6 Cobble/Sand, R7 Cobble/Sand, S3 Cobble/Sand)

The MDS method also showed the same groupings with 1990 data (‘‘0” sites in Fig. 4) grouping together in the upper half of the three-dimensional space, while 2012 sites occupied the lower half of that space (Fig. 4).

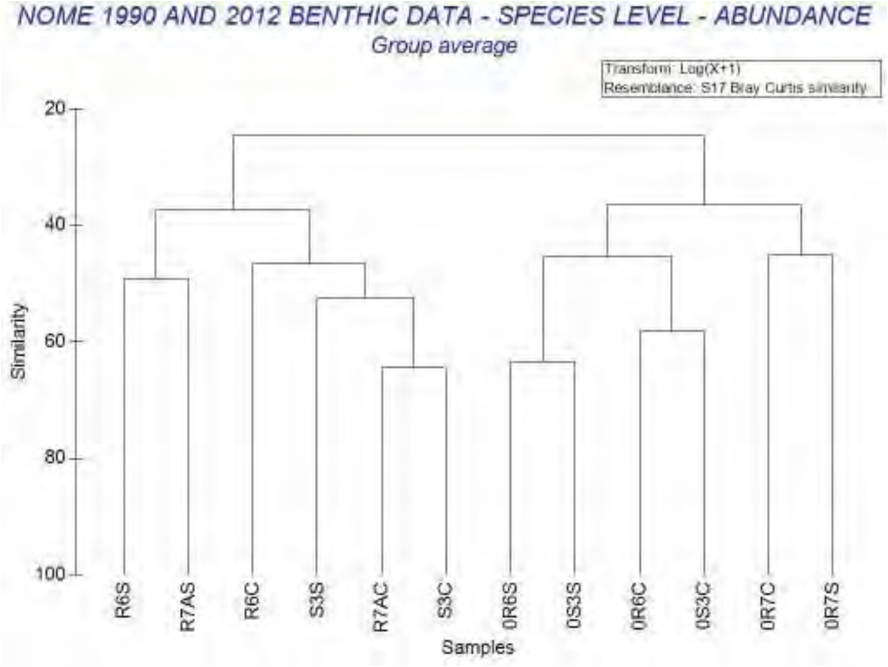

Figure 3. Cluster analysis of standardized environmental data from sites on sand and cobble substrates offshore of Nome, AK in 1990 and 2012; infauna/epifauna abundance data are at the species taxonomic level. “0” station samples were collected in 1990.

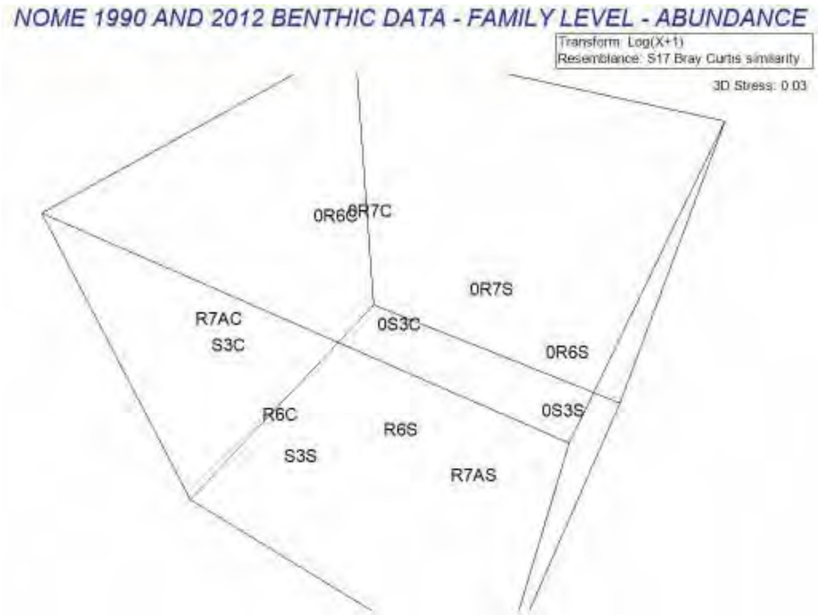

Figure 4. Non-metric multidimensional scaling (3-dimensional) of *ln*-transformed abundance from sites on sand and cobble substrates offshore of Nome, AK in 1990 and 2012; infauna/epifauna data are at the family or higher taxonomic level. “0” station samples were collected in 1990.

Because both multivariate methods yielded the same groupings this indicates there were area-wide temporal differences between these two datasets. A severe storm occurred in November 2011 just prior to freeze-up. That storm event had profound impact on the shoreline throughout Norton Sound. Because the 2012 samples were collected approximately seven months after this major storm event, the benthic environment had the time to recolonize with young capitellid and oweniid polychaetes. This is demonstrated by the higher overall abundance of animals in sand substrates at mined sites but decreased abundance at the control/reference site (Fig. 2a). This pattern was less evident in cobble substrates i.e., of the three stations, only Site R7 Cobble in 2012 had significant increases in abundance (Fig. 2b) relative to 1990 where no such major storm event had preceded the 1990 sampling.

Conclusions

Mining of 1.5 km^2 of seabed area with a bucket-line dredge occurred offshore in northern Norton Sound in 9-20 m water depths during June to November 1986-90. An associated monitoring program assessed the influence of mining on the benthic soft and hard substrates from 1986 through 1993. The benthos nearly a year subsequent to mining demonstrated benthic macrofaunal community parameters (total abundance, biomass, diversity) and abundance of dominant families were significantly reduced at mined stations in comparison to unmined stations. The recovery of the area mined once at Station R6 in 1986 and subsequently monitored for seven years by side-scan sonar imagery revealed a progressive smoothing of ocean bottom relief, decreasing size of tailing footprint and shoaling of depressions left by mining. The disturbance was best illustrated by the continued separation and slow convergence of mined with unmined stations in MDS ordinations. Recovery of the biota was apparent after four years but this process was interrupted in the fall of the fourth year (1990) by severe storms thereby restarting the recovery process. Opportunistic and/or early colonizing species dominated the nearshore (< 20 m) zone but were more prominent in mined areas.

Storms, particularly during ice-free months of late fall, are destructive to the shallow-water marine communities in northwest Alaska. This was emphasized by the November 2011 “superstorm” that battered the Norton Sound coast and left windrows of marine organisms piled on the shore. The benthic community observed at three 11-13-m stations (R6, R7A and S3) in 2012 was indicative of area-wide disturbance by this storm. No known mining activity had occurred at these stations since 1990. The cluster analysis and MDS methods revealed clear separation of the benthic communities sampled in 1990 and 2012. The preponderance of opportunist or stress-tolerant taxa on sand and cobble coupled with the absence of slow-growing taxa like bivalves reflected a widespread disturbance, especially when compared to 1990 when the benthic community was less disturbed. Further evidence of substrate change, presumably from storms, was observed at an 18.6-m station (D8) that had cobble in 1988, sand in 1991 but in 2012 coarse sediments again dominated there.

Another likely evidence of destruction from the 2011 storm was observed in a core taken during geotechnical sampling in 2012. A decomposed Arctic armored cucumber, *Psolus fabricii*, was found 0.5 m deep in a core taken in 16-m water depth and 2.5 km offshore of Nome. This organism is a surface dweller and is not capable of burrowing. Thus, it is likely this sea cucumber was suddenly buried by the 2011 storm.

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SCIENTIFIC DIVER REBREATHER FATALITY: AN INCIDENT REVIEW

Kenneth D. Johns
University of North Carolina Wilmington
5600 Marvin Moss Lane
Wilmington, NORTH CAROLINA 28409 U.S.A.
johnsk@uncw.edu

On May 5 2009 a staff diver at the University of North Carolina Wilmington's (UNCW) Aquarius Reef Base died while conducting a saturation excursion dive on a modified Inspiration rebreather. The diver was one of two staff habitat technicians assigned to a five-day saturation mission in the Aquarius underwater habitat. The four other saturation team members were part of the U.S. Navy's Specialized Research Diving Detachment. On the fatal dive, the staff diver was tasked with observing two U.S. Navy divers installing Manta Ray sand anchors using a hydraulic hammer. Approximately 34 minutes into his second dive the staff diver signaled the other divers that he was returning to the habitat. He was found seven minutes later on the bottom, unconscious and not breathing. Resuscitation efforts were unsuccessful. This paper discusses the results and recommendations of the subsequent investigations carried out by the U.S. Navy's Experimental Diving Unit, Ambient Pressure Diving and an external review board assembled by UNCW.

Introduction

The Aquarius undersea research laboratory is located 3.5 nm off Tavernier Key at Conch Reef in the Florida Keys National Marine Sanctuary. At this location since 1993 Aquarius has been utilized for scientific research, educational outreach, and as a training facility for U.S. Navy divers and NASA astronauts. The habitat is owned by the National Oceanic and Atmospheric Administration (NOAA) and is operated by the University of North Carolina Wilmington's Aquarius Reef Base (UNCW/ARB). Using saturation diving techniques, divers live and work under water for one to two-week missions, allowing for an extensive amount of underwater productivity within a short period of time. During missions a surface-based support crew monitors the aquanauts and habitat around the clock from a watch desk and command center at the shore base located 7 nm away on Key Largo. The habitat and aquanauts are closely monitored from inside Aquarius by two staff habitat technicians.

The Incident

May 5, 2009 was the second day of a planned five-day Aquarius Reef Base/U.S. Navy saturation mission onboard the Aquarius habitat. The aquanauts for this mission were from the U.S. Navy Specialized Research Diving Detachment (SRDD). The two staff habitat technicians from ARB/UNCW included the subject of this paper (the junior technician) and the lead technician who is also the Diving Safety Officer for ARB Florida Operations. The primary objective of the mission, for both the US Navy and the ARB staff, was the operational testing of rebreathers in a saturation environment. Contrary to a typical saturation mission all excursion dives out of the habitat were done utilizing a rebreather instead of open-circuit scuba.

The dive plan for that day was to set up a new aquanaut way station. Way stations allow divers to communicate back to the habitat and refill their scuba cylinders while working on nearby areas. The new way station, known as the SE way station, was located approximately 280 ft (85 m) southeast of Aquarius in 85 fsw (26 m) of water. The station site was made up of a large concrete block, an open bottom diving bell to be mounted on top of the block, and a low-pressure volume tank utilized to provide air to the bell. A 3/8 inch (1 cm) yellow polypropylene line connected the habitat to the new site, passing 10-12 ft (3-3.6 m) alongside the concrete block before terminating 40 ft (12 m) beyond it. This line was "clean" without any tie-downs or directional arrows attached to it that would interfere with a carabiner or shackle running its entire length. Surface divers would be utilized to re-position the block and the Navy dive teams from Aquarius would be tasked with removing the diving bell from the block, clearing the block of extra hardware, then installing the Manta Ray anchors and restraining straps required to secure the block to the seabed.

The morning dive team consisted of two Navy aquanauts and the lead tech. The two Navy divers were equipped with the USN Mark 16 rebreather with air diluent and AGA full-face masks. Separate 400 ft (122 m) umbilical hoses were attached to each diver providing them with air for their emergency gas supply and hardwire communication back to the habitat. The lead tech wore a modified Inspiration rebreather unit with air diluent. As with all other *Inspirations* purchased for use in the habitat, this unit had been modified by the manufacturer to enable it to be powered down while in the dry 45 fsw (14 msw) storage depth of the habitat. Standard Inspirations can only be powered down when the unit is on the surface. The lead tech was not equipped with communications. For his emergency gas supply he carried a 40 ft^3 air bailout cylinder with attached scuba regulator. He was also attached to a 30 ft (10 m) tether that was carabinered onto the 3/8 in (1 cm) polypropylene line leading to the work site. The purpose of this tether line was to ensure that the diver did not get outside the range of the two Navy divers attached to their 400 ft (122 m) umbilicals in the event the tethered diver needed assistance.

Timeline

09:08 - After completing the pre-dive checks on their rebreathers, the three divers proceeded to the SE worksite. Once out on site, the dive team removed the bell from the top of the concrete block, moved the air volume tank to a permanent location to the side of the new site, and made sure that everything was prepared and ready for the installation of the Manta Ray sand anchors by the afternoon team. After completing the remainder of the necessary preparation work the divers returned back to the habitat.

When the lead tech entered the trunk of the wet porch he was met by the junior tech who was preparing to go out on the afternoon excursion dive. The lead tech asked the junior tech if he had his rebreather scrubber canister packed and ready, and the reply was affirmative. At this time the lead tech noted his oxygen cylinder pressure (approx. 1800-1900 psi) and his diluent pressure (approx. 2000-2300 psi), completed his post-dive rebreather checks, and turned the unit over to the junior tech. The two divers had agreed prior to the mission that they would share a single rebreather in order to keep dive gear to a minimum in the confined space of the wet porch. The junior tech began the process of replacing the scrubber canister with his own, disinfecting the breathing loop, and completing the pre-dive checks. The pre-dive checklist for the Inspiration was noted as not being in the habitat on the previous evening and was unavailable at the time the junior tech was preparing for his dive.

12:16 - *R/V George Bond* and the *R/V Sabina* departed the dock at the ARB shore base in Key Largo and were en route to the Aquarius site. These vessels would be used to transport surface support divers, hydraulic tools, habitat supplies and site visitors.

12:39 - *R/V Bond* was on site

12:53 - *R/V Sabina* reported on site.

The dive plan for the afternoon excursion called for a second pair of US Navy divers and the junior tech to depart the habitat at 1300 hours in order to install the Manta Ray sand anchors and complete the set-up of the SE way station. The two Navy divers would be handling the hammer and anchors while the junior tech was to ensure their proper placement and alignment.

12:46 - Divers began to dress out in the wet porch.

12:55 - two US Navy Diving Medical Officers (DMOs) and a US Navy surface support diver, diving from the *R/V Bond*, entered the wet porch for a visit to the habitat. No medical exam was given to any of the aquanauts by the visiting DMOs, nor were any medical issues brought up by the aquanauts at that time.

13:13 - the first USN diver staged out of the wet porch and waited

13:22 - the second USN diver staged out. The equipment both divers wore was identical to that of the first pair of USN divers on the morning excursion. After checking each other's rebreather for leaks the divers followed the line out to the SE work site.

The original plan called for the junior tech to stage out immediately after the second USN diver but he was delayed because of the volume of personnel traffic in the wet porch. While preparing to stage out for his dive the junior tech was observed checking his oxygen and diluent cylinder on his rebreather as well as the 40 ft^3 bailout cylinder he would be carrying with him. The oxygen and diluent cylinders in his *Inspiration* had not been replaced or re-filled from the lead techs' morning dive with the same unit. When the junior tech had completed his pre-dive preparations, the lead tech came out to the wet porch to make sure he was ready to go and understood the dive plan. The junior tech indicated all of his surface checks were completed and he was set to dive.

13:48 – Junior tech left the wet porch, completed his in-water checks, and headed out to join the USN divers.

13:50 - The visiting US Navy DMOs and surface support diver left the habitat for the surface.

During the junior tech's descent out to the site the data recorder in his wrist-mounted display recorded decreasing PO_2 levels. Approximately two minutes after he arrived on site at a depth of 80 fsw (24 msw), a low (<0.40 ata) oxygen alarm went off on his wrist-mounted display. Within one minute of the alarm, the oxygen sensors recorded a rapid PO_2 increase and then appropriate control around the oxygen set point of 0.70 ata was established. (D. Cowgill, pers. comm.) (Note: It was determined later that the oxygen cylinder valve of the rebreather was probably closed at the start of the dive and opened after the tech was alerted to the situation by the low O_2 alarm.) The data recorded for the dive indicates that the oxygen set point remained at 0.70 ata for the duration of this dive.

At the time the junior tech arrived on site the two USN aquanauts were waiting for the Manta anchors and driving tool to be delivered from the *R/V Sabina*. The junior tech unclipped his bailout cylinder and placed it next to a large coral head. He and one of the USN aquanauts spent approximately 5-10 minutes cleaning growth from the volume tank. Approximately 10 minutes into the dive, a tender inside Aquarius monitoring the USN divers out on excursions, noticed an o-ring and black plastic spacer laying on the outside of the wet porch table. The tender knew it was not from a MK16 and immediately asked the lead tech if they belonged in the *Inspiration*. The lead tech was unsure of the origin of the parts and recalled the junior tech back to the habitat, which he did unaccompanied.

14:09 - the junior tech staged back into the habitat at the wet porch. He had a maximum depth of 83 fsw (25 msw) and a dive time of 21 minutes. Upon his arrival the lead tech asked him if he recalled installing the o-ring and spacer into his canister housing. Since the junior tech was unsure if he did, the lead tech asked if he wanted to check the unit himself or have the lead tech perform the check. The junior tech opted for the latter. At this point the junior tech closed the valves on both the oxygen and diluent cylinders to allow the hoses to be depressurized prior to opening the scrubber housing. Once the scrubber housing was opened up, the lead tech discovered that the o-ring and spacer were missing. The scrubber canister was removed by the lead tech and he noted a small amount of moisture in the scrubber canister but not any more than would be found during a typical dive. The junior tech informed the lead tech that he was comfortable diving with the existing scrubber material. The lead tech then began the process of re-installing the scrubber canister, o-ring and spacer. The canister housing was locked onto the head and the gas lines and breathing loop re-attached. Once the back cover was secured the junior tech began his pre-dive checks. The lead tech observed the junior tech reaching for his cylinders to begin the process of his pre-dive checks but no other portion of this process was witnessed by any of the personnel in the wet porch. The rebreather's data recorder indicates that power was shut off to the rebreather at 14:13 and then turned back on at 14:17. (D. Cowgill, pers. comm.) Shortly after the rebreather was powered on the junior tech informed the lead tech he was ready to resume the dive.

14:19 - The junior tech left the wet porch for the SE way station.

The Manta Ray anchors and hydraulic driving tool had arrived on the bottom shortly before the junior tech's return. The Navy aquanauts were then in the process of setting up the tool. The junior tech again unclipped his bailout cylinder and placed it against a coral head approximately 15 feet (5 m) away from the concrete block. Dive conditions at the site were ideal with 70 ft (21 m) of visibility and a slight inshore current. After about 5-10 more minutes of site and tool preparation the three divers began the process of installing one of the 8 ft (2.4 m) long Manta Ray anchors. One Navy diver operated the hydraulic hammer, the second held the anchor and driving rod in place, and the junior tech assisted in holding the anchor in position and verifying proper alignment.

14:43:28 - after approximately 10 minutes of assisting with the anchor driving operation the junior tech's data logger began recording rapid and erratic activations of the three control buttons on his wrist unit. These activations continued uninterrupted for 36 seconds at which time the rebreather completely powered down (M. Parker, pers. comm.) As was later learned, these rapid activations and power down were caused by a powerful water jet coming from the exhaust port of the hydraulic hammer in use by the divers. During the last second of operation the middle and right switches were held together and released three times - the sequence required to complete a shutdown.

There are no indications that any of the divers were aware of the shutdown at that time and the driving operation continued for another 8-9 minutes.

14:53 (approx.) - the junior tech tapped one Navy diver on the back of the shoulder, pointed to himself then pointed to the habitat, indicating he was returning to Aquarius. The Navy diver gave him an 'OK' signal that was returned. The Navy diver had a clear view of the junior tech's face at that time and did not observe any signs indicating he was under duress. The Navy diver later recalled that the junior tech's eyes and face "appeared normal."

From his vantage point higher in the water column, the second Navy diver observed the exchange of signals and saw the junior tech head in the direction of his bailout cylinder located 90° from the direction of the line leading back to the habitat. When the Navy diver returned his attention to the hammer the junior tech was outside his field of view. The junior tech never recovered his bailout as it was found the next day still tucked under the coral head where he had left it.

Using diver communications the first Navy diver notified the habitat that the junior tech was returning to Aquarius. Because of the noise of the driving tool it was very hard to hear if the habitat had understood and acknowledged their communication. The second Navy diver called for "tool cold" indicating that the hydraulic power to the tool was to be shut down, and repeated that the junior tech was heading back to the habitat. The dive tender inside Aquarius acknowledged the communication. After hearing the acknowledgement the second Navy diver called for "tool hot" re-activating the tool.

After approximately 6-7 minutes the second Navy diver repositioned himself and noticed the junior tech approximately 30 – 40 ft (10 -13 m) away lying on his back next to the line going back to the habitat.

15:00 - the second Navy diver immediately called "tool cold, tool cold" followed by "diver down." Both Navy divers moved quickly over to the junior tech who was found with his open mouthpiece floating above him and no vital signs. The divers notified the habitat that they were bringing back an unresponsive diver with a flooded rig and no pulse. Navy diver two began to carry the junior tech back to the habitat but almost immediately realized that his umbilical was fouled around the hydraulic tool. The second Navy diver handed the junior tech to the first Navy diver while he went back to clear his umbilical. The first Navy diver carried the junior tech approximately 280 ft (85 m) back to the habitat.

As soon as the personnel in the habitat heard the emergency declared they collected the oxygen administration and medic's kit and set it up in the wet porch. The *R/V Bond*, which had just entered the Key Largo canal was also contacted and told to make best possible speed back to the Aquarius site as the two Navy DMOs were needed for a serious diver emergency.

15:03 - the Navy diver appeared in the wet porch and the junior tech was lifted onto the wet porch deck. His dive gear was removed and CPR and oxygen administration were started.

15:24 - the injured diver was intubated with a Combitube airway. No vitals were present and CPR was continued.

15:27 - the Navy DMOs arrived in the wet porch. Upon assessing the injured diver they found no spontaneous respirations or pulses. An IV line was started in the right arm and an auto-injection (EpiPen) of .3 mg of Epinephrine was given.

15:44 - two more injections (EpiPen) of .3 mg each were given.

15:45 - CPR was stopped and the diver's condition assessed.

15:47 - after 42 minutes of continuous CPR, with no return of spontaneous pulses or respirations, the injured diver was pronounced dead.

Immediate Actions

The Monroe County Sheriff's Office was the first agency to be notified of the death. A Sheriff's officer and a detective were summarily dispatched to the ARB shore base facility to begin collecting preliminary information. The junior tech's family was contacted and informed immediately afterward. Key personnel at UNCW's Center for Marine Science had been notified earlier that CPR was being performed on one of the aquanauts. After the family had been contacted, those personnel were notified of the outcome. Additional University personnel that were notified immediately included the Director of Human Resources, the Director of the Environmental Health and Safety Office, and the Office of the Chancellor.

The *Inspiration* rebreather unit that the junior tech had been wearing was collected from the habitat by two US Navy divers. Before packing it was noted by the lead tech that the pressure gauges indicated 1200 psi for the unit's oxygen cylinder and 0 psi for the diluent cylinder. None of the components were altered in any way. The unit was packed into a rebreather dry bag used by the Navy for transporting their MK16 rebreathers through the water. The rebreather reached the surface and was brought onboard the *R/V Manta* for transport to the ARB shore base. The unit was taken off the boat and placed in a plastic shipping container utilized by the Navy for shipping their Mk16s. Tie wraps were used to seal the lid latches once they were secured. The sealed container was turned over to a Monroe County Sheriff's Detective for impoundment.

The decompression obligation for a saturation mission in Aquarius is just under 17 hours. The focus in the habitat and on the support crew turned toward lining up the systems and personnel required to begin this lengthy process. Once the decompression was progressing normally the remaining aquanauts were asked to provide a written statement of the events that occurred that afternoon as they witnessed them. Though a difficult task it was imperative to get these statements while still fresh in their minds. These statements would prove to be critical in piecing together the key events that occurred and the actual cause of death.

Two days after the accident the UNCW Diving Safety Officer and the UNCW Director of Environmental Health & Safety were dispatched to Key Largo for the purpose of gathering as much information as possible. Witness statements were first read then interviews were conducted with the mission aquanauts and ARB staff in order to answer any questions and obtain a clear understanding of the events that transpired. Key documents were collected such as the dive logs, diver training records, rebreather maintenance logs, watch desk logs, etc. Once collected, all of this information was disseminated and used to put together an incident narrative and timeline of the significant events that took place on that day. This remained a working document throughout the course of the investigation and was continuously updated as more information became available.

At this stage of the investigation there were many unanswered questions related to the fatality. It was anticipated that many of these questions could be answered through the forensic analysis of the rebreather and its data logger to be conducted by the US Navy Experimental Diving Unit and the results of the autopsy performed by the Monroe County Medical Examiner.

External Review Board

Shortly after the accident the decision was made by UNCW's administration to convene an external panel of experts to review all the material related to the accident. The panel was tasked with the following (J. Bozanic, pers. comm.):

1. Review and analyze the data, interviews, diving protocols, reports and other documentation related to the accident;
2. Make recommendations to UNCW, to the extent applicable, to prevent a recurrence of death or serious injury attendant to scientific saturation rebreather diving missions; and,
3. Convey recommendations on the resumption of saturation rebreather diving missions at the ARB.

This panel of outside experts was identified with particular attention made to their knowledge of rebreathers and/or saturation diving. Participants included:

• Jeffery Bozanic	Scientific diver and author of *Mastering Rebreathers*
• Paul McMurtrie	US Navy Saturation Diving System Deputy Program Manager
• Tom Mount	Rebreather and dive training expert
• Ken Johns	UNCW Diving Safety Officer, ex-officio
• Stan Harts	Director UNCW Environmental Health and Safety, ex-officio

The panel convened on August 18, 2009 in Wilmington, NC, and met for three consecutive days. Materials that were available were sent to panel members for review in advance of the meeting. Information that was not available until August 18 or information that was requested by the panel was assembled on site. The most critical piece of information that became available during this period was the button sequencing information from the diver's rebreather that was retrieved by the manufacturer of the *Inspiration*, Ambient Pressure Diving. Though it showed that the rebreather was powered down through a request from the wrist unit, the extremely rapid and random nature of the sequencing caused the panel to consider other possibilities of activation. It was through this thought process that a test with the same hammer and a rebreather wrist unit was initiated. The results of this in-water test clearly demonstrated that the exhaust ports on the hammer extruded water at a high enough concentration and intensity to activate the control buttons from a maximum distance of two feet (R. Garcia, pers. comm.) With that information in hand the panel proceeded with the tasks of completing the final summary of events and making its recommendations. The exemplary work of this panel resulted in a comprehensive 22-page report, completed entirely on their own time, which has served to improve diving safety.

Recommendations

In its final report the External Advisory Board made 15 recommendations to improve the safety of saturation and rebreather diving operations at UNCW. Though written for UNCW these recommendations also have applications for divers everywhere. Some of these recommendations, such as the synchronizing of all mission time devices to a master clock and more diligent recording of significant events observed by the watch desk, were minor. The others were more significant and brought back into focus the importance of continuously adhering to basic diving protocols. The strict adherence to the buddy system and the need for everyone in the buddy team to be familiar with each other's dive equipment is essential, even on high-tech dives with very experienced divers. When divers in a buddy team are from more than one agency or affiliation a set of uniform dive procedure should be utilized and everyone thoroughly briefed on the agreed upon signals, buddy protocols and emergency procedures to be used.

The remainder of the recommendations focused on the rebreather operations, and dealt primarily with diligently abiding by the fundamentals of rebreather operation and training. Pre-dive checklists for the rebreather must be available at the dive site and followed after the unit has been assembled and after it has been donned by the diver. Upon completion this pre-dive check should be verified by a buddy or dive supervisor. (The UNCW diving control board chose to have this verified in writing by an accompanying signature on the checklist form.) As the cardinal rule of scuba diving is "don't hold your breath", the most important rule of rebreather diving is to "know your O_2", and the only way a rebreather diver has to measure the oxygen level in the breathing gas is through instrumentation. Vigilant monitoring of the PO_2 levels at least every three minutes is critical and should be followed in spite of any distractions or increased stress levels encountered during the dive.

Although the junior tech was probably incapacitated by hypoxia when he signaled his return to the habitat the panel made the recommendation that in the event of any significant rebreather malfunction the diver should immediately switch to open circuit bailout. In order to ensure that this can be initiated quickly the carried bailout cylinder should be within arms-length of the diver. The UNCW diving control board took this one step further by mandating that the bailout cylinder must remain attached to the rebreather diver at all times. For future operations involving rebreathers and underwater tools the panel recommended that the tools be evaluated for compatibility with rebreather operation. Tools that produce significant vibrations, strong water jet output or other hydrodynamic forces that might impact the operational controls of the rebreather should not be used. And finally, the recommendation was made to find a safer alternative method of powering down the rebreathers when used out of the habitat. An electronic key or similar safeguard that could only be accessed when in a dry environment was suggested. Until such a system can be worked out with the manufacturer the use of rebreathers out of Aquarius has been put on hold.

Conclusion

The Aquarius saturation diving program at UNCW's Aquarius Reef Base has maintained a presence on Conch Reef since beginning operations in 1993. Throughout the years the program and its staff have earned a reputation for safety, professionalism and productivity. They developed long-standing relationships with leaders in the scientific diving community and played host to multiple missions with NASA astronauts and elite dive teams from the US Navy. In addition the junior tech was a highly experienced, well-respected and physically fit diver. In spite of all this an unthinkable tragedy occurred. An accident that deeply affected the close-knit staff of ARB and, needless to say, the entire UNCW and diving community. It made everyone acutely aware of the effects that even small incidents can have on an operation.

Throughout the aftermath the various entities within UNCW, including Aquarius Reef Base, the UNCW diving program, UNCW Environmental Health and Safety Office, and UNCW administrators, have all worked together to ensure that the investigative process was thorough and accurate. The recommendations provided by the External Review Board and acted upon by the ARB staff and UNCW diving control board has significantly strengthened operational safety for its diving programs. May the lessons learned from the material presented here be utilized for the benefit of others in an effort to prevent another tragic event from occurring.

THE EFFICACY OF USING A *HYPERLITE™*, HYPERBARIC STRETCHER FOR THE TREATMENT OF SERIOUS DECOMPRESSION ILLNESS: A CASE REPORT

Douglas E. Kesling[1]
John Selby[2]

[1]J.F. White Contracting Company
10 Burr Street
Framingham, MASSACHUSETTS 01701 U.S.A.

[2]SOS Hyperlite, Ltd.
1 Kelly Industrial Estate
Kirk Michael, Isle of Man, IM6 1SS, BRITISH ISLES

The risk of a scuba diver developing decompression illness (DCI) is viewed as an associated occupational hazard of compressed gas diving for the recreational, scientific, public safety and commercial diving industries. Each diving community has various techniques, procedures and approaches to help mitigate these risks in the diving populations they serve. If a diver is stricken by DCI or "the bends" and is not promptly and adequately treated, then that diver runs the risk of permanent medical or neurological deficit which could affect not only his/her future livelihood but also their quality of life. This paper explores the efficacy of the Hyperlite™, a portable "folding" hyperbaric stretcher, for field treatment of decompression illness. The deployment and use of this pressurization system is discussed in relation to an actual diving accident case where availability of a Hyperlite helped to mitigate and provide prompt therapeutic hyperbaric oxygen treatment (HBOT) to a diver suffering vestibular (inner ear) decompression sickness.

Introduction

In August of 2005, under the direction of the National Undersea Research Center at the University of North Carolina Wilmington (NURC/UNCW), a diving research expedition, supported with funding from NOAA's Office of Ocean Exploration (O.E.), was undertaken in the Thunder Bay National Marine Sanctuary (TBNMS; Figs. 1-3) as part of East Carolina University's (ECU) Diving and Water Safety Program. The expedition was aimed at documenting deep-water shipwrecks within the Sanctuary for NOAA.

TBNMS is located in the Northeastern corner of Michigan on Lake Huron and is home to more than 200 shipwrecks of which 50 have precise locations recorded. Thunder Bay became a national marine sanctuary in 2000, encompassing a 448-square-mile area of Lake Huron, and is now designated both as a national marine sanctuary and state underwater preserve.

The two week diving project focused primarily on two sites: an unidentified two-masted schooner located by Dr. Robert Ballard's Institute for Exploration in 2001 (tentatively identified as the *Corsican*) and the wooden passenger steamer *Pewabic*, which sank in 1865. Both wrecks rest in about 165 feet of water. Using mixed gas self-contained open circuit scuba diving techniques, the research diving team created high resolution photomosaics of both sites, which served as baseline data for future documentation and monitoring.

NOAA
THUNDER BAY

Figure 1. Great Lakes Maritime Heritage Center - NOAA (Photo credit: D. Kesling)

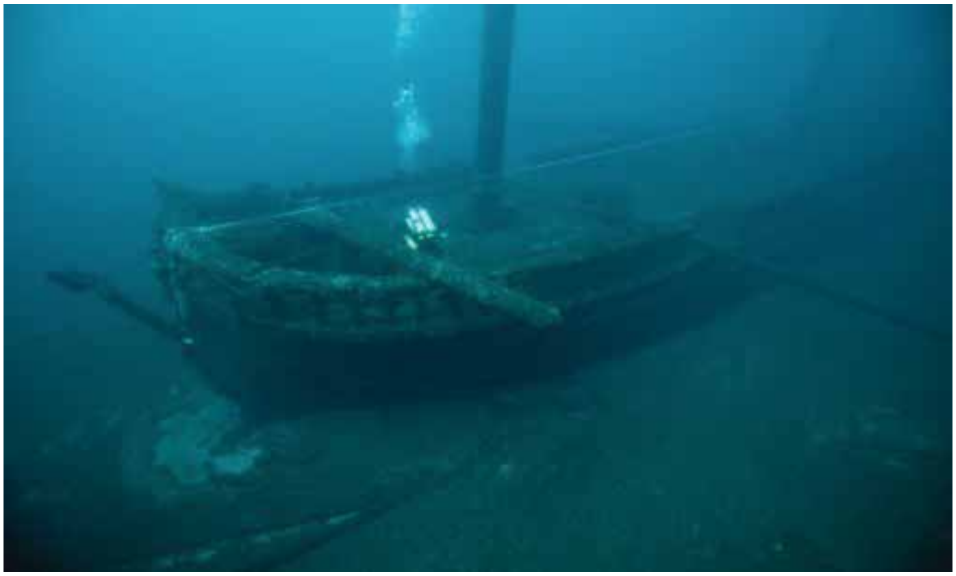

Figure 2. *Defiance* Shipwreck - TBNMS (Photo credit: D. Kesling)

During this field expedition, a diving incident occurred with one of the technical deep divers that required emergency, on-site hyperbaric oxygen therapy. Following diving protocols established by the NOAA Diving Program as outlined in the Report on NOAA's (2004) Diving Accident Management Program and Recommendations for Improvement, a recompression chamber was required within 30 minutes of the dive site for dives requiring decompression. To meet this mandate a *Hyperlite*™ Hyperbaric Stretcher (Fig. 4) was acquired by NURC/UNCW and allocated to the dive project. It had to be carried aboard the NOAA *R/V Huron Explorer*, a 12 m, ex-U.S. Coast Guard patrol vessel, in order to meet this 30-minute rule.

Figure 3. Diver 1 and unidentified shipwreck (Target 7) TBNMS (Photo credit: Joseph Hoyt /NOAA)

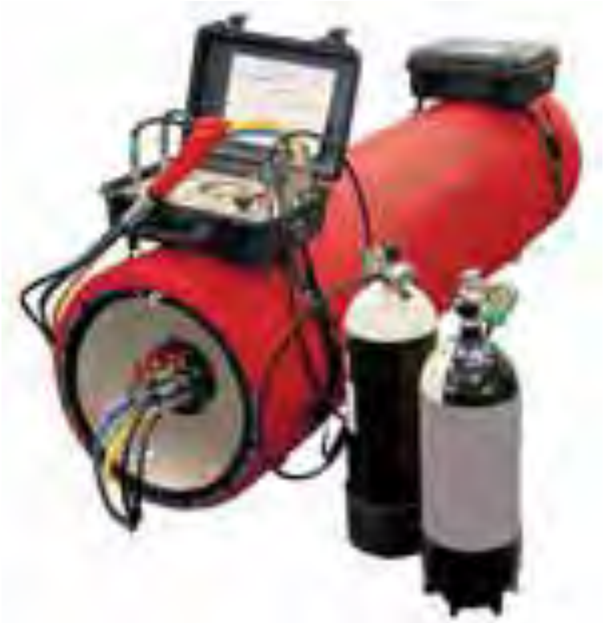

Figure 4. *Hyperlite*™ Hyperbaric Stretcher (Photo courtesy of SOS *Hyperlite*™; http://www.hyperlite.co.uk)

The SOS *Hyperlite*™ is a portable pressure vessel (or hyperbaric chamber) that provides immediate treatment for different medical conditions by supplying 100% oxygen to the patient above atmospheric pressures during transport to a medical facility. The SOS *Hyperlite*™ Hyperbaric Stretcher and Treatment System (Fig. 5) significantly improves the chances of survival and full recovery, enhancing treatment and saving lives, even in the most extreme circumstances. Hyperbaric oxygenation is the treatment of choice for individuals suffering from decompression illness related maladies.

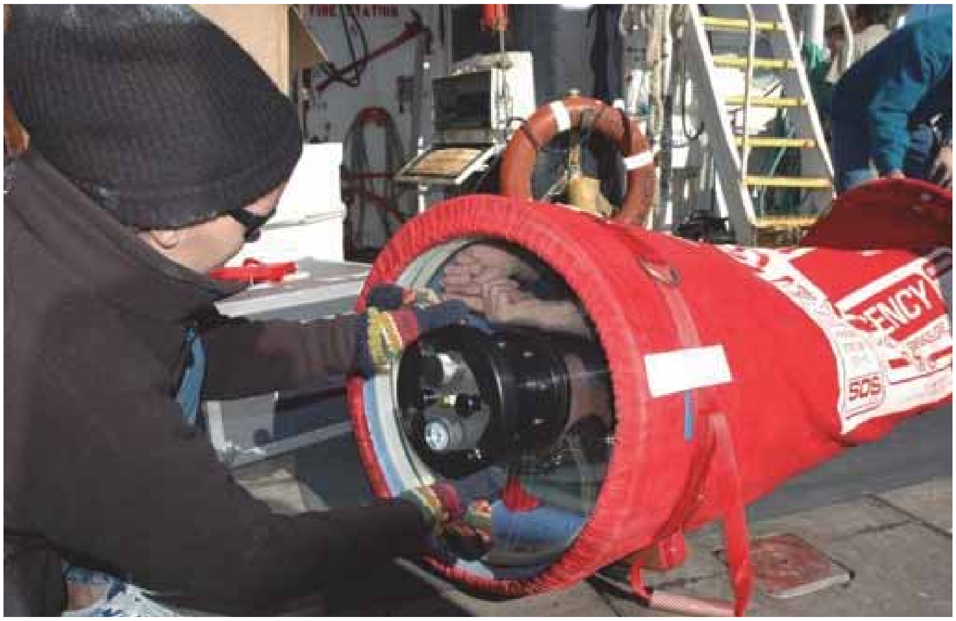

Figure 5. SOS *Hyperlite*™ Treatment System (Photo credit: Jitka Hyniova - NOAA Collection)

The SOS *Hyperlite*™ system can be assembled and operational within a matter of minutes to transport a patient to the nearest medical facility while breathing 100% oxygen under pressure. Alternatively, complete hyperbaric oxygen treatment (HBOT) can be performed at the accident site if necessary.

Background

The affected diver was a 46-year old male professional certified technical diving instructor. He had 10+ years of experience using mixed gas, open circuit, self-contained breathing apparatus at the time of the diving incident. With water temperatures averaging 42°F (5.5°C), full drysuits were utilized by all dive team members. Previous dives were conducted on 08/08, 08/10, 08/11 by Diver 1 (Table 1). All listed dives were incident-free without complications being reported post-dive.

Table 1. Diver 1 dive pre-incident profiles.

08/08	EANx 34	97 ffw (29.6 m)	24 BT	31 TDT	100% 0_2 at 20 ffw (6 m)
08/10	Trimix 22/36	161 ffw (49 m)	24 BT	54 TDT	100% 0_2 at 20 ffw (6 m)
08/11	Trimix 23/36	148 ffw (45.2 m)	25 BT	52 TDT	100% 0_2 at 20 ffw (6 m)

Emergency Assistance Plan

An emergency assistance plan (EAP) was developed for the TBNMS and approved by the NOAA Diving Center (NDC) for the regional dive location (NOAA, 2005). If an emergency situation were to arise offshore during the expedition, the Diving Supervisor would notify the Diver Medic (DMT) of the situation and take appropriate action to safely return the diver(s) to the R/V *Huron Explorer*. Once the patient was stabilized on deck, the DMT would evaluate the patient and recommend an appropriate treatment regimen. With a medical standing order in place, the DMT would be allowed to act on certain treatment protocols without direct contact with medical control. The DMT would then contact the NURC/UNCW Diving Medical Advisor (DMA) for additional treatment recommendations and evacuation protocols when possible.

The initial treatment protocol steps (Fig. 6)

1. Perform a patient assessment and begin basic life support (BLS) if necessary;
2. Administer 100% oxygen via a positive pressure device or demand valve and check vital signs;
3. Conduct a five-minute neurological examination and/or a secondary assessment of injuries;
4. Apply direct pressure to bleeding wounds;
5. Stabilize the patient, monitor vital signs, conduct a more detailed neurological examination and prepare the patient for transfer to the onboard *Hyperlite*™ recompression chamber;
6. If the patient is weak or displays altered consciousness, try to start an IV with Normal Saline or Ringer's Lactate at a wide-open rate (500-1000 ml/hour); and,
7. Place patient in the *Hyperlite*™ hyperbaric stretcher or continue evacuation to the appropriate medical facility depending on nature of the illness, severity and established standing orders as outlined by the NURC/UNCW Diving Medical Advisor (DMA).

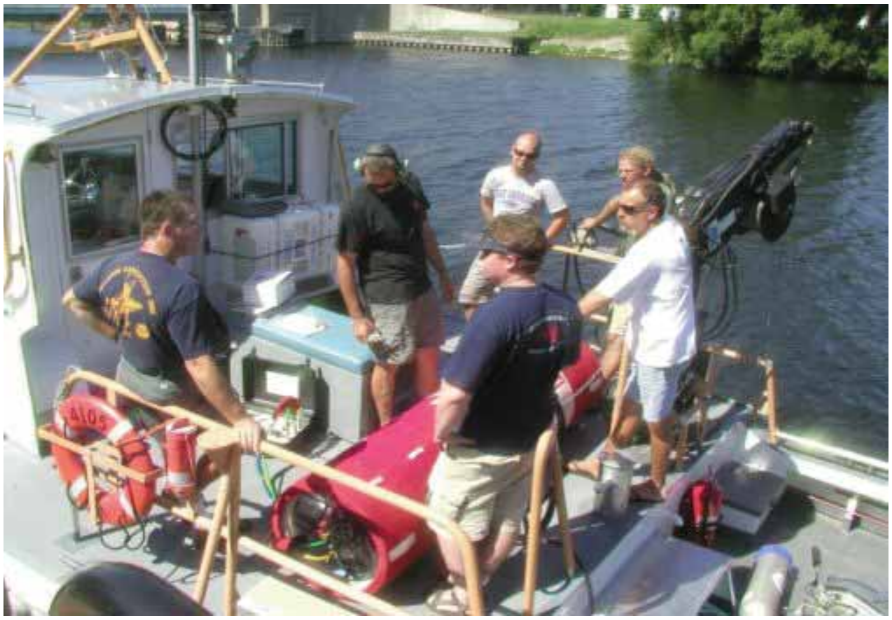

Figure 6. Mock-treatment with SOS *Hyperlite*™ (Photo credit: D. Kesling)

The Dive

Diver 1 made a 151 ffw (46 m) dive for 25 minutes with a 22/36/42 (O_2/He/N_2) mix (Fig. 7). He completed 31 minutes of deco with stops (Fig. 8) at: - Deep Stops 2 min - 104 ffw (31.7 m) & 77 ffw (23.5 m) (50 ffw/15.2 m - 1 min.); (40 ffw/12 m - 1 min); (30 ffw/9.1 m - 6 min); (20 ffw/6 m - 3 min); 100% O_2 (15 ffw/4.6 m - 16 min) surfacing on 100% O_2 on time according to plan. He entered the water at 10:52 and exited at 11:50. Following the dive, the diver returned to normal deck operations and served as dive supervisor for the next two dive rotations.

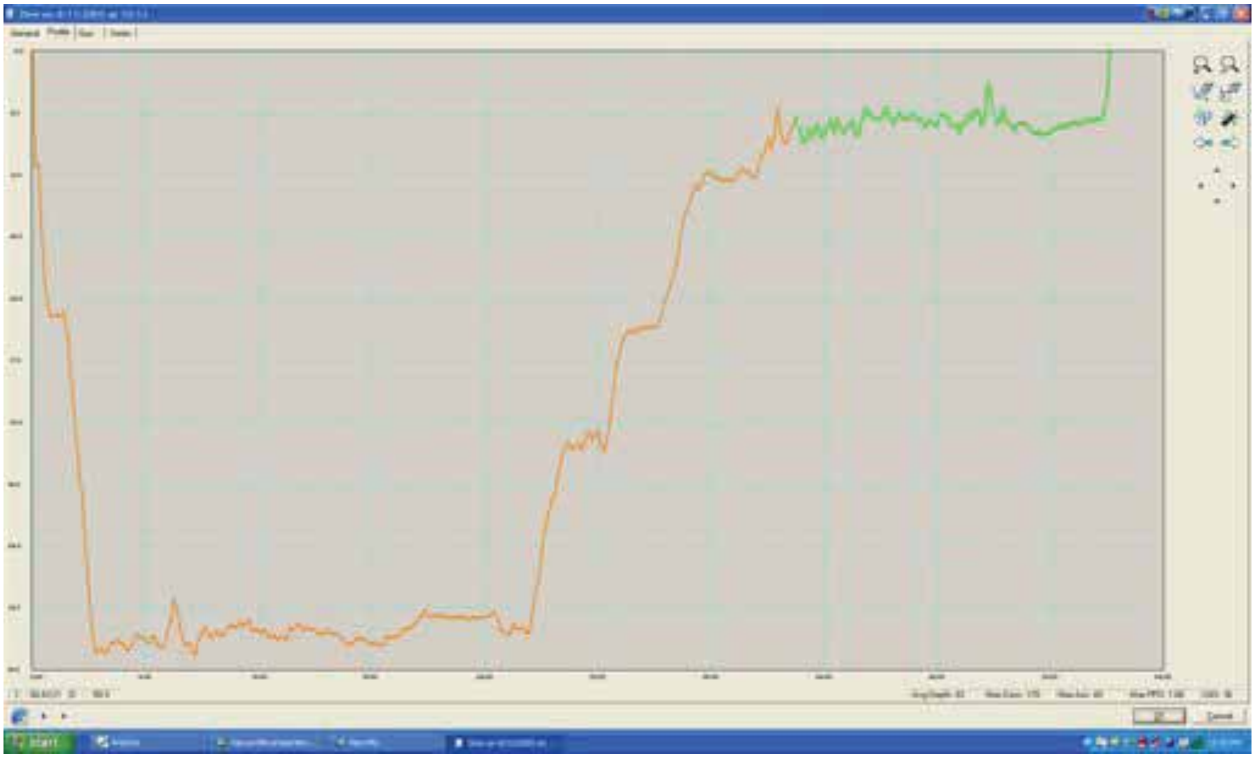

Figure 7. Diver 1 – Profile: VR3 Multiple mode/Multi-gas Dive Computer downloaded log (08/13/05)

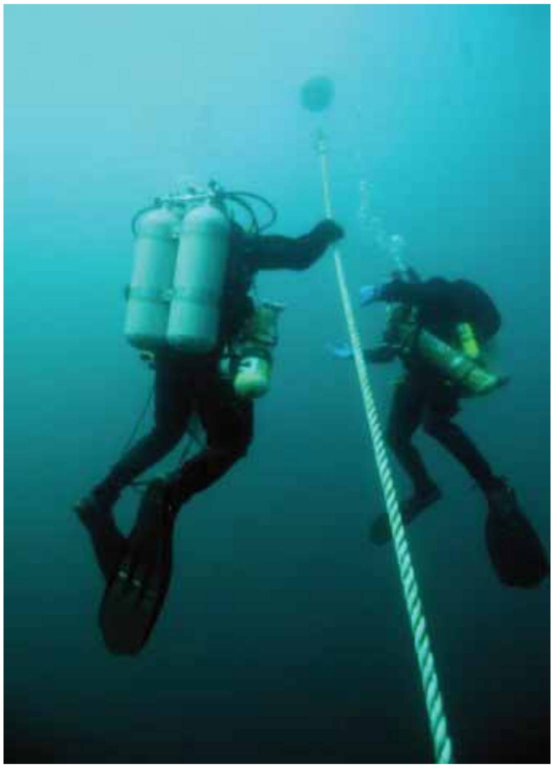

Figure 8. Staged decompression stop (Photo Credit: D. Kesling)

The Incident

At approximately 13:45, Diver 1 walked to the bow to check on the in-water ascending dive team but when he arrived was promptly struck by severe vertigo and nausea (Timeline of Events is presented in Table 2). He asked a fellow diver walking on deck to retrieve the oxygen kit and deliver it to him there on the bow. When it arrived, oxygen by demand valve was deployed and the stricken diver laid down while breathing 100% oxygen by double seal mask. Shortly after initial treatment began he became increasingly nauseous and commenced vomiting. In between bouts he continued to breathe 100% oxygen. A diver medical technician (DMT) arrived from the dive team at 13:50 and questioned the stricken diver to determine if there were other symptoms; Diver 1 reported there were none. He did report slight relief with 100% oxygen and the DMT decided that he should be placed in the *Hyperlite*™ Hyperbaric Stretcher. Given that a team of divers was still on the downline completing decompression, limited space on the vessel and the injured divers' progressing decompression illness, it was decided that treatment should start immediately after the divers were back onboard. While waiting for the rest of the dive team to surface the DMT instructed those remaining onboard to begin preparing the *Hyperlite*™ for treatment service. A brief neurological exam was conducted on the patient and no additional deficits found while vital signs were recorded by the attending DMT.

Inner Ear Decompression Sickness

Inner ear decompression sickness (IEDCS) is caused by the formation and growth of inert gas bubbles within micro vessels and otic fluids during ascent from a dive. This takes place when there is too rapid a drop in ambient pressure to a level lower than required to keep the gas in solution (Shupak et al., 2003). Signs and symptoms for IEDCS, or Vestibular DCS, include tinnitus, vertigo, nystagmus (rapid involuntary eye movements), hearing loss and associated nausea and vomiting. This is commonly called the "Staggers" because of the patient's unsteady, almost drunken gait (Ellerman, 2012). Staggers occur more often with heliox diving than air. The stricken divers' chief complaint was extreme dizziness and associated nausea and vomiting without any hearing loss complications.

Accident Management

Once all divers were back aboard the injured diver was placed in the *Hyperlite*™ at 14:35 and a U.S. Navy Treatment Table 6 (USN TT6) begun. The afflicted diver was returned to pressure at 2.8 ATA within approximately 45 minutes of initial presentation of the injury on deck. The mooring was dropped and the vessel began motoring slowly back to shore. Divers Alert Network (DAN) was notified of the treatment action and gave the attending DMT the location of the closest operational recompression chamber. While en route to shore DAN notified the local EMS for us. As the patient could tolerate it the vessel increased its cruising speed back to port. The patient reported slight additional relief at treatment depth but continued to vomit intermittently while inside the chamber. The patient tried to orally ingest as much water as possible. Some of this continued vertigo may have been induced by the vessels rolling action and/or the confinement within the small chamber. During the slide (ascent) from 60 ft/18 m to 30 ft/9.1 m, DAN was asked whether the DMT should extend treatment times at that depth as persistent symptoms were still present. The answer was *"No, continue treatment and get to the local hospital when finished* (pers. comm. Divers Alert Network). The R/V *Huron Explorer* reached the shore in approximately 90 min and the patient remained inside the chamber completing the entire USN TT6 on deck.

Hyperbaric Oxygen Therapy

Hyperbaric oxygen therapy (HBOT) was tested and developed by the U.S. military after World War I. Since the 1930s it has been safely used to help treat deep sea divers with decompression sickness.

Elevated atmospheric pressure in conjunction with intermittent 100% oxygen breathing combines to produce a number of beneficial effects that are poorly duplicated by breathing oxygen at sea level. Decompression illness bears out all the effects of Boyle's Law and of accelerated inert gas elimination, (Henry's Law) during oxygen breathing by creation of the oxygen window. HBOT involves breathing 100% oxygen in a sealed pressurized chamber. This oxygen concentration is five times higher than the ambient air normally breathed. The chamber is also pressurized to create 1.5 to 3 times our normal atmospheric pressure. These changes can improve blood circulation and its ability to deliver oxygen to the body and tissues.

Patient Transport

Once in the calm of the harbor the patient seemed to do much better. The weather was cool and overcast so there were no thermal concerns and a tarp was rigged over the *Hyperlite*™. Local EMS was standing by dockside when the vessel arrived and remained throughout treatment to receive the patient at its conclusion. At 19:45 he still reported some dizziness after reaching the surface and emerging from the chamber. He was transferred to the local EMS, examined, and taken by ambulance to the local area hospital at 20:05. The DMT rode along to help with any additional reporting of findings and to determine what the prognosis would be from the local ER doctor.

Follow-up and Medical Evaluation

A CAT Scan-Head, Chest X-ray and EKG were ordered, with a baseline medical examination performed by the ER doctor at 20:15. Intravenous fluids were administered to the patient. All findings for these three exams were normal. After initial evaluation the ER doctor concluded that this was not a case of DCS but merely an inner ear related positional/posture problem. The DMT suggested that the ER physician consult with Divers Alert Network (DAN) to discuss his working diagnosis. DAN referred him to Spectrum Health, a hyperbaric facility in Grand Rapids, Michigan, whose on-call physician informed the ER doctor at 21:00 that this was indeed a case of DCI needing prompt, follow-up HBO treatment. Ambulance transport was ordered and dispatched with the diver taken to Grand Rapids that evening at approximately 22:55. Intravenous fluids were administered to the patient to reverse his dehydration while en route to the chamber facility.

The patient was admitted to Spectrum Health in Grand Rapids and received three follow-up HBO treatments in an attempt to wash out any residual inert gas and aid in his full recovery, hence improving the prognosis of his inner ear DCI insult. He tolerated all the retreatments well and noticed slight improvement with each one.

Table 2. Timeline of Events

08.13.05 – Day 1
10:52 - Diver 1/Diver 2/ start dive
11:50 - Diver 1/Diver 2/ on surface
13:45 - Diver 1 reports possible DCS event/starts 100 % oxygen breathing via demand valve
13:50 - Neurological examination/vitals taken by Diver Medic (DMT)
14:35 - Treatment started – Compression in *Hyperlite*™ to 60 fsw/18.2 m (Treatment #1) USN TT6
19:45 - Treatment concluded. Still minor dizziness
19:55 - Diver 1 released to local EMS, transported to Alpena, Michigan regional hospital
20:05 - Arrival at Alpena Hospital, Alpena, Michigan
22:55 - Diver 1 departs for Grand Rapids, Michigan via ground ambulance evacuation

08.14.05 – Day 2
03:00 - Diver 1 arrives at Spectrum Health - Grand Rapids, Michigan

08:00 - Diver 1 reports that the HBO facility will run a USN T6 at 09:15. Little change occurred from the previous evening
09:00 - Start USN TT6 on Diver 1 (Treatment #2) in a mono-place chamber
15:00 - Diver 1 reports little change after treatment. “Feels 99%” but still afflicted by slight dizziness with nystagmus

08.15.05 – Day 3
Diver 1 will meet with the hyperbaric physician at 07:00
Diver 1 receives HBO treatment of (USN TT 5) on 100% O_2 - (Treatment #3)

08.16.05 – Day 4
Diver 1 receives HBO treatments of (USN TT 6) on 100% O_2 - (Treatment #4)

08.17.05 – Day 5
Hospital discharge

Post-incident review and dive operation continuance

The NOAA Diving Program (NDP) Manager was informed of the situation and subsequent on-site accident mitigation and reporting. After careful consideration, NOAA’s Diving Safety Board (NDSB) approved continuation of dive operations at the Thunder Bay National Marine Sanctuary. A NURC/UNCW dive supervisor imposed a 20% safety factor to be programmed into VR3 dive computers for all divers and all further diving operations. This, in effect, produced the equivalence of the *Cold and Arduous Diving Rule* using the next greater depth table to calculate decompression obligations as prescribed by U.S. Navy Air Decompression Tables. At the start of the project it was felt that this rule would not be necessary as divers would be wearing adequate thermal protection under their drysuits and the diving activity would be relatively easy with no water currents or sea states to contend with. NDP gave no indication that an additional safety factor should be added during its review of the initial dive operations plan. However, in light of the recent decompression illness incident, the dive management team felt it prudent to impose this additional safety factor for in-water decompression diving on mixed gas. The recovering diver rejoined the operation and returned to a topside support role aboard the vessel for the remainder of the mission.

Lessons Learned

- It is important to have a pre-mission set-up and demonstration of the system with a mock casualty drill to familiarize the dive team with deployment and operation of the *Hyperlite*™ system in event of a diving accident (Fig. 6). In most cases this will require 3-4 individuals to complete chamber set-up and to initiate treatment.
- A 41 ft/12 m research vessel is probably the smallest craft of opportunity capable of accommodating deployment of the *Hyperlite*™ system on deck.
- Carry more gas supplies than are recommended by the manufacturer and U.S. Navy guidelines. The USN TT 6 required three times the volume of gases recommended as the minimum in the U.S. Navy Operations Manual. Recommended: Compressed air: 174 scf; Carried: 240 scf; Required: 320 scf. Recommended: 100% Oxygen: 210 scf; Carried: 250 scf; Required = 300 scf.
- Chamber supplies for a patient should be standing by and include: a towel, bottled drinking water, a urinal, an emesis bag and patient clothing-gown.
- Appropriate sun screen/shelter apparatus for overhead cover of the chamber unit. The manufacturer does not recommend use of the chamber in direct sunlight; chamber temperature and patient comfort can then become an issue.
- Initiate treatment promptly to improve therapeutic results of HBOT. Once initiated, complete the prescribed treatment tables for improved patient outcome unless transfer under pressure can be

accomplished to a multi-place chamber system. Consider treatment table extensions if the patient is well-hydrated.

- Develop a load list for deployment which includes all components of the *Hyperlite*™ system including compressed gas cylinders, two travel cases, operators console, medical supplies, patient stretcher and chamber clean-up kit.
- Be prepared for patients to refuse treatment against medical advice (AMA) because of the constraints/size of the *Hyperlite*™ system.

Post Treatment Outcome

Upon patient discharge, the attending hyperbaric physician at Spectrum Health set a minimum of six weeks for Diver 1 to return to active diving status and then only after a follow-up evaluation with a diving medical officer (DMO).

Recovery and Follow-up

On October 14, approximately eight weeks post-incident, the diver underwent a follow-up medical exam and return–to-diving evaluation at Duke University Medical Center with Richard Moon, MD as the attending physician for the examination and consultation. He ordered a series of tests that were performed at the F.G. Hall Hyperbaric Center, including:

- Audiological evaluation (dB hearing test). Findings: Mild to moderate high-frequency hearing loss (consistent with noise-induced hearing loss)
- Vestibular – ENG with caloric stimulation. Findings: ENG within normal limits without spontaneous or positional nystagmus
- Physical Examination: Normal
- Neurological Examination: Normal

Based on the physical and laboratory findings above by Dr. Moon, limited return to diving status recommendations were issued for Diver 1 for a period of twelve months with:

- Use of enriched oxygen breathing gases on the bottom phase and high O_2 mixtures/100% O_2 as appropriate during decompression;
- Decompression procedures should be as conservative as feasible and practical;
- There is no reason to avoid air saturation diving; and,
- Restriction in the use of helium in the diver's breathing gas.

Diver 1 resumed both air and nitrox diving, conducting approximately 150 dives with minimal in-water decompression using 100% oxygen at all 20 ft (6 m) and 15 ft (4.6 m) decompression stops. During this twelve-month period, the diver did not experience any reoccurrence of DCI symptoms. In August of 2006, one year post incident, Diver 1 contacted Dr. Moon for consideration of a full return to diving status with planned mixed gas dives to 150 - 170 ft (45.7 - 51.8 m). Dr. Moon approved the use of helium as a breathing gas but felt that diving without close chamber availability would not be in the diver's best interest. If an on-site chamber could not be arranged he recommended that a shore-based chamber be located within 30 minutes of the dive site. Based on these recommendations, a chamber system was allocated to all diving operations that used helium as a breathing gas requiring in-water decompression stops. Diver 1 is still an active scientific diver within an approved diving program.

Acknowledgements

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RESEARCH AND DISCOVERIES:
THE REVOLUTION OF SCIENCE THROUGH SCUBA

Michael A. Lang[1]
Roberta L. Marinelli[2]
Susan J. Roberts[3]
Phillip R. Taylor[4]

[1]The Ocean Foundation
1990 M Street NW, Suite 250
Washington, DISTRICT OF COLUMBIA 20036 U.S.A.
mlang@oceanfdn.org

[2]Wrigley Institute for Environmental Studies
University of Southern California
Los Angeles, CALIFORNIA 90089-0371 U.S.A.

[3]Ocean Studies Board
National Research Council
500 5th St. NW, Keck 607
Washington, DISTRICT OF COLUMBIA 20001 U.S.A.

[4]University of Southern California
Dornsife College of Letters, Arts and Sciences
CGC 4100, M/C 8206
Los Angeles, CALIFORNIA 90071 U.S.A.

To highlight the scientific contributions of self-contained underwater breathing apparatus (scuba) as research methodology, *Research and Discoveries: The Revolution of Science through Scuba* symposium presented research findings by U.S. scholars with a particular focus on science. This symposium was a unique effort to demonstrate and validate the use of scientific diving techniques through high-impact results and reviews, also illuminating the impact of underwater research to our overall understanding of nature and its processes. The symposium themes focused on past, present and possible future scientific diving contributions starting with the introduction of scuba to the science community in 1951.

Important examples of the first generation of scientific diving research included Paul Dayton's groundbreaking ecological work under Antarctic ice sheets; William Hamner's pioneering studies of gelatinous zooplankton in surficial blue waters of the open ocean; multidisciplinary and long-term phycological studies by Mark and Diane Littler; crustacean behavioral ecology research by William Herrnkind; kelp forest ecological work by John Pearse and Michael Foster; and the diversity coral reef research that established important baseline understanding conducted by Ian Macintyre, Peter Glynn, and Chuck Birkeland. Some thirty years after the advent of scuba other research tools e.g., molecular biological techniques became more widely used in marine sciences. These tools used together with scuba enhance understanding of the biodiversity, systematics, and ecological processes within systems. The advances in biopharmaceutical work and the understanding of chemical defenses of marine organisms, harmful algal bloom outbreaks, and invasive species have encouraged more scientists to don scuba gear and conduct their research under water. In an era of increasing occurrences of multiple stressors on ecological systems (e.g., coral reefs), geological studies show patterns and trends of previous episodic events.

The symposium showed that scuba has indeed played a critical role in marine research; this impact continues. The papers weave scuba through the science, often with a historical and developmental perspective on methods and techniques. These accounts give credence to the assertion that it is difficult to understand many ecological processes and interactions in the underwater environment without immersing oneself in it to make direct observations and data collections. "The history of marine research has provided numerous examples of mysteries that would still have been unsolved and findings that would have been misinterpreted with confidence if not for direct observation on scuba" (Witman et al., 2013). Papers within the volume highlight research enterprises that could not have been accomplished without scuba, for example: the biology of the coral holobiont; ecological roles of major algal groups on reefs; significance of synchronized sexual reproduction in green algae; the functional role of small and cryptic metazoans on coral reefs; and, of course, the several studies developing long baselines for evaluation of the impacts of large-scale environmental change.

Nineteen chapters comprising 258 pages, 111 figures and 7 tables were written by sixty co-authors, fifty-two of whom conducted their research diving under the auspices of an AAUS-member scientific diving program. Materials from the symposium have been posted on the symposium web site (www.si.edu/sds) including abstracts, speaker bios and webcast videos of presentations. The symposium volume (Lang et al., 2013) is available by contacting Smithsonian Institution Scholarly Press (SISP) for hard copy (scholarlypress@si.edu) or accessing the pdf at SISP (www.scholarlypress.si.edu) through the Smithsonian Institution Libraries Digital Repository.

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DIVING MEDICAL RESEARCH

Michael A. Lang
[1]The Ocean Foundation
1990 M Street NW, Suite 250
Washington, DISTRICT OF COLUMBIA 20036 U.S.A.
mlang@oceanfdn.org

Peter Germonpré
Centre for Hyperbaric Oxygen Therapy
Military Hospital Brussels
Bruynstraat 200
Brussels 1120, BELGIUM
peter.germonpre@eubs.org

Karen B. Van Hoesen
University of California San Diego Medical Center
Diving and Hyperbaric Medicine
200 W. Arbor Drive
San Diego, CALIFORNIA 92103 U.S.A.
kbvanhoesen@gmail.com

Scientific diving medical certification

Scientific divers who are exposed to hyperbaric conditions must possess a current diving medical certification. In passing that examination the diver will have been declared by the physician to be medically fit to engage in diving activities as may be limited or restricted in the scientific diver medical certification. All medical evaluations are performed by, or under the direction of, a licensed physician of the applicant diver's choice, but preferably one trained in diving/undersea medicine. The diver must be free of any acute or chronic disabling disease or conditions contained in the list of conditions by Bove (1998) for which restriction from diving may be recommended. There are currently no fitness standards per se for scientific divers other than during the initial scientific diver training course, which includes in-water time and distance challenges for swimming. A stress tolerance test can be prescribed by a physician based on preliminary screening that indicates the potential of a higher than normal risk of coronary artery disease. Cardiac events are the proximate cause of more than 30% of diving fatalities in the recreational diving community (Vann and Lang, 2011). AAUS diving medical evaluations are completed before a diver may begin diving and thereafter at five-year intervals up to age 40, three-year intervals after the age of 40, and two year-intervals after age 60 (AAUS, 2011). Any major injury or illness or any condition requiring hospital care requires diving medical clearance. If the injury or illness is pressure-related, then the clearance to return to diving must be performed by a physician trained in diving medicine. AAUS diving medical standards were updated by the AAUS Medical Review Panel in 2000 and 2011.

Cardiovascular issues and other diving research

Screening for cardiovascular risk factors has taken on an increasing relevance in the scientific diving community (Bove, 2011; Douglas, 2011; Thompson, 2011). Patency of the foramen ovale is a risk factor for DCS in scuba divers, even if they adhere to the currently accepted and used decompression tables (Germonpré, 2005).

Decompression sickness

Decompression Sickness (DCS) is a syndrome that manifests itself through clinical signs or symptoms originally generated by a reduction of absolute pressure surrounding the diver. The predominant physical cause of DCS is the separation of gas in the body's tissues due to inadequate decompression, leading to an excessive degree of gas supersaturation. Rapid decompression (fast rate of ascent or omission of decompression stops) is a primary cause of gas separation in tissues, i.e., stationary or circulating bubbles. The most obvious prevention strategy for DCS is determining and observing appropriate ascent and decompression procedures, which are unfortunately largely empirical and not always reliable. Research has focused on the decompression procedures or the diver pre-conditioning and post-conditioning. Epidemiologically, there is universal consensus among the international diving medical community that the incidence of DCS is generally very low (Dardeau et al., 2012; Sayer, 2005; Sayer et al., 2007) and that there is no significant gender-related susceptibility. There is also consensus that neurological manifestations are by far the most common form of DCS amongst civilian divers and predisposing factors could include exercise, cold water, age, dehydration, and fatigue.

In an effort to explain the wide variation in individual susceptibility to DCS, other factors investigated include complement activation in the presence of gas bubbles as well as an uncertain relationship between gas bubbles, blood cells, and the capillary endothelial lining in response to bubble presence and development of DCS (Lang et al., 2013). Although the presence of Doppler-detectable gas bubbles in the blood is not necessarily predictive of clinically evident DCS, its appearance in the absence of detectable pulmonary artery and venous bubbles is rare. There is growing experimental and clinical evidence that suggests that asymptomatic “silent” bubbles in the body may be causing cellular and biological reactions that release secondary potentially damaging biochemical substances in the blood.

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PERSONAL UNDERWATER TABLETS BRING NEW POSSIBILITIES TO DATA COLLECTION, COMMUNICATION, GEOLOCATION AND DIVER MONITORING

Jouni Leinikki
Juhani Kanerva
Panu Oulasvirta
Juha Syväranta
Alleco Ltd
Veneentekijäntie 4
FI-00210 Helsinki, FINLAND
jouni.leinikki@alleco.fi

Introduction

Scientific divers use many techniques to record and take notes, photos and video footage. The wet environment and water pressure limit the adaptation of new technologies to underwater use. Cameras and other electronic devices, for example, need special housings. Annotations are usually made on plastic paper sheets by pencil. The present high-end communication techniques from diver to surface and other divers are oral and based on high-frequency sound, because of poor radio signals penetration in water.

Developing information technology is opening new possibilities for underwater scientific work. Tablet computers incorporate many functions useful in scientific data collection. Besides writing notes, a scientist can use them for taking and editing photographs, video, uploading measurement units data without taking them to surface. Many models also include a built-in compass, level and GPS. They can communicate with the internet and other devices by cellular data, wireless network and Bluetooth connections.

The standard touch screens used in mobile devices are not suitable for use in water. Several parties have developed housings for tablet computers that protect them against water but very few of them allow the use of the touch screen while submerged. With the support of Tekes, the Finnish Funding Agency for Technology and Innovation, we have launched a two-year project to develop a technology to utilize the full functionality of commercial tablet computers underwater at any depth.

Scientific divers also do large amounts of work to transfer their data from the hand-written forms to computer files for further processing. The second part of our development project tackles this problem with an eased data transfer method from the mobile applications directly to a database. This paper reviews the advantages and challenges brought on by new technology.

Methods

To meet the different requirements for depth range, weight and GPS usability, we tested aluminium and plastic as housing materials. We also developed special electronic forms with drop-menus, positioning, and map interface. The web browser-based forms can be used with tablet computers as well as smartphones. A small, portable server was used to transfer the forms to the mobile devices and afterwards to move the collected data to a database (Fig. 1).

