Data Evaluation Report on Histology of the Gonads and Analysis of Hormone Levels in the Native Bullfrog (*Rana catesbiena*) Collected from Agricultural Areas in Southern Iowa: Pilot Project.

EPA MRID Number 458677-05

Data Requirement:

EPA DP Barcode	D288775
EPA MRID	458677-05
EPA Guideline	70-1(Special Study)

Test material: Purity: not reported

Common name: Atrazine

Chemical name: IUPAC

CAS name 6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine

CAS No. 1912-24-9

Synonyms

EPA PC Code: 80803

Primary Reviewer: Thomas M. Steeger, Ph.D., Senior Biologist **Date:** March 27, 2003
Environmental Fate and Effects Division, ERB 4,
U. S. Environmental Protection Agency

Secondary Reviewer(s): Joseph E. Tietge, M.S., Research Aquatic Biologist **Date:**
Mid-Continent Ecology Division,
National Health and Environmental Effects Research Laboratory (Duluth),
U. S. Environmental Protection Agency

Stephanie Irene, Ph.D., Senior Advisor **Date**:
Environmental Fate and Effects Division, ERB 3,
U. S. Environmental Protection Agency

Mary J. Frankenberry, Senior Statistician **Date:**
Environmental Fate and Effects Division, ERB 3,
U. S. Environmental Protection Agency

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Date Evaluation Completed: 06/01/2003

CITATION: Crabtree, C.; E. E. Smith; J. A. Carr. 2003. Histology of the gonads and analysis of hormone levels in the native bull frog (*Rana catesbiena*) collected from agricultural areas in southern Iowa: pilot project. The Institute of Environmental and Human Health, Texas Technical University, Lubbock, Texas. Sponsor: Syngenta Crop Protection, Inc. Laboratory Identification Number ECORISK Number TTU-02.

EXECUTIVE SUMMARY:

This study presents the results of Phase 1 of a three-phase study where 14 pond sites in southern Iowa (3 reference and 11 atrazine-exposed) were characterized. Experimental sites were located in corn and soybean-dominated agriculture areas. Pond sizes ranged from 0.14 ha to 2.94 ha with average watershed areas ranging from 2.19 ha to 84.02 ha. Atrazine concentrations in reference ponds averaged 0.06 µg/L. Mean atrazine concentrations in corn-dominated sites over the June to September ranged from 1.07 to 19.26 µg/L; atrazine was highest in corn-dominated ponds in June/July with a maximum value of 35.07 µg/L. For soybean-dominated watersheds, the highest residues ranged from 3.19 to 3.85 µg/L. Similarly, maximum deisopropyl atrazine residue concentrations were highest in corn-dominated areas in June/July at 4.17 µg/L. Maximum desethyl atrazine (DEA) residues were highest in corn-dominated ponds at 16.55 µg/L in June/July and 16.10 µg/L in August. Residues of diaminochlortriazine (DACT) remained relatively constant during the sampling period and once again, the highest average residues (0.65 µg/L) were from corn-dominated ponds.

Bullfrogs (*Rana catesbiena*) were present at all sites in sufficient numbers for collection; however, not all life stages were collected at every site.. No significant differences were found for adult body weight or snout-vent length (SVL). Mean weight and SVL for juvenile females were significantly lower in reference sites than atrazine-exposure sites. Mean SVL for juvenile males was significantly lower in reference sites than in atrazine-exposure sites; however mean weight of juvenile males was not statistically different between sites. Gonadal somatic index (GSI) was not significantly different between sites for either adults or juveniles. No gross gonadal abnormalities were observed based on visual examination (gross morphology). The incidence of external abnormalities was less than 1% of the total frogs caught.

The number of water samples may have been insufficient to characterize the exposure potential to atrazine, particularly in reference sites. While an effort was made to characterize a limited number of herbicides, no effort appears to have been made to characterize other pesticides in general. Based on the preliminary results, none of the indices measured (weight, length, GSI or the incidence of gross gonadal deformities) in the bullfrog indicate that variable exposure levels to atrazine and other triazine degradates are adversely affecting this species. While the bullfrog appears to be clearly present, the relevancy of this species is questionable because there isn't much information available on the bullfrog relative to some of the indices of interest, *i.e.*, steroid hormone levels, aromatase levels, background incidence, and types of gonadal abnormalities.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: Nonguideline Study

COMPLIANCE: Not conducted under full GLP; however, most practices as defined by 40 CFR Part 160, August 19, 1989 were established for this study, including but not limited to:

- Written, authorized preliminary protocol
- Written, authorized Standard Operating Procedures for all key procedures.
- Organization and Personnel were sufficient in terms of number, education, training and experience.
- Facilities were of suitable size and construction
- Equipment used was of appropriate design and adequate capacity.
- Independent QA Inspection was conducted of raw data..
- Interim Report was written
- Raw data, documentation, records, protocols, and final report will be archived.