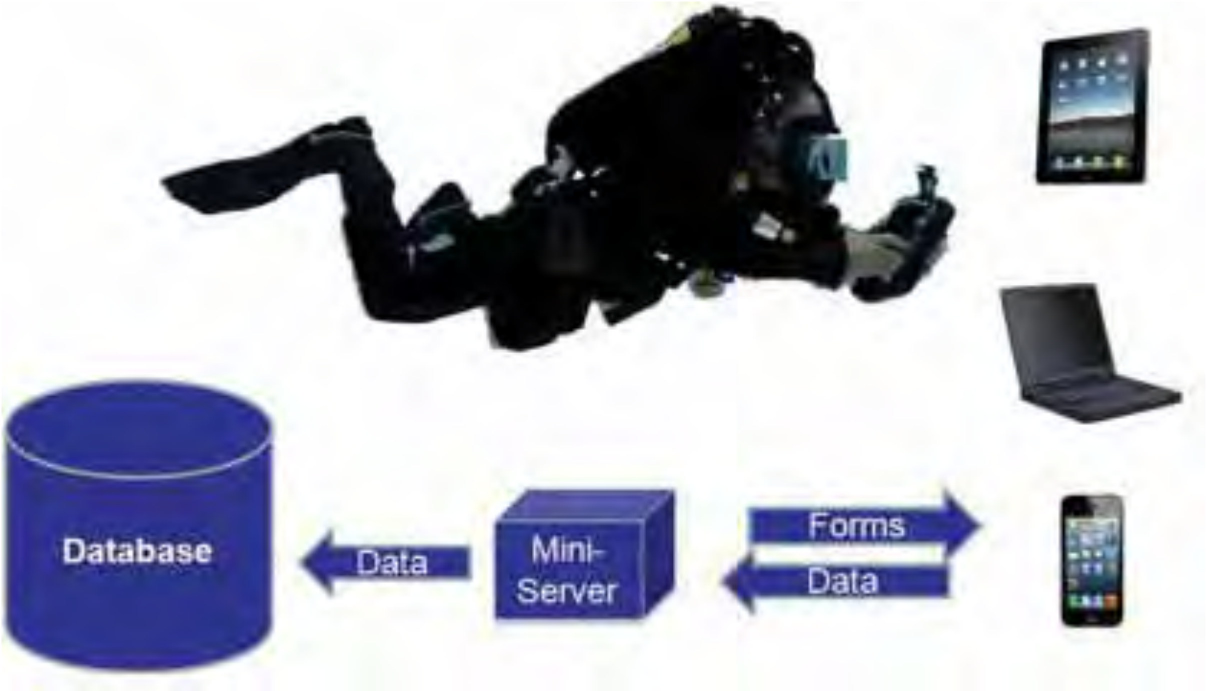

Figure 1. Data collection system.

The test was done by three scientific divers who were collecting underwater vegetation coverage data while diving on the southern coast of Finland. At the time of testing the underwater positioning capability was not yet available and so a traditional measure line method was used for positioning during the study. The diver recorded the distance on a marked line for which the starting point and direction was known. Because the divers wore mittens they used the tablet with a pen for accuracy. The collected data included depth, distance on the line, estimates of the relative (%) coverage of twelve different substrate classes, relative coverage of the algae and angiosperm species and their average heights.

Results

The general impression of the divers was that the tablet computer was easy to read under water because of the backlighting and zooming option. The full-sized aluminium housing with an Apple iPad^{TM} tablet was approximately the same size as a traditional plastic slate, but heavier. The housing made of plastic had near neutral buoyancy in the water. The camera could be used both for shooting and magnifying and the documentation for species identification was easy to access. The built-in compass and artificial horizon were also easy to use.

Typing with a single pen-point is slow in comparison to handwriting. Bare hands provide little improvement and one hand is always needed to support the tablet. Drop-menus and automatic functionalities speed up the filling of the forms and eliminate typing errors.

During the tests, a vegetation transect took an average of about 40 minutes to study, requiring the presence of 2–3 divers. The electronic forms did not substantially speed up the annotation time under water but decreased the time required for post-processing by approximately 90%. The first tests indicated that it was possible to save up to 50% of working time on collecting species coverage data in comparison with the traditional use of a slate and pencil. Further, no mistakes were found in the data indicating a decrease in typing and interpretation errors of handwriting.

Data transfer from the tablets to the server functioned well. The tablet computers would establish a wireless connection with the portable server as soon as they surfaced and the data synchronization was done immediately. The divers found this reassuring against the risk of potential data loss.

Discussion

Our test shows that it is beneficial to use common tablet computers for the collection of underwater scientific data. The experience also gives us an idea of the possibilities that lie within this technology. Within the next year, the underwater position and wireless communication will be incorporated in the tablets which will allow their use as underwater navigators with generic or user-made charts. The position signal can be sent out enabling the surveillance of divers and direct data feed to the surface. Ancillaries using wireless network or Bluetooth™ connection can transmit data under water at short distances enabling the tablet to be used for direct measurement, navigation, controlling and monitoring video cameras; this was confirmed by a pool experiment with a GoPro Hero3™ camera (Fig. 2).

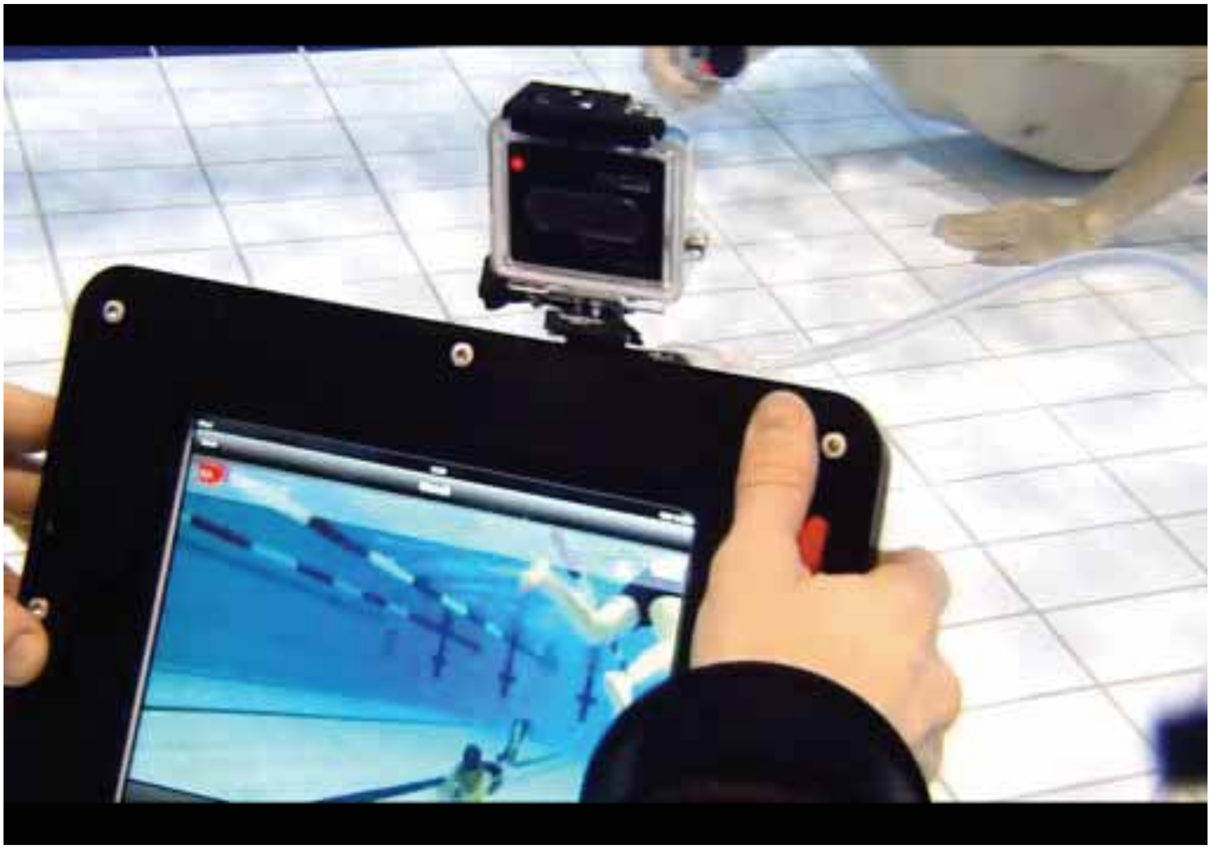

Figure 2. Pool testing GoPro Hero3™ video camera with a wireless connection to Apple iPad™.

While developing *in-situ* data collection technology, it is vital to also develop the post-processing of the data to fully utilize the new possibilities. With simultaneous development, the new technology will also best benefit science.

JUNKYARD DAMSELFISHES:
SPAWNING BEHAVIOR AND NEST SITE SELECTION

***Lisa K. Lobel*[1]**
***Phillip S. Lobel*[2]**

[1]Wheelock College
Math & Science Department
Boston, MASSACHUSETTS 02215 U.S.A.
llobel@wheelock.edu

[2]Boston University
Department of Biology
Boston, MASSACHUSETTS 02215 U.S.A.

The reproductive ecology of damselfishes is relatively well studied among coral reef fishes because of their conspicuous behavior and demersal spawning. Site-specific nesting behavior, territoriality, as well as ease in observing reproduction are attributes contributing to the use of damselfishes as indicator species for assessing anthropogenic impacts. Damselfishes including Abudefduf sordidus *spawn in demersal nests with parental care provided by the male. Even though damselfish reproduction is well studied, questions remain as to intra- and interspecific variation, geographical variation and questions related to female behaviors. The present study focused on the reproductive behavior of the blackspot sergeant major damselfish (*Abudefduf sordidus*) that occurs throughout the Indo-Pacific (This species is ecologically comparable to the Caribbean* A. taurus*). At Johnston Atoll, a former military base, several male* A. sordidus *selected submerged military debris as artificial nesting substrates including 55-gallon drums, plastic chairs, cement pilings and other submerged debris. Males engage in courtship displays using color changes and simple sounds. Spawning territories were abandoned between spawning bouts and were re-established approximately every two weeks, usually by the same male. Observations and physical evidence including scars and torn fins demonstrated that competition for nest sites occurred. Based on the atoll-wide distribution of adult fish during non-mating and mating, it appears that the few locations with a high concentration of desirable spawning substrate serve as aggregation locations for this species.*

Introduction

Johnston Atoll is a remote atoll in the Central Pacific. Johnston lies approximately 1,287 km (800 miles) southwest of Honolulu, Hawaii and 1,440 km (900 miles) north of the Line Islands of Kiribati. Currently, the Johnston Atoll National Wildlife Refuge is a component of the Pacific Remote Islands National Monument overseen by the U.S. Fish and Wildlife Service. The atoll was under military control from 1934 until 2003 and is still owned by the U.S. Air Force. During the years under military control, the atoll underwent significant geophysical changes caused by channel dredging and island expansion. The atoll was used for various activities including use as a refueling site, atmospheric nuclear testing, master LORAN station for the Pacific, storage site for unused herbicide orange (agent orange) and chemical weapons, and the incineration of chemical weapons in the Johnston Atoll Chemical Ammunition Disposal System (JACADS) (Lobel and Lobel, 2008). Many of these activities, as well as infrastructure needed to support a military and civilian workforce of up to 2,000 people, contributed to soil, sediment and biota contamination within the atoll (Lobel and Kerr, 2002; Kerr Lobel and Davis, 2002; Kerr Lobel and Lobel, 2009). Other military debris was also disposed of in the lagoon in various locations.

Abudefduf sordidus is a large (160 mm SL) benthic omnivore found throughout the Indo-west Pacific (Allen, 1991). Adults inhabit rocky lagoon and reef flat shorelines while juveniles are found in shallow tide pools (Myers, 1991). The reproductive biology of *A. sordidus* has been studied in the field (Stanton, 1985; Lobel and Kerr, 1999). Like other damselfishes, *A. sordidus* spawns in demersal nests with parental care provided by the male. The male prepares the nest site (the physical location used for spawning) by biting off encrusting material and allowing fine filamentous algae to grow. Males often chose to nest on sunken debris left over from various civilian and military activities. Males would attract females to the nest site with bright spawning coloration, exaggerated swimming and courtship sounds (Lobel and Kerr, 1999).

The goals of the present study were to locate spawning colonies or aggregations used by *A. sordidus* in the Johnston Atoll lagoon and document spawning behavior. It was conducted as part of a larger study assessing reproductive behavior and anthropogenic impacts on fish development.

Methods

Surveys for spawned activity were conducted during the peak spawning season (March-June). Reef areas surveyed on multiple occasions included all island margins (except for the restricted areas on Johnston Island), channel walls particularly around channel markers, and the majority of the inner reef. Known spawning areas were surveyed daily using scuba. Nest sites were detected by observing the behavior of male *A. sordidus* defending or cleaning nest sites. Newly discovered nest sites were given a unique identifier and labeled with flagging tape. Data collected during each survey included presence, number and size of clutches (l x w). A clutch was defined as a continuous mass of embryos of the same developmental stage, presumably spawned by one female. Data on nest site preparation and any observed spawning behavior was recorded daily. Spawning behavior was recorded using a Sony video camera in a Gates housing.

Measures of reproductive output (success), including total number of clutches spawned per male and area of nests spawned per male, were calculated (for the 1996 spawning season). The areas of all nests were measured during one reproductive season. Embryo density was calculated from 29 nests photographed with a 1:1 macro lens. The photos were scanned and counted digitally in Photoshop. A plot of the cumulative means was used to determine when a sufficient sample size had been achieved for calculating mean density. Densities for two different clutches separated by at least one spawning interval were calculated for nine nests.

Results

Spawning Behavior

Male *Abudefduf sordidus* establish and maintain territories for nesting, often returning to the same nest site multiple times. Males compete aggressively for their exclusive nest site and were observed with torn fins, scrapes, scars and other injuries, presumably acquired during securing and defending nest sites from other males. One observation was made of a small male being evicted from the nest site of a larger male. The smaller male had successfully spawned within the larger male's nest site during his absence but the larger male consumed the embryos after the takeover. The nest sites were arranged in a colonial arena layout at Sand Island, the site with the largest number of nesting males. However, other spawning aggregations within the atoll had distributions that were linear and/or more sparsely distributed (e.g. along the dredged channel). The spawning aggregation layout seemed to be dependent upon availability and distribution of suitable nest sites. Colony size ranged from seven nesting males up to 68 documented nest sites at Sand Island. Not all nest sites were occupied simultaneously and use varied from year to year.

The sexes are indistinguishable except when males are in active courtship coloration. Adult *Abudefduf sordidus* are not physically sexually dimorphic but they can be recognized by their behavior in the field. Males can be distinguished by territorial behavior surrounding nest site acquisition, preparation and defense. The nest site is an area of hard substrate used by a male for courtship and spawning and is defended from egg predators during embryo incubation. Nest sites are prepared for spawning by the male, who removes encrusting material from the substrate and allows fine, filamentous algae to grow. Once a nest site has been prepared, males will adopt bright courtship/spawning coloration with sharply contrasting black and white bars as compared to the dull gray and white bars in non-courting males and females (Fig. 1). This color dimorphism only occurs during courtship and spawning.

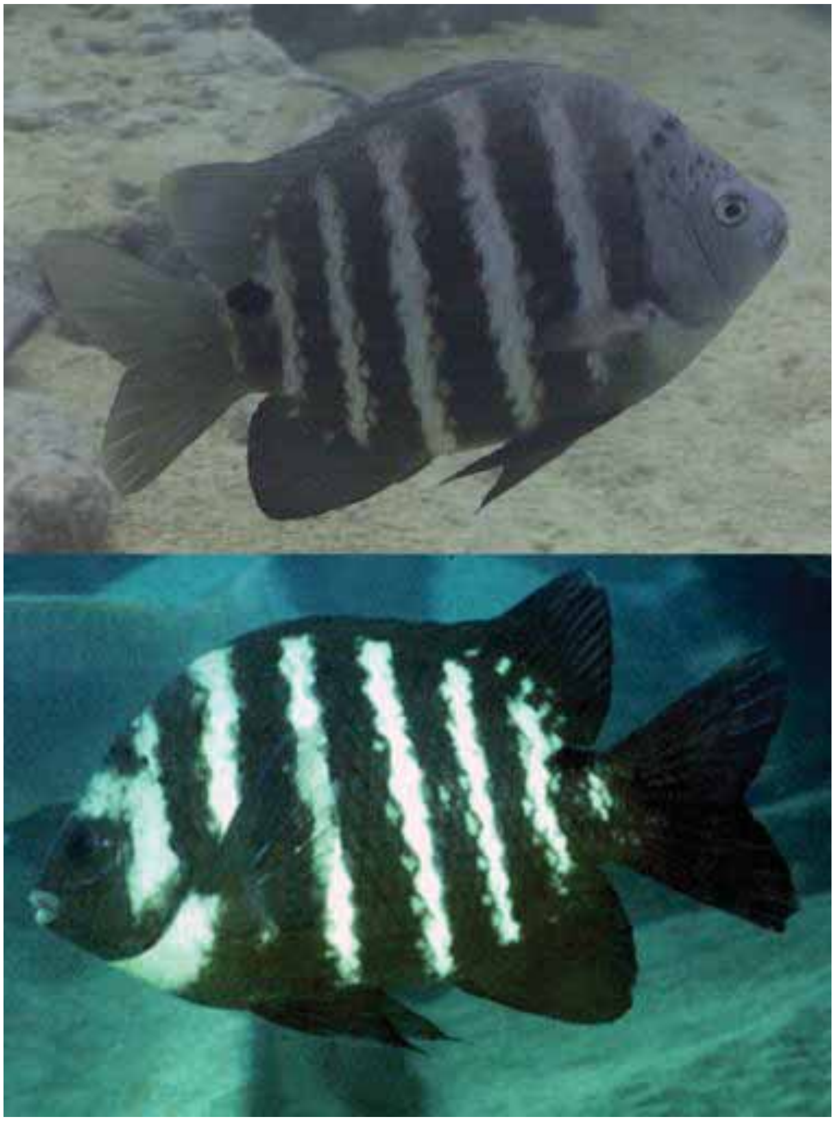

Figure 1. Color dimorphism observed during courtship and spawning of *Abudefduf sordidus*. Top panel shows the normal coloration of both the male, female and juvenile fish. Bottom panel shows the bright contrasting pattern of the males during courtship and spawning.

Courtship consists of males attracting females to their nest sites using their bright spawning coloration, exaggerated swimming motions and courtship sounds. The courtship coloration is observed in males with a nest site that has been cleaned and prepared to receive eggs. Males stay in the vicinity of their nest site and watch for females swimming through the colony. A male will dash-swim up to 8 m away from his nest to intercept a female. The male responds to a female by rapidly swimming out toward her in a zigzag pattern, swimming first canted to one side, then flipping to the other. More than one male may swim out to meet a female. One male is successful in leading the female back to his nest. Once near the nest, he swims rapidly around her in a figure-eight pattern and produces sounds (Fig. 2). The male continues this behavior and also swims into the nest site area closely over the prepared egg deposition site. Once the female enters the nest site both male and female take turns swimming with pelvic fins pressed against the nest substrate. During this time the female is presumably inspecting the nest site and may choose to spawn with the male. This behavior may last up to 20 min before actual spawning begins. If the female attempts to leave, or pauses in her passing over the nest, the male resumes the courtship figure-eight pattern and sound production. Once spawning begins the male only produces sound when chasing away a conspecific. A male will often spawn with several females over a few days. The time for a male to acquire a clutch of embryos varied but was usually one or two days after nest cleaning. Some males would not be successful in obtaining a clutch and would abandon the nest site until the next reproductive cycle.

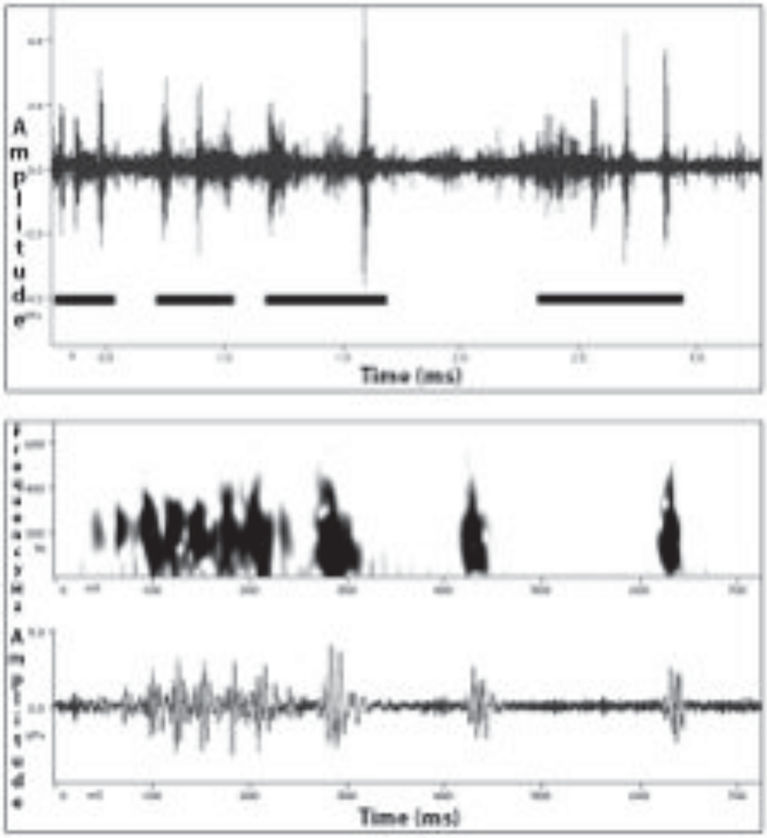

Figure 2. Sounds produced by *Abudefduf sordidus*. The top panel is a 3.5 sec oscillographic sequence of sound production with the dark bars defining each sound burst. The lower panel shows the detail on one of these sound bursts: the upper panel is a sonogram (frequency vs. time), the lower panel is an oscillogram (amplitude vs. time). The sounds are low frequency in the range of 200 to 400 Hz. (see Lobel & Kerr, 1999 for details).

Spawning occurs throughout the day and can last over three hours. The female lays the eggs in a single strand by pressing her genital papilla to the substrate. Sticky tendrils on one end of the unfertilized eggs attach them to the algae in the nest. The male watches and keeps guard as the female makes a few passes and then swims over the eggs fertilizing and fanning them with his fins. Multiple passes back and forth gradually fill in the entire area encompassed by the clutch. The male will bump or nudge the female on the side of her body if she pauses during spawning. A clutch is as continuous monolayer of eggs presumably spawned by one female during one spawning bout. The average clutch size was 30 x 30 cm (Fig. 3).

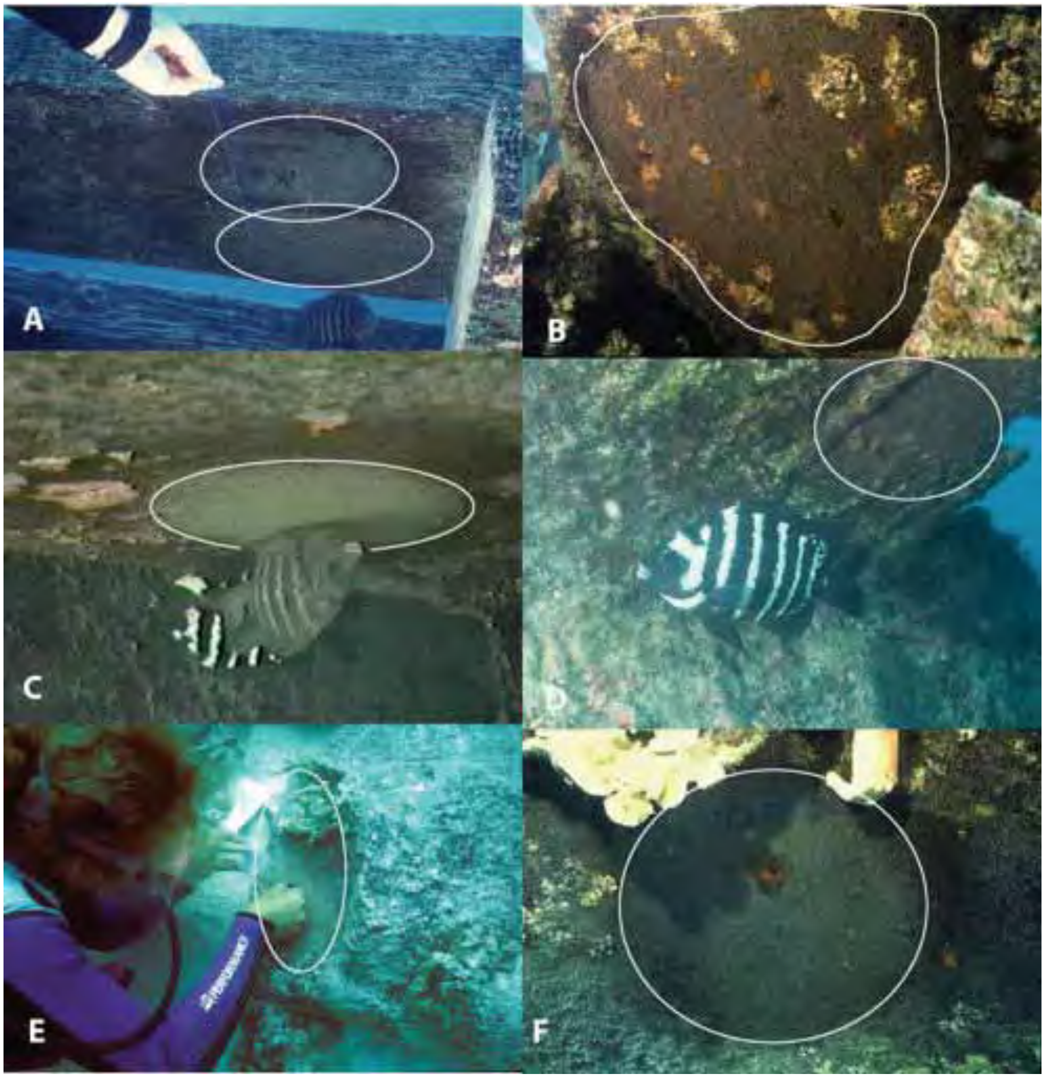

Figure 3. Nest sites with clutches of embryos outlined in white. A. Two clutches of embryos on the wood from the former wharf on Sand Island. The wharf structure fell into the water during hurricane John (1994). B. Large clutch of embryos on metal debris also shown in Fig. 4H. Note the corroding areas throughout the clutch. C. Pair of *A. sordidus* spawning underneath the sea plane ramp on the west end of Sand Island. D. Male *A. sordidus* with newly spawned clutch of embryos. An additional space of lush algae has been prepared for a second clutch. E. Collecting embryos on coral pavement along the edge of the dredged channel. F. A large clutch of embryos in a large metal cylinder off of Sand Island. Notice the corroded area in the middle of the clutch.

Clutches spawned sequentially in a nest site may share borders with older clutches or may be in a separate area of the nest site. Clutches spawned by different females or on different days can be distinguished from other clutches by location within the nest and/or differences in color as development proceeds. Nests are defined as all clutches contained within the nest site during one spawning cycle. A spawning cycle begins with nest preparation and ends when all of the clutches have hatched. Nests can contain up to five clutches of eggs usually spawned one to two days apart. The demersal embryos are guarded from egg predators and fanned to increase water circulation until they hatch on the fifth or sixth night. *A. sordidus* is not permanently territorial and males defend their nest sites only during courtship and embryo incubation. Given the time for nest preparation and embryo incubation, nesting cycles occurred approximately every two weeks.

Reproductive Output

Embryo density calculated from 29 nests photographed ranged from 45 to 104 embryos/cm^2 with a mean (± sd) of 74 ± 12 embryos/cm^2. Densities for two different clutches, spawned in the same nest but separated by at least one spawning interval, were calculated for nine nests. There was no significant difference in density between clutches (paired T test; P=0.233).

Mating success was variable: there were 50 males nesting at Sand Island (1996) and these males obtained between one to 20 clutches over 119 days during the spawning season. Three males did not receive any clutches. Nest sizes also varied depending on the number of clutches a male would receive in a given nesting cycle. The mean number of clutches received during this spawning period was 8 ± 6. The average nest size was 1,171 cm^2 with the smallest clutch measuring 22 x 30 cm or 518 cm^2 and the largest nest measured 52 x 72 cm or 2,940 cm^2. Based on the density calculations the smallest nest would contain 38,332 embryos while the largest would contain 217,560 embryos.

Nesting Sites and Substrates

In surveys of reef sites throughout Johnston Atoll spawning aggregations or colonies of *A. sordidus* were found at the west end of Sand Island, East Island, the Navy Pier, the channel adjacent to Buoy Marker 14 and on a sunken tug boat (Fig. 4).

The largest aggregation, containing 58 nest sites, was found at Sand Island. Male *A. sordidus* at this site nested on artificial materials ranging from 55 gallon drums, the tires of a jeep, plastic chairs, the cement sea plane ramp, pipes, submerged wood, metal fuel tanks and other metal debris (Fig. 5). Not all identified nest sites were used every year. The highest number of nest sites used during a given year at Sand Island was 40. Nest sites were not found on natural substrates at this location. The Buoy 14 aggregation contained 40 nest sites and nests were found on the walls of the dredged channel, coral pavement and on the buoy marker pilings. Nest sites at the tugboat (n = 16), a recreational dive site, were found on the sunken tugboat as well as dead coral surrounding the sunken boat. Nest sites (n = 15) at East Island were only found in shallow water on the western side of the island and only occurred on dead coral. All of the seven nest sites found near the Navy Pier were on the pier pilings. All nest sites occurred on surfaces that were either vertical or on the underside of a horizontal surface.

Abandoned metal structures were the most common nest substrate at the Sand Island location (Table 1), the former USCG Loran station. Areas of corrosion on the metal resulted in embryos being lost from the nest and in developmental defects (Figs. 3B, 3F, Fig. 6).

Table 1. Distribution of nest substrate types in the spawning colonies within Johnston Atoll. This distribution is a reflection of the availability of the different substrate types at the different locations. Metal debris was not found at all locations and the high number of nests found on metal at Sand Island does not necessarily indicate preference for substrate type. Dead coral also refers to coral pavement as found along the channel walls.

Spawning Site	Metal	Wood	Concrete	Tire	Plastic	Dead Coral	Total
Sand Island	43	1	9	3	2	0	58
Tug Boat	3	0	0	0	0	13	16
East Island	0	0	0	0	0	15	15
Former Navy Pier	0	0	7	0	0	0	7
Channel/Buoy 14	0	0	2	0	0	38	40

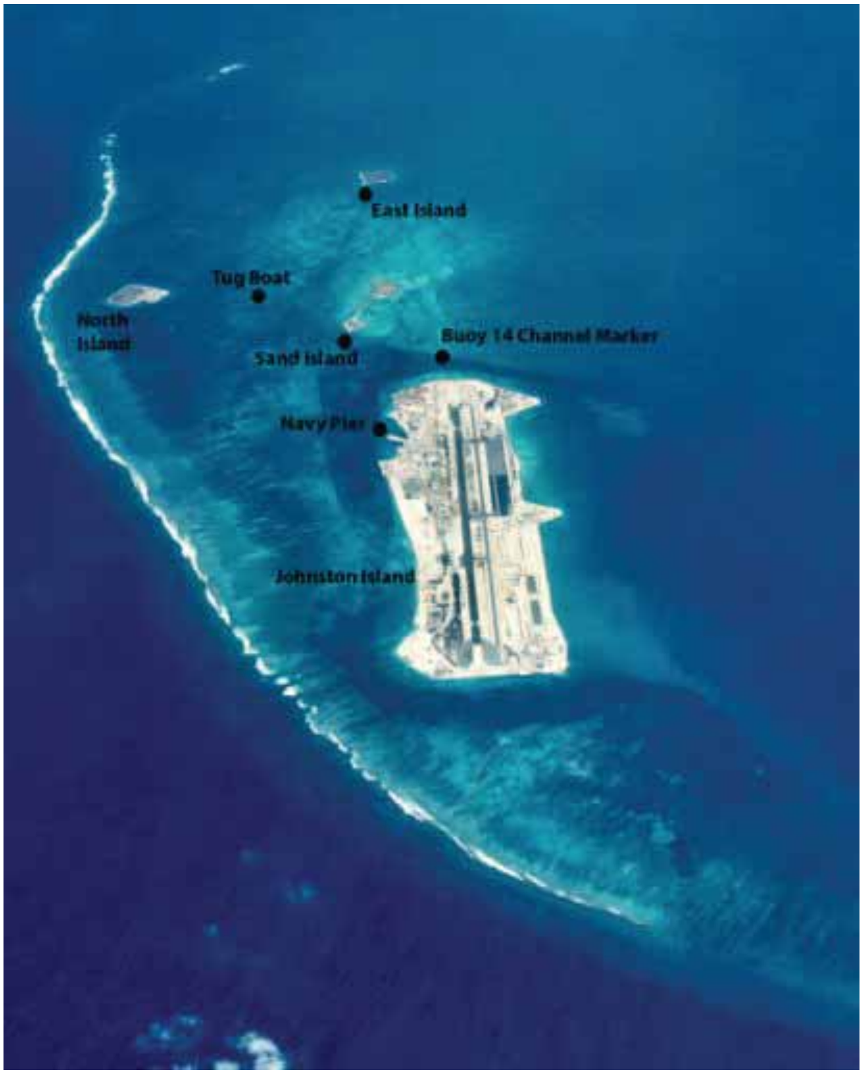

Figure 4. Aerial photo of Johnston Atoll and map illustrating locations with documented *A. sordidus* spawning. The west end of Sand Island, along the wall of the dredged channel close to Buoy marker 14, the sunken tug boat, the western side of East Island; and along the former Navy Pier on Johnston Island.

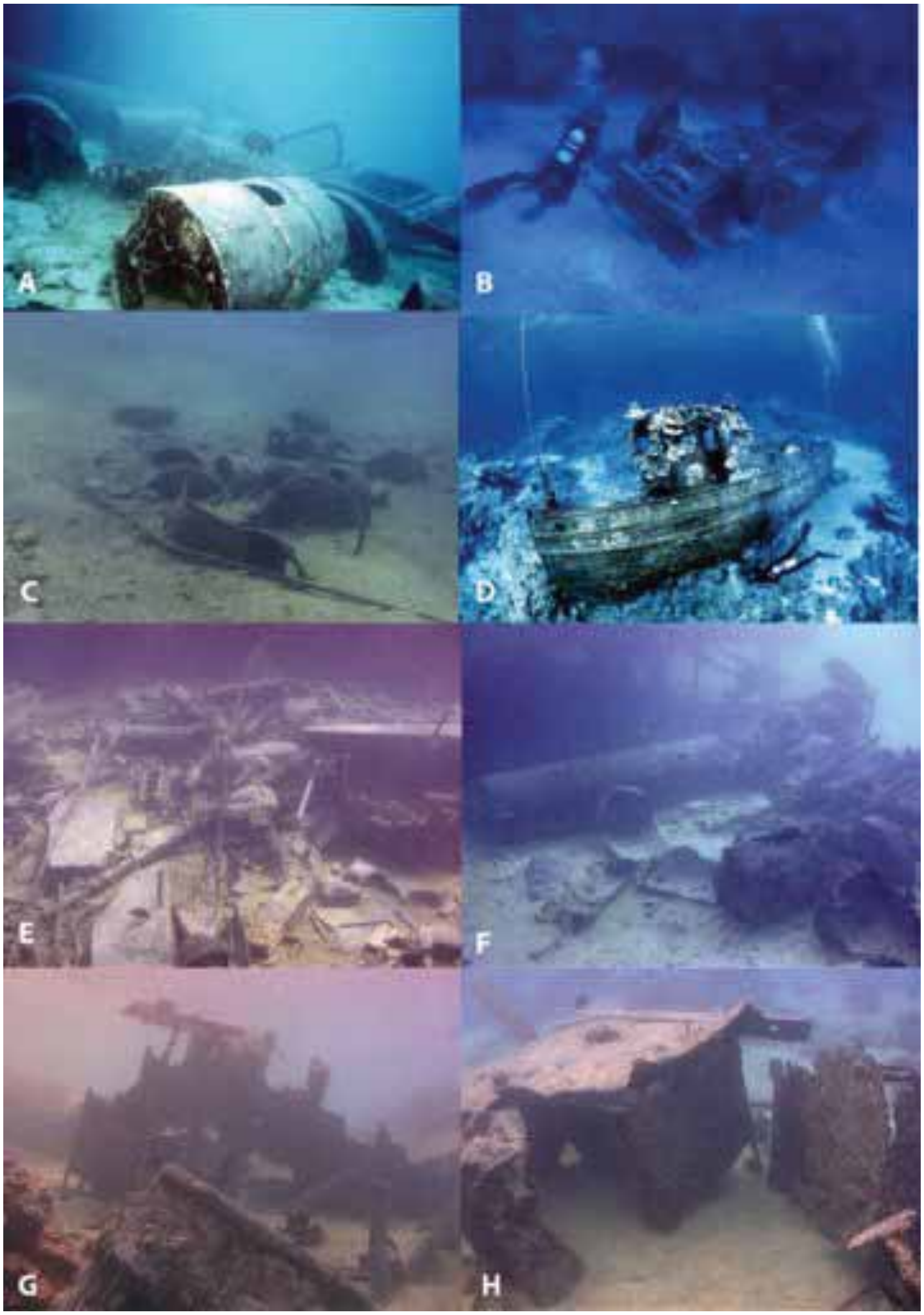

Figure 5. Debris used by damselfish for nest sites. A. Male *A. sordidus* patrolling near his nest site in a 55 gallon drum. B. A WWII jeep near the Sea Plane ramp on Sand Island. Three male *A. sordidus* used the tires as nest sites. C. Group of corroded 55-gallon drums and a plastic chair used as nest sites by male *A. sordidus*. D. The sunken tug boat. Nest sites were found on the hull and in the cabin of the boat as well on coral surrounding the sand patch containing the boat. E. Debris pile off the former wharf on Sand Island. F. Debris pile at the end of the Sea Plane ramp on west Sand Island. G. and H. Large metal fuel tanks used as nest sites at the west end of Sand Island.

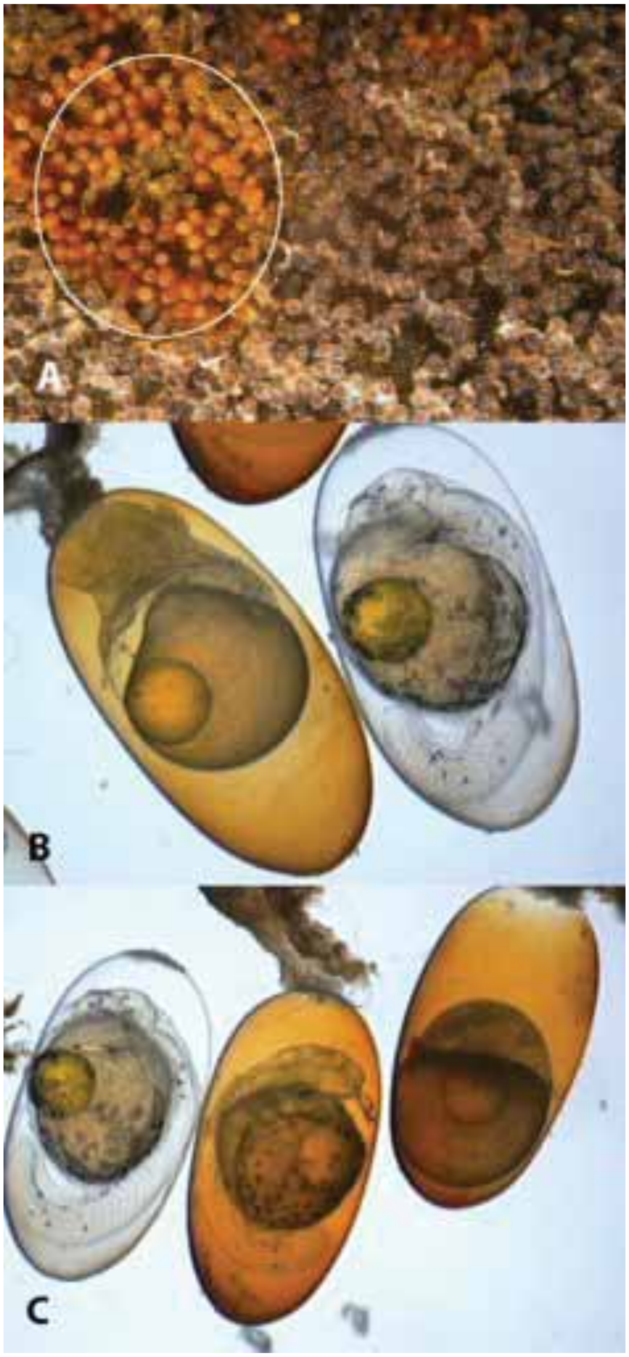

Figure 6. Photographs of *A. sordidus* embryos spawned on corroding metal. Embryos are 1 mm long. A. Macro photograph of an intact clutch of embryos. The white ellipse outlines embryos spawned on corroding metal. These embryos are not viable and have a distinctive opaque orange appearance. The embryos outside of the outlines are viable with yolk and oil globules visible. B. Micro photograph of embryos collected from the same nest in corroded and non-corroded areas. The embryo on the right is normal and has a clear chorion while the embryo on the left has an orange chorion and has severe developmental defects. C. Another micro photograph of embryos from corroded and non-corroded areas of the same nest. The embryo on the right is normal compared with the middle embryo that shows developmental delays and the embryo on the right that did not develop but was fertilized. Both abnormal embryos show the bright orange color resulting from contact with corroding metal.

Discussion

The blackspot sergeant major damselfish, *Abudefduf sordidus*, formed mating colonies or spawning aggregations at five locations within Johnston Atoll. The males of this large benthic damselfish compete for access to nest sites, prepare the nest sites, engage in courtship displays using color change and sound, and provide parental care until the embryos hatch. Spawning territories were abandoned between spawning bouts and re-established approximately every two weeks usually by the same male. Observations of scars and torn fins demonstrated that competition for nest sites occurred. Reproductive success of males was highly variable and may be caused by the quality of the nest sites or male quality or both. Based on the atoll-wide distribution of adult fish during non-mating and mating, it appears that the few locations with a high concentration of desirable spawning substrate serve as aggregation locations for this species.

The distribution of available nesting habitat appears to affect the distribution of spawning *A. sordidus*. *A. sordidus* are not common fish in the Johnston lagoon. On the majority of survey dives one adult individual might be observed during the 1-1.5 hr long dive. Even during non-nesting periods at the high-density aggregation sites only one or two individuals might be observed. Additionally, during peak nesting periods, males would be observed at their nest sites but very few free-swimming individuals would be observed in the area. The dense concentration of artificial nest substrates in shallow water adjacent to the islands served as an “artificial reef” and may have attracted males to the nesting colonies. It is interesting that nests on natural substrates were not found around Sand Island. This may be because so many smooth artificial substrates were available coupled with the lack of large smooth natural surfaces suitable for the relatively large size nests containing multiple clutches of embryos. The largest nest with multiple clutches measured 52 x 72 cm. This large area needs to be free of encrusting organisms, vertical or on the underside of a horizontal structure and fairly smooth for the fish to spawn. Many of these large surfaces used by *A. sordidus* at Sand Island were metal structures such as old 55-gallon drums, fuel tanks, girders, pipes and other very large metal structures used to support the theodolites that measured the altitude of the atmospheric nuclear tests conducted at Johnston. The disadvantage of using these structures is that embryos spawned on corroded metal have a range of developmental defects, are non-viable or are just lost because of the corrosion process (Kerr, 1996).

Additional spawning sites observed within the lagoon were also affected by or are a result of anthropogenic changes within the lagoon. The second largest aggregation of *A. sordidus* occurred along the east channel near buoy marker 14. The walls along the eastern channel were a result of the initial dredging of the channel and are cut through coral pavement. These walls provide many large and smooth surfaces for nest sites. In comparison, the walls of other channels in the atoll have a thick regrowth of *Acropora* corals, are not as steep as the eastern channel and do not provide the large clear areas necessary for *A. sordidus* spawning. The only location where all of the nest sites occurred on natural substrates was at East Island. However, East Island itself is completely artificial and it is not clear whether the dead corals on which the *A. sordidus* spawned grew secondary to the construction of the island or occurred naturally in that shallow part of the lagoon. The navy pier location on Johnston is also on the expanded portion of Johnston and all the *A. sordidus* nesting occurred on the pier pilings. The tug boat location has the sunken tug boat to serve as nesting sites but also appeared to have the only natural nest sites in the adjacent reef areas that were unaffected by dredging

In *A. sordidus*, competition for limited nest sites appears to restrict mating opportunities to the few males able to defend these sites. It is not known if the addition of artificial nest substrates increased the numbers of reproducing males or if it concentrated them into a smaller area. Male reproductive success was highly variable and limited to the nesting or territorial males. Males are known to return annually to the same nest sites (Stanton, 1985). An aging study of *A. sordidus* at Johnston found adult fish (SL of 120-160 mm; n = 25) were from 5 to 9 years old (Kerr et al., 1997). If males are able to control the most desirable nest sites over multiple spawning seasons, they will continue to dominate the reproductive output of the colony.

In nest tending species, territorial or bourgeois males gain access to females by investing in energetically expensive secondary sexual traits, elaborate courtship displays, competition for territories and nest sites, and by providing parental care (Taborsky, 2001). An alternative tactic used by small, non-territorial males is to steal fertilizations from the territorial males (Taborsky, 1994, 1998, 2001). The use of this alternative mating tactics is being investigated with molecular markers in this *A. sordidus* population. This tactic occurs often in teleost fishes when competition for access to mates determines reproductive success and successful individuals retain most of the mating opportunities (Dominey, 1981; Warner et al., 1995; Henson and Warner, 1997; Taborsky, 2001). Future investigations could assess the factors that distinguish differing qualities of nest sites and their attendant males. Some males were more successful in acquiring clutches. Determining if this differential mating success is caused by a quality found in the nest site such as defendability, nest size, location, or quality of the algae, would be important to determine as compared to the ability of the male to provide parental care.

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THE “CHORAL REEF”: THE ECOLOGY OF UNDERWATER SOUNDS

Phillip S. Lobel
Department of Biology
Boston University
Boston, MASSACHUSETTS 02215 U.S.A.
plobel@bu.edu

The sounds made by a variety of coral reef fishes were reviewed using audio-video recordings of the Central Pacific (Johnston Atoll), Western Pacific (Palau, Tahiti, Pohnpei), Eastern Pacific (La Paz, Mexico), the Caribbean (Jamaica, Belize) and of cichlids in a freshwater lake (Malawi). Movies of fishes producing specific sounds with behavior included anemonefishes, Domino damselfishes, spotted boxfish, butterflyfish, toadfish, squirrelfish, groupers, snappers and cichlids. The recorded behavior and synchronous sounds of these fishes involved courtship, spawning, aggression, swimming and feeding. The theme of this review is to emphasize the importance of the underwater “soundscape” and the role of acoustics in fish behavior. Exemplars of diver and boat noises raise the concern of the unknown impacts of underwater noise pollution.

Introduction

The soundscapes of the ocean have been under-appreciated for far too long. The notion that the sea was a "silent world" was conveyed early in the history of scuba diving (Cousteau and Dumas, 1953) and the idea has persisted. This perspective was reinforced by the fact that human hearing is poor under water and that the sounds of many fishes are not "easily" heard. Scuba divers are especially disadvantaged for hearing under water because of the near constant stream of noisy bubbles running over their ears (Lobel, 1992; 2001). The noise of scuba disappears, of course, when using “rebreathers” and this advanced diving technology is a huge advantage for conducting acoustic research on wild fishes (Lobel, 2001; 2005).

There is now a clear pattern that a wide variety fishes produce specific courtship and spawning sounds (see review by Lobel et al., 2010) and that a coral reef can also be described as a "choral reef". The total number of fish families that occupy coral reefs and adjacent marine habitats is approximately 179. Out of this total there are 48 families (27% of total 179) represented by about 1,282 species in 137 genera that are now known to produce sounds (Lobel et al., 2010). Many more sonic species will surely be discovered as more scientists use rebreathers and are equipped with the proper camera gear for recording.

Underwater research

Research on underwater sounds and its ecological role has accelerated in recent years as the result of several scientific and technical developments. First, new technology in the form of camcorders, hydrophones and computer software has made the task of quantifying underwater animal sounds and behavior much easier (Kovitvongsa and Lobel, 2009). Second, discoveries that fishes produce species-specific courtship and spawning sounds opened the feasibility for the development of passive acoustic monitoring for documenting reproductive patterns (Lobel, 1992, 2001, 2002, 2005; Mann and Lobel, 1995; Rountree et al., 2006; Luczkovich et al., 2008). Third, loud noises from ships, sonars, seismic surveys and global climate experiments such as Acoustic Thermometry of Ocean Climate (ATOC; http://atoc.ucsd.edu) have raised real concern about potential adverse impacts of loud underwater sounds on marine animals (McCauley et al., 2003; Popper et al., 2003; Popper and Hastings, 2009; Lobel, 2009). Fourth, new research is showing that larval reef fishes may be using the sounds emanating from coral reefs to navigate during their migration from the open ocean to benthic habitat (Simpson et al., 2004; Leis and Lockett, 2005; Mann et al., 2007; Radford et al., 2008). A review was recently published that emphasized the concern about noise pollution in the ocean and its impact on fish behavior (Slabbekoorn et al., 2010). While we are now beginning to determine if and which species make purposeful sounds with behavior, adverse ecological impacts may already be happening.

Knowing that fishes make noises is nothing new. Reports that fishes produce sounds date back to Aristotle in 350 B.C., who described that fishes "emit certain inarticulate sounds and squeaks." Aristotle also wrote that "the apparent voice in all these fishes is a sound caused in some cases by a rubbing motion of their gills which, by the way, are prickly or in other cases by internal parts about their bellies; for they all have air or wind inside them, by rubbing and moving which they produce the sounds" (Wentworth Thompson, 1910). Darwin was also keenly aware of fish sounds known to him primarily from the work of Dufossé (1858-1862; Pauly, 2004). Dufossé reported that some fish sounds could be voluntarily produced (by pharyngeal bones and swim bladder vibration). Darwin speculated that these sounds, like in insects, could play a role in sexual selection (Pauly, 2004).

Hearing in fishes is an early evolutionary development and most likely preceded active sonic behavior. The inner ear morphological structure of 3 semi-circular ear canals and basic sensitivity to a range of low- to mid-frequency sounds is an evolutionary innovation that is common to the gnathostome fishes (Lauder and Liem, 1983). The classic experiments by Karl von Frisch (1938) demonstrated that fish could hear. While living fishes, so far as known, maintain their hearing abilities there are many examples of fishes that have lost their sight suggesting the overall importance of the auditory sense. Sound producing mechanisms in fishes are highly varied and derived from diverse morphological adaptations. However, not all fish families with sonic species share sound producing abilities. Some fish taxa apparently remain mute (Hawkins and Myrberg, 1983; Ladich, 2000). The emerging notion is that hearing evolved primarily as a mechanism for monitoring the ambient acoustic soundscape with particular regard to detecting predators or potential prey (Ladich, 2000). Some species with advanced hearing specializations are particularly vulnerable prey species such as freshwater ostariophysians (Ladich, 2000) and marine clupeids (Mann et al., 1997). Furthermore, sound production may attract predators and some species have been found to go immediately silent when predators are detected (Luczkovich et al., 2000). Research is showing that many fishes produce courtship associated sounds and that these sound patterns are distinct and consistent in structure (see review Lobel et al., 2010). While it is no longer argued whether or not fish can hear, the level at which fish 'communicate' using acoustic signals is still relatively unknown.

Many fishes are sonic but this is not yet a particularly well-studied aspect of their behavior. The obstacle has been a combination of technology limitations for underwater recording and the fact that many fishes appear to be very discrete about where and when they produce sounds. Sound production is associated most often with reproductive activities but the courtship and mating behaviors of many fishes have been elusive. Consequently, fish sonic behavior is poorly known. Sonic behavior is often aimed discretely at nearby prospective mates. Only a few fishes make sounds loud enough to be easily heard without the aid of a hydrophone. The challenge for scuba diving scientists is to dive stealthfully, optimally by using a rebreather unit, and to record fishes engaged in natural behavior. Behavior is best documented using a camcorder-hydrophone system that synchronously records both audio and video (Lobel, 2001, 2005; Figures 1 and 2).

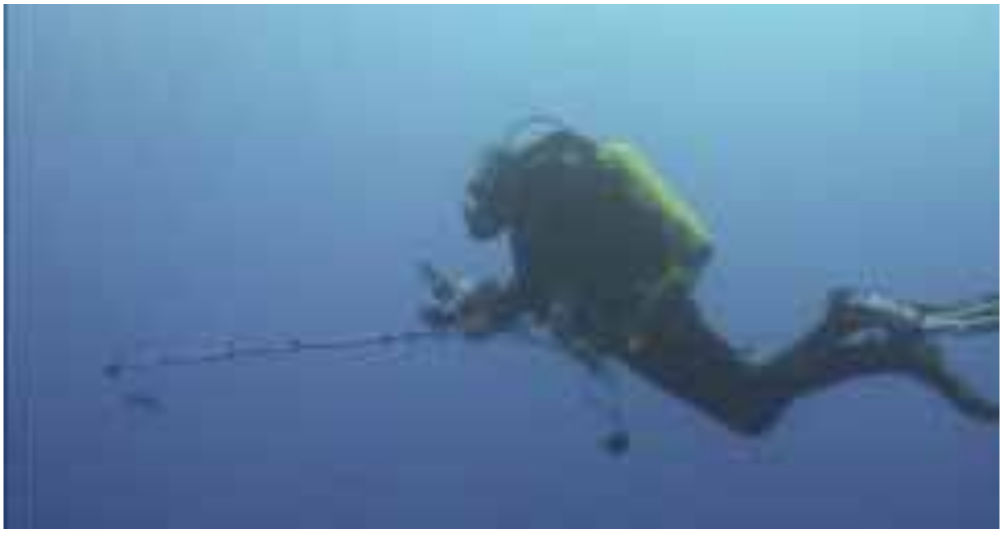

Figure 1. The author using the Evolution Rebreather and a customized camcorder in a Gates underwater housing coupled with a electronically matched hydrophone on a boom (Photo by Lisa Kerr Lobel, Belize, June 2009).

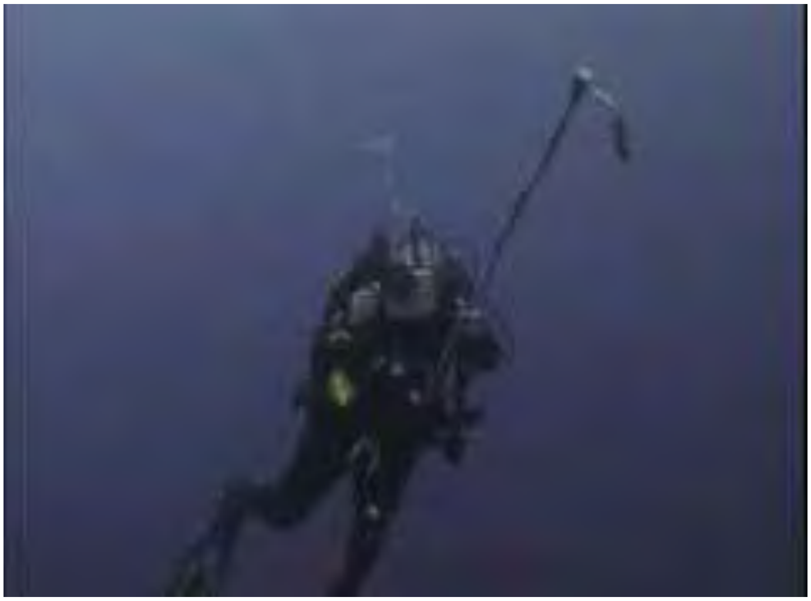

Figure 2. The author invented the first generation of this camcorder-hydrophone system in 1988 and recorded spawning fishes in Jamaica (see Lobel, 1992, Photo by Lisa Kerr Lobel, Belize, June 2009).

Environmental background sounds can be both biologically and ecologically meaningful as well. Background noises will create the ambient interference that can limit a fish's ability to discriminate sounds. Hydrodynamic sounds of fishes swimming can be detected by conspecifics and by potential predators. The mechanical sounds of marine animals disturbing substrate can be an alert signal to others around. Adventitious sounds such as scarids grinding food can signal competitors that there is a food source available. The ecology of sound has ramifications in how the fish behaviorally use it. The key issue here is the balance between the need to communicate and the risk of being overheard by a predator.

The concern is that noise pollution can mask these signals or otherwise disrupt fish spawning or other behaviors.

Relevance of acoustics

Acoustic signals are involved in much of the social and reproductive behavior of birds, insects and anurans, and the meaning and behavioral influence of sounds has been extensively studied in these terrestrial groups. In contrast, research on the acoustic communication of fishes has primarily focused on the description of sounds, sonic morphology and definition of the contexts in which sounds are produced (Rosenthal and Lobel, 2006). Progress in the field of fish bioacoustics has lagged behind that of terrestrial systems because of limitations of technology and the logistical difficulty of research in the underwater environment. Recent advancements have provided better tools to successfully conduct such studies and the number of fishes discovered making sounds has grown enormously in the last few decades (Lobel et al., 2010). Although it is now known that many fishes make sounds, only a limited number of studies have experimentally examined the behavioral significance of these sounds by using audio playback (see Lobel at al., 2010 for review). To bring the field of fish bioacoustics forward and to draw closer to the level of understanding achieved in acoustic behavioral studies of terrestrial taxa, experimental evidence that sound is behaviorally relevant to fishes is the next necessary step in this research.

One significant application for using fish sound is for tracking where and when fish spawn (Lobel, 1992, 2001, 2002, 2005; Lobel and Mann, 1995; Mann and Lobel, 1995; Rountree et al., 2006; Luczkovich et al., 2008). The scientific and technical challenge has been to develop methods that allow measurement of fish reproduction synchronously with time-series measurement of temperature, salinity and other physical oceanographic variables that are easily recorded using modern devices. If we can associate specific sound patterns that are exclusively correlated with specific fish species and behaviors (such as the courtship call of a cichlid or the mating sound of the hamletfish, e.g., Lobel, 2002) then we can develop listening devices to document their occurrence in time and space. A passive acoustic detection device that is programmed to monitor fish courtship and mating sounds becomes a "spawn-o-meter" that records data that is comparable to data from a current meter, salinity and temperature loggers at the same site. This will yield the datasets necessary to evaluate how climate change (sea temperature, salinity, flow, etc) may offset the timing and locations of fish reproduction. Overall, it is clear that being able to monitor fish mating activities is an essential tool for fisheries and conservation. The application of the "spawn-o-meter" for fisheries and conservation is that it can be used to: 1. define important breeding habitats, which is important to fisheries management and relevant to establishing protected areas; 2. establish relationship between physical oceanography and the timing of fish reproduction; and, 3. define a critical endpoint measurement in pollution studies where the courtship vigor of fish can be related to its health and fitness (Lobel, 2001).

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STALKING SPAWNING FISHES

***Phillip S. Lobel*[1]**
***Lisa K. Lobel*[2]**

[1]Boston University
Department of Biology
Boston, MASSACHUSETTS 02215 U.S.A.
plobel@bu.edu

[2]Wheelock College
Math and Science Department
Boston, MASSACHUSETTS 02215 U.S.A.

It is necessary for essential fish habitat to include the actual spawning area chosen by fishes for mating. These locations are sometimes long-term territories while other fishes choose transient sites used only when mating. The modes of reproduction among fishes range from having defined nests of benthic embryos to the random release of gametes in the water column where the embryos float and disperse in ocean currents. The spectacular annual mass spawning behavior of snappers and groupers is hypothesized to occur at locations where the underwater topography creates special flow conditions resulting in the favorable advection of embryos. The sites where fishes spawn are critical to their life history and must be included when considering the designation of marine protected areas. The key method in defining the locations of spawning sites is the direct observation by scientists of where, when and how different fishes engage in the reproduction ritual. This requires skilled scientific diving (preferably with rebreathers) and documentation by stealthy photography/videography. The first scientific challenge is knowing where and when to dive. The second challenge is to gather sufficient data, especially video, to define a species' reproductive behavior. This paper is a review of where, when and how a variety of coral reef fishes spawn.

Introduction

Much of the fascination we have with animals concerns their many intriguing courtship and spawning rituals. There is no mystery as to why animals reproduce but learning where, when, and why is sometimes challenging. Specialized knowledge of breeding habits is of interest both to amateurs who might enjoy raising aquarium fishes and to naturalists who are simply pleased by learning more about their favorite species. For scientists, knowing breeding habits is essential and forms a fundamental basis on which to develop effective management plans and species protection policies.

There is still a great deal to be discovered about the reproduction habits of coral reef fishes. The popular damselfishes (Pomacentridae), wrasses (Labridae), and parrotfishes (Scaridae) are best known. The spectacular spawning aggregations of grouper (Serranidae) and snapper (Lutjanidae) have been subjected to intense overfishing and are actively being studied by scientists and conservationists (Sadovy de Mitcheson et al., 2008; the Society for the Conservation of Reef Fish Aggregations). Other fishes are more secretive with regards to their reproductive habits – at least to the human observer. Despite numerous studies by scientists and hours of observation by sport divers, the reproductive behavior of most reef fish species remains unknown.

A partial explanation for why we have seen so little lies in our habits and not that of the fishes. Most human activities, including scientific investigation and sport diving, peak during the brightest times in the day. By dusk we settle into the comforts of home and while most of us are having cocktails and eating dinner, many reef fish are spawning.

This paper describes the reproductive behavior of a few selected fishes. The objective is to illustrate some of the diverse spawning behaviors of reef fishes and to describe what conditions to look for when exploring an underwater observation site.

Methods

A major difficulty in observing courtship and reproductive behaviors is the fishes' skittish nature. The sudden appearance of a scuba diver or a large fish can cause some fishes (but not all) to temporarily cease spawning activity (Lobel, 1978; Lobel and Neudecker, 1985; Heyman et al., 2010). The noise created by bubbles from open-circuit scuba often scares fish and may interfere with sound production and reception during courtship (Lobel, 2001, 2005, 2006). Use of rebreathers is the key method to silently and unobtrusively observe behavior. To reduce the influence of a diver's presence on spawning activities it is sometimes necessary to be in position, motionless, at least 30 minutes before courtship begins.

Results

The following case studies are provided as examples to illustrate the diverse spawning behavior of reef fishes.

Spawning Behavior of the Pygmy Angelfish, Centropyge potteri (Hawaii)

Centropyge potteri spawn predominantly at dusk. They are not alone for several other reef fishes also spawn at this time including butterflyfishes, surgeonfishes and goatfish (Fig. 1). *C. potteri* can be found within the 3 to 50 meter depth range both on patch reefs and in the vast expanse of coral and rubble of fringing reefs. It is one of the ten most commonly seen fishes in this zone.

Reef structure has an important effect on the mating organization of *C. potteri*. On patch reefs males are able to control a well-defined territory and the dominant male excludes other males aggressively. Consequently, there are two to four *more* females per male on patch reefs than in fringing reef habitat. On fringing reefs, where many territories are available, each male pairs with a single female. Males on patch reefs can, therefore, spawn more frequently than males on fringing reefs.

The most important aspect of the pygmy angelfish territory appears to be a towering rock or coral promontory over which the fish can spawn. The fish are particularly vulnerable when spawning and a high knoll may provide the nearest refuge for the fish as they spawn above the reef. If these fish are kept in an aquarium, a similar habitat should probably be constructed to provide the fish with a natural setting.

The following generalized description of *Centropyge potteri* spawning is based upon thirty-one observations of pair spawning and seven harem spawnings (Lobel, 1978a). Courtship was initiated by the male who ceased feeding and began courting about one hour before sunset. The male swam toward a female in a vertical undulating manner unlike his normal swimming motion. He stopped above her and erected all median fins while fluttering the pectoral fins. This display continued as the male drifted slowly upward with his head diagonally up or with his side parallel to the substrate. If the female failed to follow, the male halted immediately and darted back to her. The male continued courtship by swimming around the female in an undulating fashion. In this way, the male approached the female as he swam forward while rising and falling in a swooping, fluttering motion. Courtship continued until the female was enticed over to the prominent coral or rock tower and she rose above the tower with male. This usually required only a few courting passes. Spawning occurred only over the tallest coral or rock in the immediate area. During courtship and spawning the overall blue color of both fish paled as the red color intensified (Fig. 2).

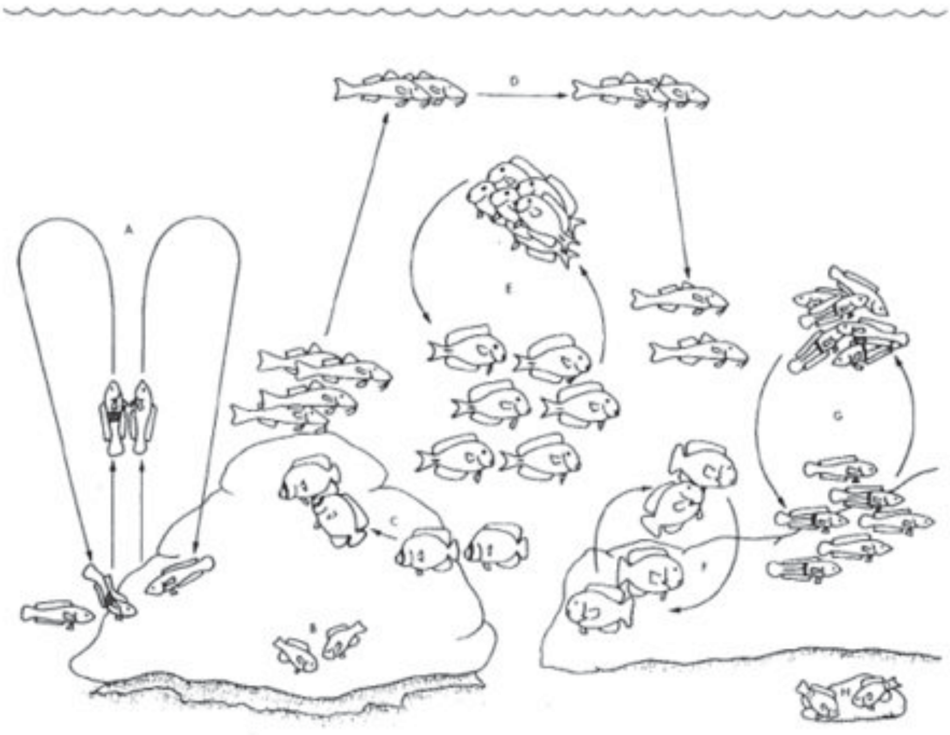

Figure 1. Reef fishes during courtship and spawning. A. Secondary male *Thalassoma duperreyi* spawns with a single female. B. *Dascyllus albisella* display courtship above the reef and spawn on the reef substrate. C. Chaeotodontids may travel in groups but they spawn in pairs. D. Goatfish display courtship in groups above the bottom and rise in pairs to release eggs and sperm high the water column. E. Acanthurids travel in schools and rush up a few meters to spawn as a group. F. Pomacanthids court near the bottom and spawn about one meter above the bottom. G. Primary male *Thalassoma duperreyi* spawn with females in groups. H. *Chromis ovalis* and *C. verator* spawn in pairs, laying eggs in nests at the fringes of the reef.

During the first few encounters, the female rose up with the male and then darted back to cover when the male attempted to move to the spawning position. The male pursued her while continuing his courtship display. Spawning climaxed when the female remained in mid-water about a meter above the coral/rock tower. The male approached from underneath and appeared to press his snout against her abdomen (Fig. 2); such contact may signal or facilitate egg release. A single burst of eggs was broadcast. Immediately after the release of eggs and sperm the pair darted to cover with the female chasing the male, apparently nipping at his caudal fin. Chasing by the female occurred only after consummation of the spawning act and not during earlier episodes of courtship. All spawning activity concluded before sunset and the fish retired into a cavity in the reef. During courtship these fish produced various sounds like clicks and grunts although the precise role for this during reproduction is not yet known.

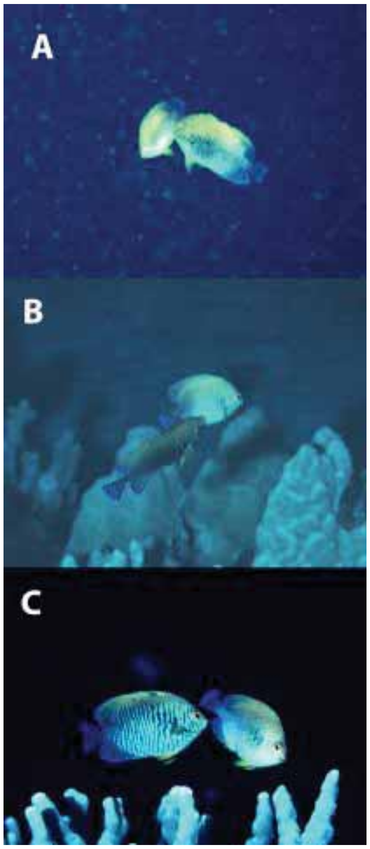

Figure 2. *Centropyge potteri* spawns in pairs although a male may have a harem and spawn sequentially with each female. Females have been observed spawning only once per evening. The male nuzzles the females abdomen as they float upward over the reef (A, B, C). Once gametes are released they dart back to cover.

The Caribbean rock beauty angelfish, *Holacanthus tricolor,* displays similar spawning behavior (Fig. 3). Further details and information are presented in Lobel (1978 a, b) and Neudecker and Lobel (1982).

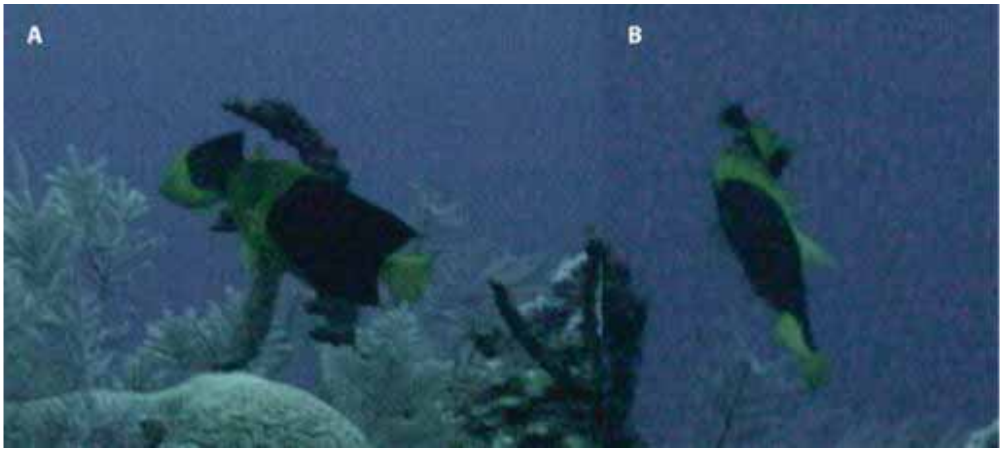

Figure 3. The Caribbean rock beauty, *Holacanthus tricolor*. Males are larger than the females. During mating behavior, the males develop a dusky mask coloration on their head. During spawning, the male nuzzles the female's abdomen in the same way as seen in *Centropyge potteri* in Hawaii.

Spawning Behavior of the Butterflyfish, Chaetodon multicinctus (Hawaii)

The reproductive behavior of *C. multicinctus* appears to be typical for the chaetodontids. Although more than two fish were present during the observed spawnings of *C. multicinctus*, only pairs engaged in courtship and initial spawning bouts (intruders will rush in at last minute, below). However, *C. unimaculatus* and *C. fremblii*, are only found in pairs during spawning. Aggregations of spawning *C. multicinctus* consisted of four individuals on four occasions and of three on two occasions (Lobel, 1989). One group of four *C. multicinctus* apparently included at least one female and two males (Fig. 4).

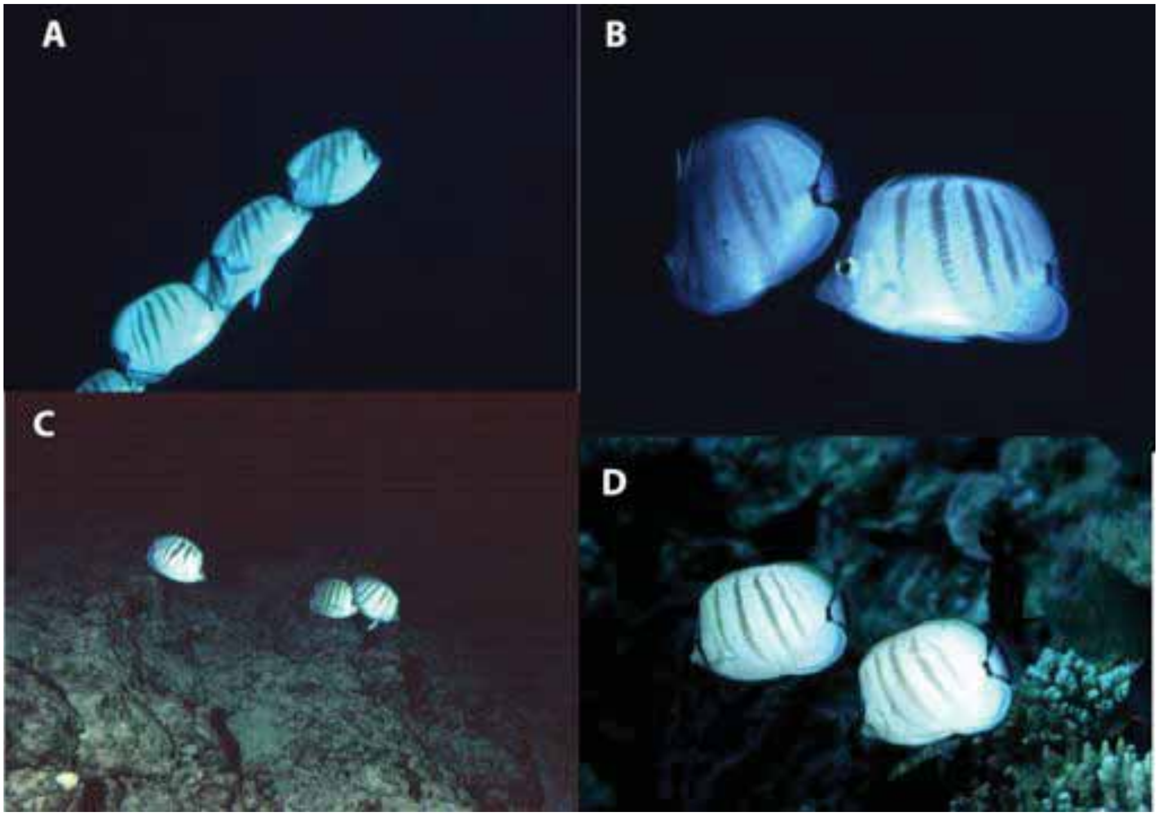

Figure 4. The Hawaiian banded butterflyfish, *Chaetodon multicinctus*, is found in pairs on the reef during the day (A). During evening this pair will begin courtship behavior and spawn (B) but other intruders will attempt to sneak in too (C, D).

Females, obviously swollen with roe, led the group or pair as they swam across the reef (Fig. 4). Courtship among these butterflyfish was less elaborate that than among *C. potteri*. As the butterflyfish swam along the reef, the female tilted her head slightly downward as she continued in front of the male. The male swam from behind and up alongside of the female. As the male reached the female and placed his snout on her abdomen, both fish quivered releasing the eggs and sperm. In one case, a male *C. multicinctus* approached a female three times in this fashion before spawning occurred. The butterflyfish spawning posture was strikingly similar to that described for *Centropyge potteri*. Again, the nuzzling by the male may signal the female to release eggs. *Chaetodon multicinctus* and *C. unimaculatus* ascended approximately a half-meter above the reef substrate.

The longnose butterflyfish, *Forcipiger flavissimus*, was observed performing the same spawning movements although actual spawning was not witnessed. The female's swollen belly with eggs indicates these fish were ready to spawn (Fig. 5). Further details and information are presented in Lobel (1978, 1989), and Neudecker and Lobel (1982).

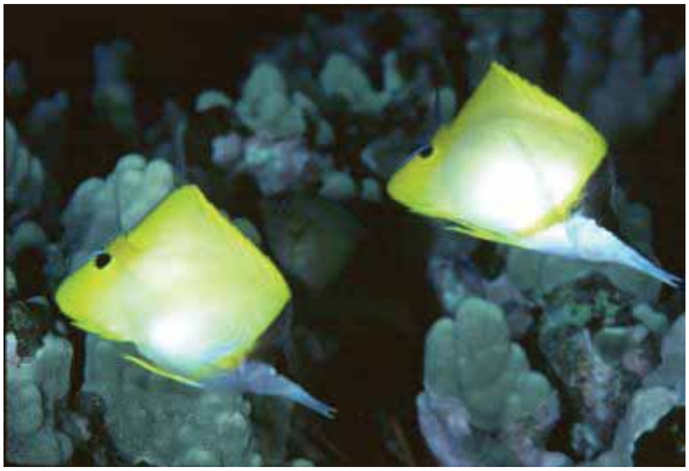

Figure 5. The longnose butterflyfish, *Forcipiger flavissimus.* The female's belly swollen with eggs (lower left) indicates these fish were ready to spawn. Sometimes this is the only means by which to distinguish sexes.

Spawning Behavior of Hamlets, Hypoplectrus species (Caribbean).

The mating behavior of the hamlets is one of the most intriguing to scientists. These fish are simultaneous hermaphrodites; they maintain a pair bond and when they spawn they switch roles as male or female between spawning bouts. These fish are even more perplexing as there are several sympatric species on a reef and although very closely related and capable of hybridizing, they generally don't. Lobel (2011) presents an introduction to the literature on Hamlets and an assessment of the species question. Hamlets are amazingly easy to observe spawning during evening twilight and they are generally resilient to disturbances by divers (Lobel and Neudecker, 1985).

Our observations of hamlet behavior provided the following general picture of spawning in *Hypoplectrus* species. Hamlets mate only in pairs; fertilization is external and zygotes are planktonic. During the daytime, mates appear loosely associated in adjacent or nearby home ranges. They begin to interact within 2 hours of sunset. They first rendezvous, remain close together, and engage in occasional short chases. The spawning act begins when one fish presents a lateral display while flicking its pelvic fins. The displaying individual slowly rises while continuing its display; the color of the leading fish fades. The two then rise together about 1 m above some towering reef structure. At St. Croix, these structures were vertical colonies of the gorgonian *Pseudoplexaura* sp. or the coral *Acropora cervicornis*. Once the proper height (about 1-2 m) is attained over a tall reef structure, the following fish folds around the pale colored leader who poses head down in an S-shaped position (Fig. 6). Release of eggs and sperm occurs as both fish quiver; quivering lasts for 2-3 sec. The individual folded around the other often opens its mouth while quivering. Following consummation, the fish cease embracing and quickly descend to the bottom. The embracing behavior of hamlets when spawning has been termed the spawning clasp (Fischer, 1980) and occurs many times throughout an evening. These fishes are simultaneous hermaphrodites and take turns releasing eggs and sperm in subsequent spawning bouts. Infrequently, fish would embrace but not quiver and the release of gametes was not evident. Each pair of hamlets initiated spawning at a specific reef location each evening. Pairs showed strong spawning site specificity. They moved between a few (from 1 to 4) particular sites each evening, and frequently a pair returned to their original site. When some pairs moved between spawning sites, other individuals occasionally interfered and attempted to steal a mate (Lobel, 1992; Lobel and Neudecker, 1985).

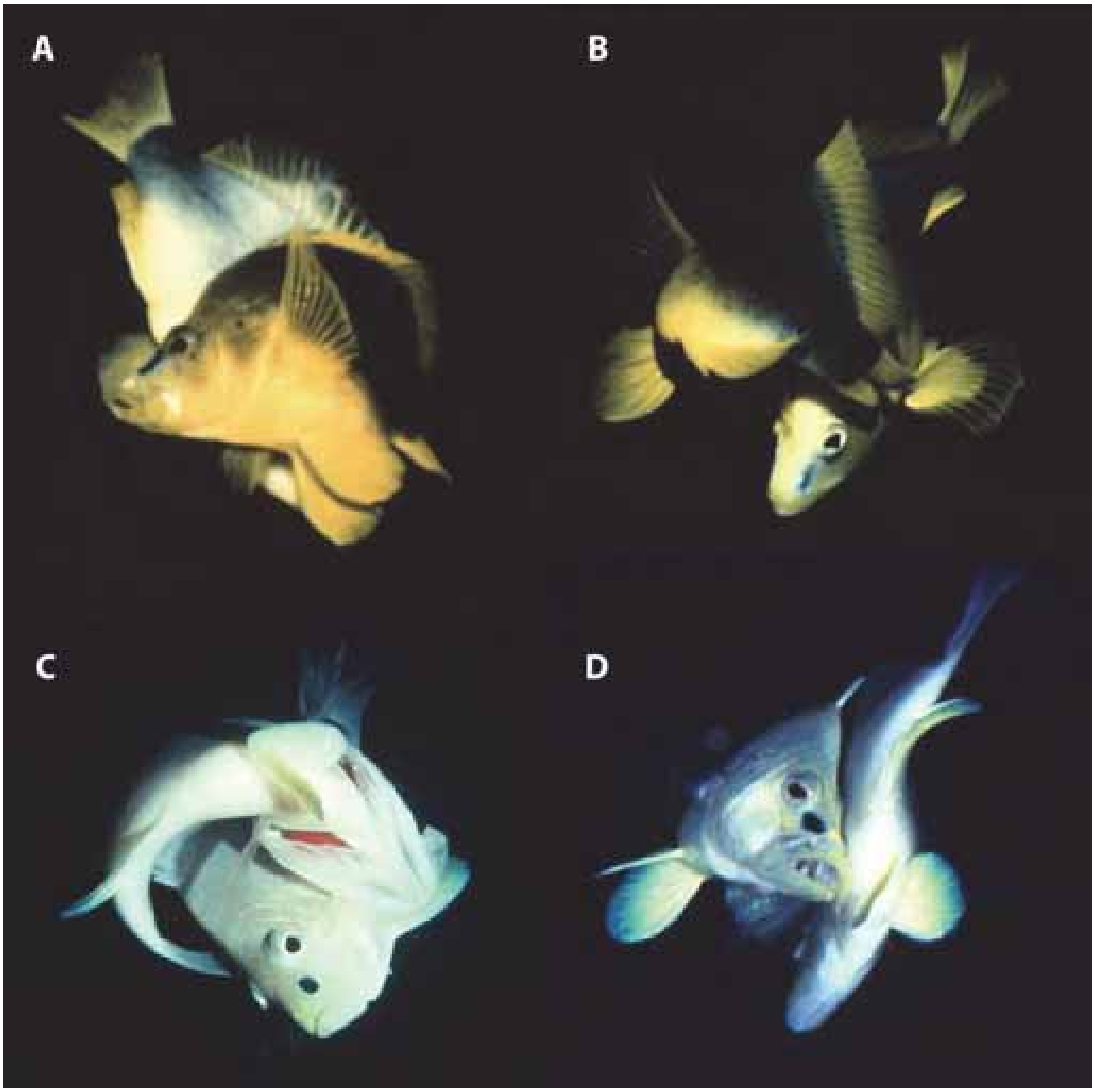

Figure 6. Spawning posture of the hamlet fishes. The mating game in hamlets is complex as these fish are simultaneous hermaphrodites. The fish releasing male gametes wraps around the fish releasing female gametes (with head down). This overall posture provides the pair with a view in all directions to see potential predators. The hamlet in A and B is *Hypoplectrus guttavarius* and in C and D is *H. unicolor.*

Spawning Behavior of the Boxfish, Ostracion meleagris (Johnston Atoll)

Ostracion meleagris is found typically in groups consisting of a male and several females. In some areas populations are large and several males co-occur and compete for mates. Dominant males will actively interfere with and disrupt the courtship and spawning of other males. Competition includes active jousting with two males facing one another and then ramming together to produce a "bump" sound.

A male courts a female by displaying and swimming around her. During this he nudges her side and back repeatedly. The pair rises together in the water column at least 2 m above the bottom but frequently higher. Spawning occurs when the pair is side-by-side, tails together and heads facing slightly apart (Fig.

7). The spawning sound of *O. meleagris* was produced only when a pair was in the mating position and releasing gametes. The sound is of long duration (about 6.3 s) with peak energy at 215 to 270 Hz (Lobel, 1996).

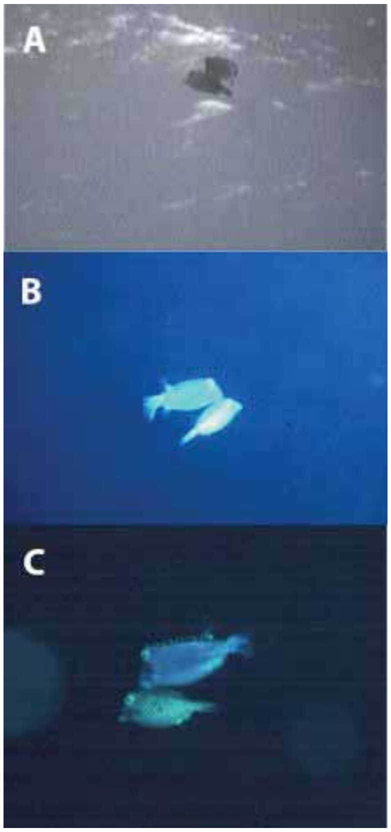

Figure 7. The trunkfish, *Ostracion meleagris*. The male is on top of the female (A, B, C) as the pair ascends near to the surface when spawning.

Group spawning and Aggregations (Caribbean and Pacific)

Many species spawn in large groups that rush upwards into the water column. The earliest observations of such group spawning were of parrotfishes (Randall and Randall, 1963). Parrotfishes and wrasses spawn by gathering together near the reef and then making rushes upward in groups which rise a few to several meters above the bottom to release gametes (Fig. 8). However, as illustrated in Figure 1, the reproductive strategies of some wrasses also involved pair spawning (e.g., Warner, 1984).

A spectacular example of spawning behavior is the annual spawning aggregations of groupers and snappers. Spawning aggregations are defined as temporally- and spatially-limited gatherings of massive numbers of fish for the purpose of spawning (Domeier and Colin, 1997). The aggregations last for less than three months and usually occur near a significant topographic feature on the reef such as a channel or seamount and can be single species or multi-species. These aggregations of large fishes releasing their gametes create a milty soup in the water that is so thick that visual cues are of no use. Large predators use this to their advantage to prey on the spawning fish (Graham and Castellanos, 2012; Fig. 9 D, E). For detailed description of the spawning aggregations of the grouper *Epinephelus striatus* see Colin (1992) and Whayland et al. (2004). Snapper spawning aggregations and the behavior of whale sharks that feed on the spawn has been well described previously (Heyman et al., 2001, 2005, 2010); the mass spawning behavior of these large fishes is illustrated in Figure 9.

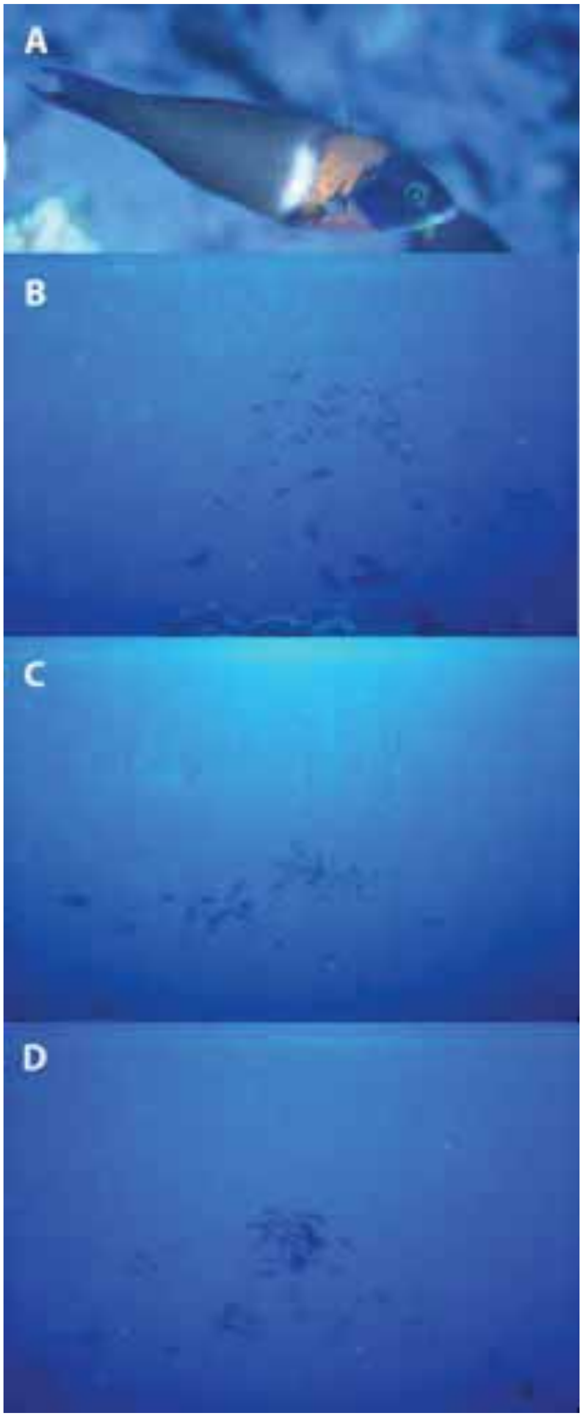

Figure 8. Wrasse group spawning. *Thalassoma duperryi*. A. Supermale; B. Fishes loosely gathering prior to spawning rush; C. The fish group tightens immediately before the rush; D. The fish rush upward in a tight group and release gametes a few to several meters above the bottom.

Discussion

The timing of reproduction among reef fishes reflects adaptation to a variety of ecological factors. Diel (the 24-hour cycle) periodicity appears to be influenced primarily by balancing of the threat of daytime planktivores feeding on eggs and newly hatched larvae and crepuscular (dusk and dawn) predators attacking spawning adults. Spawning at dusk reduces the risk to the eggs and newly hatched larvae but it is the time of peak predation upon the adult fishes. Lunar reproductive synchrony may further reduce the probability of predation on individuals by creating a swamping effect. Essentially an individual fish is safer if it is among a group of other individuals doing the same thing at the same time. The moon may act as a signal to the general population and thus synchronize their behavior.

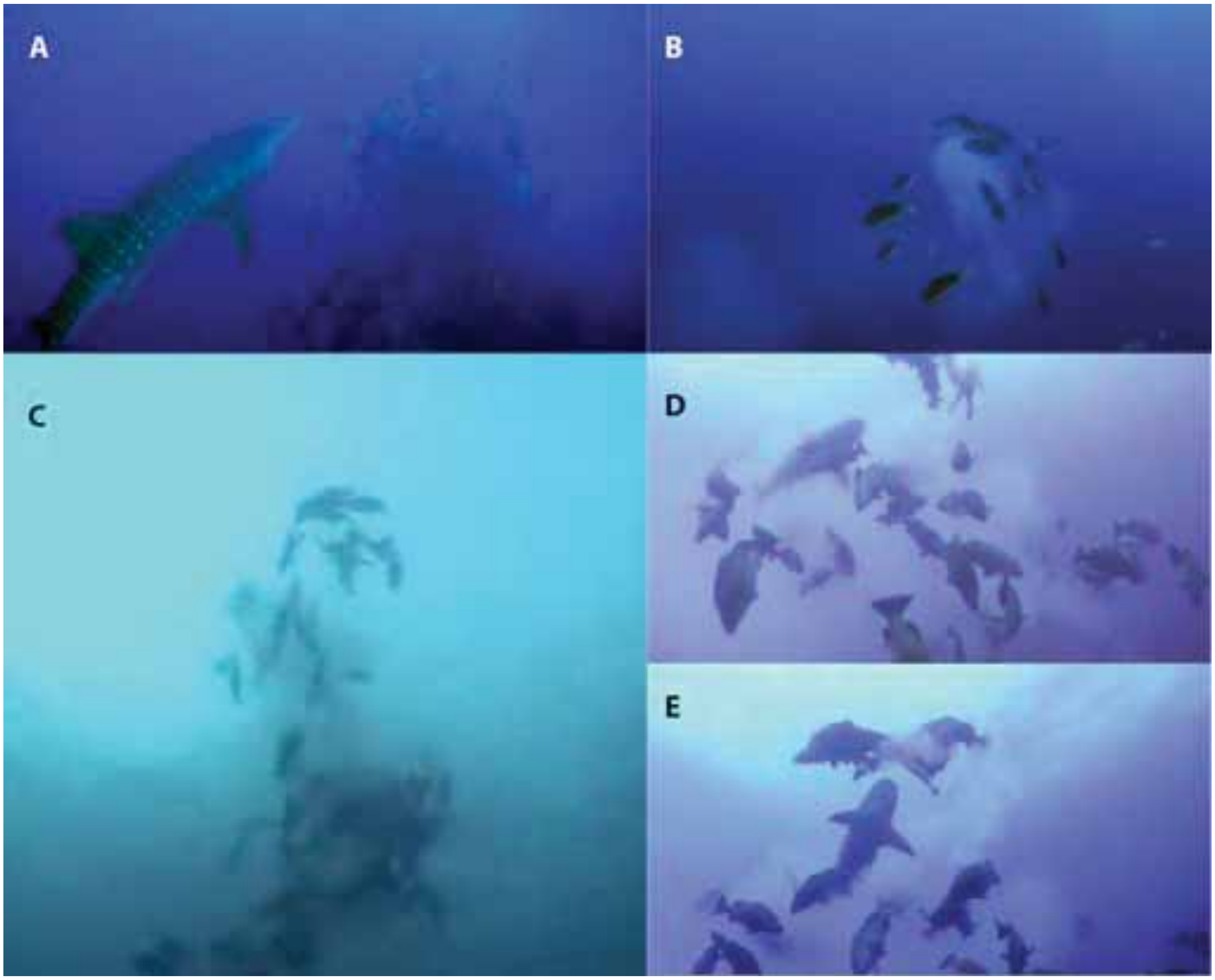

Figure 9. Scenes of mass aggregation spawning. A. Belize, Cubera snappers (*Lutjanus cyanopterus*) release gametes as a whale sharks approaches to feed on the embryos; B. Nassau grouper (*Epinephelus striatus*) spawning, Little Cayman Island; C. Dog snapper (*Lutjanus jocu*) spawning in Belize; D and E. Cubera snapper (*Lutjanus cyanopterus*) spawning attraction of a bull shark (*Carcharhinus leucas*).

Diurnal planktivores are adapted to plucking small prey from mid-water while nocturnal planktivores, such as apogonids and some holocentrids, possess large mouths and feed mainly on larger plankton-prey. By dusk most diurnal planktivores have descended to the reef for the night. Spawning at dusk may reduce the probability of eggs being eaten in two ways: (1) there are few active planktivores, and (2) any planktivores still active may be satiated quickly by the bounty resulting from the simultaneous spawning of many fishes. In addition, an outgoing tide during this time of spawning may aid in sweeping eggs and larvae from the reef and from the grasp of still-active planktivores.

The reproductive activity of broadcast-spawning species was not evident in reef areas where there were planktivores or other species spawning. The avoidance response to planktivores by spawning fishes has also been reported for fishes elsewhere. Twilight is a visually difficult time for most reef fishes and this is when predation peaks. It may be possible that low light levels from moonlight and the remaining sunlight just before sunset is sufficient for fishes to recognize mates, spawn and avoid potential predators, but not enough for planktivorous fishes, requiring high visual acuity, to continue foraging.

Spawning at dusk, however, may increase the potential for predation upon the adults by crepuscular piscivores. This time of peak activity for many reef predators and fishes would temporarily cease activity when a larger predatory fish or diver approached. Thus, while eggs may be minimally exposed to the likelihood of being eaten at dusk, adults may risk a greater potential threat from predators.

The threat from predators may explain in part why a prominent, towering reef structure is important in the home range of many species. While spawning in mid-water they are still close to a reef shelter to which they can seek refuge between encounters, after spawning and when disturbed by other fishes. The importance of such a towering coral/rock is evident on a patch reef where male *C. potteri* controlled a limited area and, consequently, access to resident females, while pairs were predominant on extensive reefs. Since females spawn only once a night, males on patch reefs spawn more often than males on extensive reefs.

Not all fishes, however, spawn at dusk. Fishes that build nests or produce many hundreds of eggs can spawn during the daytime. For example, damselfishes protect their nests vigorously from all potential egg-eating fishes. Damselfishes usually spawn during the early part of the day, but once the eggs hatch they are no longer protected by their parents (Lobel and Lobel, 2013To avoid being eaten by planktivorous reef fishes the eggs hatch predominantly after sunset (Robertson et al., 1990). This is also true for some triggerfishes that build nests (e.g. Lobel and Johannes, 1980).

Learning how and where fishes spawn and how many species follow similar patterns will lead to understanding which environmental factors, such as currents and other seasonal oceanographic variables, have shaped the behavior of fishes in so far as it impacts offspring survival. This is one aspect of fish life histories that must be known as a basis for realistic predictions of how future climate change may impact fish populations.

Acknowledgements

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BASELINE SIZE-STRUCTURE SURVEYS OF REEF-FISH POPULATIONS EXPLOITED BY A PAPUA NEW GUINEAN SUBSISTENCE COMMUNITY

***Ken Longenecker*[1]**
***Ross Langston*[1]**
***Richard Pyle*[1]**
***David Pence*[2]**
***Simon Talbot*[3]**

[1]Bishop Museum,
1525 Bernice Street
Honolulu, HAWAII 96817 U.S.A.
klongenecker@bishopmuseum.org

[2]University of Hawaii Diving Safety Program
2040 East-West Road
Honolulu, HAWAII 96822 U.S.A.

[3]University of Tasmania and Tasmanian Aquaculture and Fisheries Institute
Private Bag 5
Hobart, TASMANIA 7001 AUSTRALIA

As part of a larger effort to promote environmental conservation at Kamiali, Papua New Guinea, we used closed-circuit rebreathers and laser videogrammetry to describe the size structure of exploited reef-fish populations under traditional marine tenure. We generated size data for 33 species and compared average lengths to published estimates of maximum length and size at maturity. A typical individual in the exploited reef-fish community is 52% of its maximum length. In the subset of species for which size at maturity is known, a typical individual is 99% of female reproductive size. This baseline information will be useful in guiding future conservation efforts and evaluating their effectiveness.

Introduction

The Kamiali Wildlife Management Area, encompassing 32,000 ha of terrestrial habitat and 15,000 ha of adjacent marine environment, was established in 1996 by a village of approximately 600 residents who hold customary tenure over its natural resources. Rather than exploiting their environment for the short-term financial gain of logging or mining, which have had tragic environmental and social impacts elsewhere along the Huon Coast, the village chose to preserve a traditional subsistence lifestyle. Although the wildlife management area provides food and materials for housing and canoes, residents need cash for medicine and education; Kamiali residents contract malaria on average of about twice per year (National Fisheries Authority, 2007), and the national government pays for schooling only through sixth grade.

In 2007, to promote environmentally sustainable economic development, village leaders signed a memorandum of understanding with Bishop Museum to develop a remote research station at Kamiali Wildlife Management Area. This initiative is a self-sustaining model for environmental conservation:

continued protection of natural resources is promoted by economic compensation, this compensation is funded by research activities, and researchers will be drawn to the well-managed natural environment (Fig. 1). Researchers hire villagers for field support, are charged reasonable research fees for access to the area, and pay for room and board. Meals are prepared from locally grown or caught food, thus providing income to individuals with surplus from their gardens or fishing trips. The model thus establishes a link between economic benefit and environmental conservation, and provides a strong incentive for villagers to protect their land and water in perpetuity.

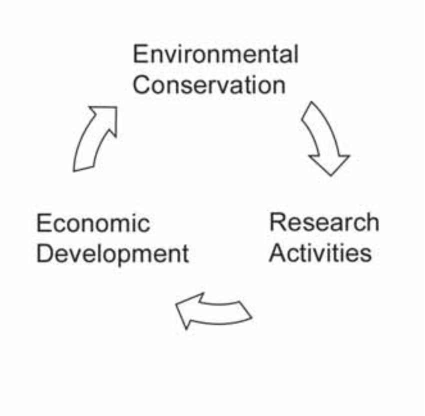

Figure 1. Conceptual illustration of the conservation model at Kamiali Wildlife Management Area.

Exploitation of coral-reef fishes may represent the biggest challenge to Kamiali's conservation model. Fish provide the overwhelming majority of dietary protein for this coastal village, and coral reefs are preferred fishing sites. For the initiative to succeed fisheries must balance the conflicting needs of conserving fish populations to attract research versus the subsistence needs of Kamiali residents. Toward this end we conducted population size-structure surveys to evaluate the current status of exploited reef fish populations.

Although the wildlife management area's fringing and patch reefs are easily accessible, the majority of fishing occurs in depths beyond the limits of conventional open-circuit scuba (Longenecker et al., 2008a). We overcame this logistical difficulty by employing advanced diving techniques to access habitats and populations targeted by village fishers.

The purpose of this study is to describe the size-structure of exploited reef-fish populations in Kamiali Wildlife Management Area and to examine size structure in light of life-history characteristics. This information will allow residents to understand whether the fish they catch have had the chance to reproduce and better gauge whether the community will have an ample supply of food fish in the future. The information will also serve as the basis for detecting changes in exploited reef-fish populations including those brought about by any conservation efforts enacted by the Kamiali community.

Methods

Study Site

Kamiali is located on the Huon Coast, approximately 64 km SSE of the port city, Lae. The terrestrial portion of the Kamiali Wildlife Management Area is remarkably undeveloped and characterized by lush vegetation. Kamiali Village is concentrated along a sandy beach in the northern portion of the wildlife management area. More-southern shorelines are protected by fringing reefs that may parallel rocky shoreline or sandy coves. The intertidal zone is dominated by mangroves, mud flats or seagrass beds. Seaward, the reef flats typically feature carbonate bench or coral beds with occasional patches of sand or rubble. The reef crest features a high abundance and diversity of corals although occasional coral-rubble beds also occur. The reef face is steep, typically descending 20 to 30 meters, and features corals, consolidated carbonate substrate and rubble. At the base, fringing reefs give way to depths in excess of 100 m featuring sandy sediment believed to occupy the majority of the marine area. Some coral outcroppings, patch reefs and pinnacles are interspersed throughout this deep, presumably sedimentary, area. These latter features are most frequently targeted by local fishers.

Fishery Surveys

We conducted 14 laser-videogrammetry surveys to describe the size distribution of exploited reef fishes in Kamiali Wildlife Management Area during the period 30 July – 13 August 2009. We used a high-definition video camera fitted with parallel laser pointers to capture images of individual fish while they were oriented perpendicular to the laser beams. We reviewed the resulting video with Sony Picture Motion Browser® and captured still frames where both lasers appeared on the fish. Because the lasers are parallel, they superimpose a reference scale on the side of the fish allowing length estimates to be generated by solving for equivalent ratios. These estimates were calculated with ImageJ software (National Institutes of Health). There is a nearly 1:1 relationship between estimated and actual fish lengths; further, a prediction interval suggested 95% of estimates will be within 0.5 cm of the actual fish length (Longenecker and Langston, 2008).

We performed laser videogrammetry surveys at preferred fishing sites most of which are beyond the depth limits of conventional open-circuit scuba. As such, we used closed-circuit rebreathers with 10/50 trimix diluent as life support to reach depths to 94 m. Because of the lengthy decompression obligations incurred while working at these depths (*e.g.*, 3.25 hours for a 15-minute dive to 94 m) we performed the work in areas with bathymetric profiles that permitted us to continue work while ascending. Thus, surveys are concentrated at pinnacles and near fringing reefs (Table 1).

The fishes included in this study met the following four criteria, they were: 1) reef fishes, 2) exploited by local fishers, 3) common enough to have been captured at least several times on video, and 4) could be reliably identified from still images. A total 33 species representing 10 families (Caesionidae, Carangidae, Haemulidae, Holocentridae, Kyphosidae, Lethrinidae, Lutjanidae, Mullidae, Serranidae and Siganidae) met these criteria.

The length information presented below is the distance between the front of the head and the end of the middle caudal ray. These lengths correspond to fork length (FL) for caesionids, carangids, holocentrids, kyphosids, lethrinids, lutjanids and mullids; and total length (TL) for haemulids and serranids. This length slightly underestimates total length for siganids, which have an emarginate caudal fin, and is called "fork" length in this report.

Life History Information

We conducted a systematic literature review using the methods of Longenecker et al. (2008b) to obtain estimates of size at maturity (L_{50}). Briefly: 1) we searched electronic resources (e.g., Google Scholar, FishBase) using key word combinations of species names plus "reproduction" or "maturity"; 2) upon obtaining these publications, we identified and obtained additional relevant literature listed in their reference section; 3) we then searched these publications and obtained any additional references.

In summarizing life history information we gave preference to studies specific to Papua New Guinea (e.g., maximum length information of Allen and Swainston, 1993). Preference was also given to length at 50% maturity (L_{50}; the size class in which 50% of individuals are mature) over other estimates of size at maturity (e.g., minimum size at maturity). We included results from studies outside the southern hemisphere only when data for southern populations were not available (e.g., reproductive size for *Caranx melampygus*).

Table 1. Sites surveyed at Kamiali Wildlife Management Area. Latitude and longitude were estimated by GPS using the WGS84 datum.

Max Depth (m)	Habitat	Latitude	Longitude
61	Offshore Pinnacle	7°19'47.00"S	147°12'24.57"E
67	Offshore Pinnacle	7°19'47.00"S	147°12'24.57"E
61	Nearshore Pinnacle to Fringing Reef	7°20'06.03"S	147°09'15.58"E
30	Fringing Reef	7°20'06.03"S	147°09'15.58"E
35	Nearshore Pinnacle to Fringing Reef	7°17'54.65"S	147°08'06.66"E
57	Nearshore Pinnacle to Fringing Reef	7°17'48.30"S	147°08'06.29"E
27	Fringing Reef	7°17'53.34" S	147°08'13.80" E
29	Fringing Reef	7°17'50.16"S	147°08'14.06"E
20	Fringing Reef	7°17'57.31"S	147°07'58.89"E
28	Fringing Reef	7°17'56.18"S	147°07'54.51"E
73	Nearshore Pinnacle to Fringing Reef	7°17'47.30"S	147°08'41.51"E
34	Offshore Pinnacle	7°18'30.11"S	147°09'58.64"E
94	Offshore Pinnacle	7°18'30.11"S	147°09'58.64"E
32	Offshore Pinnacle	7°20'37.30"S	147°10'07.51"E

Results

Mean length, also expressed as a percentage of maximum length and size at maturity, is presented for each of 33 species in Table 2. The largest value was for the sweetlips, *Plectorhinchus lineatus*, with an average length just at reported maximum length. The smallest value was for a coral trout, *Plectropomus aureolatus*, that averaged about one fifth its maximum length. The mean length of all individuals was 19 cm, representing 52% of the weighted mean maximum length of all 33 species combined. That is, an exploited reef fish swimming in Kamiali Wildlife Management Area is likely to be about one half of its potential maximum length.

Information about reproduction in these species is remarkably scant. Size at maturity is known for only 27% of the species studied. Of this subset an individual *Lutjanus carponotatus*, *Cephalopholis boenak*, or *C. cyanostigma* in Kamiali Wildlife Management Area was more likely than not to be reproductively mature. However, no individual of the larger-bodied *Caranx melampygus* or *Plectropomus areolatus* had reached maturity. But, on average, an exploited reef fish is just at (>99%) reproductive size

Table 2. Size and reproductive information for common, exploited fishes in Kamiali Wildlife Management Area. Values bridging female and male L_{50} columns (*Cephalopholis cyanostigma* and *Siganus lineatus*) indicate no sex-specific size-at-maturity values were provided. * values are %TL; they could not be converted to FL because no length-length relationship is available. Sources (+ clarifying information): (a) Allen and Swainston, 1993; (b) Froese and Pauly, 2009 (length relationships used to estimate FL); (c) Sudekum et al., 1991; (d) Randall et al., 1990; (e) Kritzer, 2004; (f) Heupel et al., 2009 (all females > 23 cm FL were mature); (g) Kritzer in Williams et al., 2002; (h) Davis and West, 1993; (i) Longenecker and Langston (unpublished data) [$FL = 0.2121 + 0.8736(TL)$, $r^2 = 0.993$, $n = 67$]; (j) Longenecker and Langston, 2008; (k) Chan and Sadovy, 2002; (l) Moss et al. in Williams et al., 2002 (no fish <14 cm were collected, all were mature); (m) Rhodes and Tupper, 2007; (n) Woodland, 1990 (smallest spawning individual was 23 cm).

Family	Species	N	Mean Length (cm)	% L_∞	% ♀ L_{50}	% ♂ L_{50}
CAESIONIDAE	*Caesio cuning*^{a,b}	164	16	38.1	?	?
CARANGIDAE	*Carangoides fulvoguttatus*^{a,b}	23	26	51.0	?	?
	Caranx melampygus^{a,b,c}	16	23	31.9	74.2	?
	Caranx papuensis^{b,d}	6	47	71.2	?	?
HAEMULIDAE	*Plectorhinchus lineatus*^{a}	10	50	100.0	?	?
HOLOCENTRIDAE	*Myripristis adusta*^{a}	13	18	56.3 *	?	?
	Myripristis kuntee^{a}	41	12	63.2 *	?	?
	Myripristis violacea^{a}	34	13	65.0 *	?	?
	Neoniphon sammara^{a}	7	14	43.8 *	?	?
KYPHOSIDAE	*Kyphosus cinerascens*^{b,d}	49	30	73.2	?	?
LETHRINIDAE	*Monotaxis grandoculis*^{a}	19	20	33.3 *	?	?
LUTJANIDAE	*Lutjanus biguttatus*^{a,b}	58	15	75.0	?	?
	Lutjanus boutton^{a,b}	66	14	50.0	?	?
	Lutjanus carponotatus^{a,b,e}	8	22	57.9	115.7	?
	Lutjanus fulvus^{a,b}	18	18	46.2	?	?
	Lutjanus gibbus^{a,b,f}	6	18	42.9	>78.3	?
	Lutjanus russelli^{a,b,g}	39	22	51.2	100.0	?
	Lutjanus semicinctus^{a,b}	13	20	58.8	?	?
	Lutjanus vitta^{a,b,h}	18	15	40.5	100.0	?
	Macolor macularis^{a,b}	10	29	52.7	?	?
MULLIDAE	*Parupeneus barberinus*^{a}	43	16	32.0 *	?	?
	Parupeneus bifasciatus^{a}	7	20	57.1 *	?	?
	Parupeneus cyclostomus^{a}	6	17	34.0 *	?	?
	Parupeneus multifasciatus^{a,i,j}	21	14	53.8	93.3	93.3
SERRANIDAE	*Anyperodon leucogrammicus*^{a}	7	26	50.0	?	?
	Cephalopholis boenak^{a,k}	10	17	70.8	113.3	106.3
	Cephalopholis cyanostigma^{a,l}	22	19	54.3	>135.7	
	Cephalopholis microprion^{a}	3	12	52.2	?	?
	Cephalopholis urodeta^{a}	3	17	63.0	?	?
	Plectropomus aureolatus^{a,m}	5	15	21.4	37.5	31.3
	Plectropomus oligacanthus^{a}	16	31	47.7	?	?
SIGANIDAE	*Siganus javus*^{d}	16	24	45.3 *	?	?
	Siganus lineatus^{a,b,n}	6	25	61.0	<108.7	

Discussion

The demographic characterizations presented above should be considered preliminary. In all cases, additional data would lead to more robust population characterizations.

Remarkably little is known about reproductive parameters for these coral reef fishes (Table 2). This is a common problem for coral reef fisheries; Longenecker et al. (2008b) report that size at maturity is unknown for 38% of the 13 most heavily exploited reef fishes in Hawaii. It is impossible to evaluate the breeding status of a population when this information is lacking.

Given the above limitations it appears a typical individual in the exploited reef-fish community at Kamiali Wildlife Management Area is 52% of its maximum length. In the small subset of species for which size at maturity is known, a typical individual is 99% of female reproductive size. These metrics suggest exploited fish populations are relatively robust despite serving as the main source of dietary protein for village residents. Perhaps most remarkable is our casual observation that the highest density of many exploited species was at the most heavily fished area, located at the southern end of the village. The current status of the overwhelming majority of these fish populations should be attractive to researchers.

Equally important in evaluating the population status of these fishes is the effort required by villagers to catch a meal. In this village of approximately 600 people, two canoes on average, are engaged in reef-oriented fishing at any one time during daylight (Longenecker et al., 2008a). Residents appear to obtain their primary source of dietary protein with relative ease.

Despite the apparent robustness of exploited fish populations at Kamiali Wildlife Management Area, residents consistently state they do not follow conservation practices. There are no gear restrictions, creel limits, minimum or maximum size limits, or seasonal closures for any species. Nor were Kamiali residents prohibited from fishing in any specific area. However, several characteristics of the village and its fishery appear to maintain robust populations of exploited fishes. First, non-residents are prohibited from fishing within Kamiali Wildlife Management Area creating a *de facto* limited-entry fishery. Second, distance to commercial markets presents a significant barrier to entry for commercial fisheries. Cinner and McClanahan (2006) suggest proximity to markets (<16 km) increases the likelihood of overfishing in Papua New Guinea. Kamiali is 64 km from the city of Lae, where fish can be sold commercially. Because there are no roads between Kamiali and Lae, individuals selling fish and purchasing ice must have a motorboat. The cost of operating these is high; a liter of fuel can cost up to USD $2.00. Therefore, economic success in commercial fishing requires that a sufficient quantity of fish be caught before ice melts and that market prices justify a costly trip to Lae. Variability in catch rate and market prices in the face of high fuel costs makes commercial fishing economically risky. Third, because cash is limited in this subsistence economy, technologies that may lead to fishery overexploitation are cost-prohibitive. Fishing is done primarily from small, human-powered, handmade, outrigger canoes. Transportation to bottom-fishing sites and propulsion while trolling requires a significant input of human energy. Hook-and-line fishing with homemade handreels and weights, or handcrafted outriggers, is the dominant fishing technique. Finally, lack of refrigeration eliminates the motivation to catch more fish than can be used within a few days. It appears the decision to maintain a traditional village lifestyle also promotes robust fish populations.

We reiterate that statements about the robustness of exploited reef-fish populations in Kamiali Wildlife Management Area are based on the limited information generated by this study or available in the literature. We feel increasing the number of site-specific surveys and increasing the number of more generally applicable size at maturity analyses would greatly enhance our ability to evaluate these populations. We further suggest that assessing the long-term viability of exploited fish populations would benefit from analyses of size-specific sex ratios. In each of four studies that examined sex ratios in species found at Kamiali (Davis and West, 1993; Kritzer, 2004; Longenecker and Langston, 2008; Huepel et al., 2009) data suggest the proportion of females in a population decreases with length. Although sex ratios were not examined in studies of serranids they are all classified as protogynous hermaphrodites (Heemstra and Randall, 1993). Because individuals mature as females, then change sex with further growth, some of these species should also be expected to have male-biased sex ratios with increasing size. In other words, conservation efforts directed at increasing the average size of individuals may not necessarily lead to increased egg production for a population. The reproductive status of any exploited fish population would be better understood if size-specific sex ratios were known.

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THE BELGIAN SCIENTIFIC DIVING TRAINING PROGRAM

Alain Norro
Royal Belgian Institute for Natural Sciences
Management Unit of the North Sea Mathematical Model - MUMM
Gulledelle 100
1200 Bruxelles, BELGIUM

Introduction

Scientific diving (SD) performed by scientists in Belgian North Sea waters (re) started in 2001. SD is operated from large research vessels because of the geographical and weather conditions of these waters. From 2001 to date some 1,200 man-dives have been conducted in the offshore conditions of the Belgian North Sea for the benefit of several research programs with no accidents reported. Belgian scientists participate in many other SD activities in foreign waters. In order to keep a high level of safety in SD operations specific training was organized in 2009 for all Belgian scientists. This training was organized by scientists and consists of a common module based on the (Advanced) European Scientific Diver ((A)ESD) standard and on other specialized modules. The common module, now accepted by Belgian law, is organized in a calm and clear water location in the Mediterranean Sea since the specialized offshore North Sea module is operated from one of the available research vessel in true field conditions. To complete the program a continuous training module, aimed at testing and developing new sampling techniques, was done in confined water (quarries) in Belgium. Moreover, a potential scientific output was proposed with the common module of the Belgian SD training. A more specific scientific experiment aimed at collecting new data using a given technique was proposed. The 2009 and 2010 experiments dedicated to sampling with differently designed airlift samplers provided data for two Master’s theses. The main lesson learned from 2009 was the appropriateness of the modular training system for Belgium. One may take into account that we address the needs of a scientific community living close to the North Sea featuring difficult to dive cold and dark offshore waters but operating mainly in foreign clear and warm waters.

The (re)start of scientific diving in Belgian waters

The Belgian waters that are situated in the Southern Bay of the North Sea are an offshore zone requiring the use of large vessels. SD was, therefore, operated from the *R/V Belgica* since the early 1980s. *R/V Belgica* belongs to the Federal Belgian Government and is operated by the Belgian Navy. Because of that, only military divers were previously allowed to dive from the vessel. That situation changed when in 1998 the author joined the research laboratory MUMM that is in charge of the management of the scientific cruises taking place onboard *R/V Belgica*. In 2000, a cruise was organized in order to test the feasibility of SD performed by civilians. Based on that positive experience other cruises followed. At the same time, a first call of interest was directed to all Belgian Universities and research laboratories using or having used the SD technique. In 2005, following the funding of the BEWREMABI research project, SD activities started aboard *R/V Zeeleeuw*, a research vessel belonging to the Regional Belgian Flemish Government.

In 2008, following the recognition by the European Science Foundation (ESF) Marine Board of the European Scientific Diving Committee (ESDP), a working group (WG) was created in Belgium among the interested parties resulting from the 1999 call of interest. The WG was created at the Federal level under the umbrella of the Federal Public Service Belgian Science Policy (Belspo). The working group was also in charge of the training and certification of scientific divers in Belgium.

Belgian scientific diving landscape

Belgian scientists were widely using SD techniques from the early days of scuba. They were operating mainly in the Mediterranean Sea as well as in the Indo-Pacific region. The Belgian University of Liége built a research station in Corsica (France) in the late 1970s. Many scientists from Belgium and over 30 countries used that research station and produced about 1000 publications of the results of studies undertaken in Calvi. Today, about 2,000 man-dive scientific dives are conducted every year from Stareso. At the same time Brussels University (ULB) had a research station in Papua-New-Guinea were scientific diving was widely used, but the station stopped activities some time ago.

The Belgian North Sea had not attracted too many studies supported using the SD techniques (Massin et al., 2002a, 2002b). Studies that were conducted recently were under the Belgian Government funded project BEWREMABI (Zintzen et al., 2006, 2007, 2008) and within the framework of the monitoring program related to the implementation of wind parks in the Belgian zone of the North Sea (Kerckhof et al., 2010).

Assessing the safety of the SD operation and legal issues

From the restart of the scientific diving operations in the Belgian North Sea, safety of the activity was a key aspect and about 1,200 man-dives have been logged in offshore conditions without accident. At the end of 2003 a new Royal Decree (RD 23-12-2003) came into force in Belgium outlining the framework, rules, and requirements for commercial diving in Belgium. Even if SD was not explicitly covered by that new legal text there was a need to clarify the situation. The Royal Decree imposed, among others: medical control, specific training, risk analysis, and notification and control of the operations by the Federal Labor Administration. The Belspo working group on scientific diving developed and organized specific scientific diver training from 2009 onward. The format of the training was based on ESDP levels of competency (ESDP, 2009) including a 5-day, 15-sessions course that was open to already trained divers already certified to Level 2, as well as a specific module for the Offshore North Sea that is operated from R/Vs in Belgian waters. The high level of this training, together with specific SOP (Standard Operating Procedures) permits, helped to achieve the zero accident levels that are reported here. From February 2012, this training is recognized in Belgium as valid level of competency for commercial diving as required by the RD 23-12-2003.

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APPROACHES TO THE ARCHAEOLOGY OF SUBMERGED LANDSCAPES: RESEARCH ON THE ALPENA-AMBERLEY RIDGE, LAKE HURON.

***John O'Shea*[1]**
***Ashley Lemke*[1]**
***Robert Reynolds*[2]**
***Elizabeth Sonnenburg*[1]**
***Guy Meadows*[3]**

[1]Museum of Anthropology
University of Michigan
1109 Geddes Avenue
Ann Arbor, MICHIGAN 48109 U.S.A.
joshea@umich.edu

[2]Department of Computer Science
College of Engineering
Wayne State University
5057 Woodward Avenue, Suite 4001
Detroit, MICHIGAN 48202 U.S.A.

[3]Great Lakes Research Center
Michigan Technological University
1400 Townsend Drive, Room 307
Houghton, MICHIGAN 49931 U.S.A.

Some of the most pivotal questions in human prehistory necessitate the investigation of archaeological sites that are now submerged. The advance and retreat of glacial ice and the associated global changes in sea level throughout the period of human development have exposed and then submerged significant coastal land masses repeatedly. As a result, questions as diverse as the origins of early human culture, the spread of hominids out of Africa and the colonization of the New World all hinge on evidence that is under water. While the discovery and investigation of such sites presents technological challenges, these contexts have unique potentials for investigating ancient sites that have not been disturbed by later human activity and for preserving organic materials that typically do not survive on land.

While sharing obvious similarities, there are fundamental differences between conducting traditional marine archaeology focused on shipwrecks and the investigation of submerged prehistoric sites and landscapes. A shipwreck presents a known and focal target for discovery and investigation while the bottom environment in which it now rests is largely irrelevant. By contrast, the bottom environment for submerged site archaeology is everything. It presents the vestiges of the ancient landscape that shaped the activities of the humans in the past, while virtually nothing is known *a priori* about the number, character, or locations of human occupation sites that may be present.

A second fundamental difference is funding. All underwater research is expensive. Yet the kinds of public and private funding that can be generated to find a *Titanic* or Spanish galleon are simply not available for the systematic investigations required by underwater prehistoric archaeology. In essence, submerged site archaeology must meet the challenges of 'big ocean' research on a 'bath tub' budget. To meet these twin challenges it is necessary to adopt approaches and technologies that can cope with large areas of bottomlands and do so within the 'bath tub' budgetary constraints of normal terrestrial archaeology.

These challenges have shaped our current archaeological research in the Great Lakes. The focus of our work is the Alpena-Amberley Ridge (AAR), a submerged feature beneath Lake Huron which, during the Lake Stanley low stand (roughly 9900 and 7500 calibrated years BP), provided a dry land corridor linking northern Michigan with south central Ontario (Fig. 1). During this time period the AAR was a natural causeway for the semi-annual migration of caribou; a setting that was actively exploited by ancient hunters. The AAR offers a Pompeii-like archaeological context in which stone hunting structures and other features are preserved without disturbance or modification from later human occupation or modern development. The AAR, because of its mid-lake location, also lacks the thick overburden of sediment or the high energy remodeling that is characteristic of nearshore areas. Yet to realize this unique archaeological potential the research must be conducted over an area of some 900 km^2 of lake bottom and 80 km offshore.

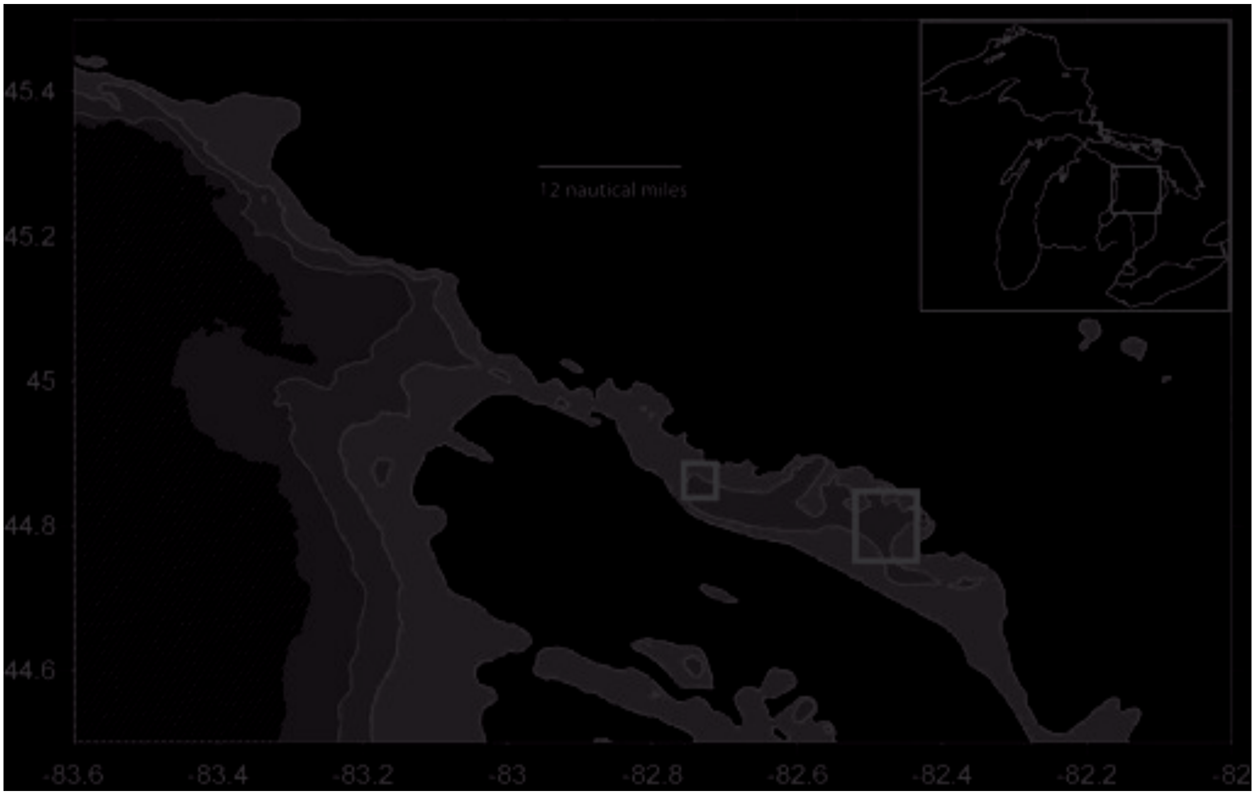

Figure 1. The Alpena-Amberley Ridge was a dry land corridor with an elevation of 140 m above mean sea level (modern lake datum is 176 m). The modern land surface is hatched and Lake Stanley stage water is shown in white. The contour interval represented by shading is 10 m. Figure coordinates are represented as degrees North Latitude and West Longitude. The map presents the western (American) half of the ridge and the black rectangles indicate the primary locations where research has been conducted.

We have developed a nested series of investigative techniques designed both to document the ancient landscape and to discover human occupation sites (Fig. 2). The research combines ethnographic research and agent-based computer simulation, with acoustic and visual search techniques, and ultimately scuba-trained archaeologists.

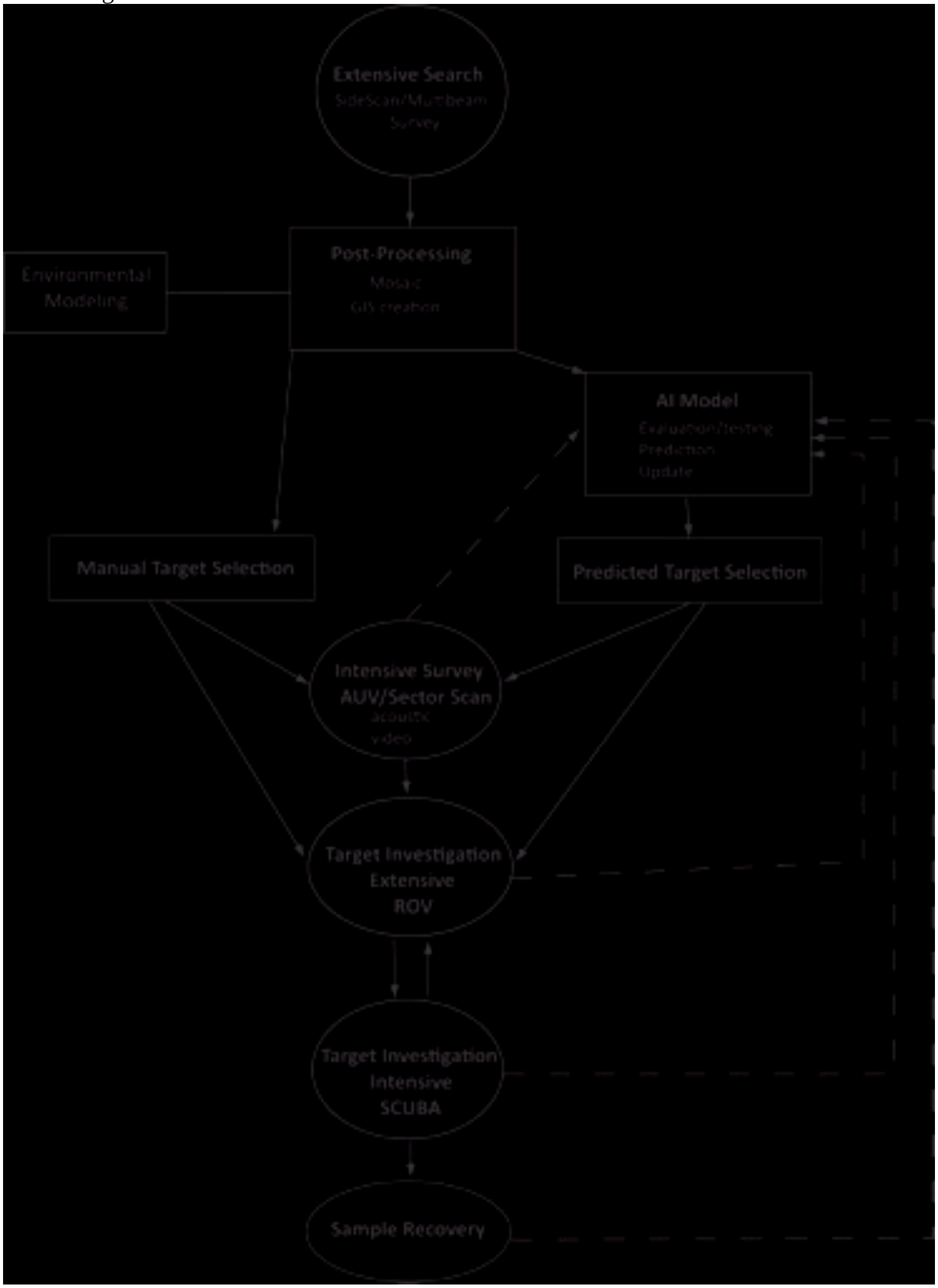
Extensive Search
SideScan/Multibeam
Survey
Environmental
Modeling
Post-Processing
Mosaic
GIS creation
AI Model
Evaluation/testing
Prediction
Update
Manual Target Selection
Predicted Target Selection
Intensive Survey
AUV/Sector Scan
acoustic
video
Target Investigation
Extensive
ROV
Target Investigation
Intensive
SCUBA
Sample Recovery

Figure 2. A schematic diagram illustrating the nested and recursive application of differing scales of search and analysis that guides research investigations on the Alpena-Amberley Ridge. Dashed lines reflect new information that is fed back into the simulation models.

The search efforts rely on the use of hand-deployable equipment that can be accommodated on a small vessel. A number of systems, including sidescan and multibeam sonars, remotely operated vehicles (ROVs), and autonomous underwater vehicles (AUVs), have been developed for small vessel use, while software developments now allow many of these systems to talk to each other and to onboard computer systems in real time. These hand-deployable systems offer unrivaled flexibility and synergistic benefits when employed together. For example, the transponder on the small ROV allows its location to be overlaid in real time on a computer representation of the ancient landscape. The boat-based operator can then lead deployed divers to specific sampling locations, make a video recording of the sampling event and location, and report back the precise three-dimensional location of the sample, which is immediately updated on the shipboard GIS.

Computer simulation is the critical second element in our approach to submerged site survey, since the size of the area places a premium on accurate predictive modeling. This involves two concurrent cycles of simulation, the first models the modern sea floor as a dry land environment, and the second uses the environmental model to predict the location of human activity. Both simulations are updated as new research data are generated, making them the effective repository of all that is known about the region.

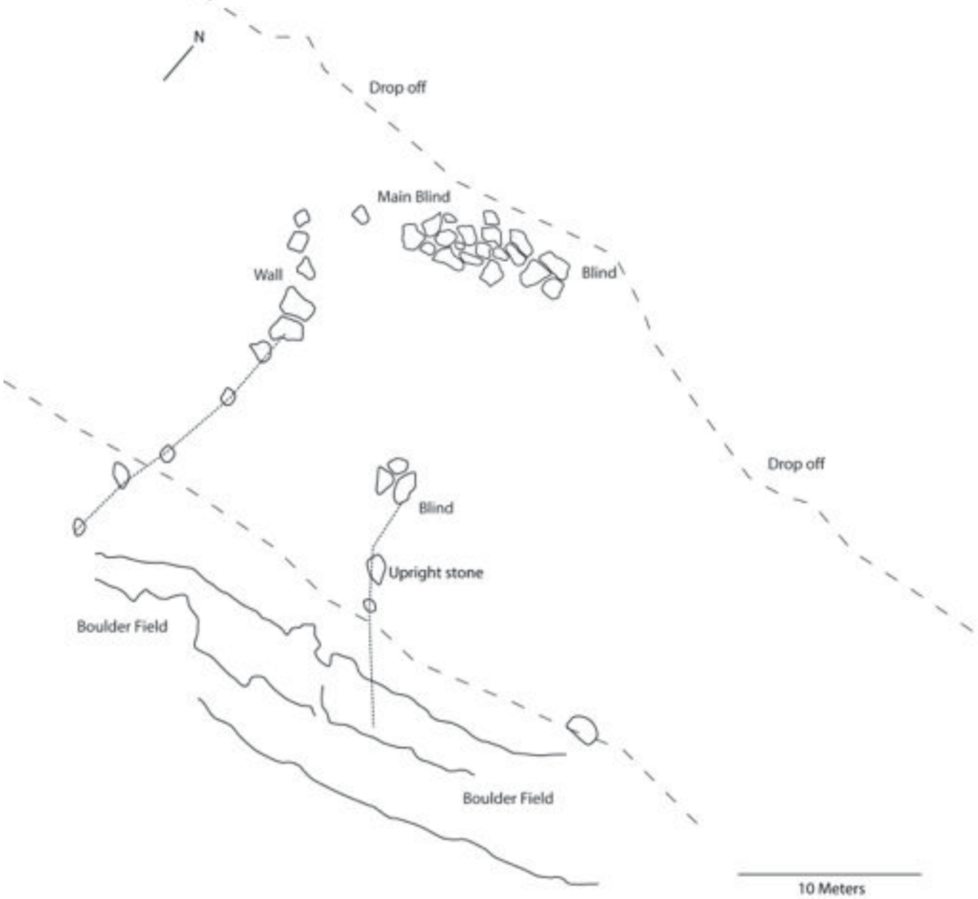

Figure 3. The Funnel Drive structure. The Funnel Drive is located at a depth of 22 m and is positioned on high ground above a marshy area. The apex of the two converging arms is at the upper left of the image. The feature is bounded by a boulder field at the bottom of the drawing that slopes upward to the south, and by a drop off behind and parallel to the main blind complex. Given the orientation of the structure, it was most likely used when animals were moving towards the northwest, which equates with the spring caribou migration. The contour lines represented in both areas are approximately 1 m.

The final step in the process is the deployment of scuba-trained archaeologists to evaluate and sample potential cultural features. By deploying divers in tandem with other surface assets the divers can concentrate on the archaeology while many of the burdens of mapping, photography and coordination are handled from the surface. One advantage of submerged-site archaeology from the perspective of diving is that most of the ancient land surfaces are relatively shallow. For our research on the ARR most areas of interest fall within the depth range of 18 to 45 m.

To date, we have been able to document the 9,000-year old environment of the AAR in detail and to identify a number of stone hunting structures and associated features. Critical aspects of the past environment such as the location of lakes, streams and marshes have been identified along with evidence indicative of the vegetation (including a series of spruce, cedar and tamarack trees dating between 8,000 and 9,000 BP) and climate. The unique ‘directional dependence’ of many of the recorded hunting features has also enabled us to determine their season of use and to provide estimates for the size of hunting groups utilizing the structures (Fig. 3).

As our research continues we are investigating ways to better integrate the acoustic and visual search systems with the simulation models in real time. A particularly promising approach provides the ROV operator with a split screen display that presents both the modern lake bottom environment and the simulated ancient land surface in real time while the ROV is in use.

SCIENTIFIC DIVING AS A RESEARCH TOOL FOR MAPPING REEFS IN THE NORTHEASTERN BALTIC SEA

Merli Pärnoja
Jonne Kotta
Kaire Kaljurand
Georg Martin
Estonian Marine Institute
University of Tartu
Mäealuse 14
Tallinn 12618 ESTONIA
merli.parnoja@ut.ee

Introduction

Reefs are ecologically among the most significant and valuable habitats in the northeastern Baltic Sea. They are characterized by lush benthic cover and high macrophyte and invertebrate diversity, thereby supporting many ecosystem functions. In the Baltic Sea reefs are formed on different types of hard substrate including granite bedrock, boulder fields of glacial moraine origin and also sedimentary material such as hard clay and limestone. To date, the knowledge of the Baltic Sea reefs is quite limited because they are shallow and can be studied only by small research boats using non-traditional sampling methods.

Scientific diving is a widely used research tool for benthic mapping on reefs in the northeastern Baltic Sea. However, the method is expensive, difficult and requires reasonably calm weather conditions. Using the remote underwater video camera is an alternative to scuba diving. Owing to its improved quality and feasibility, the video method is increasingly used in benthic community mapping. In general, the precision of two different methods is dependent both on the studied environment and the investment of resources. In the Baltic Sea range, abiotic environmental forcing factors such as salinity, water transparency and nutrient loading primarily shape reef habitats and determine community richness and species composition. Such variables can impact how well different reef mapping methods (diving and video observation) perform either through changes in visibility and/or diving comfort.

Methods

By using a standardized protocol of diving and video observation we compared these methods along major abiotic environmental gradients on reef habitats in the northeastern Baltic Sea. The mapping was performed at the depths of 0.2-14 m from 2006-2012. Altogether 764 stations were included in this study. At all stations diving and remote underwater video methods were simultaneously used. At each station the total coverage of the benthos community, the coverage of individual species and the coverage of different sediment types were estimated while the boat was anchored. Sediment was classified as rock, boulders, stones, gravel, sand, silt, clay or any combination thereof. Real-time video was captured with a digital video recorder. Depth data were recorded either by scuba diver or navigational echo sounder. Video samples were analysed in continuous and still picture modes in the laboratory.

The values of water temperature, salinity and water velocity were based on the results of hydrodynamical model calculations from early April 2011 to early August 2012. The calculations were based on the COHERENS model, which is a primitive equation ocean circulation model. It was formulated with spherical coordinates on a 1′×1′ minute horizontal grid and 30 vertical sigma layers. The inclination of coastal slopes was calculated at 50-m pixel resolutions using the Spatial Analyst tool of ArcInfo software based on bathymetry charts (available at the Estonian Marine Institute, University of Tartu). The Simplified Wave Model method was used to calculate the wave exposure for mean wind conditions represented by the ten-year period between January 1, 1997 and December 31, 2006. A nested-grids technique was used to take into account long distance effects on the local wave exposure regime, and the resulting grids had a resolution of 25 m. As a proxy of eutrophication we used the MODIS satellite derived water transparency (kd) and water chlorophyll a values. Water transparency (kd) is estimated as attenuation coefficient based on satellite imagery. The frequency of satellite observations was generally weekly over the whole ice-free period; however, several observations were discarded because of cloudiness. The spatial resolution of satellite data was 1 km. False zeroes were removed from the data prior to the statistical analysis. The ESRI Spatial Analyst tool was used to calculate the average, minimum, maximum and variance of all variables (those obtained from field sampling as well as from modelling) for local sampling scale.

Results and Discussion

The data analyses involved two stages. First, the Boosted Regression Trees modelling (BRT) was run to test for similarities in the coverage of individual species (CIS), the coverage of different sediment types (CS), presence of different species (AS) and species richness (SD) between scuba-diver and underwater video estimations along the major environmental gradients. For BRT modelling the independent variables were: sampling depth, water temperature, salinity, velocity, exposure to waves, coastal slope, chlorophyll a and water transparency (kd). They were regressed to predict the studied similarities (CIS, CS, AS and SD). For each of the similarities, multiple models were run varying both the model learning rate (between 0.1 and 0.001) and the number of trees (between 1000 and 10,000). If BRT modelling revealed large changes in similarities between estimations of scuba-diver and remote underwater video along any environmental gradients, then the Similarity Terms Analysis (SIMPER) was used to seek which species were responsible for the observed differences.

The BRT models showed that diving and video methods were relatively similar in some environments but different at others. The observed similarities of CIS, CS, AS and SD between the studied methods responded largely to the changes in water temperature, salinity, sampling depth and water transparency (kd). Specifically, the remote underwater video camera seemed to perform better at shallower depth and more saline environments compared to deeper and diluted environments. As low salinity environments are associated with high loads of turbid riverine water, the observed salinity effect is likely mediated by changes in water transparency. In addition, divers were also less efficient in more turbid and colder environments indicated by high similarities between diving and video observations in these environments.