A. MATERIALS:

1. Test Material Atrazine

Description: Not reported

Lot No./Batch No. : Not reported

Purity: NA

Stability of compound under test conditions: Not reported

Storage conditions of test chemicals: Not reported

2. Test organism:

Species: Native bull frog (*Rana catesbiena*)

Age at test initiation:

Weight at study initiation: (mean and range) Not reported

Length at study initiation: (mean and range) Not reported

Source: Field-collected at Edith Angel Environmental Center,
The Institute of Environmental and Human Health
Texas Tech University
44351 State Hwy 13
Chariton, Iowa 50049

B. STUDY DESIGN:

Objective:

1. To select sites and validate biochemical, analytical methods and sampling techniques to assess the effects of atrazine on kidney and gonad histology of bullfrogs (*Rana catesbiena*) and other species collected from various field sites in southern Iowa.
2. Develop biochemical and analytical methods for determination of blood steroid hormone levels and gonadal aromatase activity.
3. Conduct histological analysis of the gonads and kidneys of collected frogs.
4. Calculate the gonadal somatic index (GSI) of collected frogs.
5. Measure atrazine levels in the aquatic environments where frogs are collected.
6. Describe the study sites, physically, biologically, and chemically, by recording the following data for each site: shape/area, depth, plant life, approximate watershed area, major crops in the watershed, pesticides used on the major crops, and water quality indices (temperature, pH, dissolved oxygen, and conductivity).
7. Describe the morphology of collected frogs by recording the sex, weight, snout-vent length (SVL), and any physical abnormalities observed.

1. Experimental Conditions

Sampling was conducted in mid-summer (June/July 2002), late-summer (August 2002), Fall (September 2002) and Spring 2003 (no data reported as of yet) in Phase I of a three- phase study. Each pond/lake was sampled once during each sampling period. Frogs at all life stages (tadpole through adult) were collected at each sampling.

Corn/soybean rotational cropping patterns in 11 atrazine-use areas (K-1, R1 - R4, R6, S1 - S3, T1, W1) and 3 reference sites (isolated from corn/soybean culture) (F1, M1, and P1) were evaluated. Reference site watersheds were either primary hardwood forest (M1) or turf (P1, F1). Experimental site watersheds were a combination of corn, soybean and pasture/hay. Experimental sites R1, R2, R3, S2, T1 and W1 were planted in soybeans and treated primarily with glyphosate in 2002. Ponds K1, R4, R6, S1 and S3 were planted in corn and treated with atrazine in combination with one or more of the following herbicides: acetochlor, s-metolachlor, nicosulfuron, rimsulfuron or 2,4-D.

Bifenthrin was the only insecticide applied to corn. Most of the soybeans were genetically modified oganisms engineered for resistance to glyphosate. Soybeans were treated in spring with Roundup®. A variety of herbicides were used in the watersheds of corn (atrazine, acetochlor, s-metolachlor, clomazone, ethalfluralin, nicosulfuron, rimsulfuron and 2,4-D.

At each sampling, duplicate water samples were collected from each study site; two 250-mL samples were collected from 4 sites evenly distributed around each water body (N, S, E and W) and collected at a depth of 10 cm. Each set of four 250-mL samples were pooled into a 1-L composite. At the time of collection,

temperature, DO, pH and conductivity were measured. One sediment sample was collected from each site during late-ummer sampling.

Samples were analyzed for atrazine and its metabolites, simizine and metolochlor.

Frogs were collected by dip net for one hour at night. All frogs that could be collected within the 1-hour period were held in buckets of pond water until the sampling period ended. All frogs with physical abnormalities were immediately selected for analysis and the remainder were randomly selected from the pooled sample until a sample size of 10 (15 during early fall) was achieved. Tadpoles were collected using dip-nets just prior to or concurrent with frog sampling until a total of 10 tadpoles were collected. Remaining frogs were released back into the pond.

Blood samples were collected from adults by cardiac puncture. Species, sex, weight and SVL for each necropsied specimen was recorded (apparently after each of the animals had been bled and necropsied). During early fall sampling, right gonad was extracted from 5 of the 15 collected animals, frozen in liquid nitrogen for use in $^{3}H_2O$-release aromatase assay. The remainder of the carcass was fixed in Bouin's. A random sample of 340 male and female frogs (73% of all the frogs sampled in 2002) were examined visually.

II. RESULTS and DISCUSSION: [All results discussed in this section and the next are those reported by the study authors. Although supplemental data are typically used in a qualitative manner only, EFED verified spreadsheet data and ran basic statistical analyses on the major study parameters. See attached appendix. If results appeared to differ in any substantive way, the difference was reported in the text below.]