Our study also showed that more exposed areas were characterised by high similarities of the coverage of different types of sediments between the two studied methods. It is possible that at low exposure sediments contain a significant quantity of fine particles and the distinction of different sediment types by an underwater remote camera is hindered. On the other hand, the number of different types of sediments decreases along the exposure gradient resulting in higher similarities between the studied methods at highly exposed areas.

Conclusion

The results of the current study clearly indicated that relationships between estimates of species characteristics and sediments were dependent on the observed method and the results were highly context-specific. The similarities between the two mapping methods responded surprisingly well to changes in abiotic environment. In general, the quality of observations declined with the increase of

depth, water transparency and the reduction in exposure, salinity and water temperature. According to our results, the remote underwater video method is better suited for estimating the distribution of habitats or easily identifiable habitat-forming species, but scientific diving is better for describing the distribution of single species. Dissimilarities in sediment characteristics, benthic species richness and species cover obtained by the two different study methods responded well to the changes in the environmental conditions primarily indicating higher efficiency of diver-based estimates, especially under unfavourable light conditions.

VIKING DRYSUIT DECONTAMINATION STUDY

E. Rob Pedersen[1]
Stephanie Harris[2]
Sean Sheldrake[1]
Stephanie Bailey[2]
P. Bruce Duncan[1]

[1]US Environmental Protection Agency
1200 6th Avenue, Suite 900, Mailstop OEA-095
Seattle, WASHINGTON 98101 U.S.A.
pedersen.rob@epa.gov

[2]US Environmental Protection Agency
Region 10 Laboratory (Microbiology)
7411 Beach Drive East
Port Orchard, WASHINGTON 98366 U.S.A.

Government dive teams (public safety, military, resource and regulatory branches) and commercial divers work in contaminated harbors and enclosed bodies of water prone to water column and sediment contamination. Decontamination policies and practices during dive operations in polluted water vary widely. Standard dress for EPA Region 10 divers is a Viking drysuit with hazmat valves, drygloves and a full-face mask. This laboratory study examined bacteriological decontamination effectiveness of Pseudomonas-contaminated Viking drysuit material after freshwater rinsing and after treatment with Betadine.

Introduction

The EPA Region 10 dive team members work in polluted water conditions – contaminated water diving (CWD) from both biological and chemical hazards. Examples are diving near wastewater treatment plant outfalls, storm water outfalls, seafood processor waste and general chemical contaminates in the water column and sediment. The prevalence of polluted water diving was affirmed by well recognized publications (Barsky, 1999; 2002). The NOAA Diving Manual dedicates a chapter to CWD (NOAA, 2001). In recent years, the Naval Sea Systems Command has put a significant effort into their Guidance for Diving in Contaminated Waters (Pearson, 2008).

Decontamination (decon) is intended not only to rid the diver of bottom sediments but also to dilute and rinse off microbial contamination (USEPA, 2001). EPA Region 10 diver dress is typically an AGA full-face mask (mated to the hood of the Viking drysuit), drygloves, a Viking drysuit, a buoyancy compensator and scuba equipment. The divers' suits are made of vulcanized rubber for easier decontamination (Viking Pro EPDM/natural rubber (1000 g/m^2)).

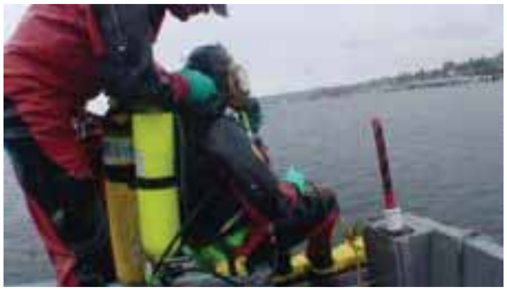

Figure 1. Trelleborg Viking Pro Dry Suit. Photo by D. Thompson, USEPA.

A researcher for the US Navy published a survey of best practices for diving in contaminated water; he found the Viking HD was a popular suit choice given its vulcanized rubber composition (Steigleman, 2002). A wide variety of decontamination solutions, scrubbing, and rinsing has been reported. Pearson (2008) stipulated the Viking HD for use in category 1 (CAT 1) or CAT 2 contaminated water. Amson (1991) also mentions the major advantage of Viking drysuits for CWD in his paper on diver protection.

Although the Viking haz-mat (HDS) suit material is more resistant to a range of strong chemicals (Trelleborg Viking, 2001), the Viking Pro suit material with hazmat valves protects EPA Region 10 dive team members from typical exposures; see EPA Equipment Page for more details: http://yosemite.epa.gov/r10/oea.nsf/webpage/dive+team. This diver dress renders the diver completely dry during contaminated water diving, apart from incidences of suit punctures or fit issues with the full-face mask.

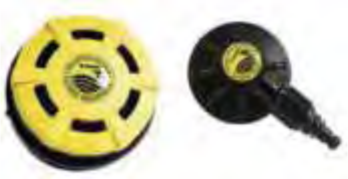

Figure 2. Trelleborg Viking Dry Suit Hazmat Valves.

Figure 3. Interspiro Divator MKII (AGA) Mask. Photo by B. Duncan.

Decontamination of divers occurs as they exit the water onto the swim step on the stern of the vessel (exclusion zone). Decon generally consists of a thorough potable water rinse. In Amson's (1991) discussion for diver decontamination after exposure to water contaminated with pathogens, the initial diver rinse should be followed by spraying down the diver with a clinical disinfectant such as Betadine surgical scrub solution. An EPA standard operating procedure from that era for decon of divers after exposure to biohazard states: “For destruction of microorganisms, a surfactant such as trisodium phosphate is used followed with a spray of Betadine, zepamine, or tincture of green soap” (Tulis, 1994).

Though the improperly equipped diver with a wetsuit and mouthpiece regulator is exposed during the dive itself, EPA diver exposure during a dive operation would be more likely after doffing contaminated equipment that has been inadequately decontaminated. Investigation as to the adequacy of potable water rinse as the day-to-day decontamination technique was needed to determine whether this is sufficient, or if more elaborate antimicrobial decontamination is worth the additional time and effort to employ. Antimicrobial solutions take significantly more effort to deploy with scuba equipment because of the limited air supply carried by the diver. If the diver must spend a significant amount of time performing decontamination, this is time that cannot be spent doing work diving. Time may also become a hazard if the diver is overheating in the sun. Consideration should also be given to the fact that rinsewater from antimicrobial solutions should be collected and disposed of properly; for example, to a publicly owned treatment facility.

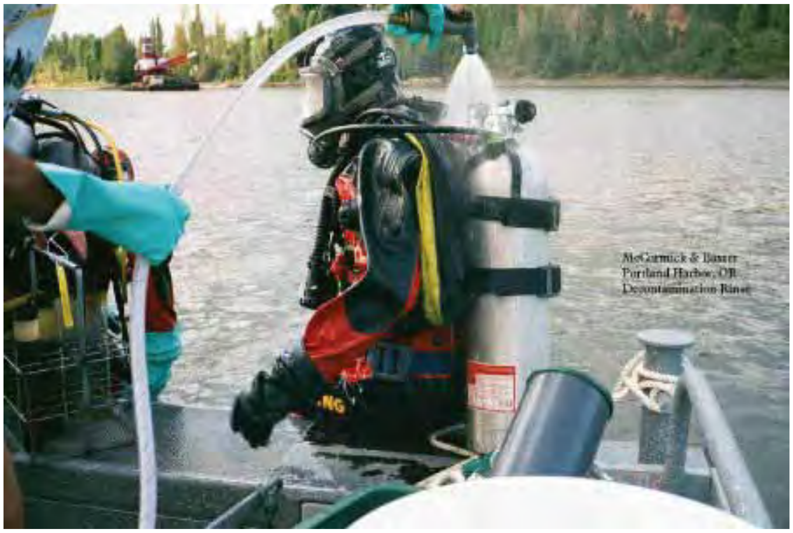

Figure 4. Diver Freshwater Rinse Decon on Swim Step. Photo by R. Fuentes, USEPA.

Many heavily contaminated water diving operations are conducted with surface supply to provide unlimited air for decontamination. However, surface supply operations severely limit the work the diver can undertake without relocating the dive platform a number of times. For long transect dive surveys use of a surface supply system becomes impractical. For these reasons, it is in the divers’ interest to have decontamination that is both very effective and of the shortest duration possible.

Whether diving under the OSHA commercial diving standards or the OSHA scientific diving exemption, cleanup workers should be working under conditions that are in compliance with OSHA standards (29 CFR 1910.120). The OSHA scientific diving exemption does not exempt scientific divers from employing personal protective equipment (PPE) and other preventative exposure measures. (USDOL, OSHA 29 CFR 1910; USDOL, OSHA 29 CFR 1910 Subpart T.)

Previously, for EPA R10 divers, if exposure occurred with a known high biological hazard then the divers were likely to be sprayed with a diluted Betadine solution (providone-iodine) as a disinfectant. Betadine is the name of Purdue Pharma’s brand of consumer-available povidone-iodine topical antiseptics. Betadine is available as a solution sold over-the counter for cleaning minor wounds and used in hospitals to prepare a patient's skin prior to surgery. http://en.wikipedia.org/wiki/Betadine - cite_note-

BetHosp-1%23cite_note-BetHosp-1Solutions are 10% povidone-iodine in water. A contact time of two minutes is recommended for some disinfection uses with the Purdue Pharam's product. The Betadine solution used by the dive team for soaking AGA masks and for Viking suit decon-spray is 9 oz. Betadine to 1.5 gal. freshwater (a 4.7 percent solution).

A common disinfectant is diluted chlorine bleach. This substance is corrosive to silicone and vulcanized rubber and is not used by the Region 10 dive team for this reason. Hydrogen peroxide (H_2O_2) may be an effective disinfectant for use on the Viking suits; however, corrosion of iron-containing metals is possible and the Region 10 dive team has not used hydrogen peroxide for disinfection purposes.

The required contact time for Betadine to be an effective disinfectant under the conditions the dive team experiences on the back of the EPA vessel is not well known. For other topical disinfectant applications with Betadine contact times of up to three minutes, or even ten minutes, are mentioned (Tulis, 1994). It may also be necessary for the Betadine solution to dry after application.

This decontamination study compares the effectiveness of freshwater rinsing of Viking suit material to rinsing suit material after a one-minute and a three-minute exposure to Betadine.

Methods

(Derived from EPA Region 10, July 9, 2008. Office of Environmental Assessment, Environmental Services Unit, Quality Assurance Project Plan for Viking Dive Suit Decontamination Study.)
http://www.epa.gov/region10/pdf/diveteam/drysuit_decon_study_data_report_030309.pdf

Objectives and Goals

The objectives of the decontamination procedure study were to:

1. determine the efficacy of the potable water rinse procedure for removal of bacteria on the Viking drysuit material;
2. determine the efficacy of Betadine to kill bacteria present on the Viking drysuit material. Two different application periods (1 minute and 3 minutes) were used; and,
3. to remove bacteria from the suit or, where this did not happen, the intent was to kill bacteria *in-situ*.

Sampling Design (Experimental Design)

This laboratory procedure occurred twice with Betadine. Hydrogen peroxide was dropped from the study when the data from the Betadine study were known.

1. Used 6"x6" patches of Viking suit material.
2. Cleaned the patches (soap/water, disinfected, air dried).
3. 4 patches were placed on a clean surface.
    - 3.1. Patch 1 was inoculated by swabbing with *Pseudomonas aeruginosa* (control patch - this would also catch any potential background that remained on the patches).
    - 3.2. Patches 2-4 were inoculated by swabbing with *Pseudomonas aeruginosa* (had a known concentration of *Pseudomonas* - this was part of the purpose of patch 1).
    - 3.3. Allowed patches to partially dry (5 minutes).
4. Hung the patches over clean pans or beakers.
5. Decon:
    - 5.1. Patches 1-2, rinsed with sterile water (patch 1 was the control and patch 2 a "test" for sterile water rinsing as appropriate for removing the bacteria).
    - 5.2. Patches 3-4, sprayed with Betadine solution (dilution ratio was 9 oz. Betadine to 1.5 gal. freshwater (4.7 percent solution)) for decon test one.
        - 5.3. Patch 3, rinsed with sterile water after 1 min. of Betadine exposure.

5.4. Patch 4, rinsed with sterile water after 3 min. of Betadine exposure.

6. Filtered a volume of the rinsates (usually 100 - 500 ml) through a 0.45 um porosity 47 mm diameter membrane filter.
7. After rinsing the filtration funnel, applied the membrane on a poured plate of mPAC agar so that there was no air space between the membrane and the agar surface.
8. Inverted plates and incubated at 41.5 +/- 0.5 degree C for 72 hours. Media - m PA - C (modified m-PA agar) available commercially. Media was prepared to manufacturer's requirements.
9. Counted colonies and reported as number of *P. aeruginosa*/100 ml.
10. Determined percent "kill" in bacteria count (on a log basis) in rinsates 2-4 relative to rinsate 1, and between rinsate 2 with rinsates 3-4. Note: there was up-front work to determine the amount of inoculum to place on the patches. In order to have logarithmic levels of removal, it was necessary to start with large numbers of organisms and then perform dilutions with the inoculum to obtain countable numbers. See "Standard Methods, 21st edition for methods reference.
11. Repeated the decon test at least twice more, on different days.
12. Determined if there was a statistically significant difference between rinsates 1-2 with rinsate 3 and rinsate 4 (and also between 3 and 4). If there were an adequate number of samples, and statistical assumptions were met, the analysis was a series of paired T-tests (for homoscedastic data) or multivariate analysis for non-normal data.

Quality Control Tests Performed

As established in the Quality Assurance Project Plan for this project, the following quality control tests were conducted as an integral part of these analyses:

1. *Negative control* – triplicate sets of pre-cleaned dive suit material were rinsed with buffer and the residue collected and filtered to check for background *Pseudomonas* contamination. A positive result (growth) from a negative control would have invalidated the data associated with that set.
2. *Negative filtration control* –Filtration of 100 ml of sterile rinse water performed to ensure that the filtration portion of the analysis demonstrates no bacterial contamination. Filters were placed on media and incubated with the test samples. A positive result (growth) on this control invalidated the data associated with the set.
3. *Positive control* – Standard aliquots of *Pseudomonas* culture were applied to triplicate swatches of dive suit material. After the timed interval without exposure to the disinfectant, the dive suit material was rinsed with the rinsate being directly filtered through 47 mm diameter, 0.45 µm porosity filters. The filters were placed on mPA agar and incubated. The number of organisms counted and factoring in the dilution used was entered in calculations determining the percent removal or log removal of organisms during the disinfection step. A negative result (no growth) on this control would have invalidated the data associated with this set.

General Conclusions and Disclaimers

For this study, two time exposure intervals were utilized to determine effectiveness of the disinfectant at reducing the levels of viable organisms. Three minutes was used initially but, once it was realized that essentially 100% of organisms were rendered nonviable at three minutes, the study was expanded to include one minute exposures. Although the results were somewhat lower for the three minute exposure (more organisms viable), both time intervals resulted in greater than 3 log (99.9 %) reduction in viable organisms and the results were not significantly different (employed paired T-tests). Laboratory procedures, analytical methods, expected range of results, and required detection limits are summarized in Table 1.

Table 1. Analytical Methods Summary.

Parameter	**Description**	**Method**	**Lab**	**Sample Container**	**Preservation**	**Holding Time**	**Precision/ Quantitation Limits**
Pseudomonas	Membrane filtration	APHA 9213E	EPA	NA	NA	8 hours	20% RSD*/ 1cfu/100 mL

* RSD – Relative standard deviation, standard deviation divided by the mean

Results were calculated based on the standard aliquot of *Pseudomonas aeruginosa* containing approximately 10,000 cfu (colony forming units) that was applied to the swatches of pre-cleaned dive suit material. The level of bacteria in the standard aliquot was determined directly with each separate analytical run by testing in triplicate (each row of Table 2 represents triplicates). Column A (D2) of Table 2 were the actually seeded amount of bacteria; it is a calculation based on the known amount of bacteria in the original aliquot and subsequent dilutions. Column B numbers were the recovered bacteria on the membrane filer (without disinfectant). The Column B figures multiplied by 100 (ml volume of the dilution bottle) gives the spiked number or the recovery from the dry suit material. The number of organisms recovered from the dive suit material after a 3 or 1 minute contact time on the dive suit material in the absence of the disinfectant was used as the bacterial load in the applied aliquot, or the "results per 100 ml." This was also done in triplicate and results were averaged to obtain the level demonstrated in the report (see Column C). These numbers can be the same, or nearly so, because they are calculations based on the known concentration of bacteria in the initial dilutant bottle.

Triplicate samples of dry suit material were used to determine the effectiveness of a 4.7 % solution of betadine at reducing the number of bacteria present on the dive suit material within a timed exposure period; this was also done in triplicate for each set of data. The first columns under the "Presumptive" section of Table 2 are averages of the triplicates after disinfection. For example, for row two (week 1b) the three counts where 15, 37, and 51 for an average of 34.3. The data table refers to log removal and percent removal.

Conclusions relative to the study objectives

The efficacy of the potable water rinse procedure for removal of bacteria on the Viking drysuit material (by comparison of Column A (D2), Table 2, (initial seeding) and column C., Table 2 in all sets of data) was apparent in that a potable water rinse will effectively remove up to 100 % of the organisms (range:74 – 100 %; rsd 13.3 %). The relative standard deviation (rsd -standard deviation divided by the mean) indicated that there was no statistically significant difference between tests. However, the organisms rinsed off the dive suit were viable and could present a biological hazard on board the vessel or in the waterway.

The efficacy of Betadine to kill bacteria present on the Viking drysuit material (1 minute or 3 minutes of exposure) was recorded at a 99.98 % (rsd = 0.15 %) reduction of viable organisms using 4.7 % betadine solution and a 3 minute exposure time. A 99.99 % (rsd = 0.006%) reduction of viable organisms was recorded using a 4.7 % betadine solution and a 1-minute exposure time. With a log 3 removal in all cases (week 1b was a log 2, but still achieved 99.71 % removal), the tests were not significantly different.

Discussion

Barsky (2002) and Pearson (2008) list extensive procedures to protect divers in waters potentially contaminated with high-risk pollutants. For diver decontamination from general biological hazards, the EPA Region 10 dive team needs an effective procedure to ensure protection of the diver and tenders. We do not dive in waters contaminated with concentrated jet fuel nor do we perform recovery of large numbers of human remains or animal carcasses from a disaster, for example. The purpose of our decon procedure is to remove the microbes from the diver and prevent contamination of the tenders and the rest of the dive platform. Decon takes place on the swim step (hot zone). Rinsed dive gear (after every dive) is kept near the transom (contaminate reduction zone). Diver tether and/or umbilical lines and other equipment lines and gear are stowed at the stern where draining occurs back into the receiving waters. Divers and tenders are not allowed in the vessel cabin (exclusion zone) unless they are clean. At the completion of dive operations, all gear is thoroughly rinsed (soaking for cameras and regulators); additionally, the boat deck is rinsed. Back in the dive locker, the AGA masks are soaked in a 4.7 percent Betadine solution for minimum of 10 minutes, rinsed, disassembled and dried.

Table 2. Consolidated data summary table.
Each row in the table represents the result for that triplicate set.

Sample Data							**Presumptive**		
			A	**B**	**C**	**D**	**mPA (48 hr @ 41.5°C)**		
Sample No.	**Date**	**Disinfection Time (min)**	**D2 results (initial spike)**	**Recovery from drysuit; MF count (ml)**	**Recovery from drysuit (spiked #)**	**Percent Recovery**	**Count (avg)**	**Percent Removal**	**Log Removal**
1a	7/15/2008	3	10,750	80 per 100	8000	74	3.7	99.95	3
1b	7/16/2008	3	10,250	119 per 100	11967	> 100	34.3	99.71	2
1c	7/18/2008	3	10,250	102 per 100	10200	99.7	9.3	99.99	4
2a	7/22/2008	1	16,750	157	15,733	94	1.3	99.99	4
2b	7/22/2008	1	16,750	157	15,733	94	0.3	99.99	4
2c	7/22/2008	1	16,750	157	15,733	94	2	99.98	3

Notes: 1 ml of “C” dilution bottle should be 10,000 organisms and “D2” (initial seeding) should be equivalent to 10 organisms/1 ml or 30 organisms per 3 ml. D = Calculate the percent recovery from the dry suit based on a 1 ml addition of dilution bottle “C”, this step will indicate the efficiency of removal and the concentration that should be used to determine the log “kill” of organisms on the test strip. E.g.: see test above. 1 ml of “C” added to strips, calculated level of spike (8 (B)) x 10000 divided by expected (30) = 2667 actual seeded amount (A). Recovery of organisms off dry suit (C) = number counted on MF (B) X volume of rinsate, or using example (17 x 150 ml) = 2550 organisms in total spike. Percent recovery off dry suit by rinsing (D) = number of organisms recovered off dry suit(C)/calculated level of spike (A) x 100. (e.g. 2550/2667 x 100= 95.6 %. Percent removal of organism from dry suit = Number of organisms recovered from dry suit before disinfection – number of organisms recovered after disinfection divided by number of organisms before x 100. E.g. (2667 – 0/2667)x 100 = 100%. Convert the percentage removed to log removal by conversion to log base 10. Based on significant figures, the log removal for this example would be 3. MF is membrane filter; mPA is the agar media used specifically for detection of PA (*Pseudomonas aeruginosa*) using the membrane filtration method.

This study has shown that it is unnecessary to spray the diver with Betadine and allow a period of time to pass before rinsing the diver again for dive sites with microbial contamination only. The standard use of Betadine as described (Amson, 1991) is not needed where removal of pathogens is the objective over killing the pathogens on the diver. Likewise, the USEPA SOP published by Tulis (1994) for diver decon after exposure to biological hazards can be considered an overly conservative procedure based on our study (comparing biohazard removal versus biohazard destruction). Using Betadine, heavy soaps, surfactants and related products that are recommended by Tulis (1994), and subsequently rinsed into the receiving water, may be problematic. A variety of disinfection products for divers are available (e.g., SaniZide Plus, Advance TB$_E$, Bi-Arrest 2, Confidence Plus). Collection of all rinsates is required in most jurisdictions. Rapid decon reduces diver stress on hot days and improves overall efficiency of the operation. With our procedure biological hazards that we carry out of the water are nearly all rinsed back into the water.

Decon of other diver gear and underwater instruments were not part of this study. Weight belts are often neglected during decon. Fins that may collect contaminated sediment must be rinsed when the diver removes them on the swim step. Decon of items such as nylon (porous) buoyancy compensators is difficult. The inside of BCDs need thorough rinsing after exposure to salt water (CWD, or not). The BCD accordion hose and inflator must also be rinsed thoroughly. Decon of divers needs updated research since the early studies and procedures such as USEPA's protocol (Traver, 1985) and NOAA's (Wells, 1984).

Disclaimer: This paper is an illustration of steps to be taken to minimize exposure to the diver and tenders in hazardous environments and does not represent the official view of the USEPA. Mention of any specific brand or model instrument or material does not constitute endorsement by the USEPA.

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OCEAN ACIDIFICATION EFFECTS ON BENTHIC MEDITERRANEAN ORGANISMS ALONG A NATURAL CO_2 GRADIENT

Fiorella Prada[1]
Erik Caroselli[1]
Francesco Zaccanti[1]
Bruno Capaccioni[2]
Giuseppe Falini[3]
Oren Levy[4]
Zvy Dubinsky[4]
Stefano Goffredo[1]

[1]Marine Science Group
Department of Biological, Geological and Environmental Sciences, Section Biology
Alma Mater Studiorum–University of Bologna
Via Selmi 3
40126 Bologna, ITALY
fioprada@libero.it

[2]Department of Biological, Geological and Environmental Sciences, Section Geology
Alma Mater Studiorum–University of Bologna
Piazza Porta San Donato, 1
40126 Bologna, ITALY

[3]Department of Chemistry "G. Ciamician"
Alma Mater Studiorum–University of Bologna
Via Selmi 2
40126 Bologna, ITALY

[4]The Mina and Everard Goodman Faculty of Life Sciences
Bar-Ilan University
Ramat-Gan 52900, ISRAEL

Introduction

Increasing anthropogenic CO_2 is predicted to be a major driver of environmental change during the current century in both terrestrial and marine ecosystems (Edenhofer et al., 2011). Climate change is a threat to marine biota because increased atmospheric CO_2 is causing ocean warming, acidification, hypercapnia, decreased carbonate saturation and hypoxia (Cao et al., 2007) leading to more corrosive conditions for calcifying organisms and making it more difficult for them to build and maintain their carbonate skeletons (Raven et al., 2005).

Most studies to date investigating how elevated CO_2 conditions will impact the function of marine organisms have been single-species laboratory experiments that last a year at most (Martin and Gattuso, 2009). Such experiments provide important information on species' responses to increased pCO_2 but fail to account for the effects of long-term exposure, ignoring full organism acclimatization. They are also unrepresentative of natural ecosystems since, for example, they remove the effects of species interactions providing little or no information on processes leading to ecosystem adaptation, such as altered reproduction, competition, food webs and disease susceptibility or genetic adaptation (Barry et al., 2010). There is, therefore, great need for empirical data documenting the long-term effects of ocean acidification on marine ecosystems acclimatized to high pCO_2 as found, for example, around submarine CO_2 vents. Vent systems are not perfect predictors of future ocean ecology owing to temporal variability in pH, spatial proximity of populations unaffected by acidification and multiple environmental parameters which may co-vary, making it difficult to resolve the influence of Ω_{arag} on calcification from that of other factors (Hall-Spencer et al., 2008). However, such vents acidify seawater on sufficiently large spatial and temporal scales to integrate ecosystem processes such as production, competition and predation.

Here we assess the effect of increasing levels of CO_2 on the abundance of the scleractinian solitary zooxanthellate coral *Balanophyllia europaea* (Anthozoa), the tube-forming gastropod *Vermetus triqueter* (Gastropoda), the brown alga *Padina pavonica* (Phaeophyceae) which deposits calcium carbonate as aragonite needles extracellularly on the surface of fan-shaped thalli (Okazaki et al., 1986), the green alga *Acetabularia acetabulum* (Ulvophyceae) whose outer surfaces of the cell wall and intercellular spaces are calcified with aragonite crystals (Kingsley et al., 2003), and the brown foliose macroalga *Lobophora variegata* (Phaeophyceae).

Methods

During several surveys pH, temperature, salinity, total alkalinity and carbonate chemistry parameters were monitored at four stations in and around an underwater extinct volcanic crater off the island of Panarea, Italy, which is characterized by continuous and localized cold CO_2 emissions creating a natural acidity gradient. Alongside, ecological surveys were made to identify the percent cover of *B. europaea*, *V. triqueter*, *P. pavonica*, *L. variegata* and *A. acetabulum* along this gradient. On every occasion the percent cover of each species was quantified at each site at a depth of 10-12 m from photographs taken at 5-10 randomly placed quadrats, 2 m away from each other. The area of each organism within the quadrat was extracted by tracing its outline with a hand-controlled mouse on the digital image, with digitizing software.

Results and Discussion

Our data indicate a decrease in the abundance of a scleractinian coral, a mollusk, two calcifying macroalgae and one non-calcifying macroalgae with decreasing pH, up to a threshold where they no longer occur, with the calcifying algae showing higher resistance to lower pH. *Balanophyllia europaea* and *V. triqueter* were found up to an average pH_{TS} of 7.72 while the three algal species were detected, in low densities, in more acidic conditions up to an average pH_{TS} of 7.38. In a previous study, *B. europaea*, *V. triqueter*, *P. pavonica* and *A. acetab*ulum were found only outside the vents where average pH_{TS} was 8.14[6]. These results may offer some cause for relief as they show that these species can survive at levels of CO_2 higher than previously thought.

Increasing levels of CO_2 can profoundly affect the abundance of a wide range of benthic organisms. Impacts on calcifiers will cascade through marine ecosystems as they play a major role in forming 3D complexity in benthic systems, including reefs. Thus, elevated atmospheric CO_2 might affect marine plants and algae in unique ways compared with terrestrial species and these interactions warrant closer examination and a research focus. Although natural CO_2 venting sites are not precise analogues of global-scale ocean acidification, they can provide essential information on high-CO_2 effects on spatial and temporal scales, which are otherwise difficult to assess. Hence, they provide valuable information for modeling future marine ecosystem trends under various IPCC scenarios.

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DIVING IN THE COLUMBIA RIVER: CHALLENGES AND TECHNIQUES

Katie Rathmell
Michael P. Wilkin
Antonio M. Baptista
Center for Coastal Margin Observation and Prediction
Oregon Health and Science University
20000 NW Walker Road
Beaverton, OREGON 97006 U.S.A.
rathmellk@stccmop.org

The Center for Coastal Margin Observation and Prediction (CMOP) operates a Coastal Ocean Observatory located in the Columbia River Estuary, which is comprised of 17 river stations, two ocean buoys and a suite of AUVs. Of the stations in the river, nine are fixed moorings that require diver access. In order to service these stations dives must be planned to coincide with the slack tide, which allows a 30-40 minute window of opportunity to complete the necessary work. River flow added to the outgoing tide produce surface flows that can exceed 8 knots (ebb). The water column is often stratified, with a surface current going one way and a bottom current going in the opposite direction. Visibility is very limited most of the year: at times it is zero. The river water temperature ranges from less than 4°C in winter to greater than 20°C in summer; meanwhile ocean temperatures remain in the 8-12°C range leading to strong temperature variations during a single dive. The combination of the tides, currents, visibility, temperature and remoteness of dive sites makes it a challenging work environment. This paper will discuss the techniques utilized by the dive team to maintain underwater science moorings in the face of challenges presented by this complex river system.

Introduction

The Columbia River flows from its headwaters in the Canadian Rockies to the Pacific Ocean at Astoria, Oregon and Ilwaco, Washington (Anonymous, 2001). The Columbia River discharges more than 244 billion cubic meters of water per year; on average a yearly flow of 7,787 m^3/s (Anonymous, 2001). The highest volumes occur during the spring freshet from snowmelt from May to June (Fain et al., 2001). The estuary has two channels, the North and South channels; the South channel is routinely dredged for navigation. The Columbia River Bar is known as one of the most dangerous in the world and has earned the name “Graveyard of the Pacific”.

Sampling stations

The Center for Coastal Margin Observation and Prediction (CMOP) operates the Saturn Observation Network, an NSF-funded science and technology center focused on the study of coastal margins. The network is comprised of stations populated with various sensors that are either moored or profiling (Figure 1). The moored sensor platforms are populated with conductivity and temperature (CT) sensors. Additionally, two stations have acoustic Doppler profilers (ADP). The moored stations are located on day marks and back range boards, which are used as aids to navigation in the river. These structures can be anything from a four-legged piling with a small crow’s nest at the top, to a larger four-legged steel tower, to a wooden tower that stands 10 m above the river bottom. Stations are located near the channels in order to characterize the estuary turbidity maximum (ETM). The salt wedge follows the channels as it comes into the river detected by the CTs at the moored stations. The stations report real-time salinity and temperature to the network, which is available to the public at www.stccmop.org. In addition to physical measurements, the Observatory measures biogeochemical parameters at two stations in the river. Biogeochemical stations are set up as pumped stations that do not require diving to service them.

Station dive planning and sampling

Each station has its own unique conditions that must be taken into consideration when planning a dive. Jetty A is the outermost station and tends to have the best visibility because of the ocean influence. There are two stations located on the Astoria Bridge, one of which requires diving. Station AM169 is located on a piling underneath the highest span of the bridge about 50 feet beneath the surface. The station is exposed to wind and waves as it is located just north of the South channel near the main navigation channel. Desdemona Sands is located near the North Channel and is a 24-foot dive; visibility at this station is commonly very bad. There is a CT and ADP located at the City of Astoria wastewater outfall that is considered a polluted dive site and special cleaning of gear is required following dives.

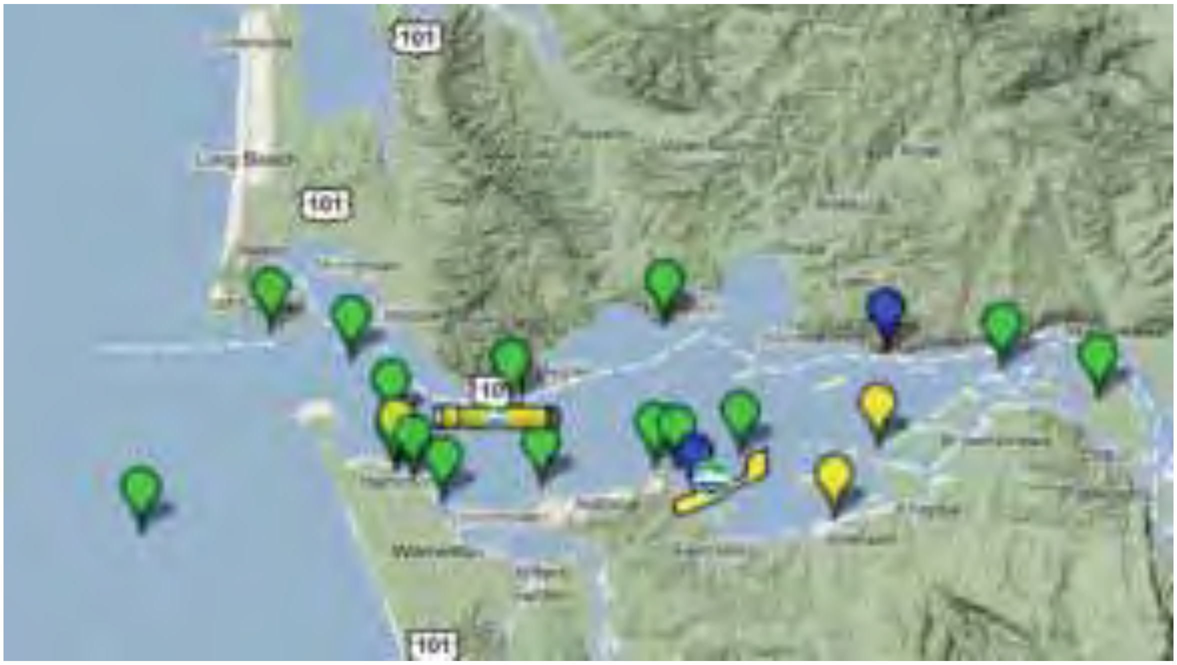

Figure 1. Saturn Observation Network.

Some stations are a bit more challenging to dive than others. Of the nine stations that are serviced by diving, Sand Island tends to offer great variability and challenges. Sand Island is a 25-minute boat ride to the North side of the river and conditions have to be quite good, with low swell and low wind waves in order to moor the boat. The general plan is to be on station before the tide stops running, just before slack tide. The boat basin where the team launches to get to Sand Island is a 30-minute car ride from the campus. The station is located on a four-legged structure with a small crow's nest at the top, shown in Figure 2. It is situated at the end of a pile dike, with the river bottom at about 7.9 m.

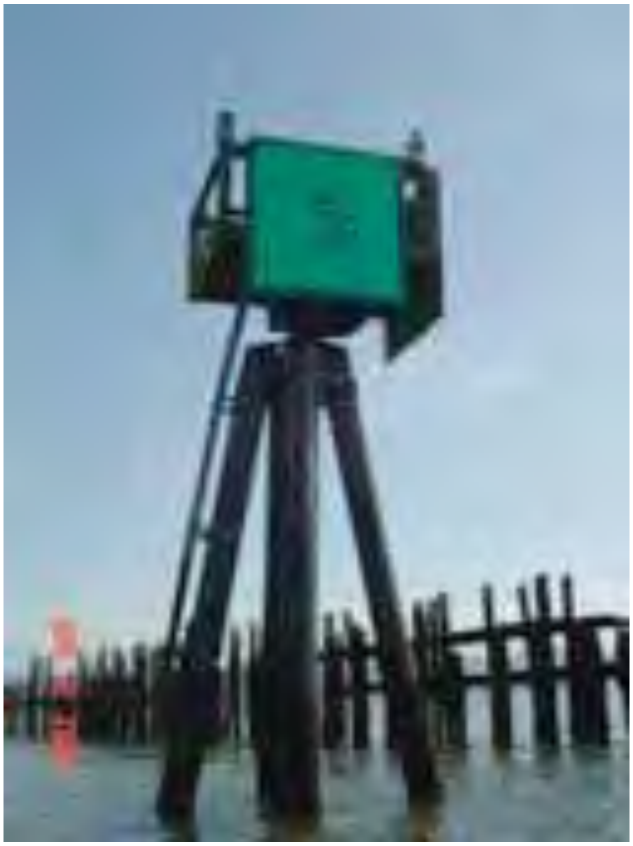

Figure 2. Sand Island.

When the tide is running out the boat swings away from the pile dike; however, when it is flooding, the boat swings toward the piles and is just about one boat length away. The dives are scheduled to coincide with slack tide; however, the tidal current rarely, if ever, stops at this site. If the water is slack at the top, it is most likely still running at the bottom. The tide will often start to run at the bottom before it starts at the top. At this station, it is highly likely that the diver will experience current at the bottom at the beginning of the dive and current at the top near the end of the dive. To overcome some of the current, divers are heavily weighted. In addition, a Kirby-Morgan full-face mask with a hard shell and two-way communications is preferred for most dives. The safety diver monitors communications and air, while a second tender controls down lines and hands off tools and sensors to the diver. The visibility at this and other stations during most of the year will be measured in cm. If the diver can see the instrument on the frame as they kneel in front of it, it is considered decent visibility. Many times that is not the case. It is for this reason that all of the moorings are configured to be the same at all sites.

The configurations of the moorings are generally kept very simple and similar to each other depending on the type of instrument. All CTs are attached to a stainless steel frame that is chained to one leg of the structure near the riverbed. Most of our CTs are attached to the frame with white zip ties and the power/data cable follows the leg up to the crow’s nest. The cable is secured to the leg with bungee cords. The legs of most of the structures are about 35 cm in diameter. A couple of stations with older sensors have a slightly different configuration requiring the diver to remove a bolted-on bracket. The zip tie attachment seems to work very well and they are easier to see than a nut and bolt, especially on seaward stations that tend to have significant hard fouling. A typical day of diving would require the diver to retrieve and replace the instrument on the mooring.

Safety considerations

As the diver drops down the leg, they follow the cable while removing it from inside the bungee cords that hold it snug to the piling. The cable is attached to the frame in a way such that slight tugging will not hurt the instrument but it is not meant to be pulled on as a down line. During most of the year, light from the surface is extinguished at about 3 m below the surface. The water is brown and full of particulates that scatter any light emitted from a headlamp and at the bottom it is pitch black. The turbidity of the water often makes it impossible to see the piling in front of the diver as they kneel down on the riverbed. With the diver positioned inches away from the CT frame they still may not be able to see anything. This is where the white zip ties come in; they do tend to catch the glow from a headlamp. When the diver finds the zip ties they cut them, pull them away from the frame and instrument, and put them in a pocket. The CT is then free from the frame and can be brought to the surface. The diver hands the instrument to a tender in the boat and waits for the CT to be switched and handed back. When the diver is given the new CT attached to the cable, along with a handful of zip ties, they descend again to reattach the CT. Once the CT is back in place the diver ascends the piling replacing bungee cords around the cable. This can get difficult, especially if the tide has started running. The diver will already be fatigued and cold and stretching an encrusted bungee around an encrusted piling with a building current pulling on them makes it especially difficult.

The water temperature in the river is highly stratified. Ocean temperatures are fairly stable ranging from 8-12°C, but the river temperature fluctuates from 4°C in winter to 20°C in summer. As the estuary is highly stratified surface temperatures can be wildly different from bottom temperatures. This also makes timing the dives difficult as the tide can be slack at the surface but already be running at the bottom. When it is slack before the flood, the tide will come in on the bottom and, at the more seaward stations, the divers may not have any opportunity to get work done as the current will already be too strong. This situation can be reversed on the slack before ebb tide. The current can be howling at the bottom while it looks completely placid at the surface. There are times when the dive is abandoned after a quick trip to the bottom and finding a roaring current.

Another component to diving in the Columbia is the danger of entanglement. There is always the possibility that the diver will encounter submerged logs, debris and fishing gear. Gill nets pose a substantial threat every year during the gill net season. On one occasion the field team set out to dive on a station located in Cathlamet Bay in the north channel of the river. As the diver descended along the piling, they noted that the entire piling leg was wrapped in gill net material, completely trapping the cable and CT. But there was more than just net, entrained within the mass were a string of floats, long pieces of crab line, sticks and other debris that had become trapped. The mass was so big that the diver had to abandon the dive because of entanglement hazards, and eventually the mooring was placed on a different leg of the structure. However, the entrapped CT and cable are still there and every time the station is serviced there is the possibility that some part of that mass will drift free and entangle a diver. Each diver carries a strong pair of shears in case of entanglement.

Safety protocols are strictly adhered to: the safety diver has gear ready to go and an extra cylinder of air is always on board should the dive team require extra time to extricate a diver. Emergency oxygen is always on board during dives and a hand held radio is available to call for assistance. The remoteness of the dive sites makes it necessary for the dive team to have standards in place ahead of time should an emergency arise. Part of the preparedness protocols involves training and fitness to dive.

The importance of proper training combined with an unflappable mentality cannot be over-emphasized. The diver must be fit physically and mentally. In order to dive in the Columbia River, any potential diver must pass the AAUS standards swim test and dive check out, and prove a mental stability that will be required for the extreme conditions they will be placed in. Part of the rigorous check out requires the diver to don a blacked out mask and perform various tasks. The reasoning behind this exercise is simple; can the diver think their way through an uncomfortable and potentially stressful situation without panicking? The conditions that a diver will experience on a real dive will be very different than a pool session. The poor visibility and cold water can put a usually fit diver into a state of stress fairly quickly. An experienced team and strict safety protocols make the dives less risky. The diver has the last word in whether or not the dive will proceed. If, at any time, the diver feels unable to continue the dive the project is abandoned and taken up at a later date. The first and most important aspect of diving in the Columbia is to get home safely.

In conclusion, diving in the Columbia River could be considered extreme diving. The currents, remote dive sites, stratified water column and extreme low visibility make it a challenging environment. The Saturn Observatory is a unique network that must be maintained on a regular schedule and the dive team in Astoria has found a way to work in the river safely.

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SUPPRESSION OF UPWELLING ALONG THE WINDWARD SHORE OF POINT REYES, CALIFORNIA

Matthew J. Robart
University of California, Davis
Bodega Marine Laboratory
PO Box 247
Bodega Bay, CALIFORNIA 94923 U.S.A.
mjrobart@ucdavis.edu

To investigate flow dynamics along a windward shore during upwelling, we used scuba equipment to deploy an array of instrumentation in shallow water near the shore off Point Reyes Beach, California, during the summer of 2011. This beach comprises the southernmost portion of the Point Arena upwelling cell which has been heavily studied in previous investigations of upwelling dynamics over the last several decades. We measured currents using an acoustic Doppler current profiler (ADCP) and also collected temperature, salinity and fluorescence data from two locations on the inner shelf offshore of Point Reyes Beach. By comparing local and regional winds and currents with in-situ instruments and satellite data, we show that upwelling is suppressed close to the coast in this area and that local downwelling is common there during regional upwelling conditions. By comparing our data with prior nearshore flow measurements elsewhere in the region, we show that this phenomenon is only found along the Reyes windward shore. Furthermore, we show that a patch of warmer water is found along this shore during upwelling comprised of aged upwelled water from within the Point Arena upwelling cell. Following relaxation, lower salinity surface water from Drakes Bay may be retained along this shore for a couple of days. This is the first detailed study of flow and water column properties along the windward shore of a major headland during upwelling, and it carries biological implications for this region and similar areas in upwelling regions worldwide such as Chile, South Africa, Namibia, Portugal and Oregon. Retention of surface water and modification of regional upwelling dynamics have important implications for larval dispersal as well as the distribution and life history of holoplankton.

Introduction

Many important studies have investigated upwelling dynamics in the Point Arena region such as the Coastal Ocean Dynamics Experiment (CODE: Beardsley et al., 1987), Northern California Coastal Circulation Study (NCCCS: Largier et al., 1993), Shelf Mixed Layer Experiment (SMILE: Dever and Lentz, 1994), Sediment Transport Events over the Shelf and Slope (STRESS: Trowbridge and Lentz, 1998), and Wind Events and Shelf Transport (WEST: Largier et al., 2006); these have led to improved understanding of coastal, wind-driven upwelling along the eastern boundaries of the world's oceans. Traditional oceanographic studies have focused on regional-scale phenomena using large research vessels and heavy mooring equipment. These methods produced a great deal of informative data that have been used to describe the physical dynamics of the upwelling process but the complex dynamics that occur very close to the shoreline are generally overlooked.

The region between Point Arena and Point Reyes is known as an upwelling cell, and there are several more along the coast of California and in other upwelling regions worldwide. Upwelling cells are anchored by an upwelling center, which is associated with the major headland at the upwind end of the cell. Where such a headland enhances wind and current speeds, as well as convex curving flow, upwelling is at its maximum (as in the case of Point Arena, Winant et al., 1988). During periods of equatorward wind, Ekman transport of surface water away from shore results in lower sea level at the coast. This causes a barotropic pressure gradient over the continental shelf that leads to a geostrophic equatorward current and also drives high density, cool, saline, nutrient-rich water toward the shoreline, feeding the upwelling. The resulting tilt of isopycnals creates a baroclinic pressure gradient in the opposite direction, which reduces alongshore flow at depth. The alongshore current parallels the coast through much of the upwelling cell but may be deflected offshore by the downwind headland, at times forming a large offshore eddy (Penven et al., 2000).

As upwelled surface water is advected away from its source (where it enters the surface euphotic layer), it ages both physically and biologically, i.e., its properties change. Physical aging is caused by warming as a result of solar heating of the cold upwelled water at the surface. From a biological view newly upwelled water is rich in nutrients but lacks phytoplankton and chlorophyll. As this water spends time at the surface, phytoplankton communities thrive on the upwelled nutrients and abundant sunlight. Through time, nutrients become depleted and chlorophyll concentration increases dramatically as phytoplankton populations bloom. This temporal aging leads to spatial patterns in surface water properties during upwelling periods consisting of cool, nutrient-rich, low-chlorophyll waters where active upwelling occurs and warm, nutrient-poor, high-chlorophyll waters away from these areas, such as offshore and in nearshore retention zones.

A marked pattern in surface temperature and chlorophyll is associated with mesoscale topographic features within upwelling cells, such as small headlands and embayments. Specifically, the retention of surface water downstream of a headland, or within a bay, has been recognized as an *upwelling shadow* (Graham et al., 1992) and observed in Monterey Bay, California (Graham and Largier, 1997), St. Helena Bay, South Africa (Penven et al., 2000; Monteiro and Roychoudhury, 2005), Drakes Bay, California (Wing et al., 1998; Largier, 2004; Vander Woude et al., 2006), Lisbon Bay, Portugal (Moita et al., 2003) and Bahia Mejillones, Chile (Marin et al., 2001; 2003). While the idea of an *upwelling trap* has been introduced for semi-enclosed bays facing into the wind, e.g., Antofagasta Bay, Chile (Castilla et al., 2002; Pinones et al., 2007), we are unaware of prior studies that focus on water column dynamics on the windward side of headlands during active regional upwelling conditions.

We expect that dynamics on the windward shores of major headlands are fundamentally different from those on the leeward side as well as those occurring along relatively linear stretches of the open coast. This is especially true for low-lying headlands such as Point Reyes where the wind is not significantly deflected by topography and the onshore component of wind stress is strong during regional upwelling. Local wind forcing may thus lead to nearshore downwelling in these areas or at least that local upwelling is significantly reduced nearshore. The absence of local upwelling would lead to the absence of newly upwelled water and the observation of increased surface temperature and phytoplankton concentration along windward shores of major headlands in upwelling areas.

It is important to note that these expectations are for conditions during intense regional upwelling periods. Upon cessation of upwelling-favorable wind, alongshore current reverses in most locations resulting in poleward transport of surface water. Since upwelling stops, surface warming results in a uniform increase in surface temperatures and nearshore thermal stratification. A buoyant surface current is often formed, as is observed in the Arena-Reyes upwelling cell (Send et al., 1987; Largier et al., 1993; Wing et al., 1995), and this produces additional warming over the inner shelf. In this region, the buoyant current may be comprised of aged (warm) surface water that is buoyant because of thermal stratification or lower salinity water from Drakes Bay (Largier et al., 2006). Originating south of Point Reyes this low-salinity water is influenced by outflow from San Francisco Bay. The additional buoyancy supplied by lower salinity and higher temperature causes the relaxation current to build strength and remain in the upwelling cell for a longer period of time. During relaxations we expect salinity at Point Reyes Beach to be lower than elsewhere in the upwelling cell because of its proximity to Drakes Bay and San Francisco Bay outflow.

It has been described that surface water in the area offshore of Point Reyes Beach tends to be warmer than most other nearshore locations in the Point Arena upwelling cell (Send et al., 1987; Vander Woude, 2006). However, previous studies have only speculated about how this patch is formed and maintained during upwelling. One possibility is that it is comprised of aged, upwelled water from within the Point Arena upwelling cell that remains nearshore because of the suppression of local upwelling caused by topographic effects. There is some evidence that surface currents are directed toward shore in this region as Kaplan and Largier (2006) found, based on HF-radar data, that approximately 20% of virtual drifters released on the inner shelf offshore of Bodega Head crossed into the nearshore zone between Bodega Head and Point Reyes. This could indicate a persistent shoreward meander in the regional upwelling jet or it could indicate shoreward transport of surface water in this area. Another possibility for the formation of the warm surface water patch at Point Reyes Beach is that it is comprised of water from Drakes Bay that was imported during relaxation and then retained through subsequent upwelling conditions.

To investigate the dynamics of this feature we deployed an array of in-situ oceanographic instrumentation off Point Reyes Beach on the windward side of Point Reyes, California during the summer of 2011. To compare flow characteristics measured during this study to those in other areas of the upwelling cell we also analyzed data from two additional deployments made during the summer of 2009 at Stewarts Point and at Bodega Head (Fig. 1).

Since salinity is conserved during the process of surface water aging, it can be used as a control to test whether regional differences in water properties are caused by aging of upwelled water or the presence of offshore or bay-outflow-influenced water types. If the warmer water seen at Point Reyes Beach originated in Drakes Bay, we expect surface water at Point Reyes Beach to be warmer than other locations within the upwelling cell but also with lower salinity because of the presence of water from San Francisco Bay. However, if the warm water originated to the north, we expect that it would also be warmer than upstream locations but with no notable difference in salinity.

The purpose of this paper is to determine whether water conditions at the windward shore of Point Reyes do indeed differ from other locations within the upwelling cell and to decide whether these differences are the result of retention of relaxed water or suppression of upwelling. The instruments we deployed allowed us to investigate flow characteristics throughout the water column, as well as physical and biological water properties such as temperature, salinity, and fluorescence.

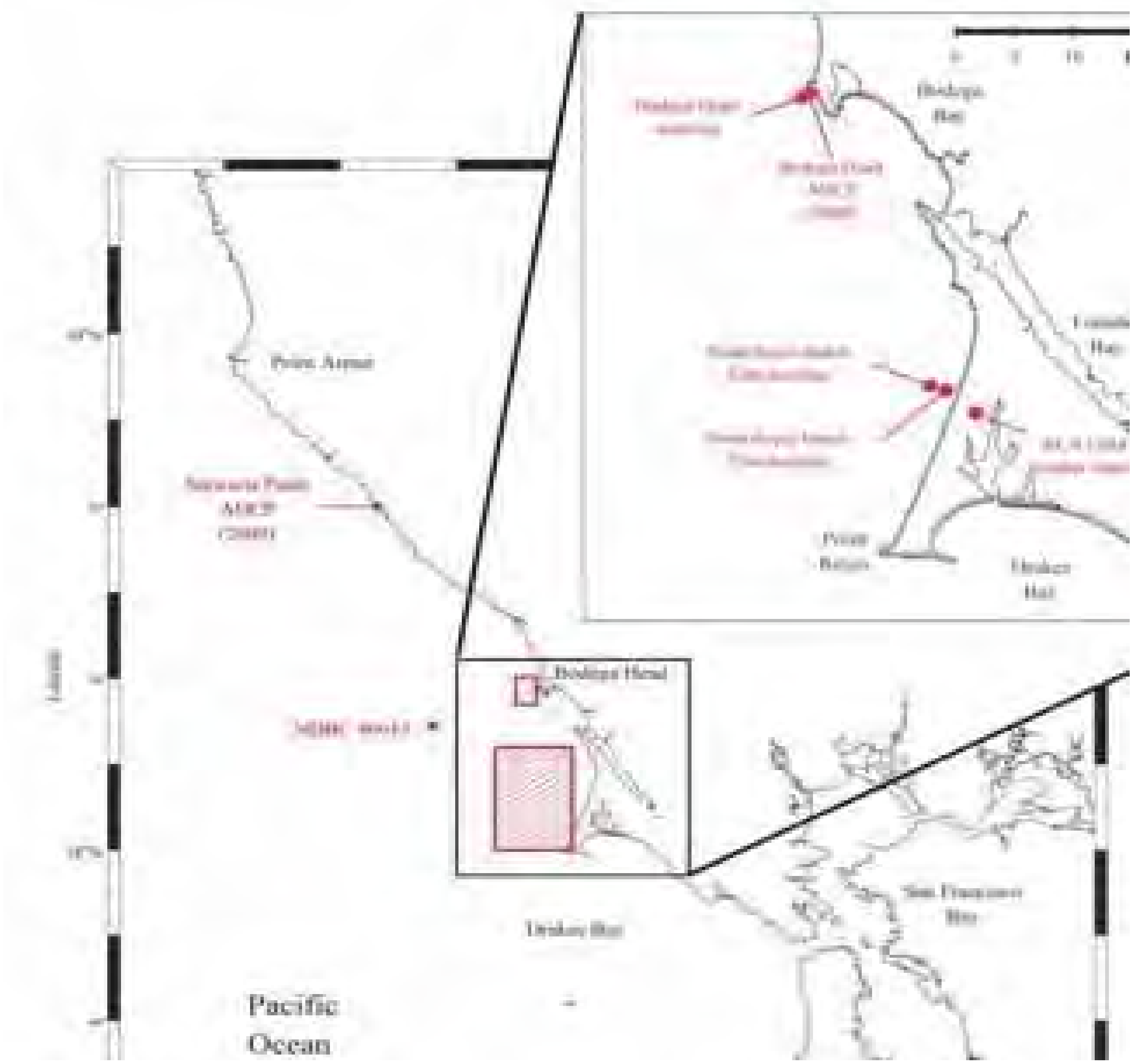


Figure 1. The Point Arena upwelling cell. Red dots and labels indicate locations where data were collected, and the red cross-hatched areas on the larger map indicate the areas where HF Radar-derived surface currents were averaged together into the flow cells used in analyses.

Methods

Point Reyes Beach mooring array: 2011

Two moorings were deployed offshore of Point Reyes Beach from 7 June 2011 until 8 November 2011. This location is on the northern flank of Point Reyes on the central California coast approximately 48 km northwest of San Francisco Bay (Fig. 1). One mooring was placed on the 22-m isobath (MLLW), approximately 800 m from the shoreline. This mooring consisted of a bottom-mounted 1200 kHz, 20^o beam angle, Acoustic Doppler Current Profiler (ADCP, Teledyne RD Instruments, Poway, CA) profiling at 120 sec intervals with a 2-m vertical resolution. High-resolution thermistors (Sea Bird Electronics, Inc. Model 39, Bellevue, WA) were placed at depths of 4 m, 10 m, and 20 m sampling at 60 sec intervals, salinity/temperature recorders (Sea Bird Electronics, Inc. Model 37, Bellevue, WA) at 0.5 m and 22 m sampling at 60 sec intervals, and a chlorophyll fluorometer/turbidity meter (WET Labs FLNTUSB, Philomath, OR) at 0.5 m sampling at 300 sec intervals. The ADCP battery failed on 13 September 2011 but all other instruments sampled for the duration of the deployment.

The second mooring was deployed on the 42-m isobath, approximately 1.6 km from the shoreline (Fig. 1). This mooring consisted of a salinity/temperature recorder (Sea Bird Electronics, Inc. Model 37, Bellevue, WA) at 0.5 m depth and high-resolution thermistors (Sea Bird Electronics, Inc. Model 39, Bellevue, WA) at 4 m, 10 m, 20 m, 30 m, and 40 m depths. All instruments on the 42-m mooring were sampling at 60 sec intervals for the duration of the deployment.

An oceanographic buoy is located on the 30-m isobath at Bodega Head, approximately 1.6 km from the shoreline (Fig. 1). At the time this buoy was not functioning as designed but was equipped with a salinity/temperature recorder (Sea Bird Electronics, Inc. Model 37, Bellevue, WA) and a chlorophyll fluorometer/turbidity meter (WET Labs FLNTUSB, Philomath, OR). Each instrument was sampling at 300 sec intervals.

Bodega Head and Stewarts Point mooring array: 2009

Two ADCP deployments during the summer of 2009 were used to make comparisons of flow dynamics elsewhere in the Point Arena upwelling cell. One deployment was in 17-m depth at Stewarts Point, near what is considered to be the center of upwelling for the region (CODE: Beardsley et al., 1987). The other was at located in 15 m of water at Bodega Head, which is roughly halfway between Stewarts Point and Point Reyes Beach (Fig. 1). Both the 2009 ADCPs were configured with a 1-m vertical resolution and 3-min profiling interval.

High-Frequency Radar

Grid maps of daily-averaged surface current vectors for this region were obtained from HF-radar data acquired by the Bodega Ocean Observing Node located at the Bodega Marine Laboratory (www.bml.ucdavis.edu/BOON). The maps used in the present study were created using data from the high-resolution (2 km) dataset. Surface current vectors are derived from radar sites located at Salt Point State Park, Bodega Marine Laboratory, and Point Reyes (Kaplan et al., 2005). The instruments operate at a frequency of 12 MHz, which results in a 2 x 2 km spatial resolution covering most of the Point Arena upwelling cell to a distance of approximately 30 km offshore.

Surface current data were averaged within each of two discrete spatial cells for analysis of time-dependent flow variability: one located just off Bodega Head (encompassing a 4 km by 6 km patch of water beginning approximately 1 km offshore) and the other located just off Point Reyes (encompassing a 14.5 km by 22 km patch of water beginning directly at the tip of Point Reyes.

Meteorological data

Wind speed and direction data were acquired from NDBC Buoy 46013 (National Data Buoy Center, www.ndbc.noaa.gov). Wind velocity was decomposed into alongshore and cross-shore components, based on the regional coast angle of 320^o from true North (following Dorman, 2006). Wind speed and direction were also obtained from the meteorological station at RCA Field near Point Reyes Beach, operated by the Western Regional Climate Center (www.wrcc.dri.edu) - these data were rotated to the local shoreline angle of 16^o from true North, producing local alongshore and cross-shore wind components.

Satellite data

Daily composite images of Advanced Very High Resolution Radiometer (AVHRR) satellite-derived sea surface temperature (SST) and chlorophyll-a imagery from the Aqua MODIS instrument were obtained using the NOAA CoastWatch West Coast Data Browser (http://coastwatch.pfeg.noaa.gov).

ADCP data processing

All velocity data from the Point Reyes Beach ADCP were converted from speed and direction to horizontal components of velocity rotated to the local 10-km scale shoreline angle of 16^o from true North (nearshore isobaths parallel shoreline). For the purposes of investigating vertical velocity shear, bottom, mid-water and surface bins were derived from the ADCP data. The mid-water bin was fifth data bin above the ADCP which represented flow from 10 m to 12 m above the bottom. The surface bin was found by tracking the surface using the ADCP's pressure record to account for tides. For each time point, the bin nearest to the surface without approaching within 10 cm of the surface itself was considered to be the surface bin. Therefore, the surface bin used in analyses was comprised of a 2-m thick band which varied with the tide but the base of which was never deeper than 4.1 m from the surface. The same procedure was repeated to find the second and third bins from the surface, each being 2 m thick. The vertical shear in cross-shore velocity was calculated as the difference in velocity between the surface bin and the mid-water bin of the ADCP data and was used as an index of local upwelling (or downwelling) circulation.

Velocities from the Stewarts Point and Bodega Head ADCPs were similarly rotated to the local shoreline angle: 320^o from true North at Stewarts Point and 330^o from true North at Bodega Head. Both of these shoreline angles were found using nautical charts. Since the Stewarts Point and Bodega Head ADCPs were configured with a 1-m vertical resolution, the surface bin used in analyses was the mean of the 2 bins closest to the surface. Likewise, the mid-water bin used to find the index of cross-shore velocity shear was the mean of the 7th and 8th bins above the bottom, which represented flow in a 2-m thick band from 7.5 m to 9.5 m above the bottom.

ADCP velocities from all sites showed little high-frequency variability and these data were hourly averaged to allow statistical comparisons with other data parameters. Cross-shore velocity was plotted as depth profiles for each hour during the study period (8 - 29 June 2011) and viewed as an animation. The data were also averaged into daily means for each depth bin and depth profiles of cross-shore velocity were plotted for each day of the deployment.

Linear regressions

Simple linear regressions using the least-squares method were performed using Matlab R2011a and R2012b (MathWorks, Natick MA). The independent variables included alongshore wind velocity at NDBC buoy 46013, alongshore and cross-shore wind velocity at RCA Field, and alongshore HF-radar surface currents for the nearshore cells at Point Reyes and Bodega Head. The dependent variables used in the regressions included the depth-averaged alongshore surface current and cross-shore shear (calculated as described above) at nearshore ADCPs.

Results

Velocity Analyses

Local wind versus nearshore currents

A comparison of local wind (from RCA Field) with data on currents revealed tight coupling of wind with surface flow at the Point Reyes Beach mooring. Alongshore wind was well correlated with alongshore flow in the surface bin (slope = 0.0663, r^2 = 0.4957, Table 1) and in the time series this coupling can also be seen deeper in the water column by comparing the surface bin with the third bin from the surface (Figs. 2a, 3a, 4a). Likewise, cross-shore velocity shear was positively but weakly correlated with cross-shore wind (slope = 0.0168, r^2 = 0.0773, Table 1). The strength of the correlation is weakened by the relaxation periods as the relationship between cross-shore shear and onshore winds is evident in Figure 2b (also Figs. 3b and 4b) by seeing that the blue (surface velocity) line is consistently above the red (mid-depth velocity) line during periods of onshore wind. Much of the time, the surface velocity is onshore and the mid-depth flow is offshore, consistent with local wind-driven downwelling.

Table 1. Results of all linear regressions. In the table, alongshore flow in the Bodega Head HF radar-derived surface current cell is named "Bodega Head HF cell" and alongshore flow in the Point Reyes HF radar-derived surface current cell is named "Point Reyes HF cell."

		Point Reyes Beach 2011			Bodega Head 2009			Stewarts Point 2009		
independent (regional)	dependent (local)	slope	intercept	R-square	slope	intercept	R-square	slope	intercept	R-square
RCA cross-shore wind	cross-shore velocity shear	0.0168	0.0348	0.0773						
RCA alongshore wind	cross-shore velocity shear	-0.0151	0.0686	0.0380						
RCA cross-shore wind	alongshore surface velocity	-0.0453	0.0864	0.3772						
RCA alongshore wind	alongshore surface velocity	0.0663	-0.0141	0.4957						
N13 alongshore wind	RCA cross-shore wind	-0.4467	-0.8891	0.5376						
N13 alongshore wind	cross-shore velocity shear	NS	0.0580	0.0000	0.0010	0.0189	0.0027	0.0043	0	0.1095
Bodega Head HF cell	cross-shore velocity shear	-0.1190	0.0067	0.0084	-0.0235	0.0133	0.0018	0.0557	-0.0245	0.0226
Point Reyes HF cell	cross-shore velocity shear	-0.0971	0.0609	0.0071	NS	0.0134	0.0006	0.0941	-0.0188	0.0527
N13 alongshore wind	alongshore surface velocity	0.0189	0.1192	0.1774	0.0113	0.0441	0.1354	0.0125	0.0710	0.1478
Bodega Head HF cell	alongshore surface velocity	0.7318	-0.0142	0.2261	0.4272	-0.0212	0.2475	0.3992	0	0.1930
Point Reyes HF cell	alongshore surface velocity	0.7156	0.0260	0.2797	0.4344	0	0.2058	0.4535	0.0269	0.2005

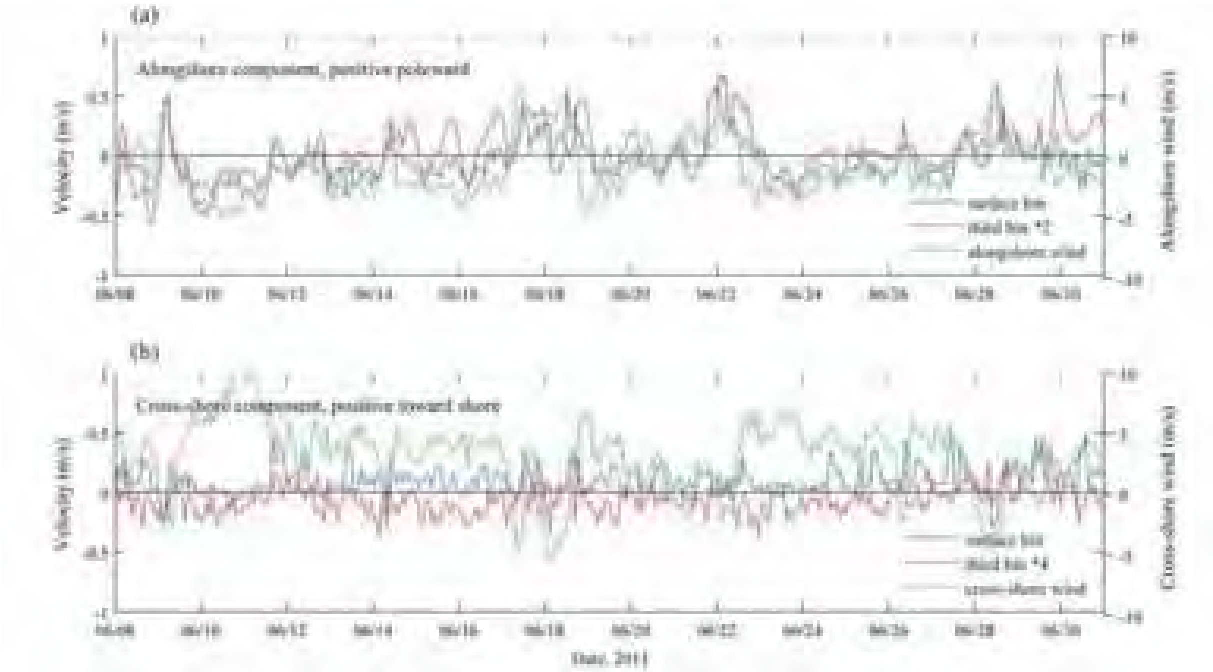


Figure 2. Time series of alongshore (a) and cross-shore (b) velocities from the Point Reyes Beach ADCP. The surface bin (blue line) and third bin from the surface (red lines) are compared with the alongshore (a, green line) and cross-shore components (b, green line) of wind from RCA Field for 08 through 29 June

2011. Alongshore flow in the third bin was multiplied by a factor of two while cross-shore flow in the third bin was multiplied by a factor of four for better visual comparisons.

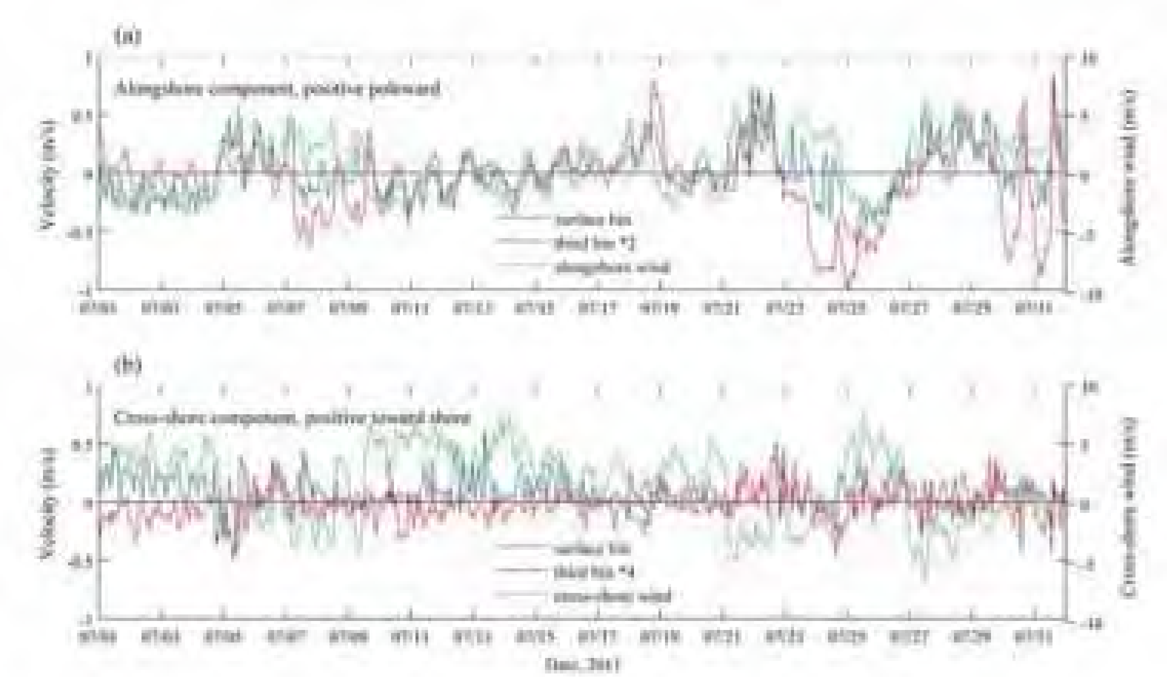


Figure 3. Time series of alongshore (a) and cross-shore (b) velocities from the Point Reyes Beach ADCP. The surface bin (blue line) and third bin from the surface (red lines) are compared with the alongshore (a, green line) and cross-shore components (b, green line) of wind from RCA Field for the month of July 2011. Alongshore flow in the third bin was multiplied by a factor of two while cross-shore flow in the third bin was multiplied by a factor of four for better visual comparisons.

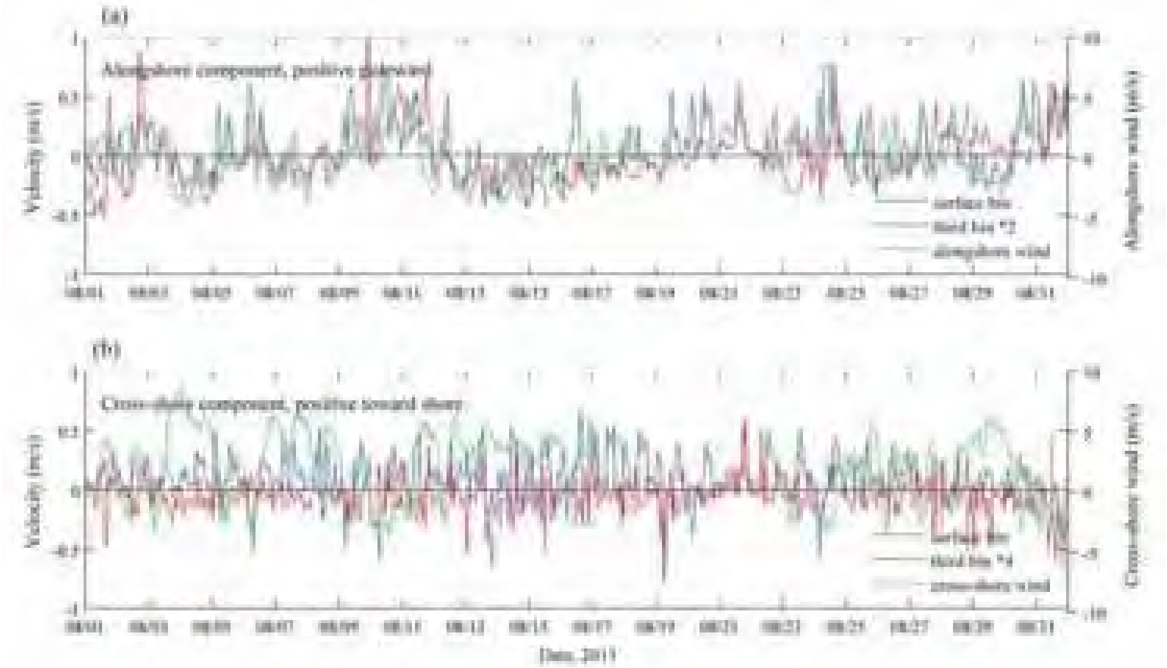


Figure 4. Time series of alongshore (a) and cross-shore (b) velocities from the Point Reyes Beach ADCP. The surface bin (blue line) and third bin from the surface (red lines) are compared with the alongshore (a, green line) and cross-shore components (b, green line) of wind from RCA Field for the month of August

2011. Alongshore flow in the third bin was multiplied by a factor of two while cross-shore flow in the third bin was multiplied by a factor of four for better visual comparisons.

Regional wind versus nearshore currents

Comparison of regional wind from NDBC Buoy 46013 with data on currents showed that flow at Point Reyes Beach was less tightly coupled to regional wind than it was to local wind. Correlations between alongshore buoy wind and alongshore current were positive at all locations (Point Reyes Beach: slope = 0.0189, r^2 = 0.1774; Stewarts Point: slope = 0.0125, r^2 = 0.1396; Bodega Head: slope = 0.0113, r^2 = 0.1270, Table 1). There was a slightly negative but non-significant relationship between alongshore buoy wind and cross-shore velocity shear at Point Reyes Beach (Table 1), yet there were positive relationships between alongshore buoy wind and cross-shore shear at both Stewarts Point and Bodega Head (Stewarts Point: slope = 0.0043, r^2 = 0.1095; Bodega Head: slope = 0.0010, r^2 = 0.0027, Table 1). The time series of alongshore flow at the surface and third bin shows that flow in the third bin is directionally consistent with surface flow at all three locations but with diminished velocity (Figs. 2a-10a). However, cross-shore flow in the surface bin was much greater at Point Reyes Beach than at the other two sites and it was the only site where cross-shore flow in the third bin opposed flow in the surface bin (Figs. 2b-10b).

Regional wind versus local wind

There was a strong negative correlation between alongshore wind at NDBC Buoy 46013 and cross-shore wind at RCA Field (slope = -0.4467, r^2 = 0.4957, Table 1), meaning that when buoy wind was equatorward, the wind at RCA Field was onshore. Examination of the time series also shows this relationship. Also, it is clear that wind is generally stronger at the buoy (consistent with Dorman et al., 2006) and that there are more wind reversals at RCA Field (Figs. 11d, 12d).

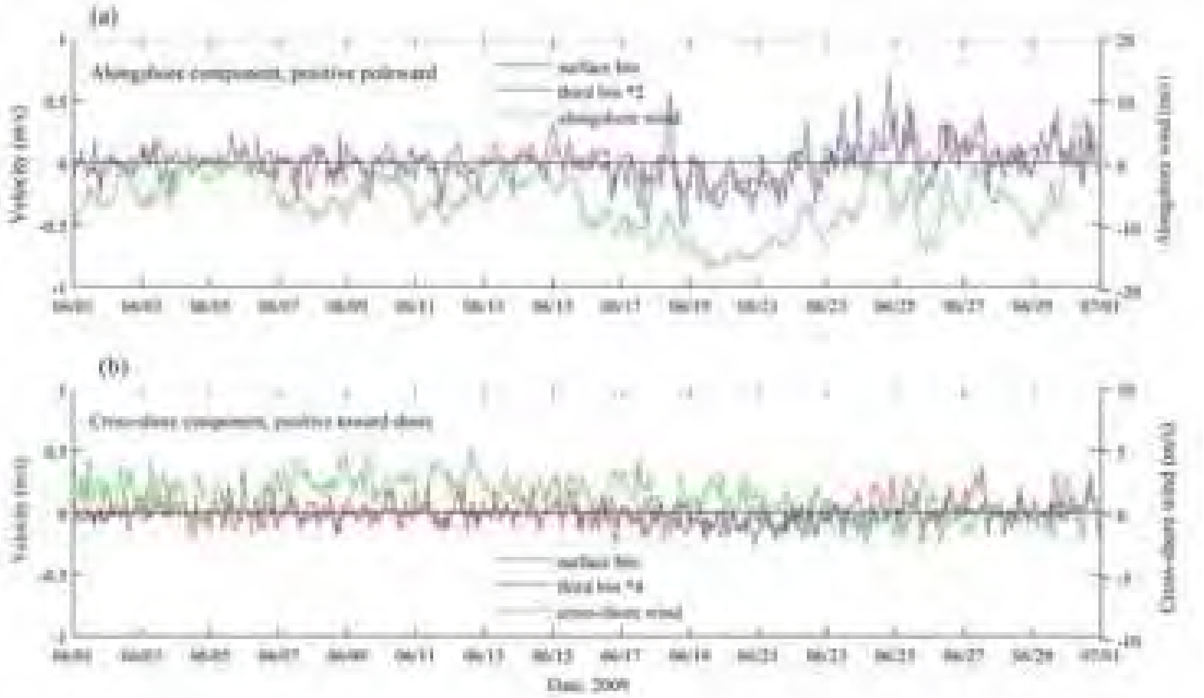


Figure 5. Time series of alongshore (a) and cross-shore (b) velocities from the Stewarts Point ADCP. The surface bin (blue line) and third bin from the surface (red lines) are compared with the alongshore (a, green line) and cross-shore components (b, green line) of wind from NDBC Buoy 46013 for the month of June 2009. Alongshore flow in the third bin was multiplied by a factor of two while cross-shore flow in the third bin was multiplied by a factor of four for better visual comparisons.

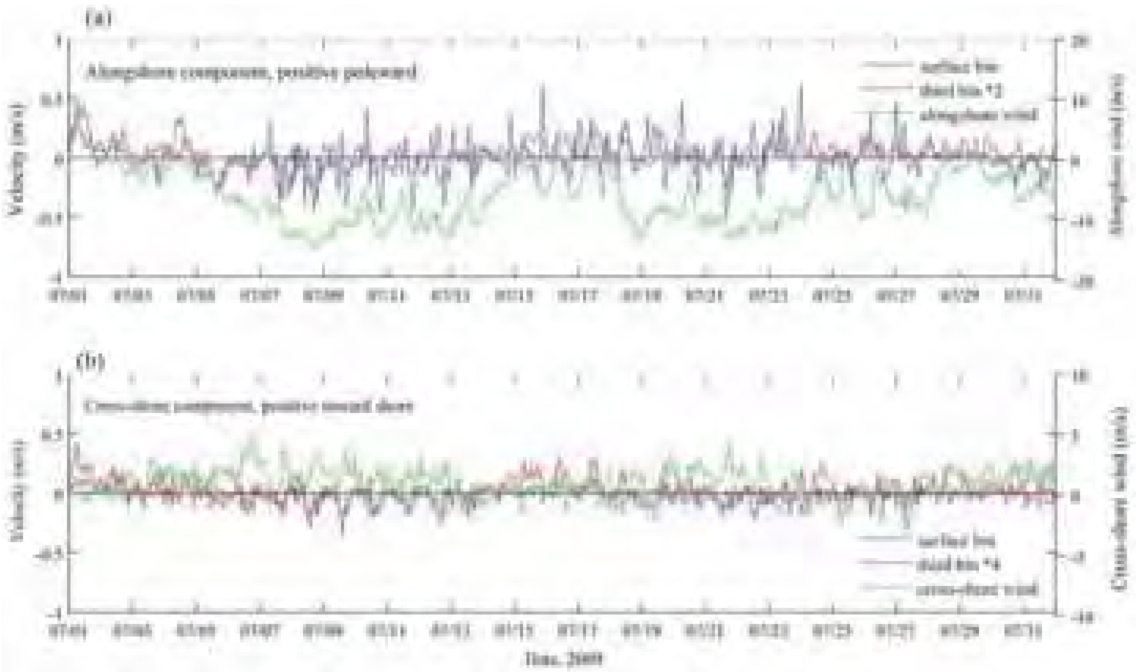


Figure 6. Time series of alongshore (a) and cross-shore (b) velocities from the Stewarts Point ADCP. The surface bin (blue line) and third bin from the surface (red lines) are compared with the alongshore (a, green line) and cross-shore components (b, green line) of wind from NDBC Buoy 46013 for the month of July 2009. Alongshore flow in the third bin was multiplied by a factor of two while cross-shore flow in the third bin was multiplied by a factor of four for better visual comparisons.

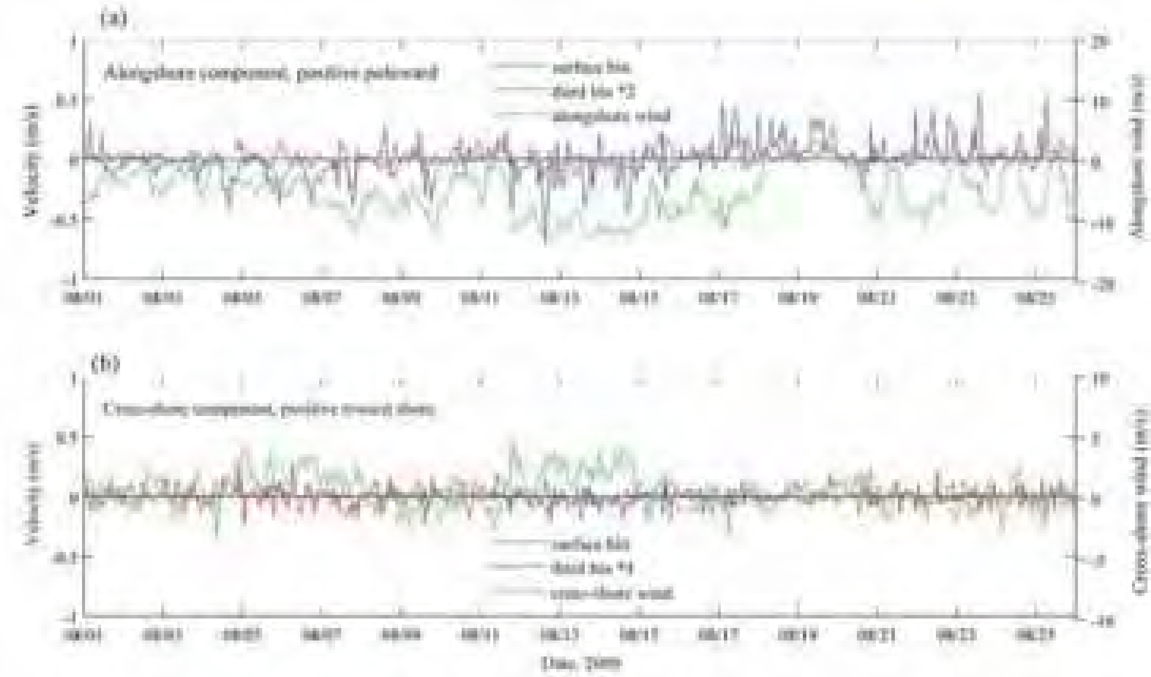


Figure 7. Time series of alongshore (a) and cross-shore (b) velocities from the Stewarts Point ADCP. The surface bin (blue line) and third bin from the surface (red lines) are compared with the alongshore (a, green line) and cross-shore components (b, green line) of wind from NDBC Buoy 46013 for the time period from 01 August through 25 August 2009. Alongshore flow in the third bin was multiplied by a factor of two while cross-shore flow in the third bin was multiplied by a factor of four for better visual comparisons.

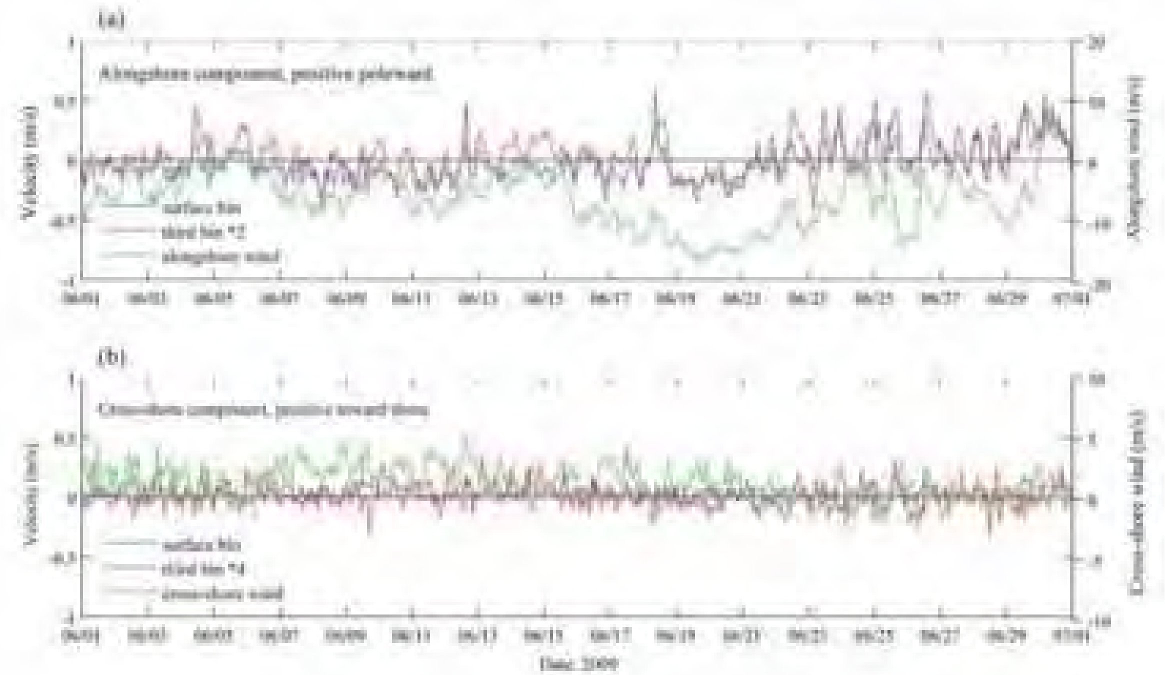


Figure 8. Time series of alongshore (a) and cross-shore (b) velocities from the Bodega Head ADCP. The surface bin (blue line) and third bin from the surface (red lines) are compared with the alongshore (a, green line) and cross-shore components (b, green line) of wind from NDBC Buoy 46013 for the month of June 2009. Alongshore flow in the third bin was multiplied by a factor of two while cross-shore flow in the third bin was multiplied by a factor of four for better visual comparisons.

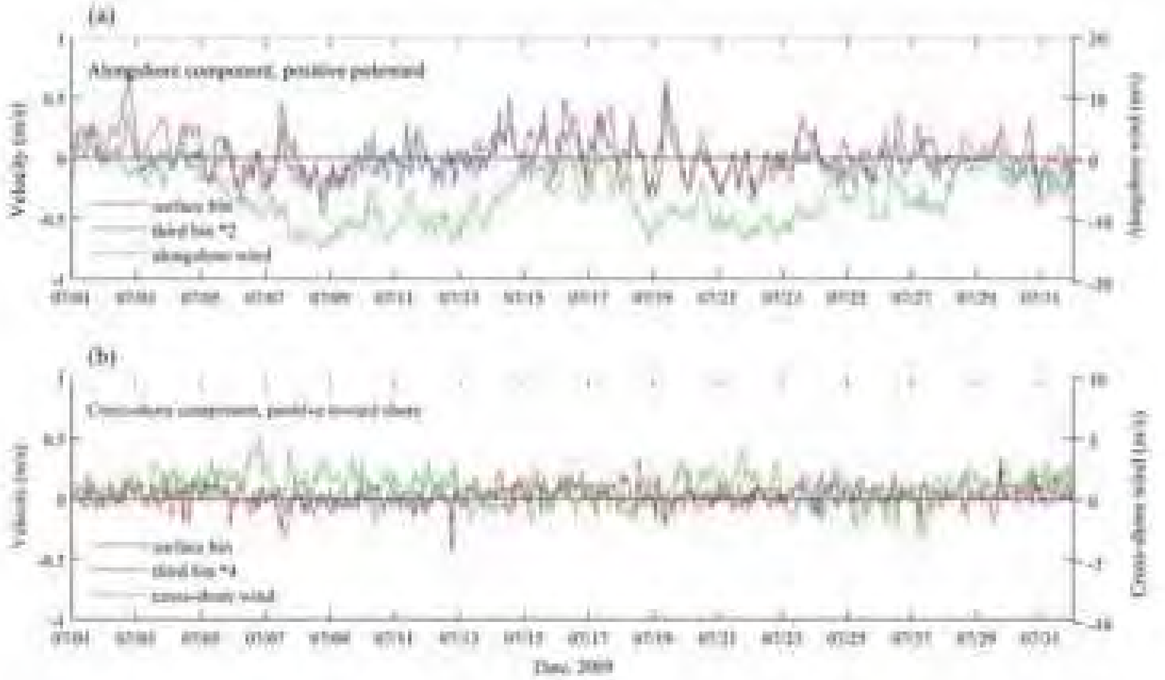


Figure 9. Time series of alongshore (a) and cross-shore (b) velocities from the Bodega Head ADCP. The surface bin (blue line) and third bin from the surface (red lines) are compared with the alongshore (a, green line) and cross-shore components (b, green line) of wind from NDBC Buoy 46013 for the month of July 2009. Alongshore flow in the third bin was multiplied by a factor of two while cross-shore flow in the third bin was multiplied by a factor of four for better visual comparisons.

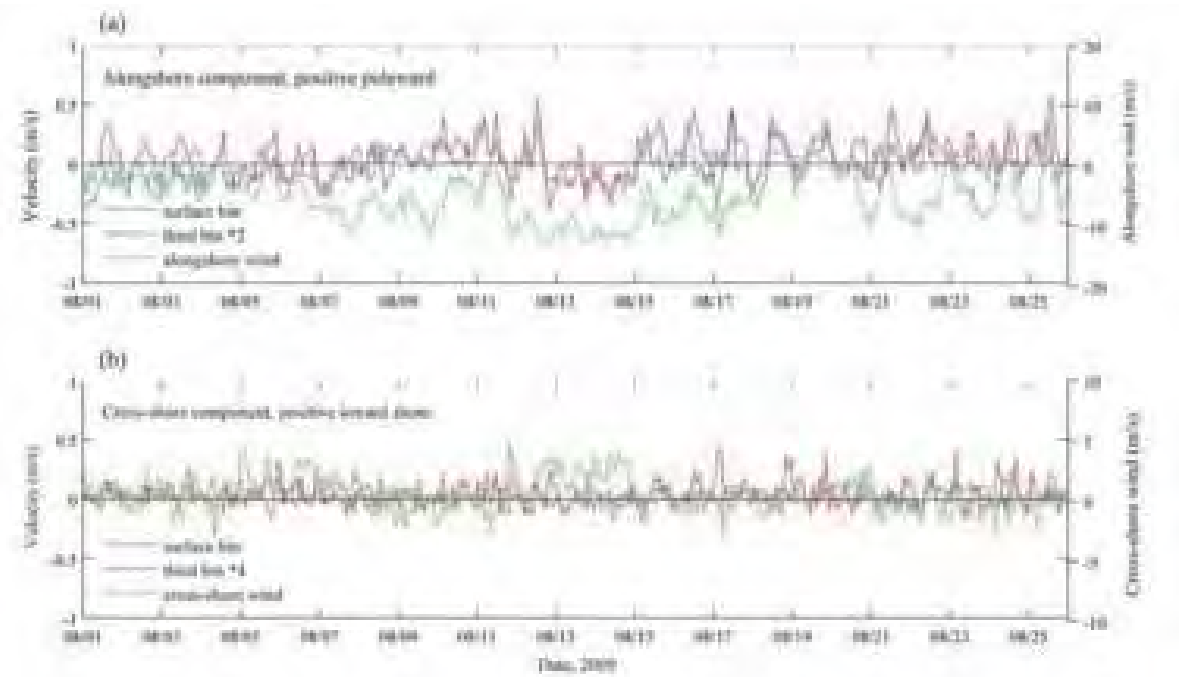


Figure 10. Time series of alongshore (a) and cross-shore (b) velocities from the Bodega Head ADCP. The surface bin (blue line) and third bin from the surface (red lines) are compared with the alongshore (a, green line) and cross-shore components (b, green line) of wind from NDBC Buoy 46013 for time period from 01 August through 25 August 2009. Alongshore flow in the third bin was multiplied by a factor of two while cross-shore flow in the third bin was multiplied by a factor of four for better visual comparisons.

Regional surface current versus nearshore currents

Alongshore surface velocities at all locations were positively correlated with alongshore velocity in both HF-radar surface current cells (Table 1). The slope of these relationships were all less than 1 (Table 1), showing some reduction in alongshore velocity nearshore, which is consistent with the idea of a "coastal boundary layer" (Nickols et al., 2012). Cross-shore velocity shear at Point Reyes Beach was negatively correlated with alongshore velocity in both HF-radar cells (Bodega Head: slope = -0.1190, r^2 = 0.0084; Point Reyes: slope = -0.0971, r^2 = 0.0071, Table 1), while cross-shore shear at Stewarts Point was positively correlated with alongshore velocity in both HF-radar cells (Bodega Head: slope = 0.0557, r^2 = 0.0226; Point Reyes: slope = 0.0941, r^2 = 0.0527, Table 1). Cross-shore shear at Bodega Head was negatively correlated with alongshore velocity in the Bodega Head HF-radar cell, but was not correlated with alongshore velocity in the Point Reyes cell (Bodega Head cell: slope = -0.0235, r^2 = 0.0018, Table 1).

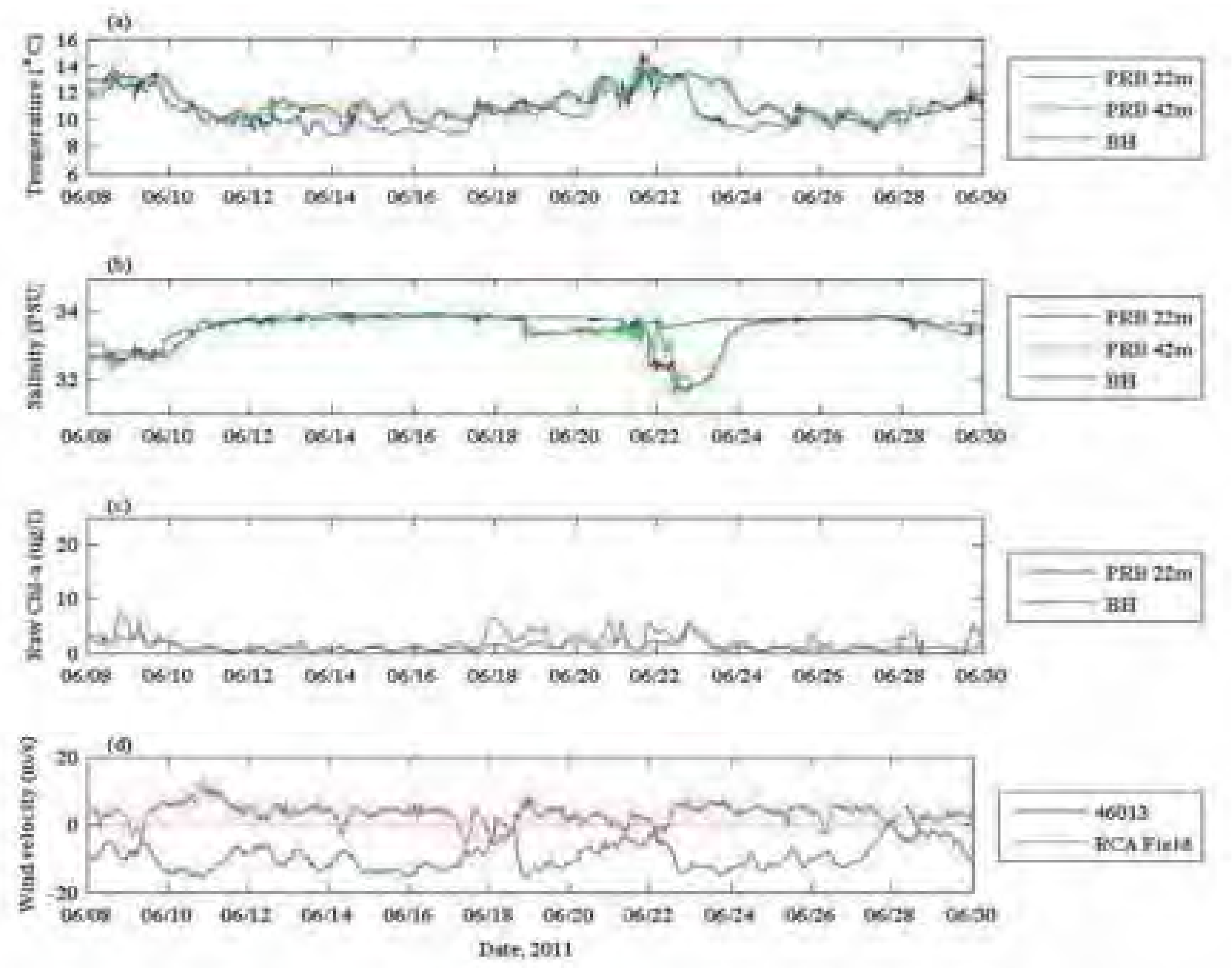


Figure 11. Time series of temperature (a), salinity (b), chlorophyll-a (c), and wind (d) from the case study period of 08 June through 29 June 2011. The red lines depict data from the 22 m Point Reyes Beach mooring, the green lines depict data from the 42 m Point Reyes Beach mooring, the blue lines depict data from the mooring at Bodega Head, the black line depicts alongshore wind from NDBC Buoy 46013, and the magenta line depicts cross-shore wind data from RCA Field.

Water Column Properties at Point Reyes Beach

Case Study: 08 - 29 June 2011

Upwelling-favorable winds began throughout the region on 9 June 2011 (Fig. 11d). This followed a period of relaxation that had lasted for several days. The satellite SST map from the first day of the event shows cool 10°C water along the coast near Point Arena and warmer 13°C water near Point Reyes (Fig. 13a). Surface water at Point Reyes Beach was 13°C on this date, while surface water at Bodega Head was approximately 12°C (Fig. 11a). Salinity was <33 PSU at the surface at Bodega Head and at both Point Reyes Beach moorings (Fig. 11b). The Point Reyes Beach alongshore current in the surface bin switched direction tidally, followed by an increasing equatorward surface flow later in the day (Fig. 2a). Chlorophyll fluorescence was higher at Bodega Head than Point Reyes Beach (Fig. 11c).

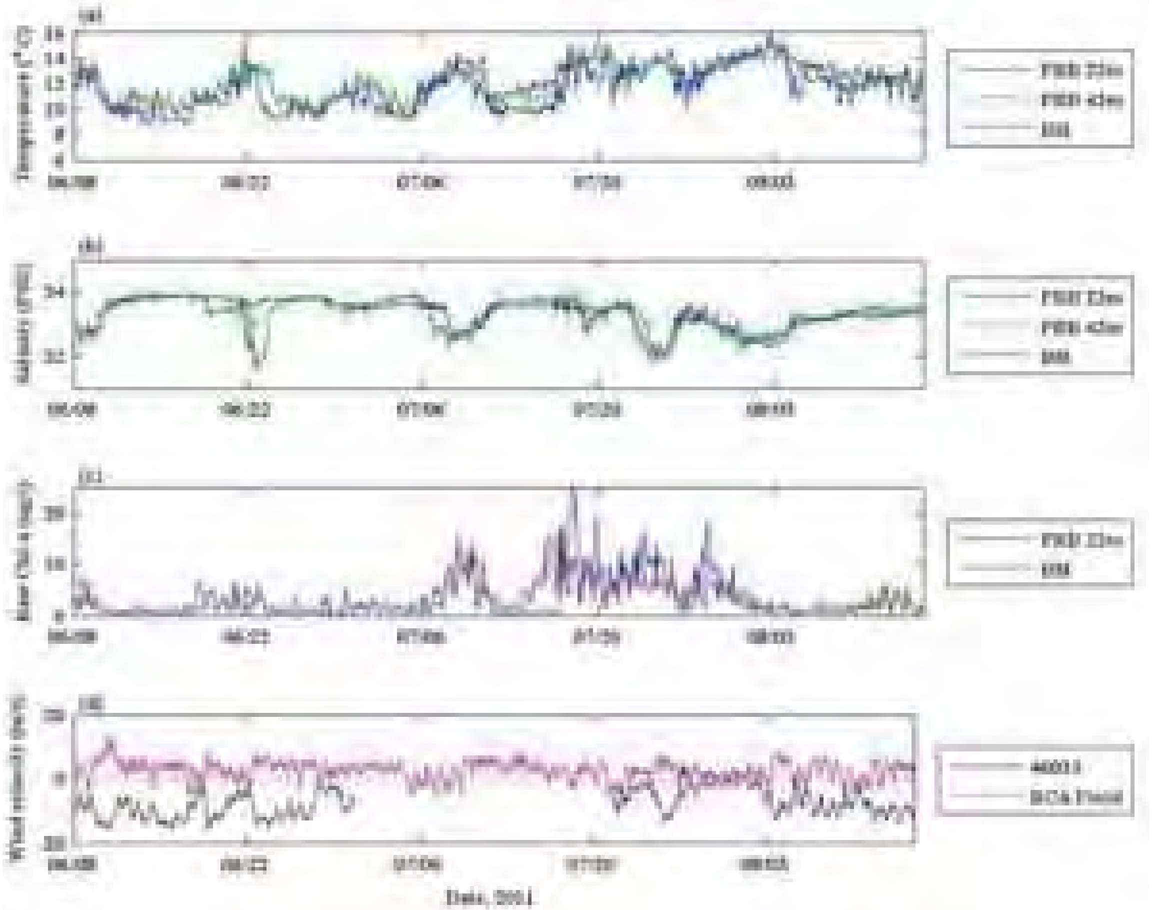
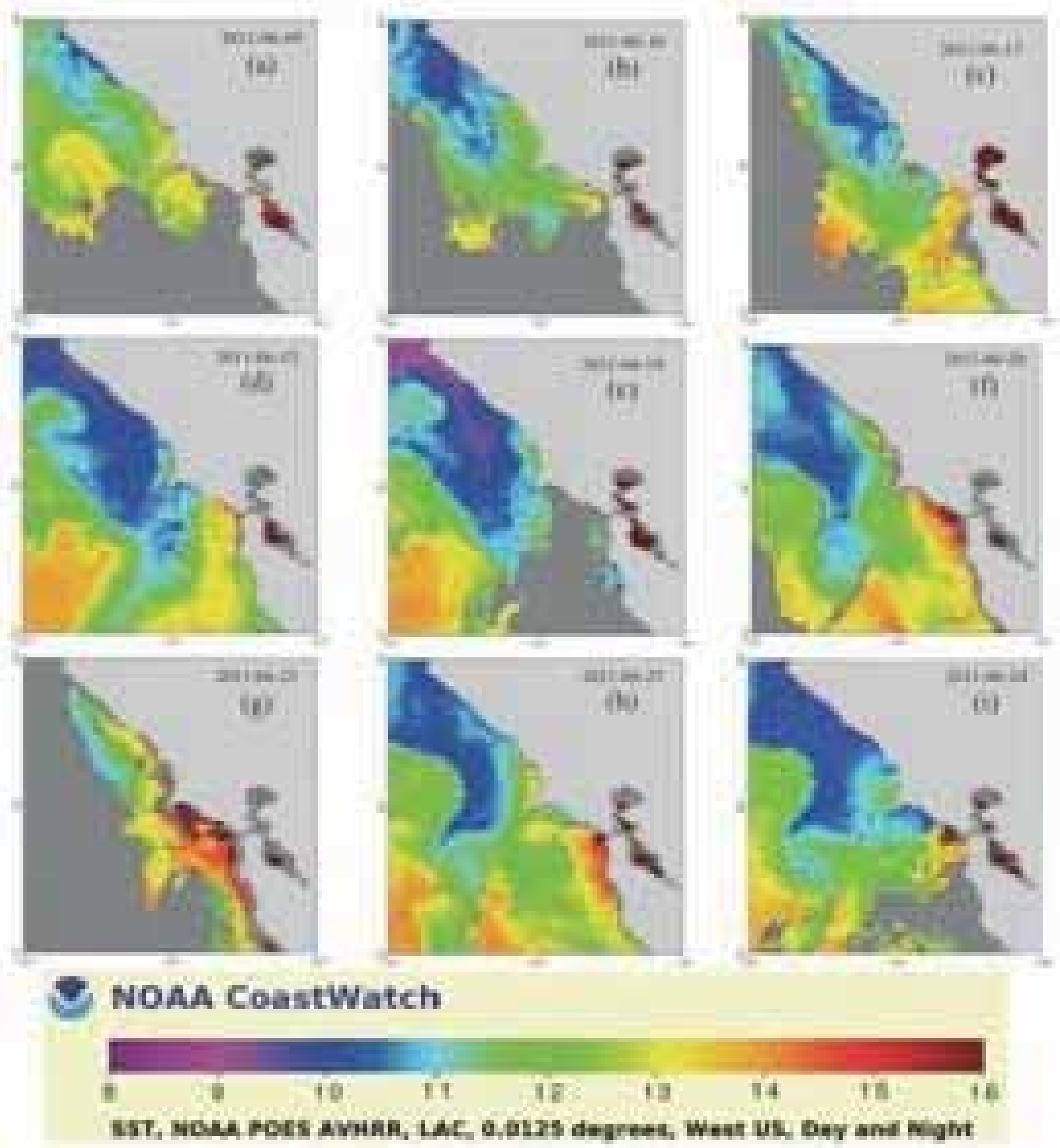


Figure 12. Time series of temperature (a), salinity (b), chlorophyll-a (c), and wind (d) from the time period of 08 June through 14 August 2011. The red lines depict data from the 22 m Point Reyes Beach mooring, the green lines depict data from the 42 m Point Reyes Beach mooring, the blue lines depict data from the mooring at Bodega Head, the black line depicts alongshore wind from NDBC Buoy 46013, and the magenta line depicts cross-shore wind data from RCA Field.

By 11 June 2011, upwelling-favorable winds had been blowing throughout the region for 2 days (Fig. 11d). The patch of 10^{o}C water near Point Arena had grown substantially by expanding offshore and toward the south (Fig. 13b), and surface water at Bodega Head and Point Reyes had cooled to approximately 11^{o}C (Fig. 11a). The cooling of surface water at the Bodega Head mooring preceded cooling at Point Reyes Beach by approximately 10 hours (Fig. 11a). Salinity at the moorings had increased and by the end of the day it was approximately 33.7 PSU at all mooring locations. As with temperature, the change in salinity at Bodega Head preceded changes at Point Reyes Beach by approximately 10 hours (Fig. 11b). Chlorophyll fluorescence dropped at both locations and a diurnal cycle of higher chlorophyll at night and lower chlorophyll during the day became evident (Fig. 11c).

Conditions remained relatively constant at the moorings for the next several days with surface temperatures in the range of 10-11^{o}C and salinity of ≈33.7 PSU (Fig. 11a, b). During this persistent upwelling a satellite image of SST showed a marked pattern of warmer surface waters along Point Reyes Beach on 15 June (Fig. 13d), consistent with the moored thermistors that show ≈10^{o}C at Bodega Head and ≈11^{o}C at Point Reyes Beach. Chlorophyll fluorescence remained low at both locations and continued to follow the same diurnal pattern (Fig. 11c). The upwelling jet was strong during this period and the plume of 10^{o}C water associated with it continued to expand southward past Point Reyes (Fig. 13c, d).


(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
NOAA CoastWatch
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SST, NOAA POES AVHRR, LAC, 0.0125 degrees, West US, Day and Night

Figure 13. Satellite SST images from 09 June (a), 10 June (b), 13 June (c), 15 June (d), 18 June (e), 20 June (f), 21 June (g), 23 June (h), and 24 June 2011 (i). Note the expansion of cold water southeastward from Point Arena in the first three images (a, b, c) during upwelling, then the northwestward expansion of warm water up the coast in the next three images (d, e, f) during relaxation, followed by cool water expanding throughout most of the region except at Point Reyes Beach in the final three images (g, h, i) during the subsequent upwelling event.

Regional upwelling wind weakened on 17 June and stopped on 18 June 2011 when local wind at RCA Field underwent a reversal (Fig. 11d). This relaxation was followed by a brief upwelling event on 19 June and a second relaxation on 20-21 June, with a return of regional upwelling winds on 22 June (Fig. 11d). These relaxation events brought warm, low-salinity water into the Point Reyes Beach study region, lowering salinity at the Point Reyes Beach moorings to ≈33 PSU during the first event and ≈32 PSU during the second event (Fig. 11a, b). This dramatic salinity drop was not seen at Bodega Head where the salinity remained above 33 PSU throughout the entire event, but both locations experienced surface temperatures of approximately 12°C (Fig. 11a, b) and the nearshore tongue of warm water extending north from Drakes Bay is evident in satellite images for 18, 20, and 21 June (Fig. 13e, f, g). Chlorophyll fluorescence increased at both locations during this double-relaxation period but the increase occurred more quickly at Bodega Head, and the maximum concentration was higher at Bodega Head (Fig. 11c). In the satellite image for 17 June a strong bloom is evident nearshore, immediately on weakening of regional upwelling winds. The maximum was in Bodega Bay which typically experiences a decrease in wind prior to that which occurs offshore at the buoy.

Upwelling-favorable wind resumed late on 22 June 2011 and at Bodega Head surface temperature and salinity responded immediately – within hours (Fig. 11a, b). The increase in salinity and decrease in temperatures were delayed at Point Reyes Beach by about a day compared with Bodega Head (Fig. 11a, b). Salinity continued to rise at Point Reyes Beach until late on 24 June 2011 when it finally reached the level seen at Bodega Head, 2 days after upwelling began (Fig. 11b). In the satellite image for 24 June, residual warm water is evident north of Point Reyes while cold upwelled waters break the surface in Drakes Bay, south of Point Reyes. Chlorophyll fluorescence dropped at both locations about a day after upwelling wind returned (Fig. 11c). Another relaxation event began on 27 June and continued to 29 June which once again brought surface warming, lower salinity, and higher chlorophyll-a concentrations to the study region (Fig. 11a, b, c).

Remainder of dataset

Throughout the summer of 2011, including the months of July and August, the cycle of upwelling/relaxation continued, with upwelling events lasting for several days at a time and generally losing strength through the summer (Fig. 12). Relaxation events were highly variable in strength, with some bringing low-salinity (<33 PSU) water to Bodega Head and others causing only a moderate drop in salinity even at Point Reyes Beach (Fig. 12b). Chlorophyll fluorescence maxima during relaxations at both locations were much greater in July than those seen during the case study in June (Fig. 12c), and at times a chlorophyll bloom was evident at Point Reyes Beach but not at Bodega Head (e.g., 15-16 July; Fig. 12c). Even though the strength and duration of events was variable there were consistent patterns to point out:

1. at the onset of upwelling, cooling at Bodega Head always preceded cooling at Point Reyes Beach by several hours up to 2 days (Fig. 12a);
2. during upwelling, temperature at Bodega Head was consistently cooler than at Point Reyes Beach by as much as 1°C (Fig. 12a), notably during upwelling on 13-16 July and 3-9 August; and,
3. at the onset of relaxation events, salinity dropped at Point Reyes Beach before it dropped at Bodega Head by approximately 24 hours on average (Fig. 12b).

Later in the record, salinity appears to be lower at Bodega Head than at Point Reyes Beach during upwelling (after 1 July). Without a clear mechanism for this phenomenon we suspect that the instrument at Bodega Head was recording erroneous values during July and August because of biofouling. We include these data here only to compare the timing of salinity changes rather than the absolute values.

Satellite imagery

Sea surface temperature (SST)

High quality images of the study region were available during the case study period from 8- 29 June 2011 because of an unusually low degree of fog cover during this timespan. The first three images from 9 June, 10 June, and 13 June show a plume of cold water beginning in the northern section of the upwelling cell near Point Arena spreading southeastward through time, eventually passing Point Reyes by the end of the first upwelling period which lasted from 9-16 June 2011 (Fig. 13a, b, c). A patch of warmer water remained just offshore of Point Reyes Beach throughout this event (Fig. 13a, b, c). The next three images from 15 June, 18 June, and 20 June show the end of the upwelling event transitioning into the relaxation event that lasted from 18-21 June (Fig. 13d, e, f). Warm water can be seen extending from Drakes Bay northward up the coast well past Bodega Head (Fig. 13d, e, f). The final three images from 21 June, 23 June, and 24 June show the transition into the next upwelling period, with the warm water being pushed back southward past Point Reyes except for a remnant patch of warmer water near Point Reyes Beach (Fig. 13g, h, i).

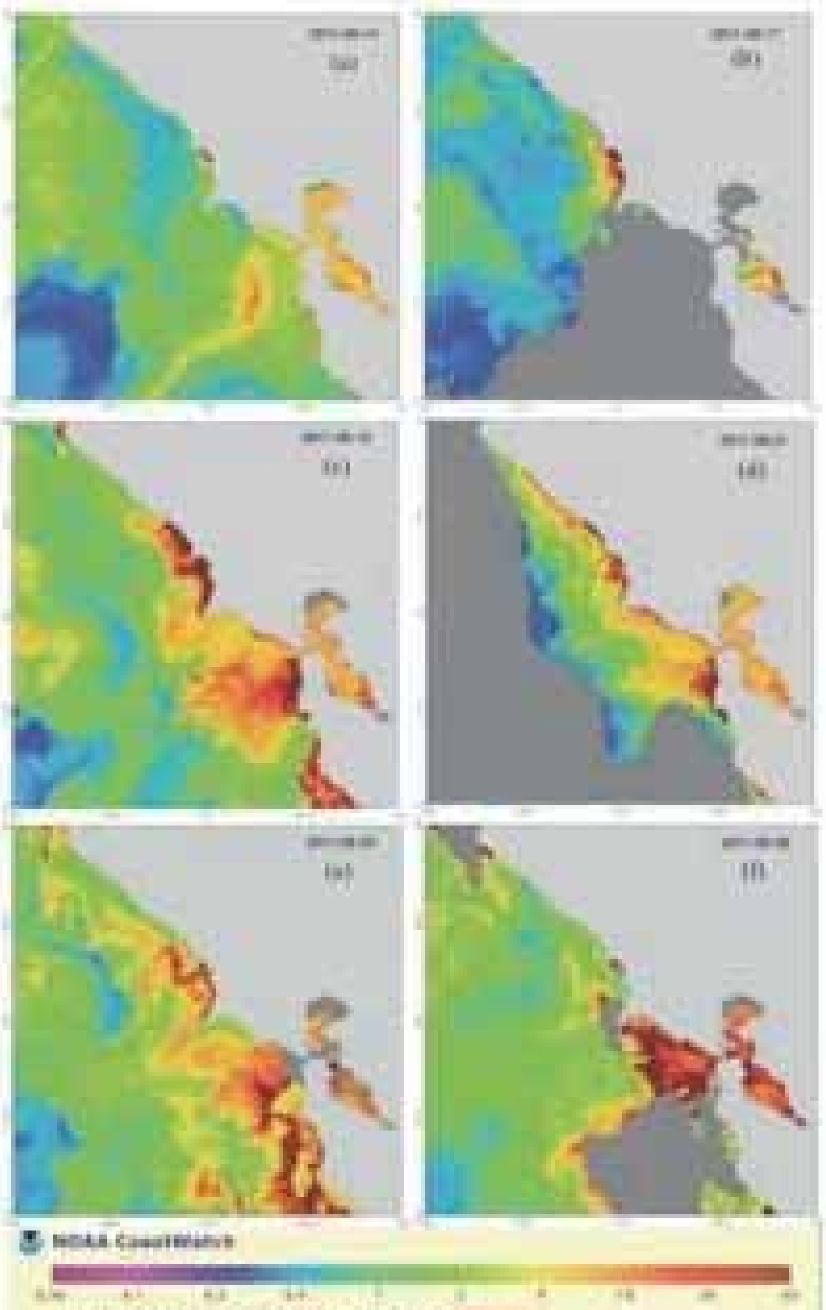

Figure 14. Satellite images of chl-a concentration from 14 June (a), 17 June(b), 19 June (c), 21 June (d), 23 June (e), and 26 June 2011 (f). During upwelling, chlorophyll-a concentration was lower near Point Arena than Point Reyes (a, b, e, f). During relaxation, high concentrations of chlorophyll-a expanded along the coast (c, d). Note the high concentrations of chlorophyll-a within Bodega Bay in all images.

Chlorophyll-a

Several good quality daily chlorophyll-a satellite images were obtained for the study period although coverage was not as clear as for SST. The first pair of images from 14 June and 17 June represents the first upwelling period, and they show generally low chlorophyll-a throughout the Point Arena upwelling cell except a patch of much higher chlorophyll-a concentration centered in Bodega Bay that by 17 June expanded to Point Reyes Beach as well (Fig. 14a, b). The next two images from 19 June and 21 June are from the relaxation period and they show that the high chlorophyll-a patch had expanded greatly (Fig. 14c, d). By 19 June it covered the area from Point Reyes to well north of Bodega Head, and it continued to expand northwestward along the coast through the relaxation period, resulting in much higher coastal chlorophyll-a levels throughout the region (Fig. 14c, d). The third pair of images from 23 June and 26 June shows the transition into the second upwelling event (Fig. 14e, f). By 23 June, a band of lower chlorophyll-a water stretched along the coast southeastward from Point Arena past Bodega Head, and by 26 June most of the upwelling cell was comprised of relatively low chlorophyll-a water except a patch in Bodega Bay and at Point Reyes (Fig. 14e, f). Unfortunately a patch of cloud cover obscured part of the image on 26 June but most of the study region remained in view (Fig. 14f).

Discussion

The bulk of the evidence compiled in this study supports the hypothesis that upwelling circulation is suppressed nearshore and often reversed on the windward shore of Point Reyes. Support for this hypothesis is twofold: 1) velocity structure measured at Point Reyes Beach shows signs of local downwelling near-surface during strong equatorward wind events which are associated with upwelling elsewhere in the region; and 2) water properties at Point Reyes Beach during regional upwelling showed characteristics of aged upwelled water from within the Point Arena upwelling cell, and also retention during upwelling of low-salinity water that had intruded during prior relaxation events.

The forcing mechanism for this phenomenon is that strong regional wind blows directly over the low-lying peninsula of Point Reyes. On a local scale, wind is onshore and surface water is pushed in the direction of the wind, especially in areas shallower than approximately 50 m where the full Ekman spiral cannot develop. When this water encounters the coastline it sets up sea level on a very local scale relative to a few km offshore. The increase in sea level at the shoreline results in a barotropic pressure gradient pointing away from the shoreline (at scales smaller than those at which Coriolis is important). Since this pressure gradient is balanced by wind forcing at the surface, the resultant flow deeper in the water column is away from shore. This positive cross-shore shear prevents upwelling and offshore advection of surface water which allows solar heating to produce warming at the surface.

Evidence from velocities

The time series of cross-shore velocity comparing local wind with the surface bin and the third bin from the surface in the Point Reyes ADCP data is a compelling piece of evidence showing local downwelling associated with cross-shore wind (Figs. 2b, 3b, 4b). This is strengthened by the positive correlation between cross-shore wind at RCA Field and cross-shore velocity shear at Point Reyes Beach (Table 1). Local wind follows a diurnal cycle with the strongest onshore wind during the afternoon and when this happens we see the surface layer begin to move toward the shore mirrored by a weaker return flow at depth with a slight time delay. The reason the return flow is weaker is that it is more diffuse through the water column and it is also likely deflected into the alongshore flow.

The positive correlations between regional upwelling conditions (NDBC Buoy 46013 alongshore wind and northward surface flow nearshore) and cross-shore velocity shear at Stewarts Point (Table 1) serves to show that the phenomenon of downwelling during regional upwelling is localized at Point Reyes Beach, rather than a region-wide phenomenon occurring in shallow areas. Even though the ADCP at Stewarts Point was deployed in 17 m of water and very close to the shoreline, it still exhibited flow patterns consistent with upwelling circulation. Kirincich et al. (2005) showed that a fully formed surface Ekman layer does not occur shoreward of the 50 m isobath, and at 15 m approximately 25% of full theoretical Ekman transport was observed. These findings are consistent with our data from Stewarts Point near the center of upwelling in this region, where we observed offshore surface transport and return flow at depth during equatorward regional wind and currents. These relationships are more tenuous for ADCP data collected from Bodega Head, where cross-shore velocity shear was only slightly positively correlated with buoy wind and was slightly negatively correlated with northward velocity in the Bodega Head surface current cell. This may be because the location of the Bodega Head ADCP was at the tip of a headland, with local flow-topography interactions not found at the other two sites, or it may be because of its location near the downstream end of the upwelling cell, suggesting that upwelling circulation is not as strong nor as persistent as that found at Stewarts Point. There may be other factors at play as well, such as localized curl in the wind field (Dorman et al., 2006; Dever et al., 2006). The true cause of the differences seen in the Bodega Head ADCP data are grounds for a separate study and is certainly outside the scope of this paper, but it is clear that cross-shore flow at Bodega Head behaves in a manner that is intermediate to the dynamics seen at Stewarts Point and Point Reyes Beach.

Alongshore surface velocity measured by the ADCPs in all three locations was positively correlated with surface flow in the nearshore HF Radar cells. This shows that the alongshore flow is coherent throughout the region and is related to the strength of the upwelling jet. Differences in the flow patterns at Point Reyes Beach are, therefore, comprised not of local flow reversal as might be expected in the vicinity of a persistent eddy. Also, alongshore flow was directionally consistent throughout the water column at Point Reyes Beach, fast at the surface and slowing with depth, showing that the shear seen in the cross-shore velocities was unlikely caused by forcing by shear in the alongshore direction.

Evidence from water properties

In general, surface water at Point Reyes Beach was warmer than surface water at Bodega Head during the summer of 2011. The lack of a clear difference in salinity between Bodega Head and Point Reyes Beach during active upwelling indicates that the surface water at Point Reyes Beach is indeed aged upwelled water rather than retained surface water from Drakes Bay. The declines in salinity seen at Point Reyes Beach during relaxation events are indicative of Drakes Bay water entering the region, but salinity climbs to ambient levels within a few days following the onset of upwelling-favorable wind conditions (indicating retention that lasts at most a few days). This delay in salinity does indicate that some nearshore surface water retention occurs in this area and temperature remains elevated throughout each upwelling period long after salinity has reached levels seen at Bodega Head. If local upwelling circulation were present at Point Reyes Beach we would not expect to observe retention of low-salinity water or elevated surface temperature during regional upwelling.

At the onset of each upwelling period in the time series surface water at Bodega Head cooled quickly, indicating that upwelling was occurring very close to the mooring location there. There was a clear delay in cooling at Point Reyes Beach which varied from approximately 10-24 hours after upwelling winds began. This is further evidence that upwelling was not occurring at Point Reyes Beach and the cool surface water that arrived was being advected there by regional currents rather than originating locally; the 0.5-1-day delay in cooling between Bodega Head and Point Reyes Beach is consistent with advections speeds of 0.3 to 0.5 m/s which are typical of observed alongshore velocities during upwelling.

Chlorophyll

The in-situ fluorometers at Bodega Head and Point Reyes Beach showed relatively low and spatially uniform surface chlorophyll during upwelling periods punctuated by dramatic rises during relaxation periods (Figs. 11c, 12c); this is consistent with previous studies that have found nearshore blooms during relaxation (Wilkerson et al., 2006). The most striking rises in chlorophyll at both locations occurred during the month of July 2011 and were characterized by chlorophyll levels many times higher than ambient levels. There also appeared to be some diurnal cycling at both locations, although it is possible this could be caused by photoquenching by sunlight because of the proximity of the sensors to the water surface. The fact that the diurnal peaks occur at night supports this idea, since diurnal vertical migration of mobile phytoplankton would be expected to cause a rise in surface chlorophyll during the day as has been demonstrated in lab studies (Olsson and Grineli, 1991) as well as in field observations in Monterey Bay (Sullivan et al., 2010).

Chlorophyll is notoriously patchy in coastal areas and blooms can be difficult to predict. The satellite imagery is limited in its temporal coverage because of persistent fog and low cloud cover during the summer, but several quality images were available during the upwelling period from 11-17 June 2011. The image from 14 June shows a patch of elevated chlorophyll contained within Bodega Bay (Fig. 14a), a known retention area (Roughan et al., 2005). Chlorophyll-a peaks were associated with relaxation events and concentrations during these peaks were consistently higher at Bodega Head than at Point Reyes Beach. This may be because of the pool of high chlorophyll-a water located within Bodega Bay. The Bodega Head mooring was very close in proximity to this source of chlorophyll and relaxation currents could bring water from Bodega Bay to the mooring site very quickly.

Implications

Suppression of upwelling at Point Reyes Beach likely affects marine communities in several important ways. First, wind-driven transport of surface water toward shore means that any buoyant organisms in the neuston will be carried toward the beach which, depending on the species, could be either beneficial or harmful. For example, buoyant late-stage larvae would be transported to shore which could allow them to find suitable habitat. However, buoyant holoplankton could be transported into the surf zone and then deposited on the beach by wave action. The very shallow nature of the shoreward flow at the surface could allow mobile larvae to maintain their position relative to the shore more easily, since only a few vertical meters separate the onshore and offshore flows. By allowing near-surface larvae to remain near to the shoreline, this phenomenon may allow for increased connectivity between populations to the north and south of Point Reyes Beach. Alongshore current at the surface showed strong tidal variability with vertical shear even during upwelling periods when regional alongshore current was strongly equatorward, such as during the period from 12-16 June 2011 (Fig. 2a). A larva migrating northward could move up in the water column during northward flow and down in the water column to avoid southward flow.

Furthermore, major headlands can be found throughout the world in other upwelling regions and in areas where the wind is not significantly deflected we expect that current dynamics would be similar to those seen in the present study. Further studies would be necessary to describe specific dynamics to each location but, in general, a strong shoreward component of wind nearshore will result in shoreward transport of surface water and suppression of local upwelling. The biological implications worldwide depend on the natural history of each region, but the ubiquity of this type of system indicates that suppression of upwelling at the windward shores of headlands may be an important part of upwelling cells throughout the world. Combined with knowledge of the dynamics associated with other mesoscale features in these regions, the present study helps to further scientific understanding of the intricacies of nearshore upwelling patterns.

Conclusions

At no point during this study did we see evidence of strong local upwelling at Point Reyes Beach, even during the strongest equatorward regional winds. On the contrary, we saw that these strong winds were associated with onshore wind at Point Reyes Beach which, in turn, caused surface flow toward the shore. This shoreward component of flow at the surface caused suppression of upwelling as was further evidenced by an offshore flow deeper in the water column. Comparisons of water properties between Point Reyes Beach and Bodega Head also indicated that upwelling occurs elsewhere in the region and then the cool water is advected into the Point Reyes Beach area. This was shown by the fact that the cooling at Bodega Head preceded cooling at Point Reyes Beach at the onset of upwelling and, during upwelling events, surface water at Bodega Head was consistently cooler yet with consistent salinity between the two locations. These factors mean that upwelled water was heated at the surface as it moved equatorward and was not infused with additional upwelled water at Point Reyes Beach itself.

Alongshore flow was reduced at the location of the Point Reyes Beach ADCP compared with surface currents slightly farther offshore, indicating that this is a region where surface water is detained. The detention of surface water allowed surface heating to maintain warmer temperatures at Point Reyes Beach in spite of strong equatorward wind and currents throughout the remainder of the upwelling cell. This is a different dynamic to what happens at the leeward side of headlands where upwelling shadows often develop as a result of retention. Surface water retention causes recirculation and much longer residence time. This sets up a buoyancy front between the warm water within the upwelling shadow and the cold upwelling jet offshore, which has been observed in Monterey Bay (Graham and Largier, 1997; Woodson et al., 2009) as well as in the northern Gulf of the Farallones (Fontana 2013) and at times at Bodega Head (Roughan et al., 2005). In the case of Point Reyes Beach, the temperature difference between warmer inshore water and cool offshore water is more subtle, and a marked thermal front is absent during regional upwelling.

Conditions found along the windward shore of Point Reyes during this study support the hypothesis that the differences in water properties in this area are caused by the lack of local upwelling rather than retention of relaxed water from Drakes Bay. This region is characterized by shoreward surface transport during upwelling events and reduced alongshore flow compared with currents farther offshore, both of which combine to result in detention and consistently warmer surface water when compared with other locations within the Point Arena upwelling cell.

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FROM OPEN TO CLOSED CIRCUIT: PROS AND CONS FOR STUDYING CORAL REEF ORGANISMS IN THE CARIBBEAN

Juan A. Sánchez[1]
Edgardo Ochoa[2]
Julio Andrade[3]

[1]Departamento Ciencias Biológicas-Facultad de Ciencias
Universidad de los Andes, Carrera 1 No 18A - 10
Bogota, COLOMBIA
juansanc@uniandes.edu.co

[2]Smithsonian Tropical Research Institute
Apartado Postal 0843-03092
Balboa, Ancon, REPUBLIC OF PANAMÁ

[3]Ecoral
Calle 10 A N 34 - 11 Ed.
Centro de Negocios Diez, Of. 3002
Medellín, COLOMBIA

Most shallow-water octocoral research and coral reef fish surveillance has been achieved using open-circuit (OC) recreational-type scuba diving. Although this equipment allows for inexpensive and practical underwater research, its limitations in terms of depth, bottom time and the noisy nature of the self-contained breathing apparatus limit the area surveyed when studying reef communities. Scuba use also affects fish behavior leading to the hypothesis that observations made with Closed-Circuit Rebreather (CCR) are not comparable with observations made with OC scuba equipment (Radforda et al., 2005). Most of the reef fish research has traditionally been done using scuba in the Caribbean, Mediterranean, Indo-Pacific and Red Sea.

Given that population biology studies are time-demanding activities relying on large individual surveys and multitemporal observations, CCR equipment poses a significant advantage. Technical diving CCR rebreather use has most often been used for deep diving. However, CCR equipment can increase the efficiency of underwater surveys in shallow water providing longer bottom times and reduced nitrogen uptake. In this study we compared the logistics and performance of CCRs in recent coral reef research, particularly of octocoral and coral reef fish surveys in the Colombian Caribbean (Barú Island, Cartagena). We evaluated the differences among data collected using scuba and CCR equipment while monitoring coral reef fish populations on shallow reefs.

Our first finding revealed how time efficient the CCR is. Using traditional OC scuba and CCR equipment (Megalodon, ISC), we conducted nine linear transects on each of the seven sites (three transects between 15-22 m, three between 8-15 m and three between 0-8 m). The objectives were to estimate reef fish abundance (cf. Friedlander et al., 2003), quantify coral cover using photo-quadrants, and to sample octocorals. Use of OC equipment required three dives (two divers) and over five hours to complete the nine transects. CCR equipment use for the same task was completed in a single four-hour dive. Maintaining optimal pO_2 (1.1-1.2) at all times greatly reduced decompression times allowing longer bottom times for research and shorter decompression stops.

The longer bottom times allowed us to cover more area for octocoral surveys. Using OC scuba and CCR, we conducted octocoral surveys at depths of 40 m, 30 m and 20 m. The result of CCR use was coverage of considerably more area and comprehensive sampling, including taking detailed close-up photographs for each species. While the use of OC equipment allowed us to perform two deep dives per day, a two-hour dive (bottom time) using CCR was enough to cover almost 50% more area.

To compare the results of reef fish surveys using OC and CCR, we conducted three linear transects on each of the shallow reefs (between 10-15 m). The data were collected on the same site at the same depth and approximately at the same time around noon. Our observations suggest that there were no significant differences between abundances estimated using either OC or CCR (*P*>0.05). Overall fish abundances did not differ between CCR and OC surveys (Fig. 1a), and that relationship was the same for both for commercial (Figure 1b) and major herbivore species (Figure 1c).

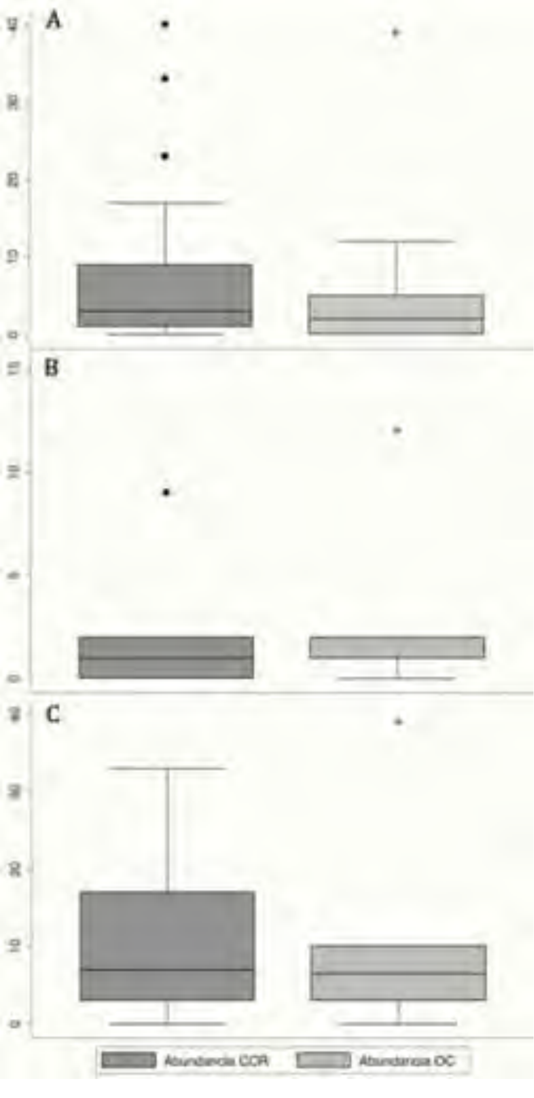

Figure 1. Reef Fish surveys comparisons between Close Circuit Rebreather (CCR) and Open Circuit (OC). A: General abundance of fish recorded using CCR and OC. B: Abundance of commercial interest species. C: Abundance of mayor herbivore species.

This finding has encouraging implications for data comparability. If data collected using CCR were not comparable with OC-collected data it would be pointless to adopt this new technology for surveys and monitoring previously done with OC. In turn, our findings suggest that CCR equipment provides valid data without overestimation of fish abundances and optimizes work hours allowing extended area coverage. Logistically, however, CCR involves greater costs and preparations than OC (e.g., training, soda lime, 100% oxygen, stages), which can be restrictive for operations and transportation. As CCR is becoming more popular, lower costs and better support are expected in the near future.

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DOCTORS UNDER PRESSURE: INTERESTING CASES AND DIAGNOSTIC DILEMMAS

Robert W. Sanders
Hennepin County Medical Center
Department of Emergency Medicine
701 Park Ave Mail code 825
Minneapolis, MN 55415
Robert.Sanders@hcmed.org.org

The Hawaiian Islands present a tremendous opportunity for scuba divers of all types. While diving accidents occur everywhere, the overall numbers and the diversity of diving accidents makes Hawaii unique. Cases are discussed that highlight the diagnostic dilemmas routinely faced by physicians at the Hyperbaric Treatment Center, University of Hawaii, John A. Burns School of Medicine. Injuries addressed include decompression sickness, air embolism, immersion pulmonary edema and near drowning.

Introduction

The role of the hyperbaric physician in the management of decompression illness (DCI) is often thought to be straight forward, and the US Navy will often use a “GMO” (only 1 year of post-graduate training) for this role. In fact, this author believes that instead, it is a position that requires knowledge, training and significant experience. The goal of this discussion is to help the reader understand a few of the diagnostic dilemmas that physicians are faced with in caring for patients presenting with possible DCI symptoms. Through a series of case studies, it will also serve as a basic review of the common signs and symptoms of DCI (arterial gas embolism: AGE, CAGE, DAE and Decompression sickness: DCS), as well as pulmonary barotrauma.

The roots of this paper are based on the author’s experience as a hyperbaric technician. It was not uncommon for a dive case to arrive that to the technician seemed to be straight forward but the physician was struggling over the treatment decision. In my later role as the hyperbaric physician, even the “simplest case” presented with serious diagnostic dilemmas and challenges to address.

There are approximately 1,000 scuba diving accidents annually in the U.S. and, on average, the University of Hawaii at Manoa, John A. Burns School of Medicine Hyperbaric Treatment Center (HTC) treats 50 cases, with a peak around 100 and a low around 35 cases annually. In 2006, the Divers Alert Network reported on 663 cases with only 380 considered “possible” or “confirmed” DCI (Pollock, 2008). That same year the HTC treated 38 patients (10% of total) and the USC/Catalina Hyperbaric Chamber admitted 22 and treated 12. The remaining 330-600 cases were seen by one of the remaining 700 chamber facilities in the U.S. At a recent nationally recognized introductory hyperbaric course, of the future care providers (doctors, nurses, and technicians) only 4 of the 48 participants had any diving experience at all. Members of the AAUS likely have more diving knowledge than most hyperbaric physicians and true “diving specialists” are rare.

Such a lack of experience is a problem for our industry. Traditional curricula make the diagnosis of DCI seem fairly straightforward. While virtually every diver has heard the description of “bloody, frothy, sputum" used when discussing the signs and symptoms of AGE, its presence does not dictate a need for recompression as highlighted through a series of actual dive cases discussed here.

Methods and case presentations

A retrospective review of case logs from the Hyperbaric Treatment Center was performed. Cases that demonstrated particular teaching points were selected for inclusion.

Case #1: "Bloody, Frothy, Sputum"

A previously healthy 35 year-old female was diving during an open water training course. She was participating in the mask clearing exercise when she panicked and shot, uncontrolled, to the surface. Upon arriving at the surface, she complained of "something in my lung" and was having respiratory distress. The paramedic report states "bloody, frothy sputum" was present and the patient was taken to the ED. Upon arrival at the ED the patient was in obvious distress; was intubated with rapid sequence intubation, and the hyperbaric physician was called.

The Differential:

The next step in a patient's care is to form a "differential diagnosis" to help focus our diagnostic workup. In this case our differential diagnosis included: arterial gas embolism (AGE), pulmonary overpressure syndrome (pneumothorax), and near drowning.

The Dilemma:

Not all pulmonary overpressure syndromes are treated in the chamber. In fact, hyperbaric therapy can cause harm in patients with acute lung injury (e.g., pneumothorax, pneumomediastinum, pulmonary edema). If, however, there is a bubble in the central nervous system (AGE) causing symptoms, hyperbaric oxygen therapy/recompression is essential. This patient was intubated using a chemical paralytic agent. As a result, there was no way to perform a neurologic exam. The decision must be made on clinical data alone.

The Process and Workup:

The workup and decision process is based on an understanding of the pathophysiology of AGE and drowning. To suffer an AGE, a diver must have air trapping in the lungs with a decrease in ambient pressure (ascending while holding one's breath). For these symptoms to be caused by drowning, it would require aspiration of seawater into the lungs, implying an open glottis. A chest x-ray in this case is the key to the diagnosis. Pulmonary edema implying aspiration of seawater was found. To aspirate water implies an open glottis, thus making AGE highly unlikely.

The Outcome:

Based on radiographic evidence and history, it was felt that near drowning was the most likely diagnosis. The patient was admitted to the ICU, remained stable and was extubated on Day 2. At that time she was found to have no neurologic deficits and was discharged in an improved condition.

Case #2: "Abdominal Catastrophe?"

A 55 year-old experienced diver presented to the ED via USCG helicopter with a chief complaint of severe abdominal pain. The patient had made five dives to 90-95fsw (30-31 msw). As this was his "routine" he was unsure of his dive times or surface intervals. Traditionally he dived "until [his] shoulder hurts" and then stops. On this day, however, after the 5th dive, he began to experience a rapid onset of severe abdominal pain and "coldness". 911 was called and paramedics were to meet the patient at the dock 1 hour away. The patient seemed to worsen rapidly so the USCG was called. The patient was lifted from the boat and flown to the UH facility. In the ED he was found to be hypotensive (61/39), and complaining of abdominal pain and weakness. He had diffuse abdominal tenderness, cold extremities, and slowed mentation. Initial workup included an EKG showing ST elevations, S-wave in lead I, Q-wave and inverted T-wave in lead III. Chest x-ray and laboratory studies were unremarkable.

The Differential Diagnosis:

The presentation of abdominal pain, hypotension and ST-elevation in a 55 y/o male is concerning for aortic dissection, ruptured aortic aneurysm, pulmonary embolus (PE) or heart attack (MI), all of which are emergent and life threatening, but not treated with HBOT. Resuscitation was begun immediately and of course DCS included in the differential diagnosis for this patient based on dive history alone.

The Dilemma:

Because of the emergent nature of these maladies, rapid diagnosis was essential. Though the history is concerning for DCS, the other issues must be “ruled-out” to ensure safety during treatment.

The Workup:

A Cardiac Echo was performed at the bedside. With no wall motion abnormalities and normal ejection fraction, MI (heart attack) and PE became unlikely, so the patient was rushed to the CT scanner for CT angiogram of the chest abdomen and pelvis.

The CT Scan:

The impact of this presentation lies in the remarkable imaging obtained from this patient. Portomesenteric venous gas in the left lobe of the liver, urinary bladder vasculature and femur marrow was found. In the abdomen a 7cm column of air was presented in another image.

The Outcome:

The patient was transferred urgently to the Hyperbaric Treatment Center and treated on a Treatment Table 60 (TT60) with extensions (comparable to USN Table 6). Because of shock and hemodynamic instability he required the use of Dopamine, a blood pressure raising medication. While it is the practice of our facility to treat patients with neurologic DCS at 220fsw (67 msw; Smerz et al., 2005), this patient was too unstable and the potential need for emergent removal from the chamber was real, so we decided to treat at 60fsw (18 msw). After the first dive, the patient’s abdominal pain significantly improved but he was still complaining of weakness, boring leg pain and an unsteady gait. The patient was treated daily with TT60s and remained in the ICU for an additional 48 hours. After the 3rd treatment, the patient had complete recovery from the diving issues and was downgraded from the ICU. On day 3, however, he developed gastrointestinal bleeding, a known and potentially fatal complication associated with DCI (Kizer, 1987). This patient, however, recovered and was discharged home on day 5. At two-month follow-up, the patient had a normal colonoscopy and resumed free diving, but not scuba diving.

Case #3: “A dizzying Experience”

A 50 year-old male “experienced diver” went diving using a rental dive computer. His first dive was to 87fsw (27msw) for 35 minutes followed by a 3-minute safety stop. Forty-five minutes later, he made his second dive to 65fsw (20msw) for 50 minutes. From about 30 to 15 fsw (10-5 msw) he had a rapid, uncontrolled ascent, got back under control and made a 3-minute stop at 10-15 fsw (3-5 msw). Approximately 10-15 minutes later, the patient reported, “it hit me,” the sudden onset of nausea, vomiting and progressive dizziness. Symptoms rapidly progressed over time and the patient could just lay on the deck. By the time he arrived back at the dock, he was physically unable to sit up without falling over. 911 was called and he was transferred to the ED. The patient stated he “felt like a pinball game bouncing off the walls”. The patient was in severe distress; as any movement caused him to vomit. Upon evaluation at the emergency department, the patient had stable vitals, normal strength, deep tendon reflexes and sensation. However, he had severe nystagmus (a rhythmical oscillation of the eyeballs, either pendular or jerky). His balance was so disabled that we were unable to assess cerebellar functions (finger-to-nose, heel-to-shin). Exam of his ears showed Teed grade 1 (injection of the tympanic membrane and along the handle of Malleus bone) with preserved ability to clear.

The Differential Diagnosis:

With the combination of this patient's scuba history and physical exam, air embolism, inner ear decompression sickness (IEDCS), cerebellar bleed, cerebellar stroke, inner ear barotrauma and benign positional vertigo was considered.

The Dilemma:

Cerebellar strokes and bleeds can be life threatening and these patients are often unstable requiring close monitoring and care. This patient was on an outer island and would have to be taken out of the hospital and flown to hyperbaric facility. If this were middle/inner ear barotrauma, it could be significantly worsened by recompression, especially to 220 fsw (67 msw). If, however, this were inner ear decompression sickness, it was completely disabling and must be treated rapidly and aggressively (Smerz, 2007).

The Workup:

While at the outside hospital, the patient had a CT scan looking for bleeding; this was negative. An MRI/MRA followed up the CT; these were negative for stroke, clot or vertebral dissection. The patient was given multiple medications, anti-emetics, anti-vertiginous medications, and benzodiazepines, and remained severely symptomatic.

The Outcome:

The patient was transferred to our hyperbaric facility and treated on a Treatment Table 220 (220fsw/67msw). By 10 minutes into the first treatment the patient had enough of an improvement in his nystagmus that he could open his eyes without vomiting. By three hours into the treatment, the patient was able to walk (though with difficulty); he had a normal heel-to-shin test, nystagmus only at the periphery but still had a positive Romberg test (unable to stand with eyes closed). After approximately 8 hours the first treatment was over and the patient was admitted to the hospital. On day 2, he was retreated on a treatment table 60 (USN Table 6). His Romberg test improved, nystagmus to the left only but continued to have unstable heel-toe walking. On Day 3, the patient signed out AMA from the hospital, but returned to the HTC for a third treatment. Treated on our CO table (USN Table 5), he had a complete recovery and was discharged to the care of his PCP.

Discussion

Decompression illness is often taught as a black and white malady. There have been journal articles that have downplayed the role of radiographic imaging beyond a screening chest x-ray (Carson, 2005), again upholding the belief that this is a straight-forward diagnosis, which it is often not. These three cases are not unique to our facility but rather just a sampling of what is almost routine in our area. While such cases are infrequent, they can mimic abdominal catastrophe, ruptured abdominal aortic aneurism, stroke, heart attack or pulmonary embolism. "Simple" cases (not life/function threatening) can mimic shoulder strain or other muscle injury, contact dermatitis, bumps and bruises. Failure to diagnose, or misdiagnoses, can lead to delays in treatment and adverse outcomes. The age and underlying health conditions of those participating is scuba diving has changed over the years (Nakayama and Smerz, 2003), and so must our approach to this patient population.

Another point that has become clear in these cases is the importance of pre-hospital and witness statements as an aid to diagnosis. With dysbaric air embolism (DAE) patients in particular, the history is important. The typical concept is that "bloody, frothy sputum" or hemoptysis, is a cardinal sign of DAE/AGE; however, one study found it in only in 5% of patients (Kizer, 1987). While it may be pathognomonic for acute lung injury, not all lung injury is DAE, and not all lung injury requires recompression. An accurate field neurologic exam would have proved invaluable in our case. Furthermore, we need to re-examine and update current teaching concepts, based on published literature.

Conclusion

It is obvious with just these few cases decompression illness is not always a black and white diagnosis and may require a specialist to diagnose and treat. Fortunately, dive cases are not a common occurrence, but this also makes specialists more difficult to find. For any professional dive entity, knowledge of the resources in their area is essential. Of course if all else fails, the Divers Alert Network is available at any time.

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DECOMPRESSION MANAGEMENT BY 43 MODELS OF DIVE COMPUTER: SINGLE SQUARE-WAVE EXPOSURES TO BETWEEN 15 AND 50MSW

Martin D.J. Sayer
Elaine Azzopardi

NERC National Facility for Scientific Diving
Scottish Association for Marine Science
Dunstaffnage Marine Laboratories
Dunbeg, Oban, Argyll PA37 1QA, SCOTLAND
mdjs@sams.ac.uk

Dive computers are used in some occupational diving sectors to manage decompression. However, there is little independent assessment of the performance of dive computers and regulatory control is lacking. Assuming that there is a significant proportion of occupational diving operations that employ single square-wave pressure exposures in support of their work, single examples of 43 models of dive computer were set to default settings and compressed to five simulated depths (nominally 15, 20, 30, 40 and 50 msw). They were maintained at those depths until all computers had registered over 30 minutes of decompression. At each depth, and for each model, downloaded data were used to collate the times at which the unit was still registering "no decompression", and the times at which 3, 5, 8, 10, 12, 15, 20 and 30 minutes of decompression were indicated or exceeded. Each depth profile was replicated three times for most models. In general, decompression isopleths for no-stop dives indicated that computers tended to be more conservative than standard decompression tables at depths shallower than 30 msw, but less conservative between 30-50 msw. There was considerable variation between models in the times permitted at all of the depth/decompression combinations; for all tests, the differences between maxima and minima times, expressed as a percentage of the maxima, ranged from 16.7 to 62.5%. Differences in results from replicated trials existed but were minor in most cases. The scale of variation in how decompression is managed by the tested dive computers suggests possible inconsistencies in how decompression stress is being calculated combined with the potential for different acceptance levels for DCS probability. Knowing the model of computer used may indicate the level of potential risk to a diver in cases of missed decompression.

Introduction

Dive computers can be accepted in some occupational diving sectors as tools for managing decompression (e.g. Dean et al., 1998). There have been a number of studies in the past that have compared the performance of dive computers in managing decompression (Lippmann, 1989; Lippmann and Wellard, 2004; Buzzacott and Ruehle, 2009). However, there are few contemporary comparative studies to guide managers when choosing models of computer that best suit their operational needs. In addition, in Europe, standards and normatives that underpin CE marking for dive computers do not stipulate operational limits for decompression management (EN 13319, 2000; Sieber et al., 2012).

The present study follows previous studies in that it compares the performance of a range of dive computers. However, all the models assessed were relatively modern and are either still being sold or are, at least, in common use in the UK (Azzopardi and Sayer, 2010, 2012). Comparisons were made of a range of potential single square-wave profiles dives for the depth range of 15 to 50 msw.

Methods

Single examples of 43 models of dive computer that are in common use in the UK (see Table 1) were set to default settings and compressed to five simulated depths (nominally 15, 20, 30, 40 and 50 metres seawater (msw)). In each test, the computers were immersed to 20 cm in a tank of seawater (SW: 33‰S) located inside a standard two-compartment therapeutic recompression chamber (Divex2000). The chamber was compressed on air to the simulated nominal depth and maintained at that depth until all computers had registered over 30 min of decompression. At each depth, and for each model, downloaded data were used to collate the times at which the unit was still registering "no decompression", and the times at which 3, 5, 8, 10, 12, 15, 20 and 30 min of decompression were indicated or exceeded. Each depth profile was replicated three times for most models.

Table 1. List of the 43 models of computer employed in the present study.

Brand	**Model**	**Brand**	**Model**
Uwatec	Galileo Sol	Suunto	Vytec DS black
Uwatec	Galileo Terra	Suunto	Vytec silver
Uwatec	Galileo Luna	Suunto	Cobra 2
Uwatec	Smart Tec	Suunto	Cobra 3
Uwatec	Smart Com	Suunto	Vyper
Uwatec	Aladin Tec 2 G	Suunto	Vyper 2
Uwatec	Aladin One	Suunto	Vyper Air
Uwatec	Aladin prime	Tusa	DC Sapience
Scubapro	Xtender	Tusa	DC Hunter
Mares	Nemo Sport	Oceanic	Veo 250
Mares	Nemo	Oceanic	VT 3
Mares	Nemo Air	Oceanic	Pro Plus 2
Mares	Nemo Excel	Oceanic	Atom 2
Mares	Nemo Wide	Oceanic	Datamask Hud
Mares	Puck wrist	Cressi Sub	Archimede 2
Mares	Puck Air	Cressi Sub	Edy II
Mares	Icon HD	Beauchat	Voyager
Suunto	D9	Apeks	Quantum
Suunto	D6	Apeks	Pulse
Suunto	D4	Seeman	XP 5
Suunto	Stinger	Uemis	SDA
Suunto	Spyder		

Depth/time isopleth relationships were generated for all the decompression end points examined over a 15-50 msw depth range for every model of computer. These were compared against isopleths constructed based on both the Royal Navy Physiology Laboratory air decompression table 11 and the Defence and Civil Institute of Environmental Medicine (DCIEM) air decompression tables.

Frequency analyses were also conducted based on the numbers of computer models falling within the time ranges required to reach the designated decompression end points.

Results

Differences in results from replicated trials within units existed but in most cases were minor. For example, for the 40 units where there were at least three replicate exposures for no-stop decompressions over the five depths tested, in 94.5% of the records variation was within 10% of the average time for all tests; variation was zero in 39% of tests. In 1.5% of tests, variation was greater than 25% in the 15-40 msw depth range.

Frequency analyses showed that there was considerable variation in the times displayed by the computer units for all the depth/decompression combinations. In general, the spread tended to be greatest at the shallower depths tested and the longer the decompression time was permitted. An example for no-decompression stop values is shown in Figure 1.

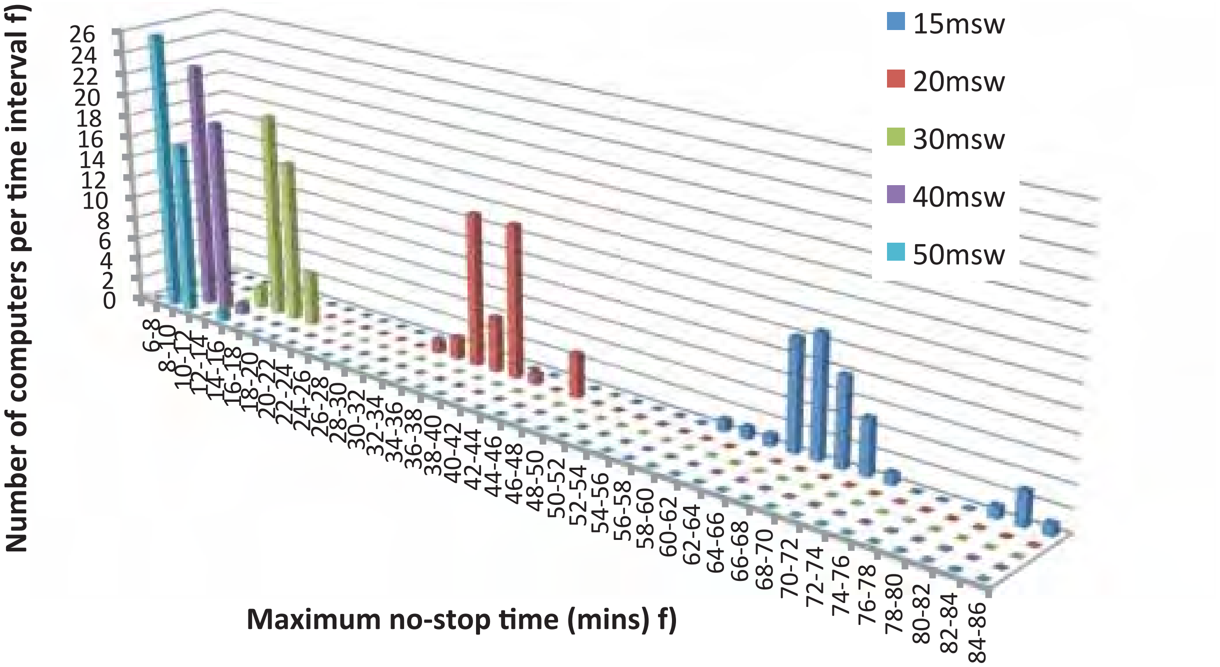

Figure 1. Frequency analysis of number of tested computer units displaying maximum no-decompression stop values for square-wave dive profiles to maximum depths of 15-50 msw. For each computer unit, n=1-3 for each depth test.

The decompression isopleths generated for no-stop dives indicated that computers tended to be more conservative than standard decompression tables at depths shallower than 30 msw, but less conservative between 30-50 msw (Fig. 2).

However, these differences were not always consistent between computer models. Whereas in some comparisons there were relatively constant differences between the computers at all depths (Fig. 3), in others there may, for example, be quite significant differences at shallower depths but these were not evident when the tests were deeper (Fig. 4).

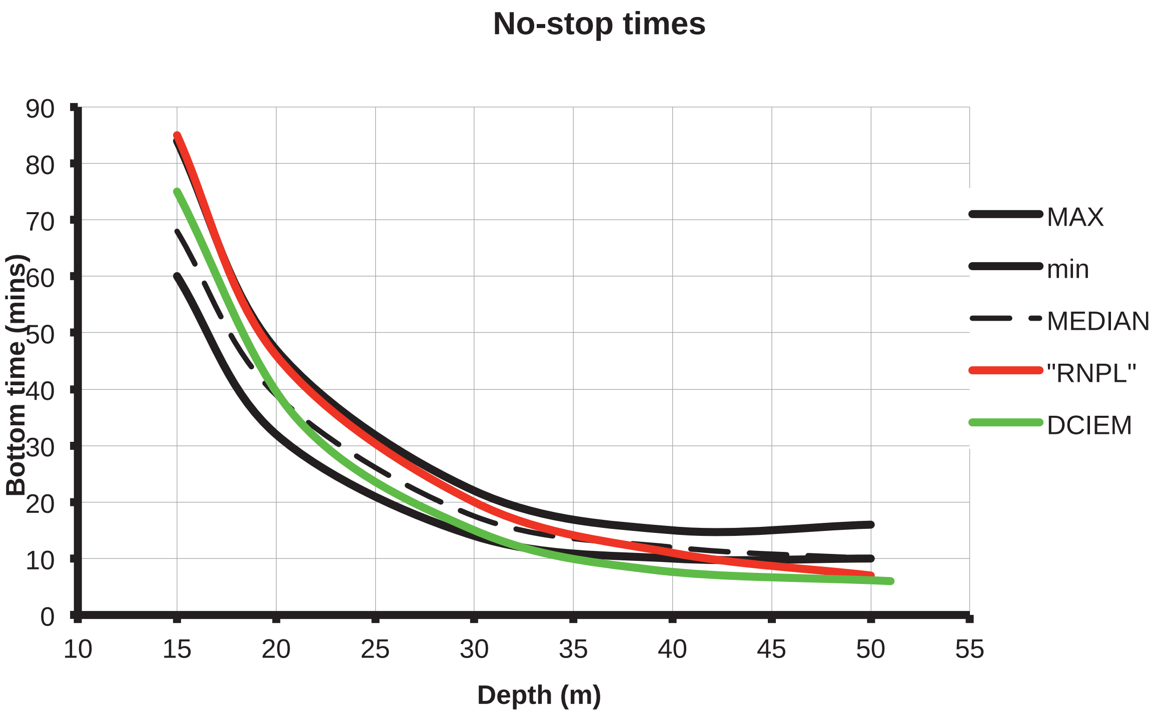

Figure 2. Isopleth relationships for the maximum times permitted by 43 models of dive computer and two air decompression tables before the dive would require any decompression over a depth range of 15 to 51 msw. All dive profiles were square-wave; isopleths for the dive computers are pooled to show maximum (MAX), minimum (min) and median values for all 43 models.

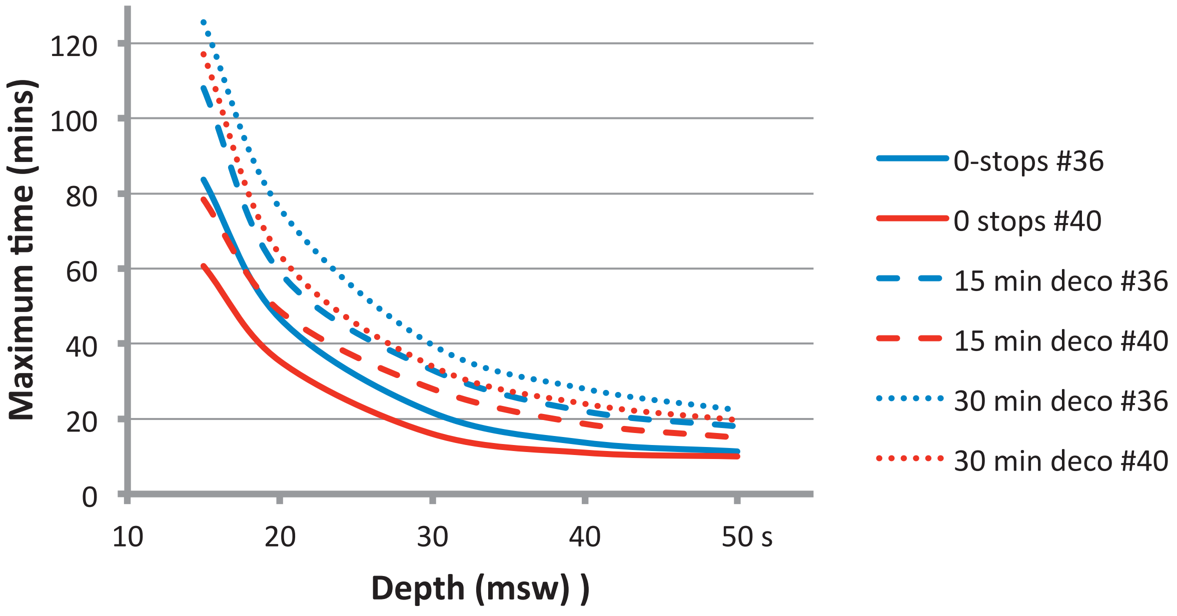

Figure 3. Decompression isopleths for two models of dive computer (#36 and #40) compared at three levels of decompression stress (no-stops; 15 min of deco; 30 min of deco).

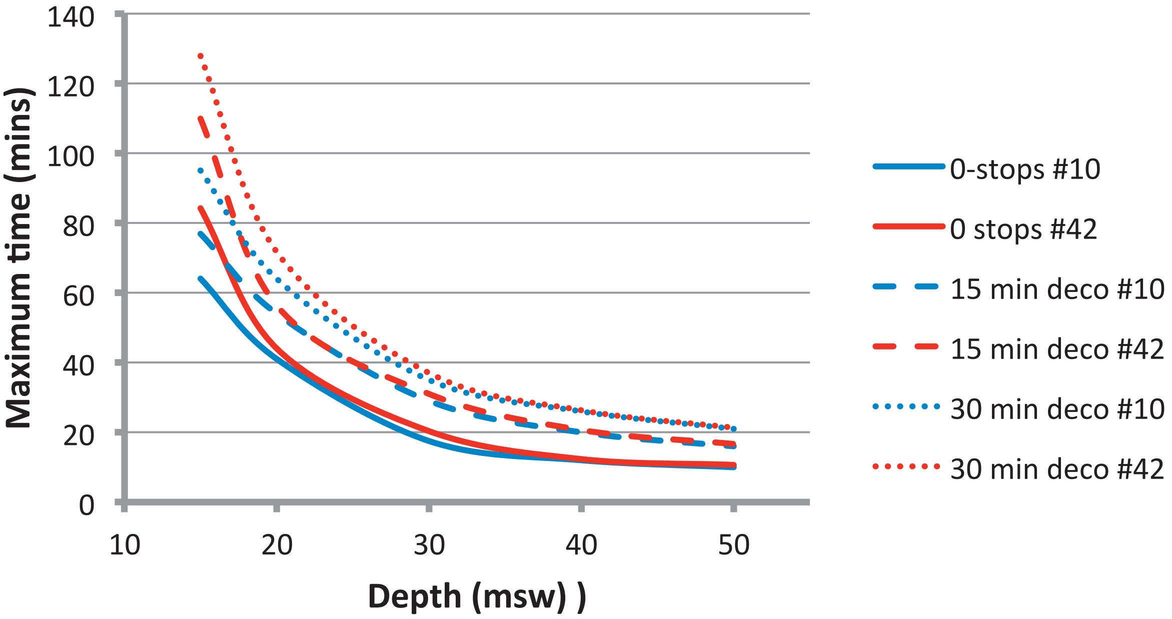

Figure 4. Decompression isopleths for two models of dive computer (#10 and #42) compared at three levels of decompression stress (no-stops; 15 min of deco; 30 min of deco).

There was a general, though weak at times, trend in the difference between maximum and minimum times permitted at each depth/decompression combination to increase with decompression stop length for the same maximum depth and at shallower depths where decompression time was standard. However, that trend did not fit between values generated between the 40 and 50 msw tests at all decompression end-points. There was a consistent difference of 10 minutes at 50 msw between maxima and minima for all decompression end-points (Fig. 5); this was an increase on the differences recorded at 40 msw (range: 3-8 min).

Conclusions

The scale of variation in how decompression is managed by the tested dive computers suggests that there may be significant differences in how decompression stress is being calculated. These differences could be increased when combined with different inherent acceptance levels for DCS probability. There may also be decreased levels of confidence in decompression management at deeper depths (greater than 40 m; see Azzopardi and Sayer, 2010; and Fig. 5). However, it must be remembered that these tests only refer to single square-wave exposures. As such, the present study both ignores the benefits of using computers to manage multi-level dive profiles and the anticipated influence of a possible second (or more) repetitive dive(s).

When using dive computers to manage decompression schedules it may be appropriate to have good knowledge of the behavior of the models to be employed. It should be remembered that in some cases, behaviors change with the depth ranges to be dived. A better overall knowledge of the levels of conservatism employed by dive computers may indicate the level of potential risk to a diver in cases of missed decompression. It may not, at this stage, be possible to make meaningful assessments that can accurately compare the operational benefits of longer dive times against possible additional decompression sickness risk.

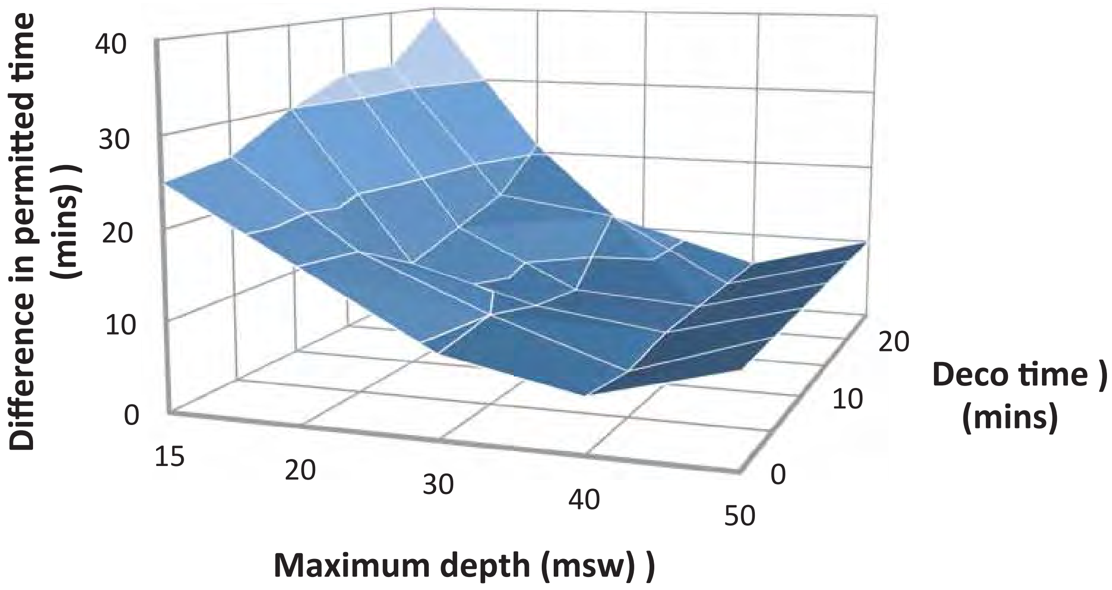

Figure 5. Area plot of the difference (maximum minus minimum) in total dive times required to generate decompression penalties of 0-30 min. Values are for 43 computer models tested across a depth range of 15-50msw.

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COMPARATIVE ANALYSIS OF FEDERAL PROGRAM POLLUTED WATER PROTOCOLS

Sean Sheldrake[1]
Rob Pedersen[1]
P. Bruce Duncan[1]
Alan Humphrey[2]

[1]U.S. Environmental Protection Agency
1200 6th Avenue, Suite 900, Mailstop ECL-110
Seattle, WASHINGTON 98101 U.S.A.
sheldrake.sean@epa.gov

[2]U.S. Environmental Protection Agency
Environmental Response Team
2890 Woodbridge Ave
Edison, NEW JERSEY 08837 U.S.A.

Recreational, government, military, law enforcement, and commercial diving occurs in contaminated harbors and enclosed bodies of water prone to water column and sediment contamination. However, many dives continue to be undertaken exposing the diver to a variety of chemicals and microbe, which may lead to adverse health effects. Standard polluted water diving protocols are examined for several federal entities including programs of the USCG, Navy (USN), Army, EPA (Environmental Response Team and Region 10) and NOAA. Standard protocols for medical monitoring, training, diver protection and decontamination amongst these various agencies are presented and compared against practices on the ground. Relevant regulatory requirements and applicable standards of industry relative to worker protection are also compared against standard practices of each agency. Recommendations are made to enhance worker protection against contaminants, and potential releases of contaminants at typical contaminated harbor work sites frequented by the federal entities listed above.

Introduction

Many federal programs utilize scuba diving to support their respective Agency missions. This diving is conducted at a variety of sites that include harbors and enclosed bodies of water prone to bacterial and other types of contamination. Some dive programs are explicitly designed towards polluted water diving, while others simply come across polluted water incidentally in support of their Agency's unique mission. Polluted water protocols have been relatively well published (Lotz, 1983; Wells, 1984; Phoel and Wells, 1991), yet are practiced in various manners, if at all, by divers and dive units in various federal programs. Often there is also a difference in dive program protocols and work on the ground. Necessary procedures for polluted water diving operations include: dive planning, diver training, medical monitoring, personal protective equipment, and decontamination protocol.

Methods

Federal dive programs were contacted to discuss a number of polluted water topics, including:

1. types of bodies of water encountered during dive operations, potential for bacterial and other contamination (e.g., harbors), and dive planning;
2. training in hazardous materials (hazmat) procedure including decontamination;
3. types of dive gear/personal protective equipment (PPE) used and decontamination (decon); and
4. medical monitoring and immunizations.

Discussion of U.S. Coast Guard (USCG), National Oceanic and Atmospheric Administration (NOAA), Environmental Protection Agency (EPA), U.S. Navy (USN), and Army approaches to their unique types of diving includes adherence to protocol, if any, in the field. Discussion also includes regulatory requirements, if any, standards of industry for various polluted water protocols, and areas where additional efforts might be focused for worker protection.

1. Dive Sites and Dive Planning

The USCG, NOAA, EPA, USN and Army all conduct dives in a variety of locations to accomplish their respective missions. For all of the above Agencies and branches of the military, harbor diving is part of the diving required to accomplish this mission. For some, this represents a significant number of dives—for the Navy alone this may constitute 40,000 dives per year (pers. comm. with Master Diver Stark, Lt Tramontin, 2010). In most harbors, divers can be exposed to contaminants in both sediment and water. In the Portland Harbor, for example, contaminants include metals, pesticides, polycyclic aromatic hydrocarbons (PAHs), volatile and semi-volatile organics (VOCs/SVOCs), polychlorinated biphenyls (PCBs), and polychlorinated dibenzodioxins, and dibenzofurans. In addition, frequent sewer overflows contribute high bacteria counts during rain events (USEPA 1998, 2009a). In any water near metropolitan areas, also frequented by these diving entities, bacteria in the water column can also be a problem from a variety of sources, including pet waste. EPA's Beaches Environmental Assessment and Coastal Health Program (BEACH) provides regular bacterial counts at popular marine recreational sites (USEPA, 2010a). Information on sewer discharge location, overflow frequency and publicly available bacterial count information can be a valuable dive-planning tool. In addition, chemical and biological contaminant trends in the water column and sediment are available through NOAA's Mussel Watch Program (NOAA, 2009). Outfalls (Figs. 1, 2) can also discharge a variety of harmful chemicals to the dive site. In Portland Harbor alone there are over 300 outfalls (USEPA, 1998). EPA's Envirofacts database presents outfall location and data that can be of use in planning for worst-case water quality at a particular dive site (USEPA, 2010b). In addition, a list of chemically impaired water bodies can be obtained from EPA (USEPA 2010c) as well as an up to date list of EPA Superfund Sites (USEPA 2010d). Even use of up to date navigation charts can inform a dive plan with some level of outfall information.

In the case of the BEACH program data, this information provides some ability to forecast various types of exposure, based on the frequency of problems detected in the past. In addition, exposures to sediment in a Superfund site can be predicted with some certainty, such as within the Portland Harbor area (USEPA, 2009a).

Some agencies did not consider their diving to be in polluted water, or thought that their diving remained "clean" if sediment contact was avoided. For one agency, polluted water guidance is pointed: "NOAA divers are prohibited from diving in contaminated water" (pers. comm. with Dave Dinsmore/NOAA, 2009).

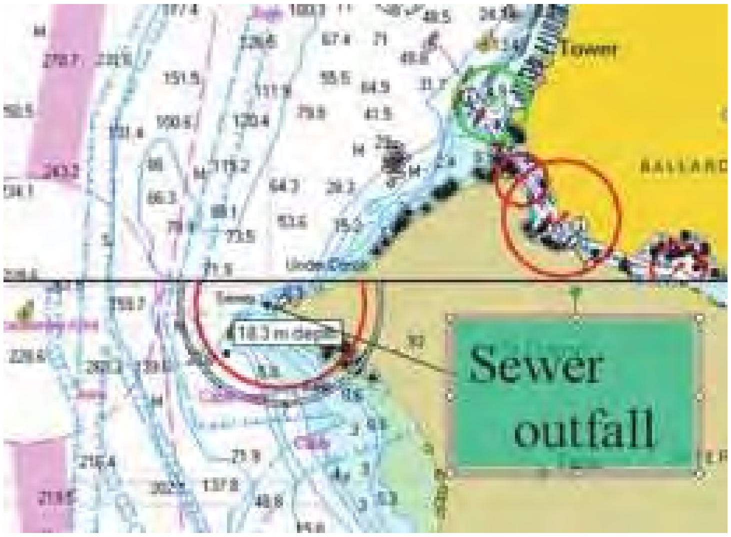
Tower
BALLARD
18.3 m depth
Sewer outfall

Figure 1. Sewer outfall near downtown Seattle in a popular charting program.

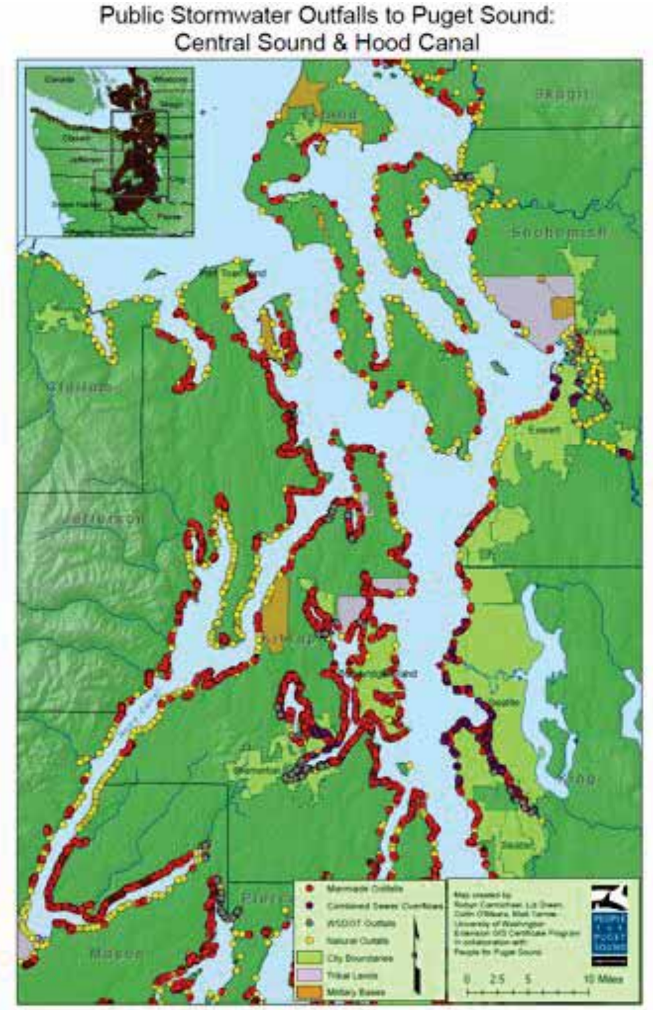
Public Stormwater Outfalls to Puget Sound:
Central Sound & Hood Canal
Skagit
Snohomish
Jefferson
Kitsap
Mason
King
Manmade Outfalls
Combined Sewer Overflows
WSDOT Outfalls
Natural Outfalls
City Boundaries
Tribal Lands
Military Bases
Map created by
University of Washington
Extension GIS Certificate Program
in collaboration with
People for Puget Sound
0
2.5
5
10 Miles

Figure 2. An example of available GIS data of storm drains and combined sewer outfalls in the Central Puget Sound.

However, the water column can change on a daily basis and even hour by hour because of sewer overflow episodic inputs to harbor areas in which NOAA, USCG, Army, USN, and EPA dive. Several years ago, two USCG divers with a maritime safety and security team (MSST) found out the dangers of a suddenly polluted water column while performing hull inspections in a harbor area that was clean until a sewer overflow occurred during their dive operation. The divers never recovered fully enough to return to their diving duties and are currently on medical disability (pers. comm. Lt. Cmdr Dennis Fahr, 2009).

At times, sewage overflows can be predicted by the presence and volume of rainfall (Seattle Times, 2006), but sewage overflows are also human-caused and can be unrelated to meteorological conditions. For example, something as simple as equipment malfunction can dump untreated sewage onto the dive site on a day without any precipitation (Seattle Times, 2010). However, as bad as that sounds, more than one hundred thousand metric tons of toxic chemicals may wash into the dive site every year in storm water if diving in Puget Sound (Seattle Times, 2009a, b, c, d). As a result, EPA divers typically dress for what could be present at a particular dive site rather than what exists there at the time gear is donned.

Other federal diving programs generally did not give examples of when they would upgrade their PPE for dives with the exception of the USN who acknowledged that heavy rains in an area that does not experience rain very often, such as San Diego, would bring a flush of contaminants necessitating PPE upgrades. Likewise, the USN noted that free product or fish kills would necessitate category I dive protocols referenced in their Guidance for Diving in Contaminated Waters. However, the USN noted that these outward signs of pollution are indications of serious pollution but did not have a specific protocol for implementing the CWD manual steps for lesser levels, which often leaves their divers either diving in wetsuits with recreational type mouthpiece regulators (USN category 4) or in drysuits mated to diving helmets (category 2).

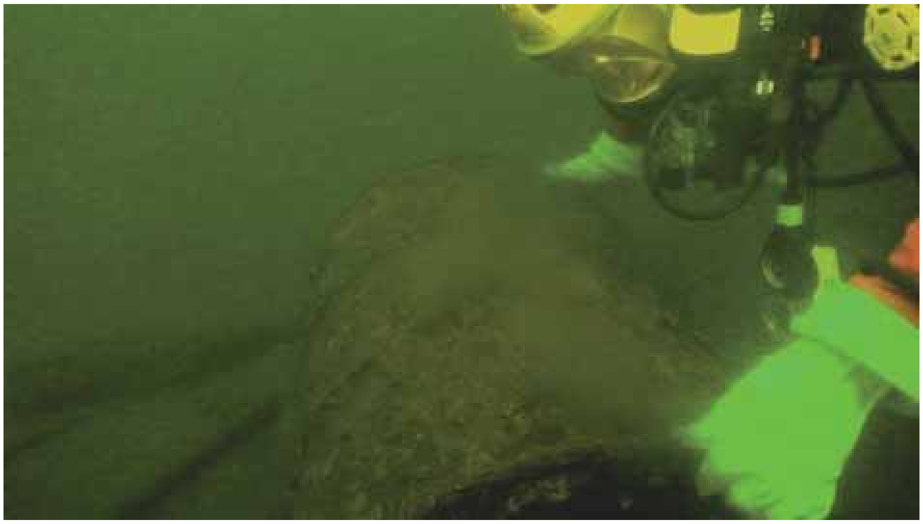

Figure 3. Diver Rob Rau inspecting a discarded 55-gallon drum encountered off a former manufactured gas plant in Seattle, Washington. Photo by S. Sheldrake.

Operational needs should also be a part of dictating the level of PPE. For example, the same site can call for different levels of protection, e.g., conducting a scientific video survey might be USN category 3 but doing a work dive with substantial bottom interaction could be a category 2 dive at the same site where sediment contamination is a driving concern, simply based on the intended interaction with contaminants. This is a focus of part of NOAA’s working diver curriculum in discerning whether a dive should be considered in “polluted water” in addition to the USN guidance.

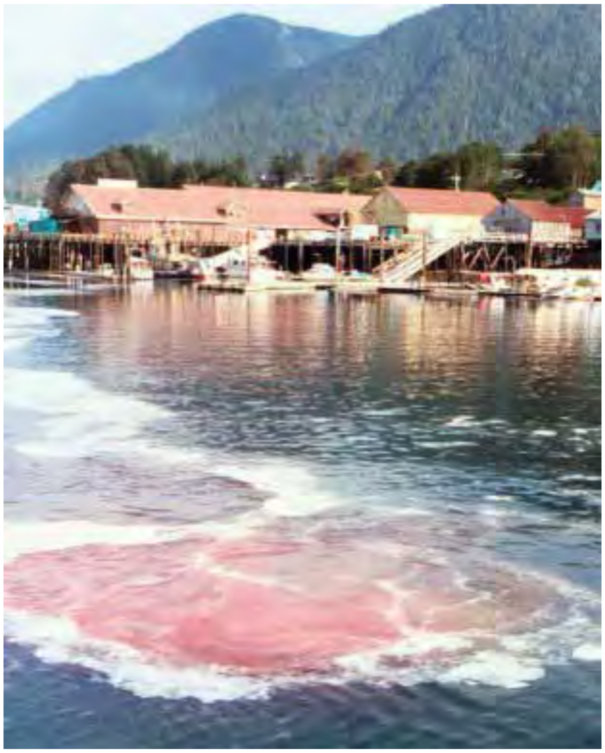

Figure 4. Photo of a plume of blood at an EPA dive site in Alaska during an enforcement inspection. Photo by S. Sheldrake.

Federal diving programs were generally unaware of the above dive planning tools and trends and/or did not use them on a regular basis to determine the level of potential contamination at their dive sites and/or proximity of potential sources of contamination. Instead, many programs will dive a site as a non-polluted water dive simply because of a lack of confirmed contamination. As the USN correctly points out, there are often too many chemicals in the water at a particular site to test them all. Further, once the test is done, the levels could change for the worse. There was also a general understanding on the part of these programs that “polluted water diving” was relegated to the most extreme of circumstances, e.g., diving in raw sewage, versus diving in more dilute contamination with lower level acute and cancer risk (USEPA, 2009b). However, it was often repeated in discussions that heat stress was a concern, perhaps an overriding concern, on why a more cautionary approach is not taken for less polluted/possibly polluted situations.

2. Training

Though there are methods to limit diver exposure to these contaminants that have been widely published and available (see review by Barsky, 1999), these methods are not always employed by divers in general, which may be because of a lack of formal training. OSHA explicitly requires that specific training be undertaken on an initial and reoccurring basis for hazardous waste site operations (OSHA 29 CFR 1910.120). EPA divers doing polluted water work undergo this initial 40-hour training along with required annual 8 hour refreshers.

Figure 5. Photo of EPA Dive Training at the Office of Research and Development Gulf Ecology Division Lab (ORD-GED) in Gulf Breeze, Florida. Photo by S. Sheldrake.

In addition, Region 10 practices decontamination techniques on training dives, including heat stress management. While this training covers tools used to plan work on contaminated sites, personal protective equipment, medical monitoring and other aspects that could apply to polluted water diving, federal diving programs such as USCG, USN, and NOAA do not generally require any time be devoted to this type of training for their dive work. However, USN divers do undergo chemical and biological warfare training that does emphasize some of the same aspects of decontamination procedure. However, this crossover application may not sufficiently cover polluted water dive planning in an explicit manner, such as what might be a causal factor for pollution at a dive site or when to upgrade PPE based on non-visual cues. Classroom and dive training should emphasize means and methods to plan a dive, how to wear and maintain appropriate PPE, techniques/types of decontamination and division of the dive platform into hot, contamination reduction, and cold zones, and techniques to manage heat stress endemic to more protective PPE.

3. Personal Protective Equipment and Decontamination

There are a number of ways to protect the diver from various levels of dive site contamination. These methods include: keeping the diver completely dry through use of a drysuit, utilization of drygloves, ensuring materials including the drysuit are constructed of an easily decontaminated material such as vulcanized rubber (USEPA, 1985), use of a full face mask that seats on a dryhood or preferably a hardhat that mates directly to the drysuit (USEPA, 1985; Barsky, 1999; USN, 2008), appropriate training, and thorough decontamination such as a potable water post-dive rinse (USEPA, 2001; 2009c). For example, a neoprene drysuit and wet gloves are inappropriate for use at a polluted water dive site (Fig. 6). A neoprene drysuit cannot be easily decontaminated and can spread contaminants onto the boat and potentially to the next dive operation (USEPA, 1991). A slick, vulcanized rubber suit is generally considered more amenable to decontamination (USEPA, 1985), though breakthrough for certain chemicals remains a concern (Trelleborg Viking, 2001). USN, Army, USCG, and EPA make decontamination compatible suits available to their divers as needed for their missions.

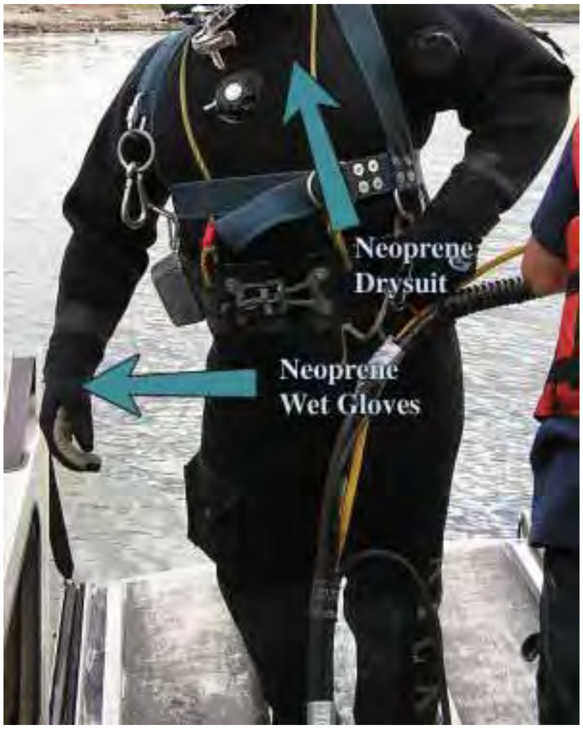

Figure 6. A diver exiting the water at a Portland Harbor Superfund cleanup site.

However, there is evidently some difference in application of appropriate PPE in the field versus dive program policy. For example, Army divers that assisted with recovery efforts in Haiti were using wetsuits and mouthpiece regulators despite the presence of raw sewage. The Army also was giving the divers simultaneous doses of Cipro and Deoxycycline (Army Times, 2010, pers. comm. with Captain Scott Sann) rather than keeping the diver dry and following an Army decon SOP, such as use of a slick rubber drysuit in conjunction with a potable water rinse. This was justified because of the "risk of hyperthermia causing far more casualties than waterborne illness."

As far as accepted PPE approach, there is some disagreement between EPA and the USN regarding use of the Interspiro "AGA" full-face mask seated on a dryhood. The USN in their Guidance for Diving in Contaminated Waters generally considers the AGA to be inappropriate for polluted water as "…full-face masks offer no protection for the Diver's head, neck, or ears, all of which are potential sites for exposure to waterborne hazards" which is only true if the full face mask is not used in conjunction with a properly fitting dryhood. The USN correctly points out that there is a droplet inhalation concern, even when using a positive pressure mask such as the positive pressure AGA. The USN notes that when using the AGA or any other full-face mask, positive pressure is preferred to minimize exposure for which there is general agreement amongst all agencies based on previous study (USEPA, 1985). The USN also points out that most commercial divers use helmets, not full-face masks. However, the USN guidance fails to cite that many, if not most, commercial divers fail to properly mate the diving helmet to the drysuit to gain the stated advantages of helmet usage. Rather, most commercial contractors use neoprene neck dams for this purpose (contrary to EPA and USN policy on proper helmet mating to the suit) which can leak profusely into the diver's helmet if it is in any position other than absolutely level, with the amount of leakage varying by tightness of the neoprene (USEPA, 2009b). In other words, incorrect helmet mating can render the helmet no more protective, and potentially less protective, than the AGA positive pressure full-face mask.

The Navy's "category 3" level of full-face mask protection allows for diver dermal (over the head and neck) and ear exposure to contamination because of the lack of drysuit and dryhood. The USN's characterization of insufficient protectiveness of the full-face mask/not using the full-face mask in conjunction with a drysuit/dryhood leads the USN to dive primarily in wetsuit (category 4) or Viking and helmet diving modes (category 2); the USN category 3 is not, or is not believed to be, sufficiently protective. This is a significant difference in PPE; essentially jumping from virtually no protection to some of the highest level of protection available, potentially causing more exposure to their divers in light to moderately contaminated environments. Likewise, the types of water for which the Navy recommends this type of gear jumps from not polluted at all for category 4 to "heavily contaminated" in the case of category 2. Category 3 dives are exposing the Navy diver through dermal and ear canal exposure to "moderately contaminated" water because of the lack of dryhood/drysuit usage in category 3. For the USN, the primary reason for this dramatic jump in levels of protectiveness seems to be because of the potential for heat stress on the diver when in a drysuit (pers. comm. with Tramontin, Stark, USN 2010) and/or lack of diver experience in using a full-face mask successfully with a dryhood.

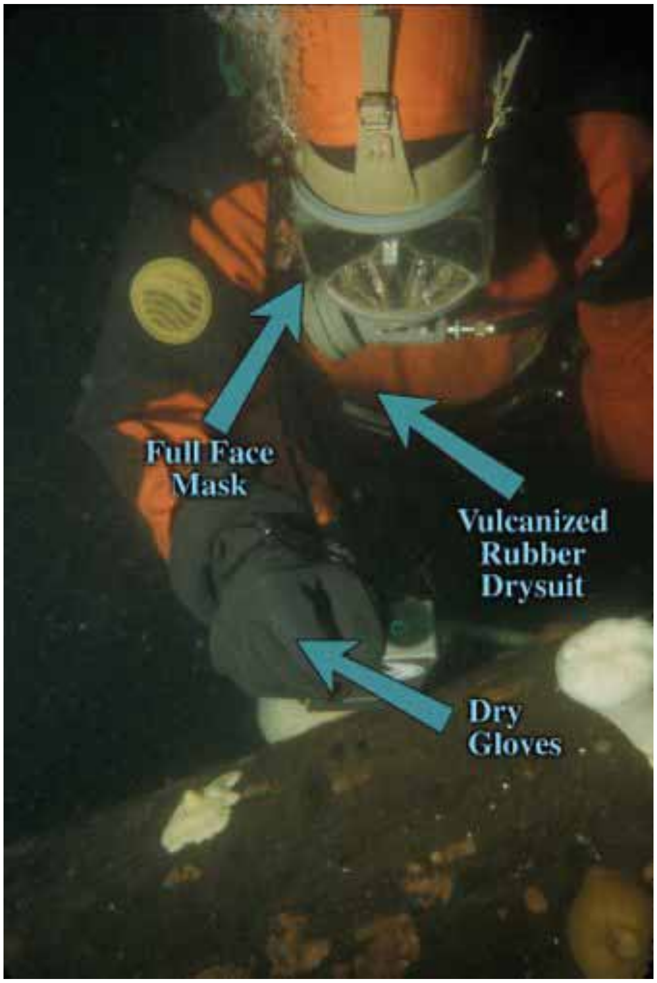

Figure 7. EPA Region 10 Diver Rob Pedersen mapping the zone of discharge along the outfall pipe at an Alaskan Seafood Processor. Note the diver is kept fully dry in decon compatible gear. Photo by S. Sheldrake.

For example, in a water body such as a harbor with many nearby outfalls that are not currently discharging, lacking available data EPA Region 10 would use a full-face mask seated on a dryhood, drygloves, and a potable water rinse for both a) unknown pollutants that may likely exist because of historical practices in the water body, and b) the possibility that conditions could change because of a sudden outfall discharge. Admittedly, keeping the diver fully dry is easier in cold water and air conditions prevalent to Region 10 inland and coastal waters in Alaska, Oregon, Idaho and Washington much of the year. However, the Region 10 dive unit effectively demonstrated management of heat stress in triple digit temperatures with varying levels of humidity for both inland (Yakima River) and Puget Sound operations (Duwamish River), primarily through the use of potable water washdown of primary and standby divers on a regular basis when on the dive platform. This has also included management of heat stress in warm water conditions (~70-75^{o}F) present in the Willamette River in conjunction with high air temperature. Therefore, even in the absence of any historical conditions, the presence of outfalls would be enough to trigger the use of the above gear since heat stress can be managed more easily than exposure to some exotic disease vectors. It is possible that a hardhat type diver dress may be most appropriate for some number of sites. However, it could also be the case that defining PPE as only including a commercial hardhat mated to a drysuit may discourage dive programs from taking some measures in PPE improvements because of the time and logistical challenges of surface-supplied helmet diving, resulting in additional diver exposure. For some dive operations, surface-supplied/hardhat measures are simply impractical because of space, cost or time constraints in response to the dive site, such as in the rescue phases of public safety diving.

Wet gloves are often inappropriately used for polluted water diving (Fig. 6), which the divers may have deemed necessary because of the impingement hazards that may compromise dry gloves. Wet gloves used in polluted water cannot be decontaminated and should be disposed of after dive operations and/or specially managed to not expose tenders and divers on this or the next dive operation. Wet gloves also potentially introduce dermal exposure to the diver during the dive, which can be significant for certain readily absorbed chemicals. Also, chemicals like polyaromatic hydrocarbons and creosote can quickly burn exposed skin when using a wetsuit or wetgloves (Fig. 8). A better course might be to put nitrile or rubber gardening-type gloves over drygloves to offer some chafing protection and then dispose of all the gloves after the dives. While the USCG and Army did not have a policy on wetglove use, the USN prohibits their use in category 2 “heavily contaminated” diving and above, where EPA would prohibit wet glove usage at and below the category 3 level “moderately polluted” water level.

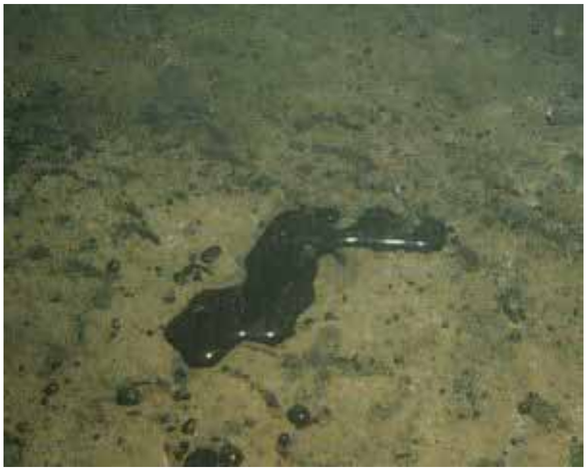

Figure 8. Photo of creosote on the bottom of Puget Sound. Photo by S. Sheldrake.

Drysuit leakage is also a substantial concern for dermal exposure, essentially rendering the drysuit to be no more protective than a wetsuit, (USEPA, 2009b). In addition to testing suits before certain polluted water dives, diving should be discontinued in the event of a drysuit leak, the thermal undergarments separately washed, the diver showered, and the suit immediately repaired (USEPA, 1985). The frequency of polluted water diving operations in Region 10 necessitates the maintenance of leak testing equipment in its dive locker such that leaks found during polluted and non-polluted water dives are immediately repaired. This equipment consists of neck and wrist clamps to allow the suit to hold air, reversing the exhaust valve such that the Viking suit can hold sufficient pressure to detect leaks, and localizing leaks via a soap and water spray.

Some diving programs may fully understand what appropriate PPE is, but may have a different view of what constitutes polluted water. Unfortunately, what constitutes polluted water is a complex issue and seems to mean different things to the federal agencies polled. More and more of the dive sites these federal programs are asked to dive in are, at times, polluted or more extensively polluted each day. To that end, there is a wide variety of what EPA considers to be polluted water where the full-face mask seated on a dryhood (e.g., Viking with a turbo or magnum hood) offers the diver a modicum of protection. For its own operations, EPA Region 10 finds that use of gear appropriate for mild to moderate polluted water is appropriate given the episodic or otherwise unknown nature of some releases into the environment. Since NOAA divers are not allowed to conduct “polluted water” dives, their standard equipment program (SEP) issue gear is not compatible with decon. For example, NOAA divers generally use drysuits with a crushed neoprene exterior that cannot be effectively decontaminated for bacteria or chemicals. Also, NOAA divers generally use regulators held in the divers mouth (mouthpiece regulators). Mouthpiece regulators promote ingestion of any chemicals and bacteria present in the water column. However, NOAA divers regularly dive in harbor areas to conduct ship husbandry at their former Lake Union facility in Seattle, which is near several storm water and other outfalls that put these dives squarely in EPA’s polluted water category.

Decontamination solutions are well understood from decades of testing. Some dive units may choose not to decontaminate their divers because of the increased logistical needs for collection of various washdown rinses, such as those noted in Table 5-1 of the USN guidance on Contaminated Water Diving, like the 5 percent bleach solution. Because of the ease of use of the potable water rinse (i.e., no general need to collect rinsewater) and general lack of wear and tear on equipment in using this solution, EPA Region 10 conducted a study of decontamination solutions on bacteria. EPA Region 10 has found that a potable water rinse is quite effective in removing bacteria when used with decontamination compatible gear, such as the Viking drysuit (USEPA, 2009c). The Army reports using potable water as a standard decontamination method for harbor diving as well, though evidently not in Haiti recovery efforts as noted above.

With certain exceptions, appropriate PPE and decontamination protocols for polluted water is well documented. What seems to vary among federal diving programs is a) what constitutes polluted water, and b) what types of dive sites trigger additional measures, such that rigorous protocols, e.g., category 2 from the USN guidance, is put into place. Too often, the absence of information and/or elevated dive site temperatures are viewed as being compatible with lower levels of protection for the diver and tenders.

4. Medical Monitoring and Immunizations

Whether diving under the OSHA commercial diving standards, or OSHA scientific diving exemption, cleanup workers should be working under conditions that are in compliance with OSHA standards (29 CFR 1910.120), as the OSHA scientific diving exemption does not exempt scientific or other divers from employing personal protective equipment (PPE) and other preventative exposure measures and monitoring, including medical monitoring for chemical exposure. However, as previously discussed, EPA's experience is that divers in Portland Harbor involved in sampling/analysis do not always dive in compliance with the OSHA standards and/or do not initially propose dive plans in compliance with hazardous waste site operation (HAZWOPER) standards (USEPA, 2009b). The reason for this is that the divers typically do not believe the dive site to be contaminated and/or do not track their contaminated site exposures against the 30-day OSHA exposure monitoring requirement. Typically, items such as basic diver environmental isolation/(PPE) and medical monitoring (1910.120 HAZWOPER items) are not proposed in the Health and Safety Plan at Superfund Sites, where EPA has purview over site health and safety. It is a reasonable presumption that contractors doing similar work not under EPA oversight may not be equipping their divers, training their divers, or monitoring their divers for hazardous waste exposure per OSHA 29 CFR 1910.120.

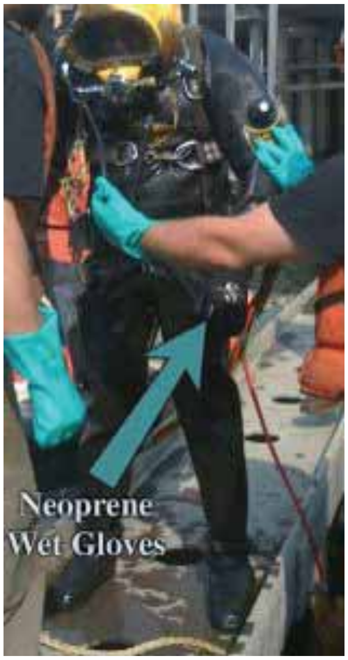

Figure 9. A diver exiting the water at a Portland Harbor Superfund Cleanup site. Neoprene materials cannot be decontaminated.

Similarly for federal divers, medical monitoring is generally only undertaken as a matter of the diver's physical fitness to dive, as most dive sites are considered unpolluted. Neither NOAA, USCG, USN or the Army provide medical monitoring steps for the diver to look for signs of chemical or biological exposures (pers. comm. with Dinsmore, Fahr, Stark, Hallman, 2009, 2010). Primarily because of the dive program's mission relative to environmental protection, which will include sampling activities at polluted sites, EPA has a different approach. EPA's standard is to inventory dive exposures for the past year and adjust blood tests based on the chemical exposures reported (See Fig. 11 below).

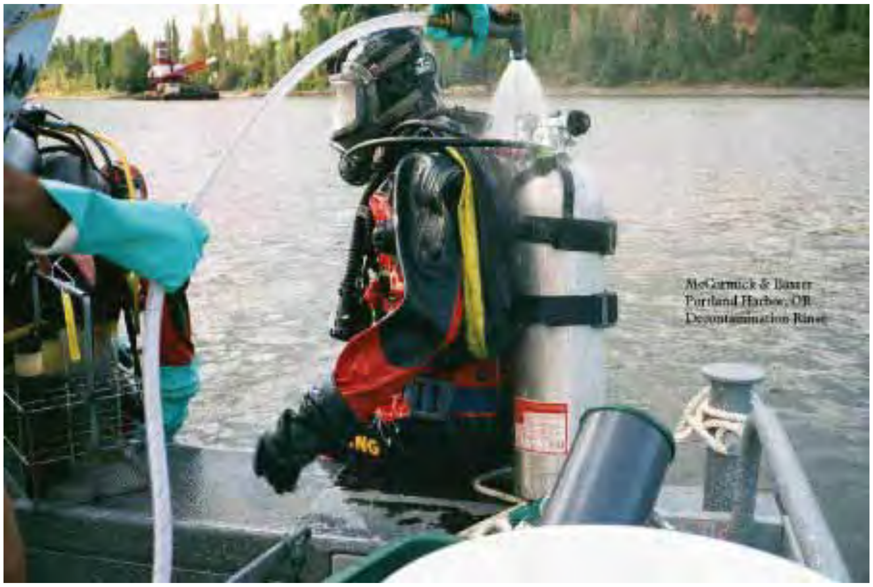

Figure 10. ERT diver undergoing potable water decontamination at the McCormick and Baxter portion of the Portland Harbor Superfund Site. The Region 10 and ERT divers were collecting contaminated bottom sediment cores for lab analysis. Photo by B. Duncan.

Site	**Date**	**Chemical and Physical Factors**	**Exposure Level**	**Level of PPE**	**Symptoms from Exposure**
1.Quendall Terminals	May 2009	PAHs	Minimal	Level B	None
2. Duwamish River	July 2009	PAHs, metals, PCBs, dioxins	Minimal	Level B	None
3.Sinclair Inlet scuttled vessel survey	February 2009	None known	None	Level B	None
4. Ocean dredge material disposal survey	September 2009	None known	None	Recreational SCUBA	None
5.Lake Pierre instrument recovery	October 2009	H_2S	Gas could be smelled through mask.	Level B	None

Figure 11. Excerpt from an exposure history form filled out for fiscal year 2009 for an EPA Region 10 diver.

Vaccinations for various disease vectors are also provided at EPA hazmat dive units, such as hepatitis A/B, diphtheria, and tetanus (USEPA, 2001). The Army also reports issuing vaccinations for polluted water dives as well in conjunction with, or in lieu of, PPE as described above. Symptoms of chemical or biological exposure are encouraged to be reported immediately so that the diver can obtain treatment. If such a case occurs, the use of PPE can be evaluated for future diving if one particular dive site can be localized as the causal factor.

Discussion

The USN has the most comprehensive contaminated water guidance, yet this manual lacks the pointed direction necessary to ensure divers at the dive site know when to implement it beyond broad generalities. Other federal agencies and military branches do not seem to acknowledge the pollution, or potential for pollution, at many of their dive sites. Knowledge of polluted water planning tools, or available sampling tools or techniques, were lacking amongst most groups interviewed. In general, comprehensive training is lacking among the agencies surveyed, focusing mainly on decontamination procedure where training is offered, and wholly lacking in ways to eliminate exposure. Many units have the capability to provide PPE and decontamination including EPA, the USN and the Army. However it is noted "*" that the Army has the capability to implement these SOPs but does not consistently, such as in the Haiti example in 2010.

Table 1. Comparison of Federal Diving Program Polluted Water Approaches.

Federal Dive Program	**Planning** includes a variety of tools to predict possible or actual contamination at the dive site and precautions as needed	*Some* **training** is provided on polluted water dive planning, PPE, decon, and medical monitoring	**PPE/Decon** suitable to the level of pollution at typical sites is available, but may or may not be used	**Medical Monitoring** is provided that looks for signs and symptoms of chemical exposure, either by SOP on a regular basis, or as a contingency
EPA	√	√	√	√
USCG			√	
NOAA		√		
Army	√		√*	√*
USN	√	√	√	

Elimination of exposures may involve boat-based or unmanned sampling techniques rather than diver-based techniques. For example, an ROV can be useful in surveying a site viewed as too polluted to dive and/or used to look for overt signs of contamination prior to diving, such as labels on leaking drums, so long as tenders are adequately protected.

Figure 14. EPA Region 10's ROV being used to survey a manufactured gas plant site. Photo by S. Sheldrake.

Heat stress was viewed as an overarching, if not overriding, concern with respect to polluted water by most. Specifically, personal protective equipment upgrades were largely viewed as overprotective given the inherent tradeoff in heat stress, despite available mitigation for such stress. Medical monitoring focuses generally only on obvious signs or symptoms, rather than making an attempt to run tests to detect more subtle signs of chemical exposure. The Army does not monitor for chemical exposures but does provide some immunizations. Specific recommendations for the federal diving community as a whole, based on the interviews and research conducted for this paper, include:

1. Development of an explicit set of dive planning tools to use as a checklist when planning a dive including items such as internet-based resources for real-time dive planning and personnel to contact with polluted water diving expertise for specific questions;
2. Provision of training to all divers in their respective diving programs in polluted water recognition, planning, PPE, decontamination and exposure monitoring regardless of mission. The importance of this is just as high for an entity that does not intend to do polluted water dives, such as NOAA, as it is for an agency who has a stated intention of conducting polluted water diving, like EPA;
3. Adaptation and broader adoption of USN/EPA polluted water PPE standards, or similar. For example, improvement of the level of protectiveness of Navy category 3 diver dress and broader application of category 3 by the federal diving community for unknown, but suspect, dive site conditions;
4. Updates to standard decontamination protocols by EPA and USN and development of decontamination protocols for other entities;
5. Development of an exposure monitoring program for those entities conducting mission oriented dives in polluted water (e.g., USN, USCG), and contingency protocols for those inadvertently conducting polluted water dives (e.g. NOAA); and,
6. Development of reporting processes to evaluate individual divemaster decision making with respect to the level of pollution at dive sites, such that feedback is given on the PPE used if under (or over) protective. A reporting procedure can also be useful in documenting when standard practices developed at the programmatic level are not applied in the field, such as in the Army Haiti example. NOAA has recently developed a reporting system for safety incident reporting (where no injury occurs) to prevent event reoccurrence on a systematic, programmatic basis, which could be an excellent vehicle for all federal entities to apply to polluted water diving lessons learned.

Though over three decades of detailed information on safe polluted water diving exists, much improvement is needed on the part of federal programs to consistently put this information into practice to the benefit of divers and tenders.

Disclaimer: This paper is an illustration of steps to be taken to minimize exposure to the diver in hazardous environments and does not necessarily represent the official view of the USEPA. Mention of any specific brand or model instrument or material does not constitute endorsement by the USEPA. The opinions expressed herein are solely those of the authors and do not represent official policy of the American Academy of Underwater Sciences.

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EXPLORING THE MESOPHOTIC ZONE: DIVING OPERATIONS AND SCIENTIFIC HIGHLIGHTS OF THREE RESEARCH CRUISES ACROSS PUERTO RICO AND U.S. VIRGIN ISLANDS

Clark Sherman[1*]
Richard Appeldoorn[1]
David Ballantine[1]
Ivonne Bejarano[1]
Milton Carlo[1]
Doug Kesling[2]
Michael Nemeth[1]
Francisco Pagan[1]
Hector Ruiz[1]
Nikolaos Schizas[1]
Ernesto Weil[1]
[1]Department of Marine Sciences
University of Puerto Rico at Mayagüez
PO Box 9000, Mayagüez 006681 PUERTO RICO
clark.sherman@upr.edu

[2]Aquatic Training Systems, LLC
5701 Wood Duck Circle
Wilmington, NORTH CAROLINA 28409 U.S.A.

Mesophotic coral ecosystems (MCEs) occur along insular shelf margins and isolated banks at depths of ~50 to 100 m. Given their depth and typical distance from shore-based facilities, specialized diving techniques and logistical arrangements are required to conduct in situ surveys and sampling of these unique settings. From 2010 to 2012, the University of Puerto Rico at Mayagüez Caribbean Coral Reef Institute and Department of Marine Sciences conducted three research cruises in order to characterize MCEs along an east-west trending transect extending from Isla Mona eastward along the southern insular margin of Puerto Rico into the Virgin Islands to Lang Bank off the eastern end of St. Croix. Ship-based operations allowed access to remote locations as well as put a multidisciplinary team of scientists on site to aid in final dive-site selection and conduct surveys, collections and preliminary processing and assessment of data. The application of technical closed-circuit rebreather (CCR) diving made extended, self-supported research cruises possible and practical. Over the course of three cruises, over twenty different sites were surveyed. CCR dives were conducted at depths of ~ 50 to 90 m with most work occurring at depths of ~50 to 70 m. Remotely-operated vehicle (ROV) surveys complemented diving operations by exploring sites prior to CCR dives, surveying beyond CCR working depths and examining sites not surveyed by divers. These cruises help to establish protocols for safely and efficiently conducting extended, self-supported, technical-diving cruises and demonstrate the scientific utility of such operations. Important scientific results from these cruises include the ecological characterization of MCEs in Puerto Rico and US Virgin Islands, documentation of systematic patterns in geomorphology of mesophotic habitats, identification of new species, extension of species *ranges, new information on genetic connectivity between mesophotic coral populations and between mesophotic and shallow coral populations, and documentation of well-established lionfish populations at mesophotic depths.*

Introduction

Compared to their shallower counterparts, reef systems deeper than ~50 m remain largely unexplored. Primarily, this has been because of the depth limits of traditional scuba techniques of ~40 m. Accordingly, much of our knowledge of reef environments, including the depth ranges of organisms and of reefs themselves, is limited to this relatively shallow range. Recently, advances in diving technology and techniques and their application to scientific research has opened a new realm for study, the mesophotic zone (water depths of ~50-100 m; Pyle, 1996; Sherman et al., 2009; Hinderstein et al., 2010). Mesophotic coral ecosystems (MCEs) are defined as light-dependent corals and associated benthic communities found at depths of ~50-100 m (Hinderstein et al., 2010). There is renewed scientific interest in these ecosystems (*ibid*.) as they often contain unique flora and fauna (Ballantine and Ruiz, 2010, 2011; Corgosinho and Schizas, 2013) and may have a potential areal extent rivaling that of shallow reefs (Locker et al., 2010). Being further removed from terrestrial and anthropogenic influences than shallower settings (e.g., Armstrong et al., 2006) MCEs may potentially serve as refugia for certain corals and other organisms during times of environmental stress and a possible source of larvae that could boost the resiliency of shallower reefs (Riegl and Piller, 2003; Lesser et al., 2009; Bongaerts et al., 2010). While the depth and typical distance from shore of MCEs may offer refugia for marine organisms, these same qualities present unique challenges to investigators; specialized diving techniques and logistical arrangements are required to conduct *in situ* surveys and sampling of these unique settings (e.g., Pyle, 1996; Sherman et al., 2009).

Technical diving has been crucial to the study of MCEs (e.g., Sherman et al., 2009; Lesser et al., 2010; Sherman et al., 2010; Smith et al., 2010). While submersibles, ROVs and AUVs have important applications (e.g., Armstrong et al., 2006; Bare et al., 2010; Rooney et al., 2010; Bridge et al., 2011; Bridge et al., 2012), they also have limitations. Submersibles are obviously costly and logistically complex, placing limits on study locations and the frequency that sites can be visited. ROVs and AUVs offer greatly reduced cost and increased flexibility versus submersibles but can be limited by on-site conditions such as sea state, currents and bottom topography (Singh et al., 2004). Additionally, in submersible-, ROV- and AUV-based research, the observer is removed from the surrounding environment making detailed observations and sampling difficult. Only diver-based research has the capability and flexibility to make detailed, high-resolution observations (including photography) and conduct carefully targeted collections. Mixed-gas, closed-circuit rebreathers (CCRs) provide scientific divers unparalleled access to mesophotic habitats. CCRs provide significant advantages over open-circuit diving with respect to gas use, flexibility and equipment needs (Sherman et al., 2009).

In the fall of 2007, scientists from the University of Puerto Rico at Mayagüez (UPRM) Caribbean Coral Reef Institute (CCRI) and Department of Marine Sciences (DMS) began an intensive study of the MCEs off of La Parguera, southwest Puerto Rico. Using a combination of technical rebreather diving (cf. Sherman et al., 2009) and ROV observations, this study was focused primarily on characterizing mesophotic communities, understanding their distribution with respect to depth and geomorphology, and determining the extent of connectivity with shallow reef systems. This work has resulted in a thorough characterization of MCEs off of La Parguera and the development of conceptual models of the physical and biological factors controlling their makeup and distribution. From 2010 to 2012, UPRM-CCRI-DMS conducted three research cruises to expand these studies over a broader geographic range within Puerto Rico and US Virgin Islands. The cruises took place from 9-23 January 2010, 15 April – 5 May 2011 and 24 April – 10 May 2012. General objectives of the three cruises were to 1) further characterize MCEs over a broader geographic range and in a variety of geomorphic and oceanographic settings; 2) collect data to test and refine conceptual models describing the character and distribution of MCEs; and, 3) determine the horizontal genetic connectivity between mesophotic coral populations and vertical connectivity between mesophotic and shallow coral populations. This paper seeks to: 1) provide a descriptive summary of cruise diving operations that can help to establish protocols for safely and efficiently conducting extended, self-supported, technical-diving cruises; 2) demonstrate the scientific utility of such operations; and, 3) provide a brief summary of important scientific results and accomplishments.

Study Area

The islands of Puerto Rico and the US Virgin Islands lie along the northeastern margin of the Caribbean between approximately 64° 33’ and 67° 57’ W longitude and 17° 40’ and 18 °31’ N latitude. It is a tectonically active region with generally narrow insular shelves and steep margins that plunge to oceanic depths (Hubbard et al., 2008). The main islands of Puerto Rico and the northern Virgin Islands are connected by the Puerto Rico-Virgin Islands (PR-VI) Platform, while St. Croix and Isla Mona are separated from the PR-VI Platform by deep basins. Dive sites extend along an east-west trending transect from Isla Mona eastward along the southern insular margin of Puerto Rico into the Virgin Islands to Lang Bank off the eastern end of St. Croix (Fig. 1).

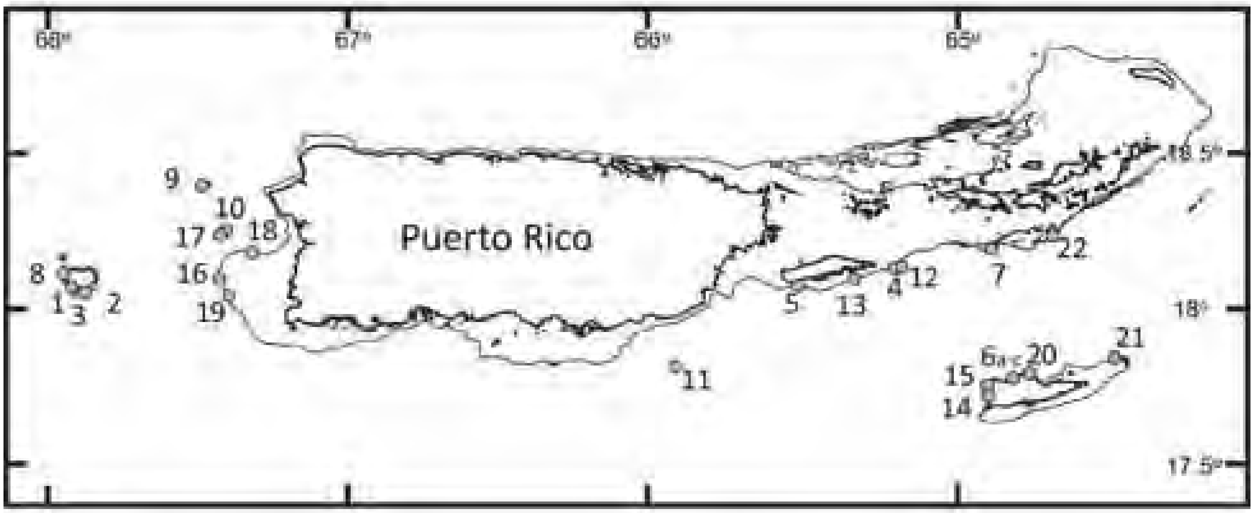

Figure 1. Map showing locations of dive sites for the 2010-2012 UPRM-DMS research cruises. Numbers correspond to site locations listed in Table 1.

Dive sites were chosen to cover a broad geographic range across Puerto Rico and the US Virgin Islands. They were also selected to cover a broad range of geomorphic and oceanographic settings with regard to factors such as slope gradient, bottom topography and degree of exposure to prevailing seas. Preference was given to locales where NOAA multibeam bathymetry was available as this aided in site selection and dive planning and allowed for a more quantitative and systematic assessment of the physical characteristics of each site.

Methods

Cruise platforms

Each cruise lasted from 2 to 3 weeks and consisted of 14 to 18 participants in the scientific party, including divers. Appropriate vessels had to have the capability of accommodating groups of this size, supporting technical diving operations and operating self-sufficiently for at least a week at a time. There were opportunities for resupply between successive (week-long) legs of each cruise. Private live-aboard dive charters were contracted for each cruise as they offered the best combination of cost and flexibility. For the first cruise in 2010 a 24 m by 12 m SWATH (Small Waterplane Area Twin Hull)/catamaran hybrid was chartered and made an exceptional cruise platform. The twin-hull design resulted in a smooth ride and copious space for the scientific party and equipment. A dive platform along the stern of the vessel that could be raised and lowered facilitated recovery of technical divers laden with gear. Large deck space, both covered and uncovered, provided an area for initial processing of samples. The stability of the vessel and large dining area also allowed for setup of a temporary "lab" with computers and a microscope for additional description, photography and cataloging of samples and data. Unfortunately, this same vessel was not available for the later cruises. For the 2011 and 2012 cruises, a 30-m aluminum crew boat refit for open-ocean diving was chartered and made another exceptional cruise platform. It had several large dive benches on the stern deck that could accommodate technical divers, along with boarding ladders and a large swim platform that facilitated recovery of the divers.

Safety considerations

The combination of technical diving and remote locations required that extra steps be taken to ensure the safety of the divers and all participants. This included having the appropriate equipment and personnel on hand to respond to emergency situations as outside assistance would not be readily available. To help achieve this, personnel from the University of North Carolina at Wilmington (UNCW), Advanced Diving Technology Program (ADTP) were brought in to oversee all scientific diving operations and provide first responder support in the event of a diving emergency at sea. Members of the UNCW-ADTP team were fully trained and certified in CCR operations and able to participate actively as members of the scientific dive team. UNCW-ADTP staff also served as the topside dive supervisors when not participating in the dive rotation. They were specifically trained in conducting extended shipboard operations and oversaw all the day-to-day scientific diving operations. Each assigned UNCW-ADTP staff member was also certified as a Diver Medical Technician (DMT-A) with the National Board of Diving and Hyperbaric Technology. They also held certifications as *Hyperlite*™-Hyperbaric Stretcher operators.

Because of the remote nature of these diving operations, an emergency assistance plan (EAP) was developed and posted that outlined the accident management plan including the location of the nearest hospital(s) and hyperbaric chamber(s) for each dive site. Additionally, because the first two cruises (2010 and 2011) were partially supported by UNCW-ADTP through NOAA's Cooperative Institute for Ocean Exploration, Research and Technology, an SOS *Hyperlite*™-Hyperbaric Stretcher for field use was allocated. The SOS *Hyperlite*™ is a portable pressure vessel (or hyperbaric chamber), that provides immediate treatment for different medical conditions by supplying 100% oxygen to the patient at above atmospheric pressures. This on-site chamber allowed for complete self-sufficiency of the dive team in the event that a diving emergency occurred while at sea. Emergency response would be immediate because of the proximity of this portable chamber system. The SOS *Hyperlite*™ and UNCW personnel were not available for the third cruise (2012) because of the unfortunate termination of the UNCW Advanced Diving Technology Program. For the 2012 cruise, appropriate personnel were privately contracted to serve as dive supervisors and first responders.

Diving operations

Diving operations were based on protocols established at our shore-based facility, the Magueyes Island Marine Laboratories in La Parguera, Puerto Rico (cf. Sherman et al., 2009). The technical CCR dive team consisted typically of 5 divers. Divers used Ambient Pressure Diving Ltd./Silent Diving Systems LLC *Inspiration* closed-circuit rebreathers with *Vision Electronics*. Most dives were made to depths of ~50 to 70 m, with a few dives to just over 90 m (Table 1).

Table 1. Generalized dive log of three UPRM-DMS mesophotic research cruises (2010-2012).

Site #	**Date**	**Site Name**	**Coordinates (Latitude°) (Longitude°)**	**Depth (m)**	**Accomplishments**
2010 UPRM-DMS Mesophotic cruise					
Leg 1 - Mona					
1	10 Jan 2010	SW Mona	18.05148 -67.90917	71	• Geomorphic characterization • Fish survey • Sample collection: o Corals, algae, lithic substrates
1	11 Jan 2010	SW Mona	18.05148 -67.90917	71	• Benthic photo transect • Fish survey • Sample collection: o Corals, algae, sponges, lionfish
2	12 Jan 2010	SE Mona	18.04553 -67.87821	62	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, lithic substrates
2	13 Jan 2010	SE Mona	18.04553 -67.87821	75	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, black coral, lithic substrates
3	14 Jan 2010	Carabinero, Mona	18.06250 -67.92223	68	• Geomorphic characterization • Fish survey • Sample collection: o Corals, algae, lithic substrates
3	15 Jan 2010	Carabinero, Mona	18.06250 -67.92223	53	• Benthic photo transect • Fish survey • Sample collection: o Corals
Leg 2 – Vieques and USVI					
4	17 Jan 2010	El Seco, Vieques (west)	18.12357 -65.20160	71	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Lithic substrates, algae
5	18 Jan 2010	SW Vieques	18.07065 -65.52224	71	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, algae, lithic substrates
6a	19 Jan 2010	Cane Bay, St. Croix	17.77327 -64.81383	70	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Algae, lithic substrates
6a	20 Jan 2010	Cane Bay, St. Croix	17.77327 -64.81383	55	• Benthic photo transect • Fish survey • Sample collection: o Corals, algae, sponges, lithic substrates
6a	20 Jan 2010	Cane Bay, St. Croix	17.77327 -64.81383	70	• Sample collection: o Sclerosponges
7	21 Jan 2010	Grammanik Bank	18.18200 -64.87860	70	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, algae, sponges, lithic substrates

2011 UPRM-DMS Mesophotic Cruise

Leg 1 – West Puerto Rico

8	16 Apr 2011	NW Mona	18.10627 -67.94888	70	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, sponges, lithic substrates
8	17 Apr 2011	NW Mona	18.10627 -67.94888	50	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, lithic substrates
9	18 Apr 2011	W Desecheo	18.38588 -67.49560	73	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, algae, sponges, lithic substrates
9	19 Apr 2011	W Desecheo	18.38588 -67.49560	57	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, algae, sponges, lithic substrates
10	20 Apr 2011	Bajo de Sico (east)	18.24491 -67.41272	70	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, algae, sponges, lithic substrates
10	21 Apr 2011	Bajo de Sico (east)	18.23105 -67.42367	51	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, algae, lithic substrates

Leg 2 – Grappler, Vieques and USVI

11	23 Apr 2011	Grappler Bank	17.81460 -65.92705	71	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, lithic substrates
11	24 Apr 2011	Grappler Bank	17.79458 -65.90825	65	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, lithic substrates
12	25 Apr 2011	El Seco, Vieques (east)	18.12723 -65.17777	70	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, algae, sponges, lithic substrates
13	26 Apr 2011	SE Vieques	18.09117 -65.33367	55	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, sponges, lithic substrates
14	27 Apr 2011	W St. Croix (Sub Mooring)	17.71743 -64.89382	81	• Sample collection: o Sclerosponges
14	27 Apr 2011	W St. Croix (Sub Mooring)	17.71743 -64.89382	57	• Fish survey • Coral colony characterization • Sample collection: o Corals
15	28 Apr 2011	W St. Croix (Armageddon)	17.75062 -64.8978	52	• Geomorphic characterization • Benthic photo transect • Fish survey

					• Sample collection: o Corals, sponges, lithic substrates
6a	30 Apr 2011	Cane Bay, St. Croix	17.77398 -64.81403	83	• Sample collection: o Sclerosponges
6a	30 Apr 2011	Cane Bay, St. Croix	17.77398 -64.81403	60	• Fish survey • Sample collection: o Corals, sponges, lithic substrates
6b	1 May 2011	North Star, St. Croix	17.76985 -64.82173	52	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, black corals, lithic substrates
6b	2 May 2011	North Star, St. Croix	17.76985 -64.82173	62	• Fish survey • Sample collection: o Corals, sclerosponges, lithic substrates
6a	3 May 2011	Cane Bay, St. Croix	17.77398 -64.81403	55	• Sample collection: o Corals

2012 UPRM-DMS Mesophotic Cruise

Leg 1 – West Puerto Rico

16	25 Apr 2012	Abrir la Sierra, PR	18.09083 -67.43467	52	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, lithic substrates
16	26 Apr 2012	Abrir la Sierra, PR	18.76197 -67.15696	70	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, lithic substrates
17	27 Apr 2012	Bajo de Sico (west)	18.23075 -67.43177	70	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, lithic substrates
17	28 Apr 2012	Bajo de Sico (west)	18.23075 -67.43177	52	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, lithic substrates
18	29 Apr 2012	Tourmaline, PR	18.17530 -67.32730	54	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Sponges, octocorals, lithic substrates
19	30 Apr 2012	N of Buoy 4, PR	18.03939 -67.40445	70	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, sponges, lithic substrates

Leg 2 – USVI

20	4 May 2012	Salt River Canyon, St. Croix	17.78689 -64.75856	70	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, sponges, lithic substrates
20	5 May 2012	Salt River Canyon, St. Croix	17.78689 -64.75856	54	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, black corals, lithic substrates
21	6 May 2012	Lang Bank, St. Croix	17.83421 -64.47584	55	• Geomorphic characterization

					• Benthic photo transect • Fish survey • Sample collection: o Corals, black corals, lithic substrates
6c	7 May 2012	Davis Bay, St. Croix	17.76600 -64.83100	92	• Geomorphic characterization • Fish survey • Sample collection: o Sclerosponges, black corals, octocorals, sponges, lithic substrates
22	8 May 2012	E St. John	18.22186 -64.67596	54	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, lithic substrates
22	9 May 2012	E St. John	18.22389 -64.66849	71	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, lithic substrates

Equipment setups were centered on two-person buddy teams, with each team carrying enough open-circuit (OC) bailout gas to safely bring one diver to the surface in the event of CCR failure. Given the high reliability of the equipment being used and the built-in redundancy within the CCR unit (e.g., dual oxygen controllers, dual displays, dual batteries, etc.), it was thought to be highly unlikely that both CCRs on a buddy team would completely fail on a given dive. Both members of a buddy team carried OC bottom bailout mix that matched the diluent being used in the rebreather. In addition to the bottom mix, one member of the team carried EAN 32% deco mix, while the other member of the team carried 100% O_2. In the event of a three-person buddy team an additional EAN 32% deco mix was carried by the third member of the team. Based on calculations, the EAN 32% deco mix was the gas needed in highest volume in case of a bailout. Two standard mixes for diluent/OC bailout were used depending on the planned dive. For dives from 49 m to 61 m (160 ft to 200 ft) an 18/30 trimix was used (i.e., 18% O_2/30% He/52% N_2). This mixture has a maximum operating depth (MOD) of 68 m (224 ft) at a PO_2 of 1.4 atmospheres absolute (ATA). At 61 m (200 ft) and a rebreather PO_2 setpoint of 1.3 ATA, the mixture provides an equivalent air depth (EAD) of 37 m (120 ft). For dives from 61 m to 91 m (200 ft to 300 ft), a 12/50 trimix was used, which has a MOD of 107 m (352 ft) at 1.4 ATA PO_2 and an EAD of 38 m (126 ft) at a rebreather setpoint of 1.3 ATA PO_2. Divers used two decompression computers, the integrated *Inspiration* computer with a peak PO_2 setpoint of 1.3 ATA and high and low gradient factors set at 85 and 15, respectively and a *VR3* set at a constant PO_2 of 1.25 ATA using the Bühlmann decompression algorithm and a safety factor of 10. At these settings the *VR3* was the more conservative of the two decompression computers and would always clear after the *Inspiration* computer. Divers always cleared both computers before exiting the water.

Both cruise platforms were well-equipped liveaboard dive vessels with their own compressors and were able to provide EAN 32% for the duration of the cruises. All other breathing gases, e.g., oxygen and trimix, needed to be brought onboard. For each approximately two-week cruise, the following complement of diving gases was carried onboard: three (3) Type-H 337 ft^3 cylinders of diving-grade oxygen, two (2) HC4500 434 ft^3 cylinders of premixed 18/30 trimix, and two (2) HC4500 434 ft^3 cylinders of premixed 12/50 trimix. This was more than enough gas to top off the CCR onboard cylinders after each day of diving for a CCR team of 5-6 divers. Premix diving gases were prepared at the technical dive facilities of the DMS Magueyes Island Marine Laboratories. During the cruises, CCR onboard cylinders were topped off from the storage cylinders with the aid of a portable pneumatic booster pump. For the most part, OC bailout gases were never used and only needed occasional topping off caused by minor incidental loss of gas during dive operations. The OC EAN 32% was regularly used to fill lift bags but could be easily topped off using the ship's onboard compressor.

All dives were conducted as live-boat drift dives. Most dives were made to depths of approximately 50 or 70 m with a planned bottom time of 20 minutes. Representative dive profiles are shown in Figure 2.

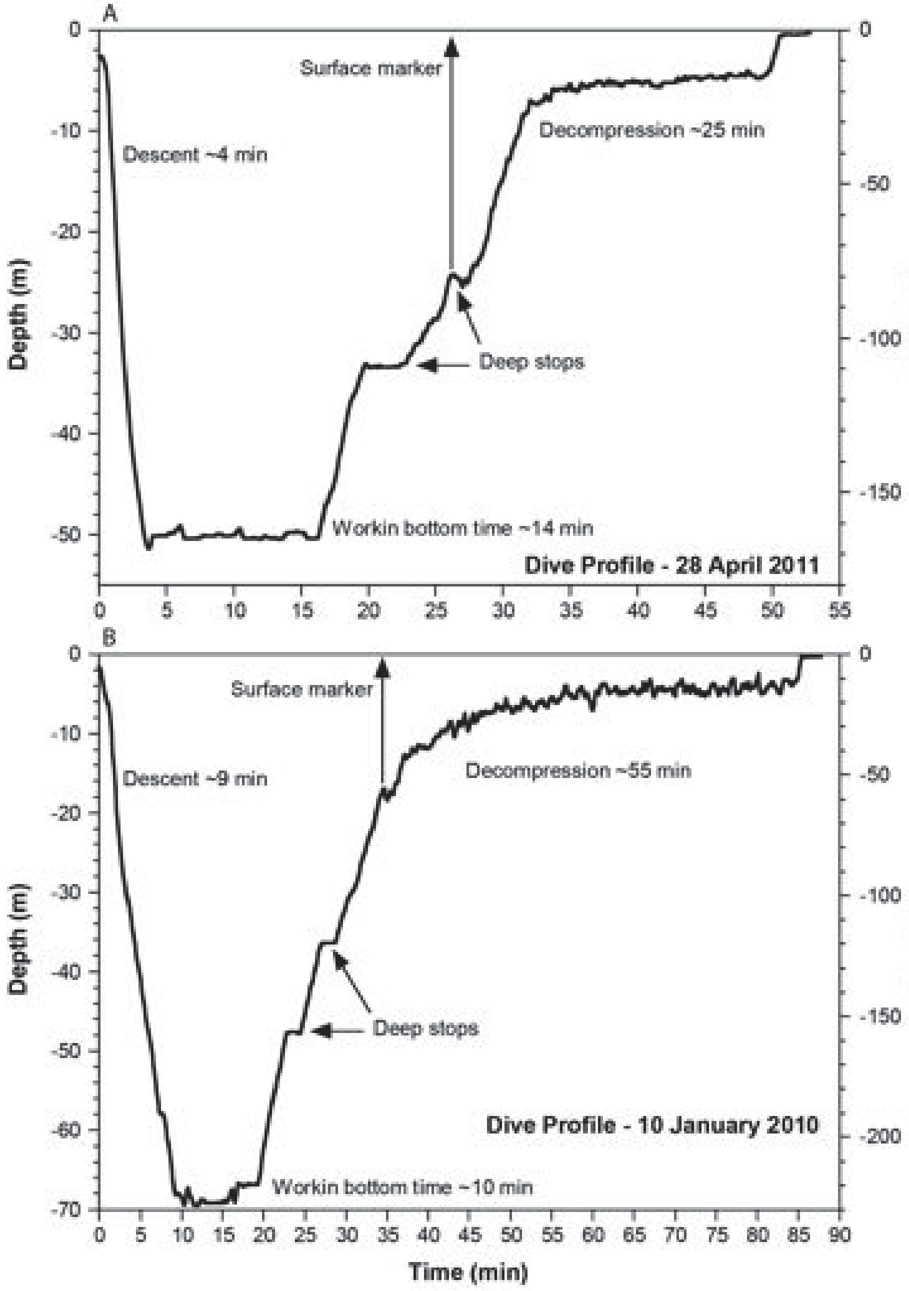

Figure 2. Typical CCR dive profiles for a dive to 52 m (A) and 71 m (B).

Divers were dropped at a suitable location above the target site to minimize descent times to the target depth. This typically entailed dropping in at shelf/slope breaks and descending down steep insular slopes to the target depth. Descents generally took from 5-10 minutes leaving approximately 10-15 minutes of working bottom time before beginning the ascent at a run time of ~20 minutes. During the ascent, two two-minute deep stops were conducted according to the *VR3* dive computer. At an appropriate time during the ascent, typically at a run time of ~25-30 minutes, a surface marker was deployed to alert topside support of the divers' location. Divers would then drift along with the surface marker and complete the remaining decompression. Upon surfacing, divers were recovered by the cruise vessel. Total run times were ~60 minutes for a dive to 50 m and 90 minutes for a dive to 70 m. One decompression dive was conducted each day for up to six consecutive days followed by one offgassing day. Following deep CCR dives, the remainder of each day was used for ROV operations and, if possible, shallow-water dives (<30 m water depth) by a second team of divers.

Sample and data collection

Each member of the CCR dive team had a specific task on each dive. These tasks included geomorphic characterization of the site, photo documentation (including general photography and benthic transect), fish surveys, sampling (live benthic cover and lithic substrates) and in-water divemaster. The geomorphic characterization took place typically during the descent and involved a general description of site topography and nature of the seafloor and benthic cover. Upon reaching the target depth, this same diver would assist the photographer in completing a continuous high-resolution benthic photo-transect ~10 m long by 40 cm wide (cf. Sherman et al., 2009; Sherman et al., 2010). Individual photographs from the transects cover an area of ~40 by 60 cm and were analyzed using CPCe point-counting software to determine the relative abundances of major groups of benthos and substrate types (Kohler and Gill, 2006). Fish surveys were patterned after the techniques of Brock (1954). Fish were identified and counted for ~15 minutes along 10 m long by 3 m wide (30 m^2) belt transects. In addition, in order to quantify target species observed within MCEs but outside of the transects, large predators and important (commercially or ecologically) fish species observed during each dive were recorded (cf. Bejarano, 2013). Divers also made careful and targeted collections of lithic substrates and benthic macrofauna, including corals, algae and sclerosponges, for more detailed studies (e.g., genetics and morphometrics) and positive species identifications in the laboratory. Once samples were collected, they were sent to the surface with a lift bag to be recovered by the cruise vessel. This allowed sample processing and cataloging by topside scientists to begin immediately while the divers completed decompression. The in-water divemaster observed the rest of the team and was able to act in the event of any difficulties and lend assistance with science objectives when needed.

ROV operations

Additional surveys were conducted using a Seabotix LBV200 remotely operated vehicle (ROV) rated to 200 m and equipped with color video camera (570 lines), LED lights, parallel lasers for size estimation, a sampling arm and tracking unit. The purpose of these surveys was to characterize mesophotic communities and geomorphology beyond typical depths reached by the CCR divers and over the full depth range of MCEs down to 100+ m, taking advantage of the greater bottom time available. ROV dives were scheduled to last at least 1 hr. All ROV dives were scheduled after deep CCR diving activities for the day were completed. Video documentation of key components of the ecosystem, especially corals, provided additional information on species composition, size and depth distribution. All video was recorded to digital tape for permanent archiving. During each dive a running log was also kept of key observations (e.g., species, depths, geomorphology), based on expert observers. Matched with the video recordings, these key observations could be quickly referenced and reviewed as necessary. While the sampling capability of the ROV was limited to only one specimen per dive, even this could be significant when the divers were time-limited and tasked with multiple objectives. Weather permitting, ROV dives were made at each location sampled by divers. In addition, five ROV dives were made at sites not visited by divers: (2010) Elbow – Isla Mona PR, Buck Island St. Croix, (2012) Tourmaline PR, eastern Lang Bank St. Croix, and western Grammanik Bank, St. Thomas.

Results

Diving statistics and accomplishments

Over the course of three cruises 24 different sites were surveyed along an east-west trending transect that stretches ~370 km from Isla Mona to Lang Bank, St. Croix (Fig. 1). A total of 42 decompression dives were made to depths ranging from 50 to 92 m, with most dives made to ~50 and 70 m (Tables 1, 2).

Table 2. Summary dive statistics for three UPRM-DMS mesophotic research cruises (2010-2012).

		Cruise I-2010	**Cruise II-2011**	**Cruise III-2012**	**Total**
Number of Sites		7	10	8	24*
Dive Days		11	16	12	39
No. of Team Dives#	Depth				
	50-59 m	2	8	6	16
	60-69 m	2	3		5
	70-79 m	8	5	5	18
	80-89 m		2		2
	90-99 m			1	1
Total Number of Team Dives#		12	18	12	42

* Does not include sites revisited on multiple cruises.
# Teams consisted of two to six divers.

Dive totals reflect the number of team dives (not individual dives), with teams consisting of two to six CCR divers. In most cases, these surveys represent the first *in situ* characterization of mesophotic habitats (>50 m water depth) at these sites. To our knowledge, the dives on Grappler Bank were the first documented dives on this isolated bank south of Puerto Rico. A total of 32 continuous, high-resolution, 10-m long, benthic photo-transects were completed, with most done at depths of ~50 and 70 m. An attempt was made to conduct transects at depths of 50 and 70 m at each site. However, topography and conditions at some sites did not allow for this. Transects were analyzed to identify species present and determine the relative abundances of major groups of benthos and substrate types. Numerous specimens of stony corals (primarily *Agaricia* sp. and *Montastrea cavernosa*), macroalgae, sponges and antipatharians were collected and are currently being described, cataloged and further analyzed for genetic and morphometric studies. Sclerosponges (*Ceratoporella nicholsoni*) were collected from depths of ~70-90 m. These will serve as important paleoceanographic archives of factors such as water temperature and water column structure over centennial time scales.

ROV accomplishments

The use of the ROV provided important information both from sites visited and not visited by the CCR divers. Examples of the former include establishing the depth limits of corals and other benthic macrofauna beyond the range of the divers and the observation of unique features, such as a zone of diverse coral development located at 80–90 m at eastern Grammanik Bank, St. Thomas. At slightly shallower depths (70 m) visited by divers live coral cover was sparse. A pre-CCR dive survey off Sub Mooring, western St. Croix discovered one of the largest coral colonies (*Agaricia undata*) observed at a depth of 57 m. This same colony was subsequently documented and sampled by divers to assess its genetic composition. Notable observations made at sites not visited by divers include (1) the broad plain off Tourmaline, western Puerto Rico, which contained adult queen conch down to at least 59 m and a small outcrop at 73 m that supported over 100 lionfish, (2) the use of ledges and ghost traps between 60 – 70 m as key nursery habitat for blackfin snappers on the otherwise featureless sandy slope off Buck Island, St. Croix, and (3) the spectacular extent off western Grammanik Bank characterized by 100% live cover of *A. undata*.

Scientific highlights

Important scientific results from these cruises include the ecological characterization of MCEs in Puerto Rico and US Virgin Islands over a broad geographic range and over a broad range of geomorphic and oceanographic settings. These surveys are allowing for further documentation of systematic patterns in the occurrence and distribution of MCEs related to geomorphology and oceanographic factors. Well-developed MCEs are shown to be distributed patchily and dominated by agariciid corals. Surveys have expanded the number of scleractinian species for Puerto Rico, with *Leptoseris cailleti* and *Agaricia undata* as new reports for the area. Collections are resulting in the identification of new species of algae and invertebrate meiofauna (Pesic et al., 2012; Petrescu et al., 2013) as well as new information on genetic connectivity between mesophotic coral populations and between mesophotic and shallow coral populations. Paleoceanographic sea-surface temperature records have been generated from collected sclerosponges that provide important information on factors controlling regional climate variation over the last 500 years (Estrella Martinez, 2013). Both diver and ROV surveys have documented well-established lionfish populations at mesophotic depths.

Discussion

Scientific utility of cruises to study MCEs

Extended, self-supported diving cruises provide numerous benefits for the study of mesophotic habitats. Extended cruises provide unparalleled access to remote and varied settings that would be difficult or impossible to reach via day-use shore-based facilities. The mobile cruise platform also facilitates visiting multiple sites over a broad area thereby simplifying logistics for regional studies relative to shore-based work. Cruises place a multidisciplinary team of scientists on-site that can help with final site selections, conduct surveys and sampling, and provide immediate insight on and assessment of data and sample collections. This allows for significant processing and interpretation of data to be completed over the course of the cruise. Field operations are necessarily compressed during a cruise resulting in an efficient use of time and resources. The visiting of multiple sites within a short time also facilitates site comparisons. Technical CCR diving requires considerable equipment, including both in-water dive gear and topside support equipment (e.g., custom-mixed breathing gases, CO_2 absorbent, replacement parts, etc.). A cruise vessel can easily transport equipment and personnel from site to site. It would not be practical to transport this equipment and personnel from one shore facility to another, especially in an island setting. The extent of diving and number of sites surveyed on the UPRM-DMS mesophotic cruises would not have been accomplished from shore-based facilities. Samples collected on the cruises to date have formed the basis of one PhD dissertation and two MS theses. The copious data and samples collected over the cruises will continue to generate numerous studies for some time to come.

Value of CCR diving

Previous work has demonstrated the value of technical diving in the study of MCEs (e.g., Sherman et al., 2009; Lesser, 2010; Sherman et al., 2010; Smith et al., 2010) as well as the advantages of CCR diving over open-circuit (OC) techniques (Sherman et al., 2009). The UPRM-DMS research cruises further demonstrate the value and advantages of CCR diving in the study of MCEs (Fig. 3).

The UPRM-DMS CCR divers were able to make detailed *in situ* observations of mesophotic habitats, collect high-resolution photographs and make precise and targeted collections of biological and geological samples. This level of precision and detail is not possible using remote techniques. The advantage of CCR versus OC techniques is especially apparent on extended cruises. On a given dive, a CCR diver uses about an order of magnitude less gas than an OC diver. After a day's diving during the cruises, the 3 L CCR diluent and oxygen cylinders could be easily topped off from the bank of storage cylinders brought along for the cruise (see above). OC bailout cylinders only needed occasional topping off caused by incidental loss of gas during diving operations. If these cruises were done using OC techniques, more than ten times the amount of breathing gas would have been required to support the diving. This would have greatly inflated the cost of the cruises and may have required larger cruise platforms to accommodate storage of the additional breathing gas. CCR also offers more flexibility than OC diving. During the cruises, the CCR divers switched between two standard breathing mixtures depending on the planned depth of a dive. This was accomplished by each diver having two sets of cylinders, one for each gas mixture. Each cylinder set consisted of a CCR diluent cylinder and corresponding OC bailout cylinder. The CCR diver simply used the appropriate cylinder set/breathing mixture for the planned dive. Making similar changes using OC setups is much more involved and costly and would require either having multiple double-tank setups for each diver, or having to empty and refill a set of double cylinders each time a different breathing mixture is used. Either option would result in increased equipment needs, increased costs and increased setup time. CCR equipment is less cumbersome making both entry into the water and, more importantly, reboarding the cruise vessel following a dive much easier. CCR techniques offer distinct scientific advantages as well. Because bubbles are not continuously released by the CCRs, marine life behavior (e.g., fish, marine turtles, etc.) in the presence of divers is more natural and surveys can provide more accurate information on the composition and behavior of the community.

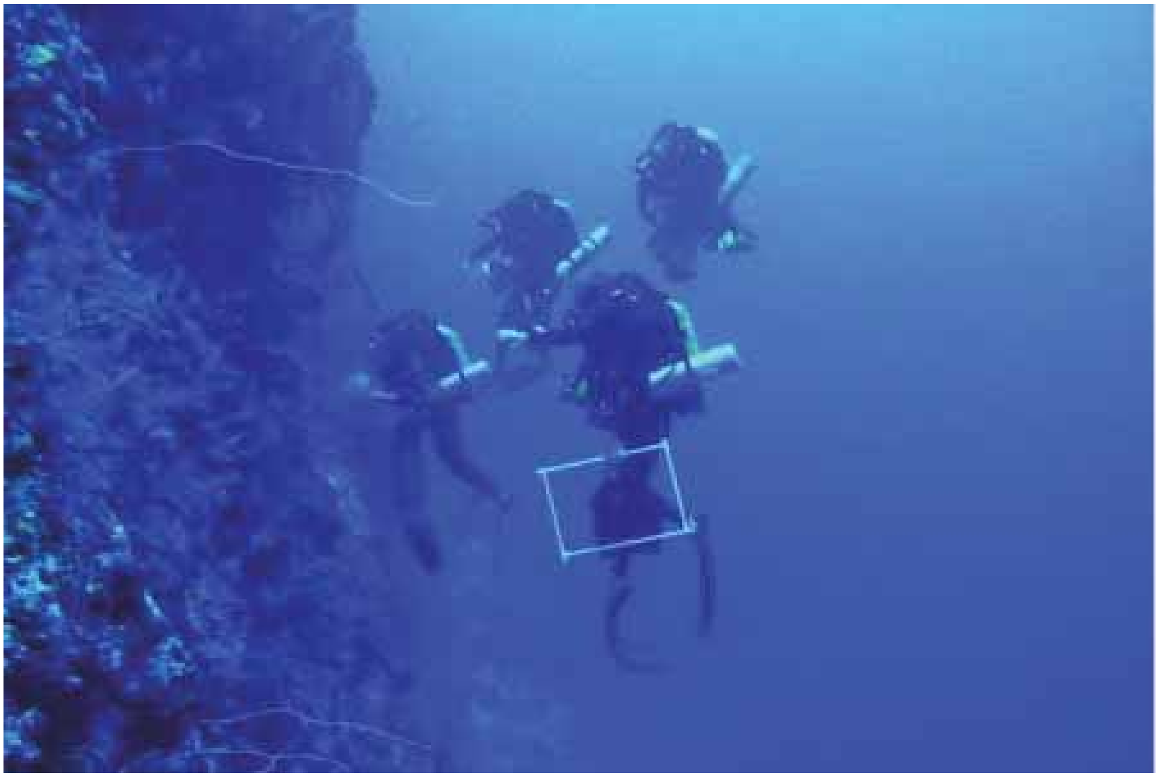

Figure 3. UPRM-DMS CCR divers explore a steep wall off Isla Mona, Puerto Rico.

Difficulties encountered

As with all marine field operations, weather and sea conditions represented the greatest obstacle to completing the planned operations and frequently dictated final site selection and scheduling. As a whole, the majority of sites are located along the more sheltered southern and western margin of the PR-VI Platform. The northern margin of the Platform remains to be explored. ROV operations were especially limited by weather and sea conditions. Strong winds prevented the ship from holding position over the ROV, while strong currents drove both the ship and the ROV too rapidly over the bottom to conduct operations. In moderate conditions, the ROV was deployed with the cable secured to a weighted line at depth to reduce the effects of cable drag. Use of the ROV in high seas was avoided to reduce the chance of impact on the hull of the ship during deployment and recovery. CCR divers were able to operate over a broader range of sea conditions than the ROV.

Mandatory off days were planned into the cruise schedule for divers to relax and reenergize between each subsequent at-sea research legs. Successive deep, decompression dives whether on open-circuit or closed-circuit apparatus is physically demanding and mentally taxing. The mandatory day off allowed divers to rest and be better prepared for the next series of research dives.

There are inherent risks in all types of diving. Deep, decompression diving compounds these risks. Even when diligently following conservative and well-established decompression dive profiles, accidents can occur. During the 2010 cruise, a UPRM CCR diver suffered a mild case of decompression illness (DCI) following a dive to a depth of 71 m with a bottom time of 20 minutes and a total run time of 99 minutes. The dive was uneventful, with a normal ascent. The diver reported severe right shoulder pain immediately upon surfacing and was placed on 100% oxygen for 30 minutes. Vital signs were stable and a complete neurological exam was conducted with negative findings. During the course of the 100% surface-oxygen breathing period, the patient reported his symptoms improving. The decision was then made by the Lead DMT to initiate treatment in the *Hyperlite,* Hyperbaric Stretcher for a USN Treatment Table 6 (USN TT6). The patient received 100 % oxygen therapy with intermittent air breaks throughout the treatment. When the chamber was returned to ambient atmospheric pressure and the patient removed, he was examined and the Lead DMT felt that the recompression treatment was successful and the diver continued to remain asymptomatic, post-treatment. No further treatment was warranted at that time. Working diagnosis was a case of undeserved - Decompression Sickness Type I, with full symptom resolution with recompression therapy. Diving activity was suspended for this individual for the remainder of the cruise. He was instructed to follow-up with a diving physician following the cruise for a thorough examination and determination of return to diving status. The cruise was able to continue on its original at-sea operational schedule.

This incident highlights several important points. Even with the utmost planning and precautions, accidents can occur. Thus, it is critical to have appropriate personnel and equipment on hand to deal with a variety of situations. If the DMTs and *Hyperlite* had not been onboard, this situation would have been very different. The diver would not have been able to receive immediate treatment, significantly increasing his discomfort level and jeopardizing full and timely recovery. He would have required immediate transport to the nearest hyperbaric facility (in this case San Juan, PR), which would have been logistically complex and would have jeopardized the continuation of the cruise. It is, therefore, crucial for those planning extended or remote diving operations and for the agencies supporting these operations to recognize the critical need of having appropriate equipment and personnel to deal with emergency situations.

Conclusions

1. Over the course of three UPRM research cruises from 2010-2012, 24 sites were surveyed along an east-west trending transect stretching ~370 km from Isla Mona, PR to Lang Bank, St. Croix.
2. A total of 42 decompression dives were made to depths ranging from 50 to 92 m, with most dives made to ~50 and 70 m.
3. In many cases, these represent the first *in situ* observations and samplings of mesophotic habitats (>50 m water depth) at these sites.
4. Scientific accomplishments include the ecological characterization of MCEs in Puerto Rico and US Virgin Islands across a broad geographic range, documentation of systematic patterns in geomorphology of mesophotic habitats, identification of new species, broadened geographical and depth range information for several species, new information on genetic connectivity between

mesophotic coral populations and between mesophotic and shallow coral populations and documentation of well-established lionfish populations at mesophotic depths.

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IT’S JUST A FLESH WOUND:
NON-LETHAL SAMPLING FOR CONSERVATION GENETICS STUDIES

***Derek J. Skillings*[1,2]**
***Robert J. Toonen*[1]**
[1]Hawai‘i Institute of Marine Biology
University of Hawai‘i at Mānoa,
Honolulu, HAWAII 96744 U.S.A.
derekjs@hawaii.edu

[2]Department of Zoology
University of Hawai‘i at Mānoa
Honolulu, HAWAII 96817 U.S.A.

The Papahānaumokuākea Marine National Monument (PMNM) is a chain of coral reef atolls and small basalt pinnacles, home to the most beautiful reefs in Hawai‘i. The size, remoteness and near “pristine” condition of PMNM make it a unique and ideal natural laboratory for conservation and scientific studies. Because PMNM enjoys the highest level of protection under U.S. law, every effort is made to minimize impacts when performing research and collecting samples within the borders of the Monument. An overview of underwater, non-lethal, tissue-sampling techniques is given for a wide range of invertebrates used for genetic population studies. Many invertebrates pose a special challenge for non-lethal sample collecting as defensive chemical and physical structures can make downstream genetic procedures difficult or impossible if they are not sampled correctly.

Introduction

The Hawaiian archipelago stretches more than 2,500 km, from the Big Island of Hawai‘i at its southeast point, to Kure Atoll in the northwest. The Archipelago is commonly divided into two regions: the main Hawaiian Islands (MHI) consisting of populated, high volcanic islands and the Northwestern Hawaiian Islands (NWHI), an uninhabited chain of basalt pinnacles, atolls, shoals and banks. Although the inhabited MHI are about on par with reefs in other inhabited areas, partly because of their isolation, the coral reefs of the NWHI are among the healthiest and most extensive remaining in the world (Pandolfi et al., 2003). The reefs of the NWHI represent a nearly undamaged coral reef ecosystem with virtually no human impacts by comparison with the heavily populated MHI (Selkoe et al., 2009), abundant and large apex predators, and an extremely high proportion of endemic species across many taxa (Friedlander and DeMartini, 2002; DeMartini and Friedlander, 2004). The coral reefs in the MHI, by comparison, are under considerable anthropogenic pressure; reefs near many of the urban areas and popular tourist destinations show significant negative impacts (Friedlander et al., 2004; Rodgers et al., 2009).

The Papahānaumokuākea Marine National Monument (PMNM) was created to protect the nearly 140,000 square miles of the NWHI; home to more than 7,000 species of fishes, invertebrates, marine mammals and birds. While the full extent of its biodiversity is still unknown, an average of about 27% of the known species are endemics found nowhere else on Earth (Eldredge and Evenhuis, 2003). The diverse habitats found in the PMNM present both opportunity and duty. Its size, remoteness and near pristine condition make it a unique and ideal natural laboratory for conservation and scientific studies. As one of the last bastions of minimally impacted coral reefs we have a duty to preserve the NWHI. In order to inform ecosystem-based management of the PMNM, understand the role of NWHI in the management of the MHI, and evaluate the potential for spillover of fisheries species from the protected area, our group has embarked on a genetic survey of reef fishes, corals and other invertebrates. This survey was designed to address the issue of population connectivity between the Northwestern and Main Hawaiian Islands. An extended overview of this effort and preliminary research results can be found in Toonen et al. (2011).

In keeping with the conservation ethic every effort was made to minimize impacts when performing field research and collecting samples for laboratory analysis. This paper reviews underwater, non-lethal, tissue-sampling techniques for a wide range of invertebrates used by our research group for population genetics studies. Many invertebrates pose a special challenge for non-lethal sample collecting; defensive chemicals and physical structures can make downstream procedures difficult or impossible if an appropriate sampling methodology is not followed. We present here common downstream problems and methods for navigating the line between safe, non-lethal sampling and the effective and efficient use of collecting time.

Methods

Sampling preparation for genetic population studies starts well before getting into the field. The end needs for the study in question must be kept in mind when deciding on the appropriate sampling methodology. This is especially important for our research group because of the isolation of the NWHI. Following a roughly 6-month permit application process, any field expedition is expensive and extremely time-constrained with typically only 1 day to perform collections, and at best 4 days possible at any given site. Thus, in addition to the ethical responsibility to avoid wasting anything collected, it is also important to know exactly what is needed for the study because there may not be a second chance for getting samples. Final sampling methodology is dictated by the following questions, each addressed in turn:

What laboratory procedures will be used?

Lab methodologies for population genetics studies utilize genetic marker variability, or infer genetic variability, through fragment length or electrophoretic differences in order to understand population structure. Genetic variability is determined generally using either direct sequencing of DNA, or size-based scoring of the allele frequencies of genes or proteins. Our research group uses both direct sequencing of mitochondrial (mtDNA) and nuclear genes (nDNA), and the scoring of length variability within nDNA microsatellite markers. The required sample size for using the different types of markers is dependent on the variability both between and within the chosen markers, and is beyond the scope of this paper (reviewed by Ruzzante, 1998; Kalinowski, 2005).

Sampling considerations come from three laboratory processing stages necessary for obtaining genetic information: 1) the preservation of the DNA molecules within the whole tissue; 2) the clean extraction in order to isolate molecules of interest; and 3) the amplification of target genetic material. Details for these procedures can be found in Iacchei and Toonen (2013). The quality of the final product depends largely on type of tissue that is used in the initial extraction. Most extraction procedures are optimized for vertebrate skin, muscle or blood. Invertebrate muscle or epithelial tissue is likewise usually suitable for DNA extraction, depending on the organism in question. Protein extraction may call for specific tissues that have a higher concentration of the protein of interest, and requires fresh tissue or specialized storage conditions (-80ºC).

How much tissue is needed?

Most DNA extraction protocols call for only a few milligrams of tissue per extraction. Generally, the smaller the amount of tissue that is biopsied the greater the chances are for a quick recovery for the organism, but this is also a trade-off in that very tiny biopsies do not leave a tissue backup for re-extraction if the first sample is lost or contaminated. Thus, it is almost always a good idea to take more tissue than is needed for a single laboratory procedure. A biopsy of 50-500 milligrams of tissue generally allows a reserve to be stored in the case of failed extractions, lost product, multiple analyses or for an independent extraction should the results need secondary confirmation.

Small tissue biopsies can also make it very difficult or impossible to correctly match a tissue sample to the species from which it was taken at any point downstream. This easily overlooked consequence of tissue biopsy sampling can completely derail a research project. Larger tissue samples, or samples with important identifying features, can help for later identification. Regardless, good field photos, notes and proper labeling of all tissue are essential and can mitigate this risk almost entirely.

A greater amount of tissue is needed for protein extraction than is needed for DNA extraction; the total amount of tissue needed will depend on the concentration of the protein in the tissue. Preliminary preservation and extraction tests should be run prior to field expeditions, especially for marine invertebrates that frequently show taxon-specific difficulties for preservation, extraction or DNA amplification (reviewed by Dawson et al., 1998). Other considerations include whether the tissue will be shared across multiple labs, if multiple types of analyses will done using the tissue and, if so, how much tissue will be needed for each analysis. Any of these factors will increase the amount of tissue that will be needed.

What type of tissue is needed?

The type of tissue needed will depend on both the desired end product and the organism in question. The tissue needed for protein extraction will depend on the type of protein that is targeted. If DNA is the target then cellular tissue is required generally in the form of epithelial, muscular, visceral or reproductive tissue. Structural, skeletal and connective tissues rarely contain enough DNA or protein to work with easily. Although it has been done successfully with special effort, coral skeletons, crustacean exoskeletons/molts, molluscan shells and echinoderm tests or spines are not ideal samples for subsequent genetic analyses.

Many invertebrates also have defensive chemicals that can interfere with the amplification or sequencing of DNA. These chemicals can be concentrated in tissues or diffused throughout the body. The tissue selected for biopsy will then depend on whether the tissue contains compounds that interfere with downstream procedures and whether the interference can be removed or mitigated.

Can the study organism be non-lethally sampled?

The amount and type of tissue needed for molecular procedures, taken together with the idiosyncracies of the target organism, will determine if non-lethal sampling is viable. Tiny organisms are more difficult to sample non-lethally as the amount of tissue that is needed might be immediately lethal or significantly decrease the survival rate post-biopsy. Molting, regenerating or colonial organisms are prime candidates for non-lethal sampling because they can usually recover quickly from biopsy injuries. Segmented organisms might also be good candidate if there are replicates of the body part chosen for sampling or the natural defense strategy of the organism includes autotomy (self amputation). Finally, the ability to successfully capture, biopsy and safely release the organism in a reasonable timeframe will also determine if non-lethal sampling is possible. For example, this last factor is one of the key reasons why small reef fishes are difficult to sample non-lethally.

How will the tissue be preserved?

After the size and location of tissue biopsy is decided, the method of tissue preservation must be determined. Two of the more common preservatives for genetic studies are dimethyl sulfoxide (DMSO) salt-saturated buffer (Seutin et al., 1991) and >70% ethanol (EtOH). Although specimen collection is time consuming and expensive, few laboratories test preservation methods for tissues before setting out on field expeditions. Particularly for marine invertebrates, there are substantial differences among taxa in the efficacy of tissue preservation among methods, and species-specific differences indicate that preservation comparisons should be undertaken for any long-term storage of samples destined for PCR study (Dawson et al., 1998). In general, DMSO is non-flammable and makes a good preservative for samples that require shipping or denser tissue; soft tissues can be broken down into a thick, mucous-like texture, making tissue handling difficult. Ethanol dehydrates tissue, often hardening it in the process, making it good for soft tissues that must be manipulated downstream. Ethanol is flammable, however, so samples preserved in EtOH require special storage, and shipping samples stored in ethanol can be difficult. Protein analyses usually require deep-frozen (-80ºC) tissue that has not been chemically preserved; this fact alone makes studies focusing on proteins impractical in many tropical locations, especially when sampling takes place in remote locations.

Discussion

Marine organisms comprise an extremely diverse category for study and each new group presents new challenges. Our research group has experience with about 1/3 of marine phyla but this represents only small fraction of the multitude of marine species. Despite this we have found some general rules-of-thumb that have been helpful when starting work on new organisms.

Sponges and Corals

Sponges and corals are colonial organisms that quickly regenerate damaged tissue and are straightforward to sample non-lethally. Sponges are totipotent and contain equivalent tissue throughout whereas corals are usually comprised of thin tissue covering a dense skeletal structure unsuitable for genetic study. Care must be taken so that the colony attachment point to the substrate is not damaged. Most of the downstream problems involving sponges come from skeletal elements and the myriad array of defensive chemicals that interfere with DNA extraction and amplification. Additionally, we have found that EtOH preservation of both corals and sponges yields poor quality DNA relative to DMSO (Gaither et al., in review). Troubleshooting generally involves extended cycles of cleaning the DNA or diluting the extraction solution to a point where inhibition drops off but there is still enough template DNA for amplification. Downstream problems for corals include extreme amounts of mucous production and similar interference from defensive chemicals or metabolites (Concepcion et al., 2006).

Crustaceans

All crustaceans are segmented with articulated joints, and most use autotomy as a defensive mechanism of self-amputation to escape predators. Likewise, most shed their exoskeletons multiple times throughout their lifetimes and have the ability to completely regenerate lost limbs. Many small crustaceans are difficult to identify to species in the field and are too small to effectively sample non-lethally. Larger benthic crustaceans such as lobsters, crabs and shrimp can often be hand-caught and many will drop a limb for distraction in the effort to escape capture. This makes sample-collecting easier because the whole organism need not be captured. Even for those who do not autotomize a limb, once caught, a leg joint can be clipped and will often regenerate within a single molt cycle. The sampling goal is to obtain muscular tissue and not the strong, chitinous exoskeleton. We often come across empty exoskeletons, or molts, that have been shed in the growing process. Though DNA can occasionally be salvaged from these molts it takes special effort and is not a preferred sampling strategy. Muscle tissue from a live specimen is the ideal tissue sample.

Echinoderms

Echinoderms are a very diverse group with both physical and chemical defenses to be overcome in the post-sampling analyses. Most echinoderms are capable of extensive regeneration. In the case of urchins and sea cucumbers, most regenerate quickly so long as the body cavity is not exposed and damaged, and in the case of brittle stars and sea stars, most regenerate quickly so long as the oral disk is left intact. Many sea cumbers also have defensive chemicals in their skin that interfere with DNA extraction and/or amplification so it is important to get muscle tissue from the body wall when biopsying. Care must be taken to not puncture the muscular body wall completely, which will expose the viscera and frequently result in death via infection or predation as a result. Whole arms or individual tube feet can be taken in the case of sea stars or brittle stars (Fig. 1). Urchins can be difficult to sample non-lethally, because most of the exposed portion of an urchin is skeletal material that does not contain much tissue. One strategy for urchins that can be picked up is to place them on a hard, clean surface until they attach themselves with their tube feet. They can then be quickly pulled away from the surface; this usually leaves behind a few tube feet that can be collected for subsequent DNA analyses. Alternatively, urchins with large spines will have muscle tissue and skin at the base attachment point that can be taken after pulling or twisting out spines. It is important to not puncture the test while removing spines or tube feet; this will usually kill the urchin because, in our experience, damaged urchins on a reef usually suffer predation before the diver reaches the surface.

Molluscs

By comparison to the groups above, most molluscs are fleshy and either slowly regenerating or non-regenerating. Biopsies can create permanent damage but if care is taken the damage can be repaired or remain very minor. For example, seemingly healthy individuals are seen in the field with old wounds from predators that are far more extreme than damage done by tissue biopsy. Thus small, non-lethal tissue sampling is possible and pieces of mantle, foot, or in the case of cephalopods, arm tips are preferred.

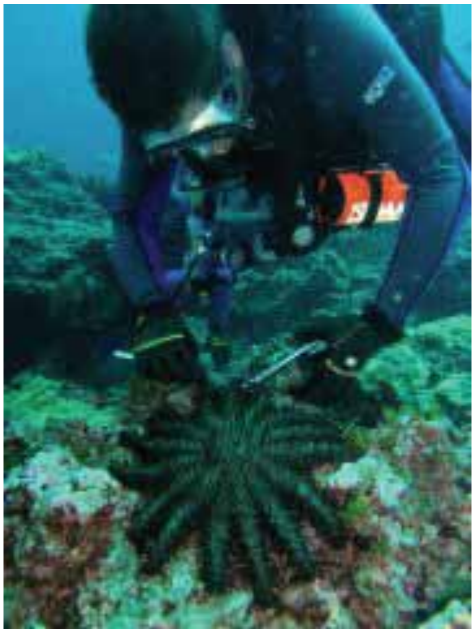

Figure 1. Non-lethal sampling of a Crown of Thorns sea star (*Acanthaster planci*) without removing the animal from the substrate. Roughly 1cm is cut off the tip of an arm *in situ* to minimize impact to the animal.

Conclusions

Studies of genetic population connectivity are important for the successful management and conservation of coral reefs (see Toonen et al. 2011). When performing a study aimed at conservation and management, we believe every effort should be made to minimize impacts on the natural resource we are studying. In the case of molecular genetic studies, the tiny tissue sample needed for research makes non-lethal sampling possible for many species. In order for any study to be successful, it is important to plan sampling methodology and preservation method ahead of time, especially when considering the expense associated with field research across a large and remote geographical area. In the case of remote locations such as the National Monuments in the Pacific, field expeditions are often one-time opportunities within the career of a graduate student. Thus, knowing the physical and chemical characteristics of target organisms is important to minimize the impact of collections and the chance of insurmountable problems with downstream laboratory procedures while simultaneously maximizing the scientific return on the time and financial investment in the research.

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LIFE SUPPORT INVESTIGATIONS

***Gregg Stanton*[1]**
***Joerg Hess*[2]**
***Jonathan L. Conard*[3]**
***Paul Haynes*[4]**

[1]Wakulla Diving Center
2784 Coastal Highway, Rt.98,
Crawfordville, FLORIDA 32327 U.S.A.
greggstanton@wakulladiving.com

[2]Rebreather Solutions, LLC
12 Cager Court
Crawfordville, FLORIDA 32327 U.S.A.

[3]Memorial Hospital
1400 E. Boulder Street
Colorado Springs, COLORADO 80909 U.S.A.

[4]Haynes Marine Ltd.
Polbare Close, Stonehaven
Kincardineshire, AB39 3LG UNITED KINGDOM

What began as a request to develop investigative protocols for Closed Circuit Rebreather fatalities by the ad-hoc committee attending the DAN Workshop on Technical Diving in 2008 soon evolved into a request to test rebreathers and provide this service. This paper outlines the model developed to investigate rebreather fatalities. The group follows a two-deep operational management policy with five compartments, which includes: Medical, Engineering, Behavioral, Forensic, and Legal. The group members analyze the data of the case in their respective fields of expertise. After consensus opinion was collected and used to form the protocol for rebreather investigation, the Divers Alert Network requested its removal from sponsorship, leaving its application in jeopardy. Repeated requests by USCG to test the protocol resulted in the formation of a pro-bono team of highly qualified rebreather specialists. Anonymity of team members was considered a part of the effectiveness of the investigation. To protect its goals and minimize personal and collective liability, the group incorporated as a not-for-profit company known as Life Support Investigations, Inc. Nearly all team members are rebreather divers who seek to return a necessary and unbiased service back to the community.

Introduction

What began as a protocol to secure a rebreather (RB) for later failure analysis became the basis for a team approach to diver fatality investigations. During the 2008 DAN Technical Diving Conference (Vann et al., 2008) a group that was asked to evaluate rebreather fatalities met to organize the project. Conflict soon emerged between manufacturers and organizers that compromised the project. G. Stanton proposed to look forward rather than only backward by designing a protocol to secure data from an incident that could later be carefully evaluated. R. Vann asked G. Stanton to form an ad-hoc group to write such a protocol.

Within a year, G. Stanton had collaborated with a group of practicing investigators, consolidating their recommendations into a single document. These findings were presented to R. Vann who supported the concept. However, because of shifting legal considerations DAN distanced itself from the project and their name was removed. We were subsequently asked to provide the protocol to investigative units in the USA and Europe. Bozanic and Carver (2011) incorporated these protocols in a paper presented at the DAN Recreational Diving Fatalities Workshop (Vann and Lang, 2011).

In 2008 we were asked by the Pensacola Medical Examiner (ME) to test the protocol on a cold case (over 2 years old). The transition from writing a protocol to testing it required the formation of a team of specialists. The requirements were simple: G. Stanton envisioned a departmental approach involving several disciplines: Recovery (first responders for whom the protocols were written), Engineering (technical evaluation of the RB once secured), Forensics (police reports, eye witness, etc.), Medical (interface with ME, review of dive profile), Behavior (training, human issues) and, at a later date, Legal. This paper is structured around those departments.

The team was selected from professionals who were specialists in their respective disciplines, willing to work pro bono and with no interests in litigation; these were necessary elements for both political and logistical reasons. All participants were active RB divers who, during their diving careers, had lost someone they knew to a RB incident with an unsatisfactory explanation or a pathway to preventing a reoccurrence. At a later date, the team expanded to incorporate a two-tiered management philosophy such that when one member was not available the back up stepped in. Philosophically, the team did not chase incidents but rather responded to law enforcement only when invited. We often received calls from the field during recovery by first responders asking for assistance. We provided the protocol but awaited invitation to engage in spite of urging from the responders.

We found that the ME or Coroner was more willing to discuss medical issues with a peer physician than with a forensic specialist or engineer. Likewise, law enforcement was more likely to work with peers in law enforcement than engineers or dive instructors. A RB diving attorney was brought onto the team for the same reasons. Law enforcement is seldom knowledgeable of the nature of diving fatalities, let alone RB incidents. Local Sheriff Departments often seek assistance from local dive shops that are usually ill equipped for the task. Body recovery, a component of the underwater crime scene investigation, was addressed by Stanton (2003) and Kelly et al. (2008).

Once the equipment has been secured, it often is stored in a back office (for days to years) until they can figure out what to with it. A policy to ship equipment to the U.S. Navy Experimental Diving Unit in Panama City, Florida, (NEDU) worked until the retired volunteer retired yet again. Because of military agenda priorities, civil investigation at the NEDU has a low priority. During this waiting period, RBs often remained on, draining batteries and with caustic cocktails digesting components. What remained was conjecture on the part of manufacturers, training agencies, law enforcement and the family of the deceased. Clearly a change was in order.

As the coordinator of the team, G. Stanton is usually the first person contacted. If contacted by a bystander, Stanton provides details of the equipment stabilization protocol and encourages contact with local law enforcement. If they wish to engage the team, he provides a phone number. If law enforcement is calling, Stanton is asked about availability for an investigation, rates and next steps. He notes critical incident information, asks regarding the storage and condition of the technology and contact points. Law enforcement usually must secure permission to engage our team. Stanton creates a description of the events as presented to him and copies it to everyone on the team. He usually also asks the Medical Department to contact the Coroner's Office to provide immediate assistance with Autopsy Protocols. Team availability to assist is then requested. When contacted for a second time to engage the case, Stanton seeks recovery of the volatile equipment as soon as possible. Shipping RBs is expensive and not easily done, considering they are usually full of water with caustic chemicals and flashing lights. Crating the rig is possible but airlines often require waterproof boxes and security then becomes a challenge. In every case we have engaged in, one of the team went to the location of the RB or met law enforcement half way and took possession. In one case, Stanton flew to a Caribbean Island to ensure that the battery was recharged in time to preserve vital data. He then conducted a preliminary inspection with members of the team over the Internet and the department lead investigator before sealing the rig up in a water proof box and returning it to the stateside lab and engineer for more detailed work. Usually we must drive west or east to the coastal city where law enforcement has the RB locked up.

The purpose of this paper is to document the formation of our team and it's code of conduct, it's history and reasoning, and to provide guidance to the formation of other teams, as requested by law enforcement and volunteer groups in other regions.

Forensics

Virtually all human deaths become an investigation and are handled accordingly until determined otherwise. From the time a body has been discovered until the ME has filed a determination, a close chain of custody protocol is followed. We developed a protocol for stabilizing a RB that is available for use by law enforcement and emergency responders that may better preserve forensic data later used by the ME to determine the cause of death. Each time the life support technology changes hands, the paperwork documents who is responsible for its security. Obviously people with vested interest in the case, such as manufacturers and relatives, must not be allowed access to the equipment.

When our team is engaged in a case everything is photo documented and serial numbers recorded with component descriptions to be included in the final report. As the RB is disassembled during the course of the investigation, each step is also photographed. One case was solved by returning to the photo journal to verify a missing component. One manufacturer-recommended procedure we now follow is to video tape the entire study of the technology from start to finish.

The final report is assembled by the forensics section of the team, often discussing details with other members to be sure we do not exceed our mission. It was not uncommon for another section to interpret what was found, if only for discussion purposes. Such comments were restricted to a discussion portion of the report, if at all. Both the procedures and the report must stand up to legal scrutiny should it ever be necessary, and the forensic member must be familiar with those requirements. The forensic member must have experience in crime scene investigations, ideally underwater crime scene investigations, and have an understanding of the technology and environment in which this activity is conducted.

Medical

Fatalities during diving have many variables to consider, but RB diving adds to those variables. As the engineering side evaluates the "diving machine", i.e., the RB, for faults, the medical side must evaluate the "human machine" for possible faults and to include the RB:human interface.

The leading cause of death in diving incidents is by drowning (Vann and Lang, 2011). Drowning is death secondary to hypoxemia caused by asphyxiation by immersion in a liquid. This does not usually help in the root cause analysis of the incident but only defines the final end point. Many other aspects need to be evaluated to understand what led to the drowning. This is why a full autopsy is done along with toxicology. The medical side will speak directly to the pathologist and/or request a copy of the final report. The medical side requests the results of the autopsy with any relevant past medical history and toxicology report. This information is then reviewed with the history of the incident to evaluate whether there were medical reasons contributing to the RB fatality.

Background information is important when evaluating the incident. Certification level and experience are important pieces of information. Was the diver certified and also experienced to do the type of diving involved? How current was the diver, had there been a recent lapse in consistent diving and/or conditioning for the particular diving environment? The history of the dive accident is important. How was the diver "feeling/acting" before and during the dive? Was this a solo diver, with a buddy or in a buddy team? What were the events leading up to the fatality? Was there separation from the dive buddy/team? How was the diver found? Were they breathing, convulsing, on the bottom, in between the bottom and surface, or on the surface? During which phase of the dive did problems start, i.e., descent, bottom, ascent, deco or surface? Were there any signs of incident triggers, i.e., entanglement or trauma during the event? Was any RB alarm state noticed by the buddy? Were there any rescue attempts tried at depth or on the surface and when did they occur? Who performed the resuscitation? Was there any response to the resuscitation?

Past medical history is an important part of the evaluation of the diving fatality. This would include history of: seizures, cardiovascular disease, diabetes, asthma, chronic obstructive pulmonary disease, etc. Medication history is important as it is useful to know what was or was not taken prior to the dive. Did the diver start any new medications recently and did they have any significant side effects or drug reaction with the current medications?

The autopsy findings are evaluated; the external examination is important to evaluate for any signs of external trauma and subcutaneous emphysema. Other signs of trauma are important including, for example, signs of entanglement or marine animal interaction. The internal examination is important to look for any signs of intravascular gas. It is important to document if pneumothoraxes were evident signifying an overpressurization injury to the pulmonary system. Further, the lungs need to be examined for abnormalities, i.e., bullae, blebs and hemorrhage. The cardiovascular system should be examined for signs of cardiovascular disease that could lead to functional compromise. Also, atrial and ventricular defects as well as other cardiac anatomic abnormalities need to be evaluated. Cranial evaluation is important to evaluate for cerebral hemorrhage and other catastrophic findings and also for significant deep intravascular bubble formation. The intraoral examination is important to evaluate for any signs of mucosal burns from "caustic cocktails". A toxicology evaluation of the blood and urine is important to check for any contributing drugs leading to the fatality.

After all of the information is compiled and reviewed, a root cause analysis is done with a medical emphasis. The medical side tries to determine if there was any medical reason for the fatality to occur. This is also evaluated in terms of what was found on the engineering side to see if there was a combination effect. This information is added to the completed report.

Engineering

The engineering aspect of the investigation is most often misunderstood as a technician's role, but goes far beyond it. Engineering will encompass system analysis and troubleshooting to at least the level of the original manufacturer.

Skill requirements

The investigator should have a background in mechanical engineering and fluid dynamics. On the electrical/electronic portion of the investigation, they must be knowledgeable in embedded systems both hardware and firmware. Additionally, a solid understanding on the concept of oxygen sensors, as well as experience in the oxygen sensing failure modes, is required. Experience and training in regulator repair, as well as experience and training in scuba inspection (e.g., Professional Scuba Inspectors) and gas safety, is essential (Fig. 1).

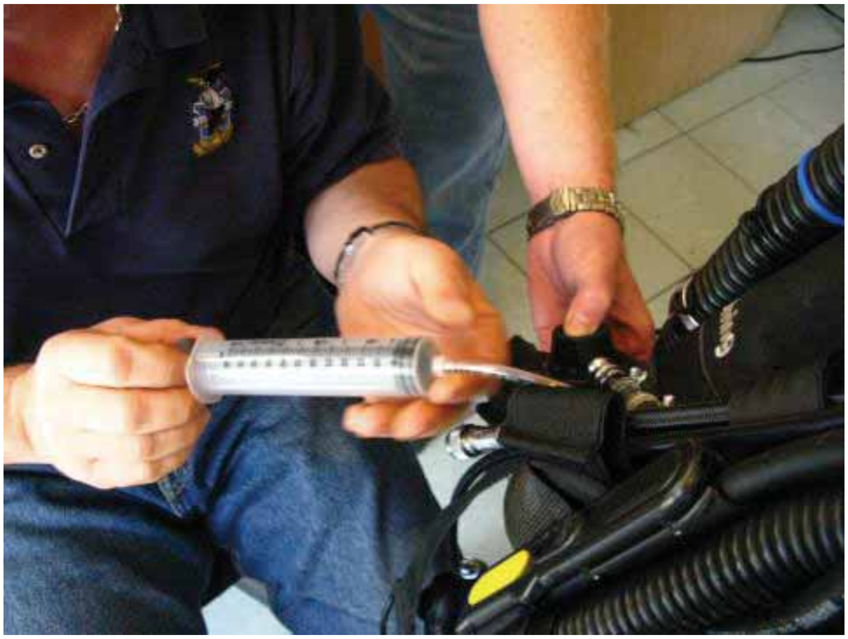

Figure 1. Gas samples pulled from a counter lung for content analysis.

A good feel for numbers and the ability to interpret their meaning is mandatory. Experience has shown that downloading data from a RB (if applicable) will require IT (information technology) knowledge at the level of system administrator when interfacing with a PC (Fig. 2).

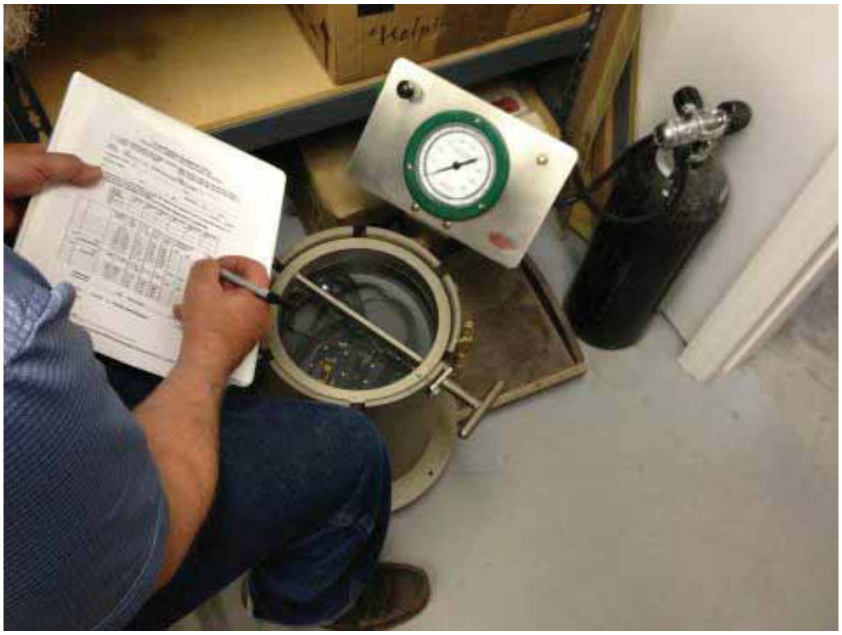

Figure 2. CCR head in pressure pot for test run on sensor and computer performance

The range of commercially available RBs is considerable and continues to grow. As a consequence, the engineering and human interface nuances between designs are also considerable. The investigative team should, therefore, include individuals experienced in the broad range of RB technologies currently available, including the plethora of after-sales accessories. The investigator must be capable of interfacing and sustaining a working relationship with manufacturers. This can be challenging as the situation has the potential to be perceived by the manufacturer as an adversarial engagement. Diplomacy is, therefore, required to help convince the manufacturer that the motivation of the group is not litigation-orientated but instead is focused upon helping improve safety for the wider RB community.

This spectrum of skills may be overwhelming for a single investigator, especially in the case of a newly-formed team. The experience described in this paper is based on the input from several individuals with their main expertise in other areas. It is the overall expertise of the whole team that satisfies the requirements mentioned here.

Analysis environment, tools required

Of particular benefit to an investigation are RBs with data acquisition capability (‘black box recording’). The sophistication of data acquisition and the man-machine data upload interface varies considerably between RBs that incorporate this capability. Typically, as a minimum, depth, dive time, oxygen sensor output and alarm status are recorded. In addition, where incorporated into the RB design, digital output pressure transducers provide oxygen and diluent pressures from which gas consumption and/or loss can be deduced. However, caution is required when interpreting uploaded information that by definition is simply data that may not necessarily accurately reflect or record what actually occurred. Not all RBs incorporate a data acquisition facility and so access to such data cannot be exclusively relied upon but is considered an additional device that forms part of a broader investigative toolbox. It is imperative that the RB’s user manual is available during the investigation as well as documentation for any accessory. Recalls or user and/or technician advisory notices should also be taken into consideration. Ideally, a properly maintained unit should be available for side-by-side comparison (Fig. 3).

Figure 3. Review of the data during joint discussion of the case.

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A secure space for storage and investigation is required including:

- Temperature/climate controlled laboratory
- Availability of multiple PCs, running Linux and Windows, virtual machines are recommended each with a “clean” system.
- Safe network, possibly untethered PC to maintain security and chain-of-custody
- RS232 (serial port) connection capability
- Bluetooth connection capability
- USB connection capability
- Data package sniffer, data package analyser
- Variable stabile power supplies
- Oscilloscope: multi-channel, 100Mhz
- Multimeter (multiple)
- Solder station: variable temperature, grounded, ESD safe
- Sensor emulator: 3 channels, with adapters for various connector types
- Pressure pot, 80 msw minimum
- Batteries: various types and sizes
- Flow-resistance metering system
- Digital recording: still and video
- Optical magnifiers: digital preferred
- Gas sampling capabilities
- Gas-contents analysis system (at a minimum for oxygen and helium but CO_2 and CO are desirable)
- Gas chromatography if possible for *in-situ* measurement
- 3rd party laboratory collaboration
- Calibrated pressure gauges

Evaluation process

Stanton and Hess (2011) outlined points to be considered in the evaluation process. The sequence of investigation can follow this outline but in all cases it must be documented thoroughly. A running camera augmented with notes and detailed still pictures as required serves that purpose well. Very often it will be tempting to jump the sequence since there may be obvious clues. However, each step taken must be considered for its potential destructiveness to the evidence. Jumping the sequence may lead to irreproducible results. Additionally, each step must be considered for its necessity at that point in time. A specific situation may require postponement of a step until further clarification is acquired. This method may sound contradictory but, as an example, consider the initial approach to the high-pressure gas supply for a RB, be it oxygen or diluent. Ideally, the RB should arrive with the handles turned off, but with a note showing if the handles had been turned during the phase of securing the equipment. Most often there is no annotation, the pressure gauge might show pressure, but most often it will not. A faulty pressure gauge may show zero pressure despite high pressure present. Purging the system would alter the state of any regulator or inflator used to do the purging, making it impossible to determine whether a potential fault was present prior to purging. Disconnecting an inflation hose from the manual override buttons (if present) may alter their state and either hide or cause leaks that were not present previously. Purging into the loop will alter the existing loop’s contents. Eventually, the regulator’s first stage will need to be disconnected from the tank in order to evaluate all the take-offs independently. This step will have to be taken, and it could potentially alter the condition without documentation. Thus, the sequence needs to be considered carefully based on the individual circumstances. Any such consideration must be justifiable, must co-align with the overall purpose and goal of the investigation, and must be agreed upon by the whole investigative team. The point illustrates that no ready-to-go recipe can do justice to all possible cases. Further information from the other disciplines contributing to the investigation can aid in making the decisions for an adequate sequence of steps. It also illustrates that the decisions must not be taken by any one single member of the investigative team alone.

Behavior/Training

When a diving fatality is investigated not only are the medical and technical contributions to that event carefully evaluated but so too is the victim's training and skill level. Labels on databases such as Expert or Novice are as misleading as branding the fatality by the manufacturer's name. When was the last time an auto accident was labelled as a Ford or Honda event driven by an expert?

Fatalities on underwater life support equipment are often proven to result from human error, which can be the result of environmental or technical conditions not anticipated by the victim. Failure to monitor these conditions is not the fault of the environment or the technology. Such failures are the result of human limitations found in distractions, complacency, attitude and inexperience. There is no exposure limit adequate to describe who may be exempted or exceptionally risk prone.

To help interpret eyewitness accounts and/or assess the possible actions taken prior to the demise of the victim, a high level of RB instructional experience is considered necessary to complement the technical, legal and medical aspects of the investigation team. Deviation from established RB training protocols and safe practices can then potentially be determined based upon current training agency standards and good practices.

In an effort to optimise a diver's response to a range of routine and emergency scenarios, an appropriate level of model-specific RB training is required to educate the user in safe practice and to establish conscious and sub-conscious action (muscle memory). Product manuals that contain recommended operating procedures are usually (but not always) provided by the RB manufacturer. Such documentation serves as a standard by which the designer visualizes the life support system to function and be applied. When available, product-related documentation and software helps the investigation team to determine whether there has been any deviation from manufacturer recommended user procedures and/or whether an aftermarket or 'home build' modification to the original build state has been adopted; this may have had a bearing on the outcome.

In order that safe RB diving procedures are imparted to the trainee, the instructor must break down and structure life support system routine and emergency operating procedures into a series of skills that, through rational progression and repetition, develop effective 'muscle memory'. RB management proficiency is then measured by the trainee's ability to competently, confidently and consistently conduct each skill under a range of scenarios that will likely be encountered within the diving environment. Through a gradual cognitive adaptation process the trainee will move from conscious incompetence to unconscious competence. This enables greater concentration to be focused upon critical life support failure mode analysis and response. Ideally, training should take the diver to the point where repetition of the emergency response to a potentially life threatening failure mode (human or machine) has become habitual. However, it must be borne in mind that despite the training level of the diver diving skills, particularly emergency response skills, quickly degrade unless routinely exercised. This again is where the diver's currency with the specific RB unit may have a bearing on events.

Our investigation includes researching the victim's environmental conditions leading up to the event, their experience and level of formal training, and linking specific failures that together contribute to the death to safe practice models. Take a recent fatality where a diver carried a cylinder into a cave labelled oxygen to 30 msw, telling buddies it was nitrox, and breathed on it until they convulsed. The cylinder was correctly labelled as pure oxygen so the technology was not at fault; the victim was mistaken. They also assumed a higher risk by needlessly taking pure oxygen deep into the cave. This case represents a human tragedy, not a technical one.

Legal

While exposure to litigation is always a concern for investigative teams, adherence to established protocols, restrictive policy on divulgence of case data outside of law enforcement, and professional integrity speak loudly to the credibility of the mission. This team has steadfastly maintained anonymity, staying away from social media, and remaining dedicated to understanding the nature of RB diving fatalities in particular. The team's attorney advises the team in legal matters and fully participates in the investigation.

To that end the team has formed a not-for-profit corporation registered in Atlanta and assigned the task of Treasurer to the diving attorney of the team. The team charges a flat fee for open-circuit investigations of $1000 and for closed-circuit investigations of $2000 per case to cover expenses. Unusual expenses, such as travel, are separately covered. These funds go to pay for gas analysis, investigative tools, limited travel for team members and documentation supplies.

Vested interest in the investigation of their product on the part of the manufacturer is part and parcel to their required due diligence. Our mission is to uncover contributory facts leading up to a diving incident that should be forwarded to the manufacturer for future use in better product development. We always request that the confidential report should be released to the manufacturer as soon as possible. We also ask that sanitized information be released to the Divers Alert Network for their analysis on diving accidents.

Earlier diving fatality investigative teams rejected manufacturer participation for a variety of reasons including detrimentally influencing the outcome, fear of interference and legal threats. We began following that model but have since worked with a National Transportation Safety Board (NTSB) concept of limited cooperation, which has proven very beneficial to our mission. It is understood that in involving the manufacturer will not always be an easy task, and is subject to potential conflict of interest. While we do not suggest or assume unethical behavior on part of the manufacturer, they must never be allowed to attain a position where such a conflict is allowed to dictate the investigation or its outcome.

Root Cause Analysis

Root cause analysis is a well-known method in engineering, medicine and other industries. Wikipedia provides the following definition: "Root cause analysis (RCA) is a method of problem solving that tries to identify the root causes of faults or problems that cause operating events. RCA practice tries to solve problems by attempting to identify and correct the root causes of events, as opposed to simply addressing their symptoms. By focusing correction on root causes, problem recurrence can be prevented. RCFA (Root Cause Failure Analysis) recognizes that complete prevention of recurrence by one corrective action is not always possible."

In order to assist law enforcement in performing a RCA, three perspectives are discussed based on the findings of all disciplines as described above. All investigators contribute to this analysis of the case:

1. Document what was found
2. Document what was not found but should have been there
3. Document what was found but should not have been there

Since the contents of these findings are specific to each individual case, the format of documentation will vary. The team only assists law enforcement in their understanding of possible scenarios. The team, however, does not form an opinion of its own.

Discussion

The team successfully analyzed a number of cases and was praised by the respective law enforcement agencies for the assistance rendered. Each case improves the capability of our team and we are very happy to expand the model, and share experiences and lessons learned with future teams.

There remain a number of unresolved issues. We are working on a protocol of engagement for law enforcement. This will have to include a contractual obligation of law enforcement for the disclosure of information, if present, beyond the equipment itself. This protocol also includes the terms of non-disclosure of the team's findings to third parties. It remains our intent to encourage law enforcement to ultimately make the sanitized findings available to manufacturers, training agencies and the RB diving public. A trust relationship with the manufacturers has not been established. The team will continue to approach manufacturers in order to establish "rules of engagement" that satisfy all sides involved. We still struggle with an urgency of engagement, i.e., establishing the awareness in law enforcement, that the timely response of the investigation is critical to the outcome. Data recorders, batteries and dissolved components degrade quickly in this equipment, mandating early documentation.

Summary

Six years in development, the Life Support Investigation model continues to evolve as we seek cost effective and efficient investigative mechanisms. Our efforts have been most effective in the Southeast USA but we encourage and will collaborate with other teams to organize in other regions of the country and beyond.

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MAPPING OF ARTIFICIAL REEF HABITATS BY SIDE SCAN SONAR AND ROV IN THE NORTHWESTERN MID-ATLANTIC COASTAL OCEAN

Peter F. Straub
Steven Evert
Tara L. Harmer Luke
Mark C. Sullivan

Richard Stockton College of NJ
School of Natural Sciences and Mathematics
Galloway, NEW JERSEY 08205 U.S.A.
pete.straub@stockton.edu

Coastal artificial reef sites near Little Egg Inlet, New Jersey (39.472°N/074.198°W) were surveyed with side scan sonar and remotely operated vehicle (ROV). Digital side scan sonar L-3/Klein 3900 dual frequency (455/900 kHz) was used to collect geo-referenced sea floor swath data. Sonar swaths were processed and mosaic maps produced with Chesapeake Sonar Wiz 5 software. A Seabotix LBV-s-6 ROV equipped with a BlueView P-900, 130° field imaging sonar and video was used to investigate sonar mapped structures associated with habitat. Depth of mapped sites ranged from 15-25 m on the New Jersey Department of Environmental Protection's Little Egg reef site or adjacent shipwrecks. Reefed ships, barges, armored vehicles, reef balls and concrete castings were observed. Mapping allowed these sites to be followed for repeated observation. Characterization of the communities on these habitat structures has included habitat preference and productivity of black sea bass and tautog, which are of particular interest to the recreational fishery. Seasonal and year-to-year variation was seen in percent coverage and number of sessile attached invertebrates. Video capture and laser scaling allowed mobile species identification and size discrimination. Tautog appears to prefer larger structures while black sea bass generally were less discriminate. Tautog was observed throughout the end of the year (Dec.) while black sea bass exhibited a seasonal move off the reefs in late fall to deeper water.

Introduction

One of the main rationales for artificial reef development in New Jersey and throughout the country is to enhance sport fisheries. The goal of this project was to survey and map artificial reef habitats for the purpose of evaluation of reef functions. Surveys and mapping were undertaken using digital side scan sonar and a remotely operated vehicle (ROV) with sonar and video capability. Small, lower cost, observation class ROVs are revolutionizing access to important fishery habitats located between shallow near-shore zones easily accessible by scuba and deep water sites more suitable for working class ROVs (Pacunski *et al*., 2008). Many New Jersey artificial reefs (small tanks and workboats, concrete castings, reef balls) occupy these shallow-to-intermediate depths that limit scuba bottom time and are frequently of a scale not amenable to traditional line transects (i.e., discrete structures with organisms aggregating at different depth intervals). Accurate mapping of the distribution of organisms and habitat utilization will facilitate the assessment of artificial reefs of varying structure and the idea that they enhance local productivity.

Methods

Study Site

The site selected for this study is the Little Egg (LE) reef, a part of the New Jersey Department of Environmental Protection (NJDEP) Artificial Reef Program. This 8.4 km^2 reef is convenient to access from Stockton's Marine Science and Environmental field station (~7 km offshore), is relatively shallow (~15-20 m depth), and is well established with most structures added from 1996-2005. Structures on the reef include reef ball fields, concrete castings, armored vehicles, deck barges and the *Jessie C*, a 20-m crew boat.

Instrumentation

Stockton's 9-m vessel the R/V *Gannet* was used as a work platform to deploy Stockton's ROV (the ROV *Shearwater*) and other instruments including an L^3/Klein (Salem, NH) 3900 high resolution digital side scan sonar with 455 kHz and 900 kHz frequencies. The *Shearwater* is a Seabotix (San Diego, CA) LBVs^6, six thruster, tethered vehicle rated for 300 m (Fig. 1).

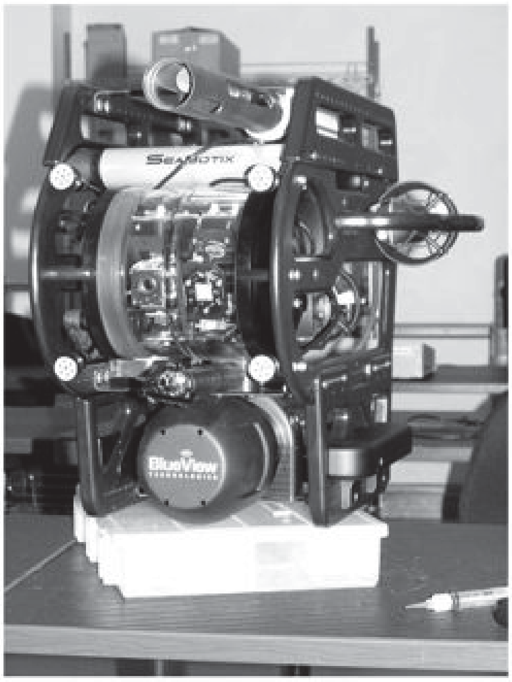

Figure 1. The ROV Shearwater, a Seabotix (San Diego) LBVs^6 remotely operated vehicle fitted with a Blueview (Seattle, WA) P900-130 2-D sonar on the lower tool skid and a Hach Hydromet (Loveland, CO) MS5 water quality sonde on the top tool skid.

The vehicle is equipped with two internal video cameras, color and low level black and white, paired red light scaling lasers, external lighting, a Blueview (Seattle, WA) P900-130 900 kHz 2-D imaging sonar, a Tritech (Aberdeenshire, UK) echosounder, a Tritech ultra-short baseline (USBL) tracking navigation system, a fixed position grabber claw and an integrated Hach (Loveland, CO) MS5 water quality sonde for dissolved oxygen, pH, temperature, depth, salinity and chlorophyll determination in flight. The ROV was operated using a Seabotix (San Diego, CA) integrated navigation console (INC) with navigation data, GPS positioning, echosounder and video captured and logged and saved to the computer hard drive with Tritech (Aberdeenshire, UK) Seanet Pro 2.1 software and ROV sonar viewed and recorded with Blueview (Seattle, WA) Proviewer 3.

Surveys

A side scan survey was initiated to characterize a section of the Little Egg reef of approximately 0.25 km^2. Side scan sonar data was collected with L^3/Klein (Salem, NH) SonarPro 12.0 and post-processed using Chesapeake Technologies (Mountain View, CA) Sonar Whiz 5.0. Seven 0.5 km x 80 m overlapping bottom swaths were collected at 455 kHz low frequency and mosaiced to produce a geo-referenced map (geo-Tiff) which was converted for use (KMZ format) in Google Earth (Mountain View, CA). Additional high frequency sonar swaths were collected (900 kHz) for better target visualization. ROV survey missions visited structures on the mapped sites over a two-year period to collect video transect data, seasonal data and size distributions of attached and mobile fauna. Where transects were not appropriate, point count methods (modified Bohnsack and Bannerot diver method; Bohnsack and Bannerot, 1986) were used to efficiently survey reef fishes and associated invertebrates outside, above and inside a given structure within a predetermined volume of water column (Patterson *et al*., 2009). This method generally involves constructing a virtual cylinder around a given habitat and pivoting the ROV at fixed locations to conduct fish counts. The actual diameter and shape of the cylinder may vary somewhat depending on the type of structure being sampled (i.e., tank versus reef ball versus casting) – regardless, this methodology allows one to quantitatively calculate fish abundance (which can then be compared with other sampling dates, sites, structures, etc.) A "tiered" approach with respect to the height of the ROV reduces the likelihood of "double counts" (as opposed to simply circling the structure with the unit or running multiple, line transects at a fixed depth). A laser scaling system was incorporated to collect fish length data or size information of the structure itself. Video analysis for counts and sizing used Media Cybernetics' Image Pro Plus v 6.3 (Rockville, MD) for frame capture and calibration of images to scaling points.

Results

Side scan swath data was used to create a geo-referenced bottom mosaic of the LE reef study site to accurately map the position of bottom structures of interest to the survey (Fig. 2). Geo-referenced video was collected during a number of missions to the reef site structures. In general, the low light level black and white video camera was utilized as particles in the water and water color made the color video camera useful only at extreme close-up range and with external lighting. A "proof of concept" inventory using a modified version of the Patterson *et al.* (2009) technique (two tiers only) was conducted on an M578 armored recovery vehicle on the LE reef (Fig. 3). In this instance, the ROV Shearwater was initially flown ~1 m above the seafloor and pivoted ~180 degrees to survey fishes in and around the tank's running gear. A second segment was recorded ~1 m above the turret to capture individuals aggregating further away from the structure in the water column. This survey captured high quality video that was later quantified frame-by-frame in the laboratory (Fig. 3). A more traditional ROV transect survey was conducted at Southwick's Barge on the LE reef because of the flat, elongated nature of the structure (Fig. 4). In this case, average frequency of occurrence was calculated over three discrete digital video intervals. Both techniques proved to be minimally invasive and allowed for multiple, rapid, cost-effective surveys throughout the sampling season, in some cases capturing multiple life stages of a given species, i.e., juvenile, male and female adults (Fig. 5). With respect to the armored recovery vehicle, tautog (above turret) and cunner (alongside running gear) showed clear preferences for different regions of the overall structure (Fig. 3). The armored recovery vehicle generally harbored more attached organisms than Southwick's Barge and displayed a higher concentration of fish as well. Point counts were also made at reef ball sites and other structures including a large crew boat, the *Jessie C*, within the LE reef system. Reef balls, in general, were highly variable in terms of attached epifauna and fish diversity (tautog, black sea bass, cunner, etc.) and site, seasonal and annual variation in standing biomass of epifauna, particularly blue mussels, was noted at several sites (Fig. 6). Laser scaling from video frame capture allowed size and year class determination of mobile fauna (Fig. 7).

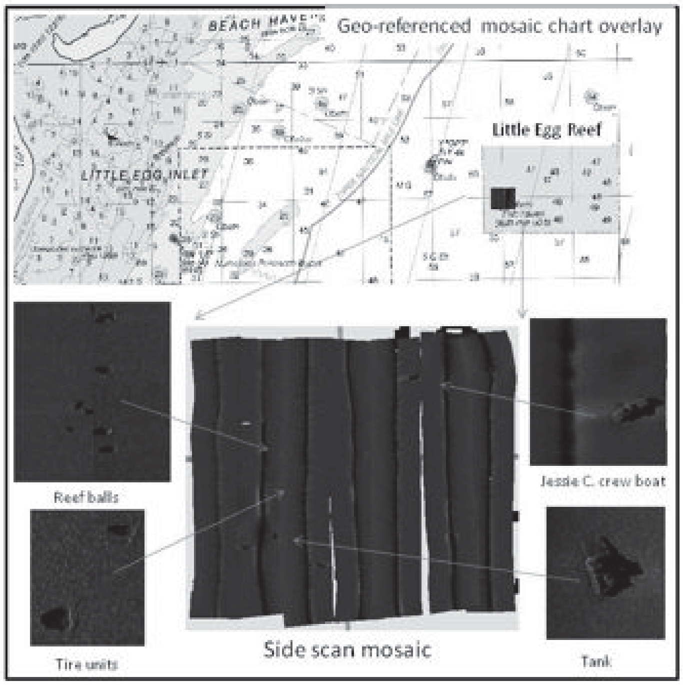
BEACH HAVEN
Geo-referenced mosaic chart overlay
Little Egg Reef
LITTLE EGG INLET
Reef balls
Jessie C. crew boat
Tire units
Side scan mosaic
Tank

Figure 2. Geo-referenced map and chart overlay (NOAA Chart 12323) of the Little Egg (LE) reef study site off of southern New Jersey, USA, produced from a mosaic of 455 kHz side scan survey bottom swaths. Side photos show higher detail of reef structures.

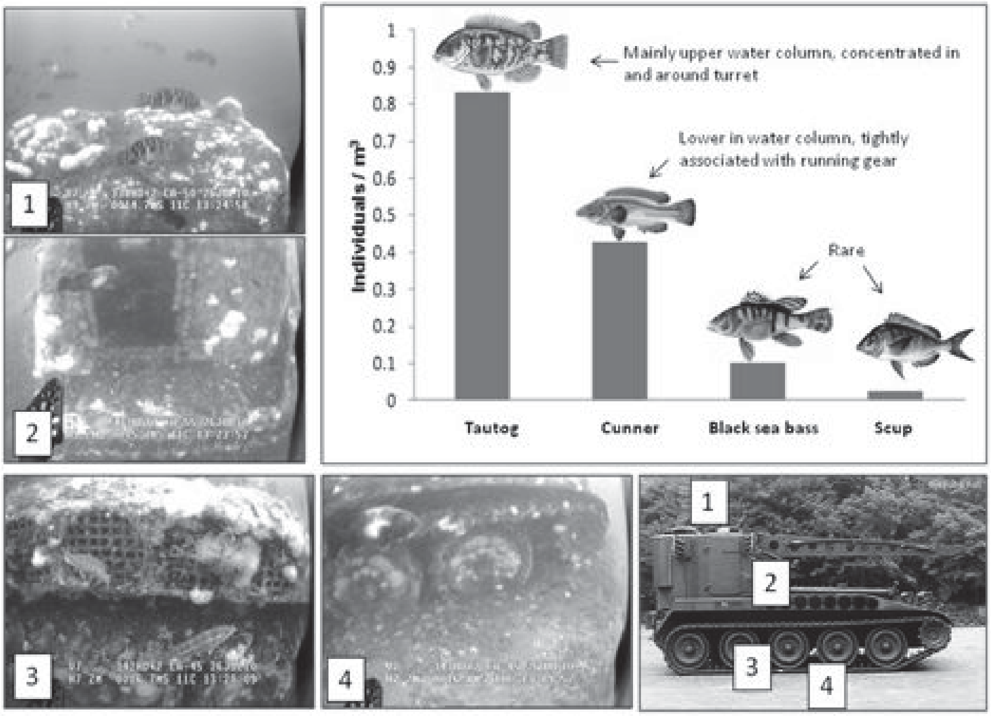

Figure 3. Results of M578 armored recovery vehicle survey with ROV Shearwater (Little Egg Artificial Reef) for four common reef species using a tiered approach and counts made in a modified “cylinder” transect. Numbered “habitat” boxes refer to details on pre-submerged armored vehicle image at lower right.

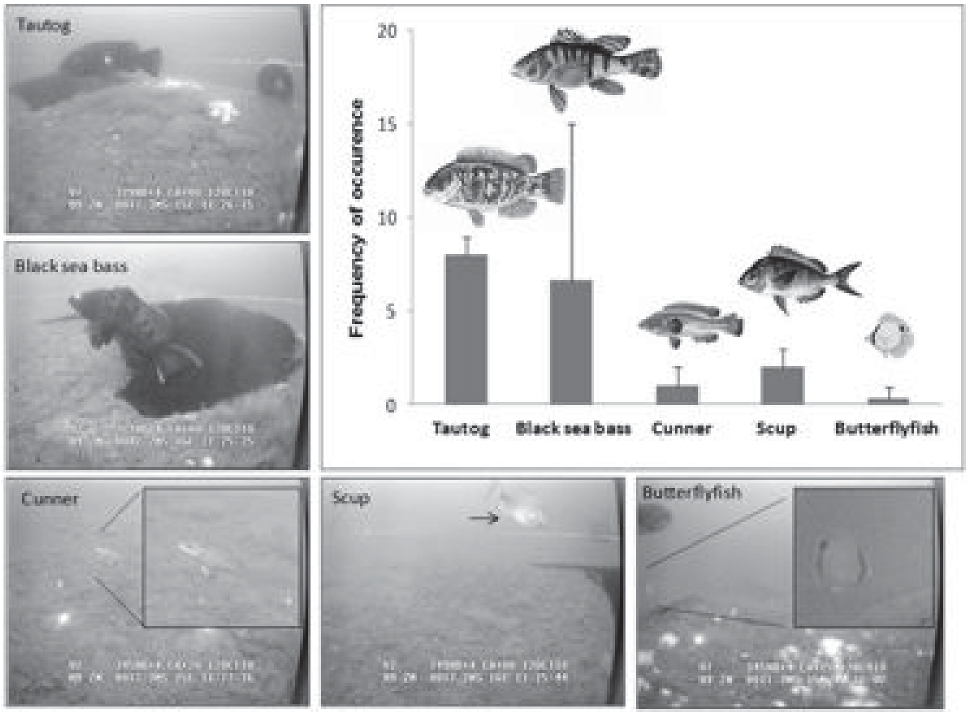

Figure 4. Results of Southwick's Barge traditional transect survey with the ROV Shearwater (Little Egg Artificial Reef). Digital video stills of each species are shown for reference purposes.

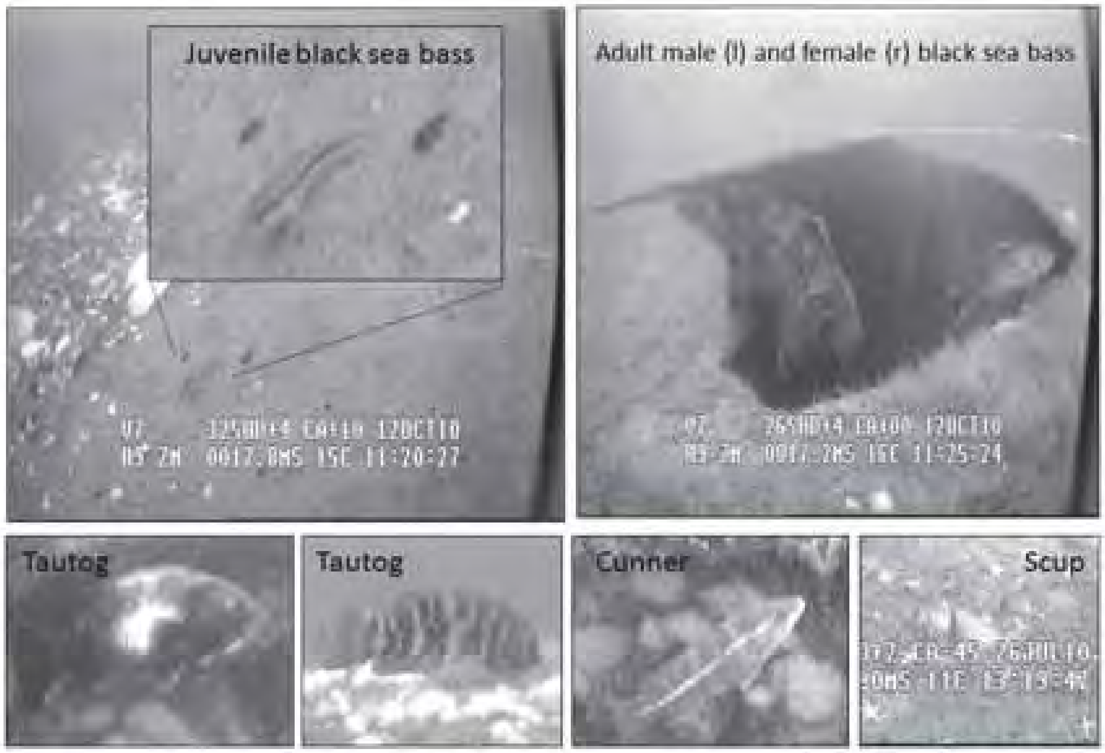
Juvenile black sea bass
Adult male (l) and female (r) black sea bass
Tautog
Tautog
Cunner
Scup

Figure 5. Top (left to right): Multiple life stages of black sea bass (*Centropristis striata*), imaged by the ROV Shearwater during Southwick's Barge and M578 armored recovery vehicle surveys, Little Egg Artificial Reef.

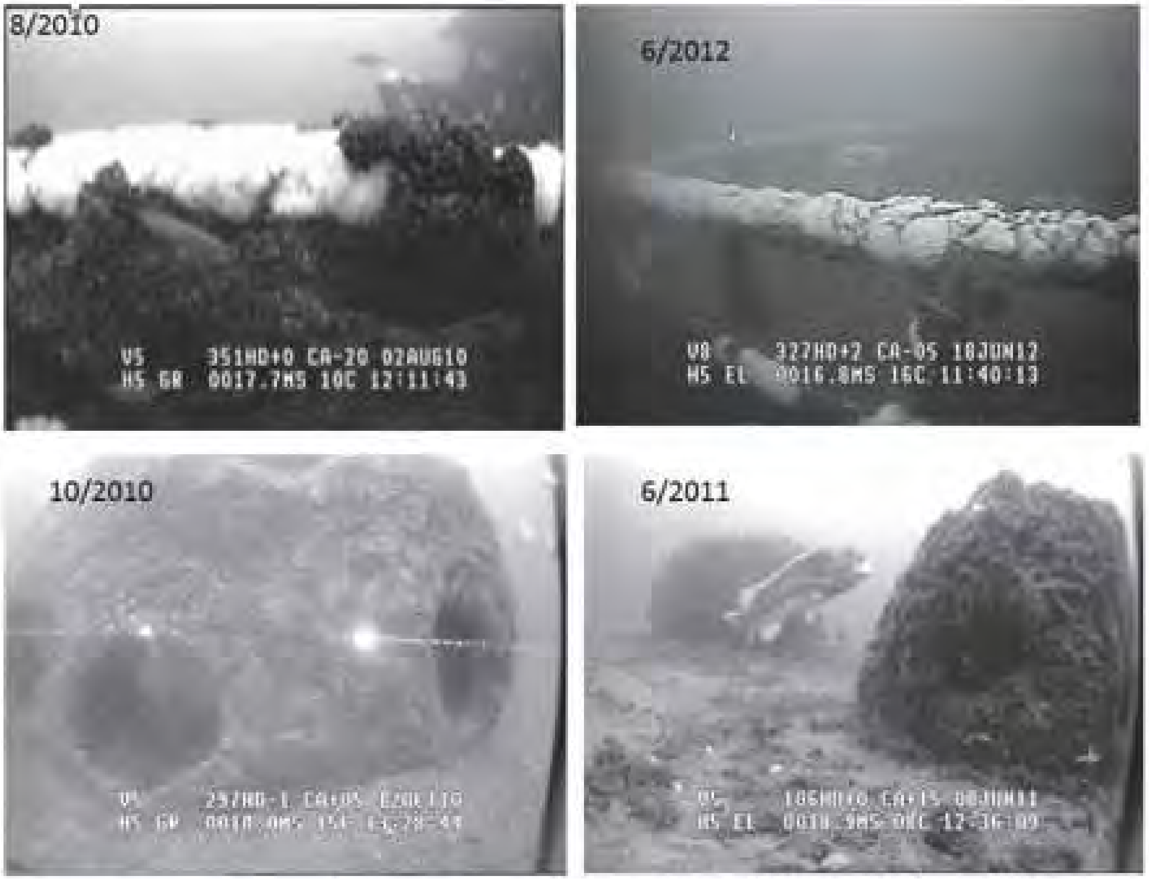
8/2010
V5 351HD+0 CA-20 02AUG10
H5 GR 0017.7MS 10C 12:11:43
6/2012
V8 327HD+2 CA-05 18JUN12
H5 EL 0016.8MS 16C 11:40:13
10/2010
6/2011

Figure 6. Top: video capture of the bow of the *Jessie C*, a 20 m reefed crew boat. Note the annual variation in attached organism cover. Bottom: video capture of reef balls from different sites and years. Note association of mobile fauna with high biomass of attached organisms. Note scaling laser is an older version and is 28 cm.

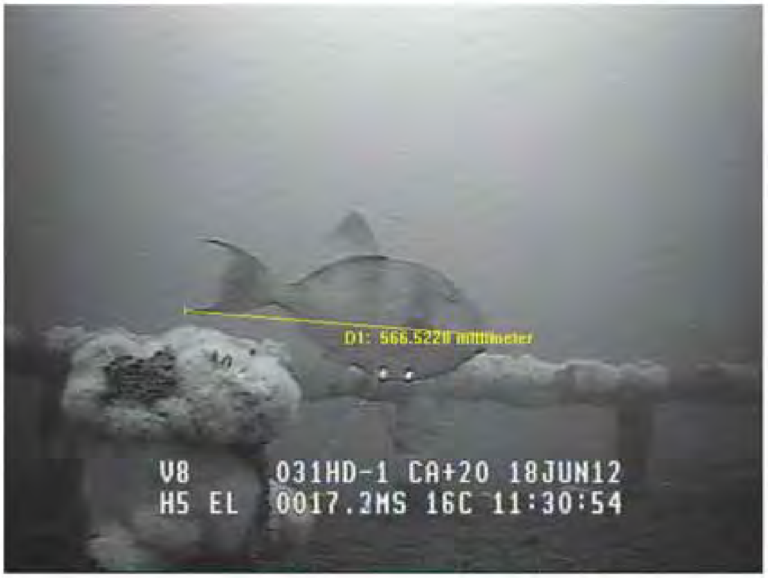
D1: 566.5220 millimeter
V8 031HD-1 CA+20 18JUN12
H5 EL 0017.2MS 16C 11:30:54

Figure 7. Video capture and laser scaling (30 mm) of grey triggerfish (*Balistes capriscus*) on the *Jessie C* site of the LE reef.

Discussion

Utilization of geo-referenced, digital side scan sonar mapping of the underwater sites allowed for precise location of structures and repeated observation over a number of seasons and years. Catch and effort surveys among fishers and experimental colonization studies have done much to define the increase in standing stocks of artificial reefs (Figley, 2003) but productivity determinants are more difficult to measure given seasonal and annual variability (Powers et al., 2003). Diver observation, while valuable, has the potential bias of disturbance of the observed fauna, in particular by bubbles of open circuit scuba and is limited by diver available gas supply and bottom time. However, small observation class ROVs have the potential to overcome some of the limitations of divers in an economic and scientifically valid manner. Direct observation and data collection in a non-intrusive manner by ROV has the potential to detect linkages among fine scale processes such as reef utilization throughout different life history stages, feeding and behavior of mobile fauna, territoriality, and predator prey interactions that otherwise might be missed. This would contribute greatly to the current goals of ecosystem based management in fisheries (Latour et al., 2003) and to the definition of Essential Fish Habitat (EFH) and Habitat Areas of Particular Concern (HAPC), (NOAA, 1996) particularly for black sea bass (Drohan et al., 2007) and tautog (Steimle and Shaheen, 1999).

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NUTRITIONAL EXCHANGE IN THE ANEMONEFISH-ANEMONE SYMBIOSIS: WHAT NEMO DID NOT TELL YOU

E. Alan Verde
Ann Cleveland
Raymond W. Lee

Corning School of Ocean Studies
Maine Maritime Academy
1 Pleasant Street
Castine, MAINE 04420 U.S.A.
alan.verde@mma.edu

Present assessment of symbiotic interactions reveals that these associations range from “loosely bound” to “highly integrated” with regard to nutrient exchange (for both organic and inorganic molecules) and evolutionary driving forces at the cellular, organismal and ecosystem levels of integration. Such symbiotic associations between host anemones (with zooxanthellae) and anemonefishes are both classic icons and quintessential models of mutualistic symbiosis. Since the anemonefish-anemone-zooxanthellae are in such close association at both the extracellular and intracellular levels, we investigated the process of nutrient transfer directly from fish to anemone to algae; this process was unequivocally demonstrated using chemical tracers ^{15}N and/or ^{13}C stable isotopes. However, the question remained as to whether the process of nutrient transfer occurs in the opposite direction. In other words, do nutrients obtained by the zooxanthellae or anemone host ultimately find their way to the tissue of the resident anemonefish? Consequently, the central focus of the present study was to determine whether such transmission of nutrients from the anemone holobiont to the resident anemonefish takes place or not.

Experiments were designed in both laboratory (2007) and field (2010) conditions to ascertain whether nutrient transfer occurs from the host anemone (*Heteractis crispa*) and/or endosymbiotic zooxanthellae to resident anemonefishes (*Amphiprion perideraion* and *A. clarkii*). One group of anemones was hand fed two times per day with ground fresh shrimp mixed with Isogro (Sigma-Aldrich; double-labeled with both ^{15}N and ^{13}C isotopes); anemones were fed for three days by placing the shrimp paste directly onto the oral disc twice each day (0900 and 1600); the anemones swallowed the shrimp paste by manipulating the tentacles to move the food into the mouth and subsequently the coelenteron over the course of several minutes. The control anemones were only fed the original shrimp paste that had not been mixed with Isogro. Both these anemone treatment groups were allowed to regurgitate all indigestible material over the course of 48 hours prior to association with any anemonefish.

A second group of anemones was incubated (during daylight hours) in seawater where the natural bicarbonate ion (HCO_3^{-1}) was replaced with ^{13}C-bicarbonate ion isotopes or bicarbonate ion (control). The pH of the “initial” seawater was noted (pH = 8.18-8.23) and continuously measured with a Mettler-Toledo (Model 120) portable pH meter calibrated to both pH 4.0 and 7.0. Between 1.2 - 1.3 ml of 12 M HCl was added (in 250 µL increments) until a pH of 4.0 - 4.4 was achieved to convert all dissolved natural HCO_3^{-1} into gaseous CO_2. An aeration stone was lowered to the bottom of the container and molecular N_2 gas was vigorously bubbled into the seawater for 10 min to displace all gaseous CO_2. A 600 µL aliquot of 10 M NaOH was added (in 100 µL increments) until a stable pH of ≈ 9.6 was achieved. Approximately 2.6 g of $NaH^{13}CO_3$ stable isotope (Sigma-Aldrich) was added to the seawater and allowed to dissolve completely and the final pH was adjusted to ≈ 8.2 (the original seawater pH) with drop wise additions of either 10 M NaOH or 12 M HCl. This “artificial” $H^{13}CO_3^{-1}$ labeled seawater was transferred into 20 L bottled water containers that were resealed, covered with two layers of thick black plastic bags (to prevent phytoplankton uptake of $^{13}CO_2$ via photosynthesis) and stored for 2 days until utilized. The experimental anemones were directly exposed to either overcast sunlight (< 1000 µmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}) or covered with neutral density screens during full sunlight (> 1000 µmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}) from 0800 to 1800. Both temperature (°C) and light intensity (µmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}) were monitored at 30 min intervals throughout the day using thermometers and a LI-COR Spherical Quantum Sensor (UWQ 6695). After exposure to sunlight, the anemones (both ^{13}C-labeled and controls) were placed in separate outdoor seawater raceways for 2 days to allow them to clear any seawater within their coelenteron prior to deploying in aquaria (2007) or in the field (2010). Subsequently, all anemones were provided with freshly-caught adult anemonefish of either species and incubated for 10 days (laboratory experiments during 2007) to 3 weeks (field experiments during 2010).

Mass spectroscopy analyses (GV Instruments Isoprime isotope ratio mass spectrometer) revealed that ^{15}N and/or ^{13}C concentrations were highly elevated in both the anemone and zooxanthellae fractions as well as significantly higher quantities of ^{15}N and/or ^{13}C in experimental fish tissues (intestines, liver, gills, and reproductive organs) compared with control fish; this was true for both species of anemonefishes. Based on the highest magnitude of stable isotopes in the fish’s digestive track and liver, ingestion, digestion and absorption of anemone tissue is the parsimonious mechanism employed by anemonefishes to obtain anemone and/or zooxanthellae-derived organic products. This interpretation is supported by both eyewitness accounts and photographic documentation in the field from various anemonefish and anemone species; as such, anemonefishes may be obtaining a “significant” portion of their daily caloric or nutrient input from host anemone tissues and zooxanthellae. Consequently, these stable isotope experiments provide the first empirical evidence that nutrient transfer of nitrogen and/or carbon products occurs from the anemone and zooxanthellae to anemonefish. Such direct transmission of organic molecules from endosymbiotic dinoflagellates and host anemone highlights the fundamental, yet innovative, role that nutrient dynamics play in the evolution of this truly mutualistic symbiotic association.

MESOPHOTIC SURVEYS OF THE NORTHWESTERN HAWAIIAN ISLANDS WITH NEW RECORDS OF BLACK CORAL SPECIES

Daniel Wagner[1]
Robert J. Toonen[2]
Yannis P. Papastamatiou[3]
Randall K. Kosaki[1]
Kelly A. Gleason[1]
Gregory B. McFall[4]
Raymond C. Boland[5]
Richard L. Pyle[6]

[1]NOAA Papahānaumokuākea Marine National Monument
Honolulu, HAWAII 96825 U.S.A.
Daniel.Wagner@noaa.gov

[2]Hawaii Institute of Marine Biology
Kaneohe, HAWAII 96744 U.S.A.

[3]Florida Museum of Natural History
University of Florida
Gainesville, FLORIDA 32611 U.S.A

[4]NOAA Gray's Reef National Marine Sanctuary
Savannah, GEORGIA 31411 U.S.A.

[5]Pacific Islands Fisheries Science Center
National Marine Fisheries Service
Honolulu, HAWAII 96822 U.S.A.

[6]Bernice P. Bishop Museum
Honolulu, HAWAII 96817 U.S.A.

Most marine science is performed within the depth limits of traditional SCUBA diving (<40 m). Mesophotic coral reef ecosystems are found just below these depths and as a result have been vastly under surveyed, especially in remote locations like the Northwestern Hawaiian Islands (NWHI). During a research expedition in the summer of 2009, 37 trimix technical dives were performed at depths between 50-80 m across the NWHI. Surveys for the invasive octocoral Carijoa *sp. and the invasive red algae* Acanthophora spicifera *did not record these species from any of the inspected sites. In contrast, the two black coral species* Antipathes griggi *and* Myriopathes ulex *were recorded from the NWHI for the first time, despite of over three decades of dedicated research in these remote islands. The new records of these highly conspicuous marine species highlight the utility of deep diving technologies in surveying the roughly 2/3rds of the depth range of coral reef ecosystems that remain largely unexplored.*

Introduction

After presidential proclamation in 2006 the Papahānaumokuākea Marine National Monument surrounding the Northwestern Hawaiian Islands (NWHI) became the largest marine protected area under U.S. jurisdiction encompassing over 362,000 km^2. With the creation of the monument there has been an increased emphasis on understanding what marine species live within the NWHI, both at shallow and deep depths. Most of the previous marine surveys in the NWHI have been performed within the depth limits of conventional scuba diving (< 40 m) (Dana, 1971; Grigg and Dollar, 1980; Grigg et al., 1981; Coles, 1998; Maragos et al., 2004). Additionally, some information on the deep-water fauna (> 100 m) of the NWHI was added through trawling performed by the Albatross and Sango Expeditions (Nutting, 1902; Grigg and Bayer, 1976) as well as more recent surveys using deep-sea submersibles and remotely operated vehicles (Baco, 2007; Parrish and Baco, 2007). However, as in many regions worldwide little is known about the marine fauna found between these two depth ranges. This intermediate depth range hosts mesophotic coral reef ecosystems (MCE), which are warm water, light-dependent coral reefs found below conventional scuba depth limits (40 m) and extending to the deepest portion of the euphotic zone where light levels fall to 1% of those found at the surface (Kahng, 2010). The lower depth limit of MCE varies by location but may be over 150 m in some tropical and subtropical regions with high water clarity (Kahng et al., 2010). These ecosystems are notoriously under surveyed around the globe (Kahng et al., 2010) and especially in remote locations like the NWHI (Rooney et al., 2010).

In the summer of 2009 trimix technical diving was used for the first time to systematically survey the NWHI fauna below conventional scuba depths (> 40 m). The primary objective of the mission was to survey for two invasive species that are abundant in the inhabited Main Hawaiian Islands and have either not been recorded from the NWHI (the octocoral *Carijoa* sp.) or recorded in very low numbers (the red algae *Acanthophora spicifera*) (Kahng, 2006; See et al., 2009). Special emphasis was placed on surveying for black corals because they have similar habitat requirements as the invasive octocoral *Carijoa* sp. in the Main Hawaiian Islands (Kahng, 2006). Black corals are important in Hawai‘i because they represent the state’s official gemstone and are commercially harvested for the black coral jewelry industry (Grigg, 2001, 2004). Consequently, surveys for Hawaiian black coral populations have been numerous, although historical surveys are restricted to the Main Hawaiian Islands (Grigg, 1974, 2001, 2004; Parrish and Baco, 2007). Furthermore, black corals constitute some of the major habitat-forming fauna on Hawaiian deep reefs (40-110 m) and provide critical habitat for many species and associates (Grigg, 1964; Barnard, 1971; Castro, 1971; Montgomery, 2002; Boland and Parrish, 2005).

Methods

All surveys were performed using open-circuit trimix technical diving on a research expedition to the NWHI aboard the *R/V Hi‘ialikai* in the summer of 2009. This research vessel is particularly well equipped for this type of advanced diving as the ship is outfitted with a decompression chamber, multiple small boats to carry divers to and from working areas, and multibeam sonar for seafloor mapping. Dive sites were chosen by selecting for areas with steep vertical drop-offs and hard substrate at depths between 50–80 m, which are the typical habitat requirements for the octocoral *Carijoa* sp. (Kahng, 2006) as well as several antipatharian species in Hawai‘i (Grigg and Opresko, 1977). A total of 37 dives were conducted off of Ni‘ihau Island, Nihoa Island, Necker Island, Laysan Island, Pearl and Hermes Atoll, Midway Atoll and Kure Atoll. Dives were performed to maximum depths of 80 m, with bottom times ranging from 15–25 minutes. Antipatharian colonies were photographed *in situ* and were clipped and preserved in 10% formalin. Antipatharian species were identified by comparing samples to museum specimens housed at the National Museum of Natural History, Smithsonian Institution in Washington, D.C. (USNM), and the Bernice P. Bishop Museum in Honolulu (BPBM).

Results

The octocoral *Carijoa* sp. and the red algae *A. spicifera*, which are both considered invasive in the Main Hawaiian Islands (Kahng, 2006; See et al., 2009), were not recorded at any of the surveyed sites. Two commercially valuable antipatharian species were recorded from mesophotic depths in the NWHI for the first time: *Antipathes griggi* Opresko, 2009 from the Islands of Necker and Laysan in 58–70 m, and *Myriopathes ulex* (Ellis and Solander, 1786) from Necker Island and Pearl and Hermes Atoll in 58–70 m (Fig. 1).

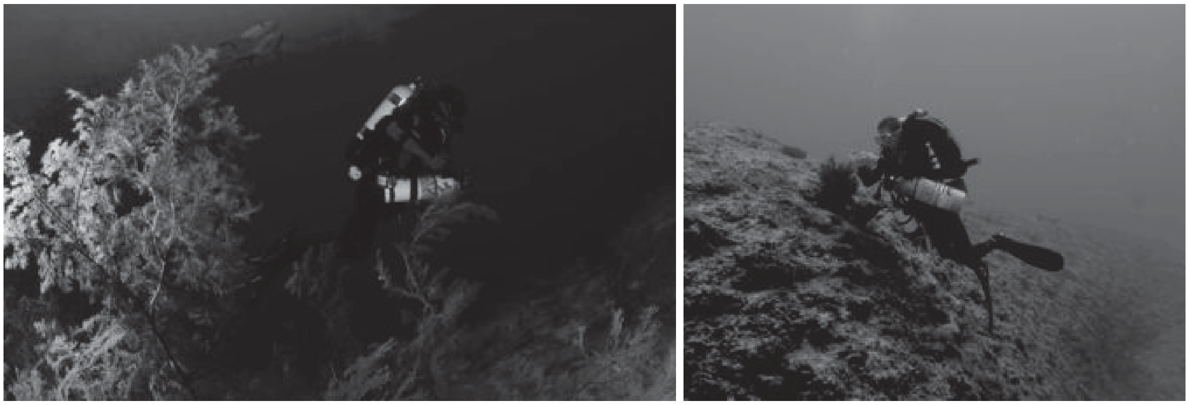

Figure 1. Technical diver collecting the black coral *Myriopathes ulex* (left) and *Antipathes griggi* (right). (Photo credits: Gregory McFall, left; Kelly Gleason, right)

Discussion

The new record of *Antipathes griggi* from the islands of Necker and Laysan is the first report of this species north of Ni‘ihau Island (Grigg and Opresko, 1977). This Hawaiian antipatharian species was initially identified as *Antipathes dichotoma* (Bayer, 1961), a species originally described from the Mediterranean Sea (Opresko, 2003). However, recent comparisons of Hawaiian specimens with those found in the Mediterranean Sea have revealed significant morphological differences and as a result the Hawaiian “*A. dichotoma*” has been assigned the new name of *A. griggi* (Opresko, 2009). *A. griggi* is the main species targeted by the Hawaiian black coral fishery (Grigg, 1993, 2001, 2004; Parrish and Baco, 2007). Therefore, surveys for this species have been numerous in the Main Hawaiian Islands where it is reported from the islands of Hawai‘i to Ni‘ihau (Grigg and Opresko, 1977; Grigg, 2001, 2004). Here we extend the known geographic distribution of this commercially valuable black coral species to the NWHI of Necker and Laysan, representing a range expansion of over 1,250 km to the northwest.

Myriopathes ulex is also commercially harvested in Hawai‘i (Grigg, 1993; Parrish and Baco, 2007) although it is rare at the 40–75 m depths where black coral is currently harvested (Grigg, 2001; Boland and Parrish, 2005; Parrish and Baco, 2007). *M. ulex* was originally described from Indonesia (Ellis and Solander, 1786) but subsequently reported from throughout the Indo-Pacific (Brook, 1889; Van Pesch, 1914; Grigg and Opresko, 1977; Colin and Arneson, 1995; Chave and Malahoff, 1998; Rogers et al., 2007; Bo 2008). Unfortunately, the original species description is brief and the type material is lost; therefore, the true geographic distribution of this species will be uncertain until a neotype is designated and a thorough taxonomic study is undertaken. The morphology of the corallum and skeletal spines of specimens collected as part of the present study are very similar to those of other *M. ulex* specimens previously collected from around Hawai‘i. Therefore, we continue to use the previously published name of *M. ulex* here, pending future taxonomic surveys. *M. ulex* has previously been reported from the Main Hawaiian Islands as well as around Brooks Banks in the NWHI (Grigg and Opresko, 1977). Here we extend the range of this species in Hawai‘i to include Necker Island and Pearl and Hermes Atoll, thus extending the geographic range of this species to over 1,020 km to the northwest.

Mesophotic coral reef ecosystems remain scarcely surveyed around the world and especially in remote locations like the NWHI. The reports of substantial range expansions of conspicuous marine species like black corals highlight the utility of deep diving technologies in surveying the roughly 2/3rds of the depth range of coral reef ecosystems, which remain largely unexplored.

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DO EPIFAUNAL COMMUNITIES IN BRITISH COLUMBIA SEAGRASS MEADOWS VARY WITH ENVIRONMENTAL GRADIENTS?

Ross Whippo
Mary I. O'Connor

University of British Columbia
Bamfield Marine Sciences Centre
100 Pachena Road
Bamfield, BRITISH COLUMBIA V0R 1B0 CANADA
whippo@zoology.ubc.ca

Many marine ecosystems are spatially structured and contain species whose potential dispersal distances exceed the spatial extent of local patches they occupy. In these systems, metacommunity processes (e.g., dispersal, abiotic filtering) contribute to spatial and temporal patterns of community assembly. Seagrass habitats in the Pacific Northwest are spatially structured within and between meadows; however, processes that determine assembly and persistence of faunal communities in seagrass meadows across spatial scales (i.e., m to km) and abiotic gradients (e.g., temperature) are not well understood. We studied epifaunal mesograzer communities in seagrass (Zostera marina) meadows of Barkley Sound, BC, to address the following questions: 1) How does spatial and temporal assembly of epifaunal community composition vary within and among meadows? 2) How much spatial and temporal variation in community structure can be explained by abiotic factors? We documented variation in epifaunal community composition within and between meadows that occur along an environmental gradient. However, species identity did not predictably vary with the average temperature and salinity, suggesting biotic interactions and relative exposure may play an important role in structuring seagrass communities over relatively small spatial scales (< 1km).

Introduction

The spatial and temporal assembly of ecological communities is contingent on processes that occur at both the local (e.g., trophic interactions) and regional (e.g., abiotic filtering) scales (Witman et al., 2004). Determining how these processes structure communities is a major challenge for modern ecology, which has been addressed in part by the 'metacommunity' concept (Leibold et al., 2004; Henriques-Silva et al., 2013). Metacommunity theory integrates across spatial scales and provides testable hypotheses to disentangle environmental and biological interactions that drive the assembly and persistence of ecological communities.

Seagrass meadows are globally distributed, nearshore marine habitats that provide resources for a wide variety of endemic and migratory species (Hemminga and Duarte, 2000). These meadows provide habitat for many species during specific life-stages, consist of geographically isolated metapopulations and interact across large and small spatial and temporal scales. Additionally, the spatial distribution of seagrass meadows across regions and abiotic gradients (e.g., temperature regimes) suggests metacommunity principles are applicable.

By synthesizing our current view of seagrass habitats across various scales, we allow the potential emergent properties in seagrass community structure to become apparent. This may clarify our understanding of processes that structure seagrass communities over local and regional scales.

The purpose of this study was to fill conceptual gaps in our knowledge of seagrass community assembly and persistence in the Pacific Northwest by answering the following questions: 1) How does spatial and temporal assembly of epifaunal community composition vary within and among meadows? 2) How much spatial and temporal variation in community structure can be explained by abiotic factors?

Methods

Sites

The present study was conducted at four sites along Trevor Channel in Barkley Sound, BC, on the west coast of Vancouver Island. Barkley Sound experiences mixed semi-diurnal tides and ranges in temperature from 5^{o}C to 20^{o}C with an average of 9^{o}C (pers. obs.), and salinity ranges from 5 ppt to 32 ppt with an average of 21 ppt (pers. obs.). The sites are oriented from Northeast to Southwest from Alberni Inlet to the Pacific Ocean (Fig. 1). The sites consisted of highly exposed locales, subject to wind waves and swell (Wizard Islet, Numukamis Bay) and sites sheltered from wind and wave action (Dodger Channel, Robber's Passage). All sites were sampled twice for meadow characteristics and community composition (May 2012, August 2012).

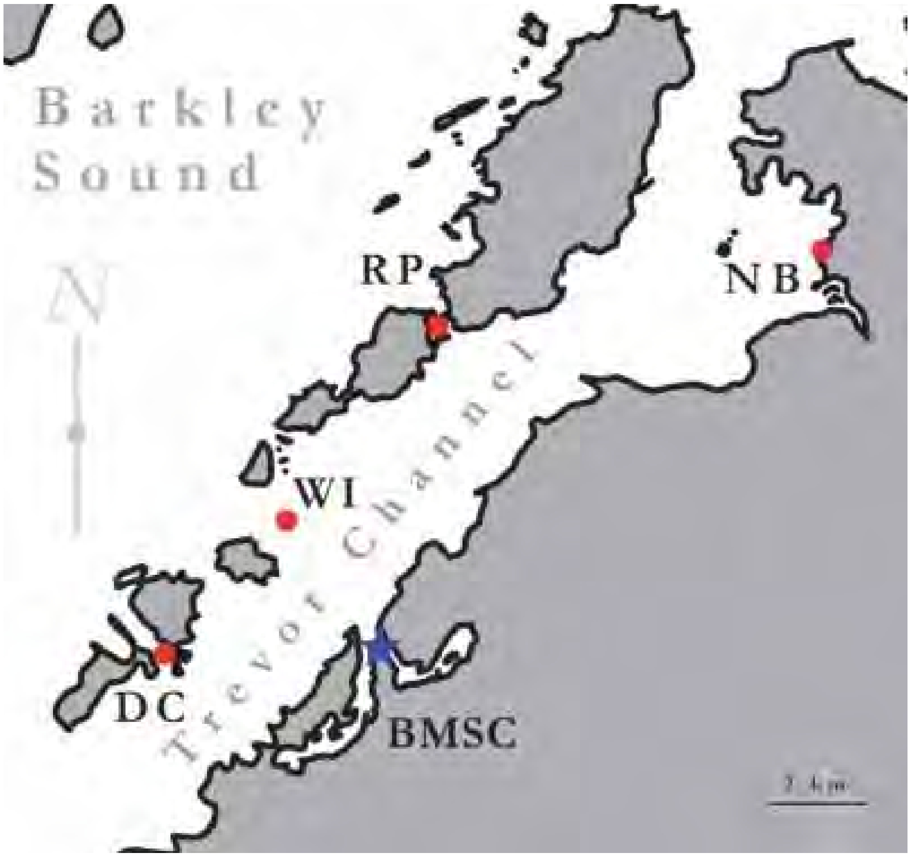

Figure 1. Four sites were used for epifaunal seagrass community sampling. NB = Numukamis Bay (48^{o}54.391'N, 125^{o}00.740'W); RP = Robber's Passage (48^{o}53.648'N, 125^{o}07.104'W); WI = Wizard Islet (48^{o}51.495'N, 125^{o}09.520'W); and DC = Dodger Channel (48^{o}50.023'N, 125^{o}11.720'W).

Abiotic Monitoring

All sites were monitored for temperature and salinity by boat using a handheld temperature/salinity probe (YSI Inc., OH USA). Measurements were taken opportunistically at various times of day and at different points in the tidal cycle to capture the greatest variation.

Sampling Regime and Organism Identification

Epifaunal communities were sampled subtidally using scuba in a 4 x 4 design for a total of 16 samples per site. Each plot was 1 m away from its nearest neighboring sample. This distance was held constant across seagrass meadows to control for differing size and configuration of meadows. For each sample, all seagrass within a $0.28m^2$ quadrat was cut away at the sediment-water interface and placed into a 250µm mesh bag. Seagrass was transported back to the lab in seawater and processed within 24 hours of collection. All epifauna was scraped from seagrass blades and then size-sorted using 8, 4, 2, and 1 mm sieves. Organisms were preserved then identified to the finest taxonomic resolution possible using light microscopy.

Statistics

All statistics were run in R (R core team, 2013, v. 3.0.0) computing environment. Multivariate analyses used the “vegan” package (Oksanen et al., 2013, v. 2.0-7).

Average temperature and salinity were calculated for each site and differences were detected using multi-way ANOVA and post hoc Tukey tests. Overall differences in community composition between sites and sampling times were tested using adonis (PERMANOVA).

Results

Abiotic conditions

Surface measurements regularly over-predicted water temperatures and under-predicted salinity in the water column (data not shown); therefore, averages from multiple depths were taken for each site throughout the sampling season. On average, the sites fell along a temperature gradient ranging from cooler (near the outer coast) to warmer (toward the inlet) ($F_{(3,255)}=8.73$, $p<0.0001$, ANOVA; (Fig. 2).

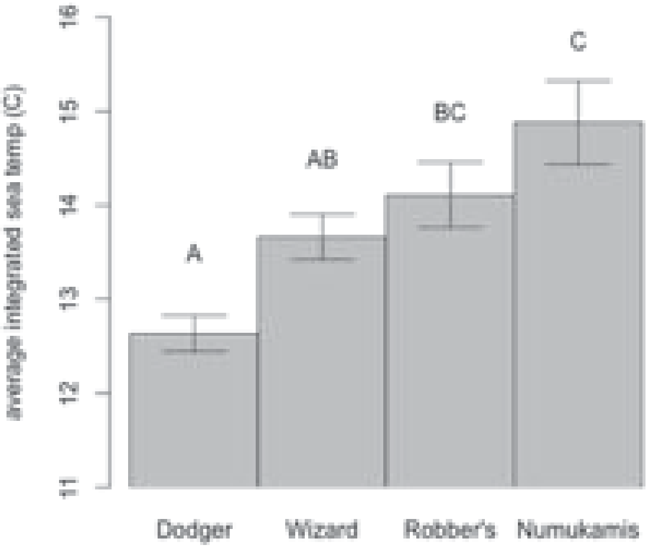

Figure 2. Average water temperature at four sample sites along Trevor Channel. Values are calculated from surface and mid-water averages between May and August 2012 and shown with standard error.

Average temperatures did not differ between adjacent sites. However, a significant step-wise increase in temperature was seen along the channel (post hoc Tukey). Salinity tended to be higher towards the outer coast and decreased up the Channel toward more fresh water inputs ($F_{(3,255)}$=46.84, p<0.0001, ANOVA; Fig. 3). Average salinity differed by at least 3 ppt between each site (*post hoc* Tukey).

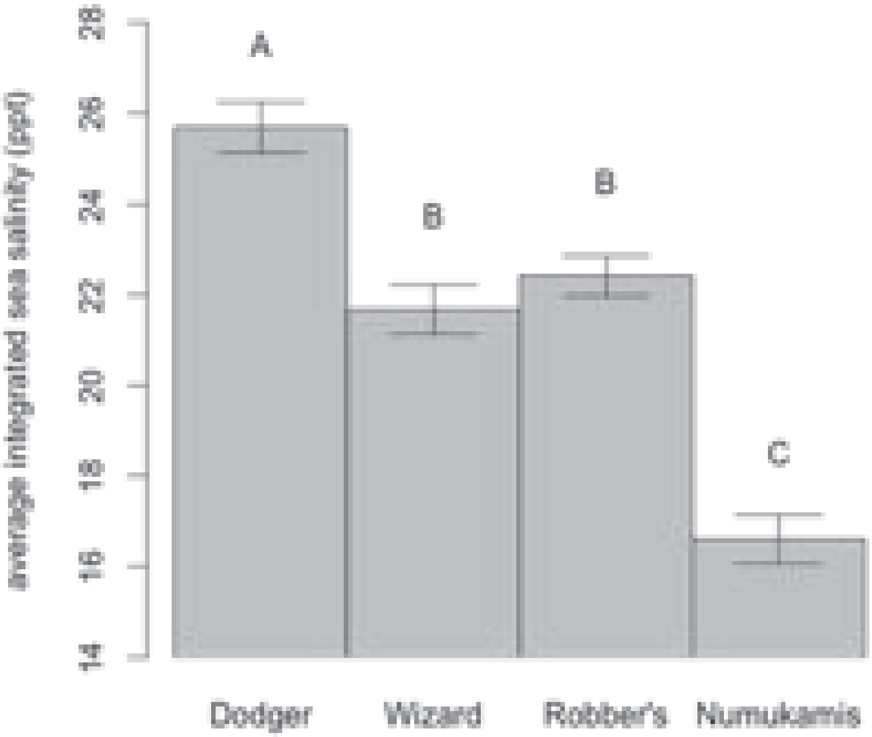

Figure 3. Average water salinity at four sample sites along Trevor Channel. Values are calculated from surface and mid-water averages between May and August 2012 and shown with standard error.

Community Composition

Overall community composition differed amongst sites and between sampling periods (PERMANOVA, pseudo-$F_{(3,115)}$=16.95, p=0.0001; Fig. 4), with a significant interaction between site and time. The most common organisms identified across all sites and time periods were: *Caprella sp.* (8338), *Phyllaplysia taylori* (6248), Order *Amphipoda* (1114), Class *Polychaeta* (968) and *Idotea resecata* (704). Overall organismal abundance was 500% higher in August than in May with the largest increases in *Phyllaplysia taylori* (>9000%), *Caprella sp.* (>500%), and Class *Polychaeta* (>300%).

Discussion

Seagrass meadows of Barkley Sound foster unique assemblages of epifaunal invertebrates across space and time. *Caprella sp.*, *Phyllaplysia taylori*, and *Polychaeta* abundances increased markedly through the summer, suggesting bottom up processes (e.g., epiphytic algal production) may control epiphytic grazers and their potential predators. Although distinct temperature and salinity gradients were apparent, organisms did not track with these gradients as would be predicted by the species-sorting paradigm of metacommunity theory. This indicates that trophic and competitive interactions may play a stronger role in structuring seagrass communities. However, similarities in community composition between relatively exposed and relatively sheltered sites suggest abiotic energy regimes (e.g. – wave and wind action, current exposure) determine abundances of some taxa.

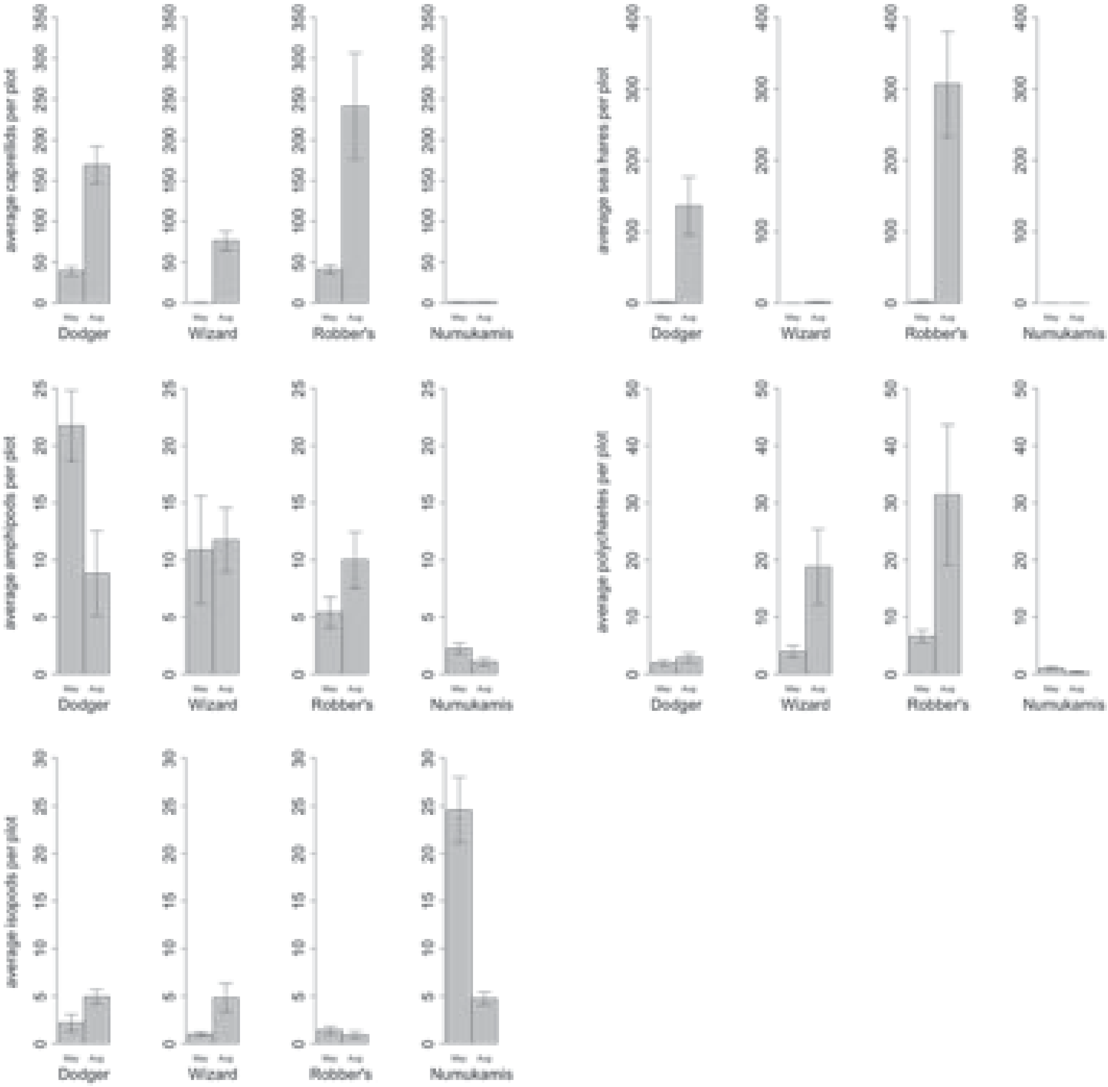

Figure 4. Average abundances of common seagrass epifauna from four sample sites in May and August 2012. Samples were taken from 16 28cm^2 quadrats and are shown with standard error.

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