Pond sizes ranged from 0.14 ha to 2.94 ha. The average watershed areas ranged from 2.19 ha to 84.02 ha. Dissolved oxygen in terms of percent saturation was surprisingly low during the late-summer and fall samplings dropping as low as 1.9% saturation. In pond T1, pH is reported to have dropped to 1.31 and is likely a typo.

Although atrazine concentrations in corn-dominated sites over the June to September sampling period averaged 5.52 µg/L (Table 1), the means from each of the ponds ranged from 1.07 to 19.26 µg/L. Atrazine was highest in corn-dominated ponds in June/July with the maximum value of 35.07 µg/L; experimental pond R6 (corn) exhibited the highest atrazine levels ranging from 5.7 to 35.07 µg/L. For soybean-dominated watersheds, pond W1 had the highest residues ranging from 3.19 to 3.85 µg/L. Similarly, deisopropyl atrazine concentrations were highest in corn-dominated areas and averaged 0.89 µg/L; however maximum residues were recorded in June/July at 4.17 µg/L. Desethyl atrazine (DEA) residues were highest in corn-dominated ponds; however residues from June through August were relatively consistent. Maximum residues for DEA were at 16.55 µg/L in June/July and 16.10 µg/L in August. Residues of diaminochlortriazine (DACT) remained relatively constant during the sampling period and once again, the highest average residues (0.65 µg/L) were from corn-dominated ponds. The maximum residue detected (2.33 µg/L) occurred twice, once in June/July and again in September.

Metolachlor residues averaged between 0.05 and 0.06 µg/L across all sample sites while simazine residues averaged 0.05 µg/L. The detection limits for both metolachlor and simazine are 0.1 µg/L and thus residues were at or below detection limits.

Table 1. Mean atrazine, diaminochlorotriazine (DACT), deisopropyl atrazine, and desethyl atrazine residues in pond water collected at reference[a], corn-dominated[b] and soybean-dominated[c] sites.

Chemical Residues	Reference µg/L	Corn µg/L	Soybean µg/L
Atrazine	0.06	5.52	1.05
Diaminochlorotriazine	0.10	0.65	0.24
Deisopropyl atrazine	0.05	0.89	0.21
Desethyl atrazine	0.05	3.18	0.65

[a] reference site include ponds M1, P1 and F1
[b] corn-dominated sites include ponds R4, R6, K1, S1 and S3
[c] soybean-dominated sites include ponds R1, R2, R3, S2, T1 and W1

Frogs without tails but less than 70 g were classified as juveniles, while frogs without tails but weighing ≥ 70 g were classified as adults. More females than males were collected in sampling period 1 (50% vs 35%) and in period 2 (54% vs 40%); however, in sampling period 3, the percentage of males and females was roughly similar 50% vs 49%). Overall, for the three ponds combined, females outnumbered males (51% vs 43%) and juveniles outnumbered the adults (52% vs 44%). Species other than bullfrogs represented 6% of all collected frogs.

T-test comparisons between reference sites and experimental sites for adult males and adult females resulted in no significant difference for either weight or length (**Table 2**). In general there was a trend for male and female adults to weigh more at reference sites. However, mean weight and SVL for juvenile females was significantly lower in reference sites than experimental sites. There were no differences in the gonadosomatic index for males (weight of the right gonad ÷ frog's weight) or females collected at reference versus experimental sites (**Table 3**).

No gross morphological abnormalities were observed (n = 340) in either male or female gonads collected across reference and experimental sites.

Table 4 summarizes the total number of bullfrogs caught and sampled in reference and experimental sites and the sexes of those animals over the three sampling periods. This information was taken from Tables 10 through 12 of the report. Based on these data, the percentage of males in reference samples ranged from 11 to 56% over the three sampling periods, while the percentage of males in experimental site samples ranged from 41 to 49%. In reference sites, the percentage of frogs where the sex could not be determined based on gross morphology ranged from 0 to 22% while in experimental sites, the percentage ranged form 1.4% to 5.9%.

Table 2. Necropsied carcass weight and snout-to-vent length (SVL) for male and female, adult and juvenile bullfrogs collected from reference and experimental sites.

Site Type	Sex	Stage	Weight			SVL		
			N	Mean	SD	N	Mean	SD
Reference	male	adult	14	147.33	47.79	14	119.1	11.9
	female		16	195.61	104.58	15	126.6	20.7
	male	juvenile	22	18.65	19.03	22	55.5	18.8
	female		32	15.63	13.13	32	53.7	15.5
Experimental	male	adult	78	134.40	57.74	78	114.9	15.9
	female		68	160.23	98.84	65	117.7	17.9
	male	juvenile	78	26.56	17.58	78	65.6	15.3
	female		103	26.99	16.75	103	65.6	14.5

Table 3. Gonadosomatic index for bullfrogs collected during sampling period 3

Site Type	Sex	Stage	N	Mean	Standard Deviation
Reference	male	Adult	6	0.07%	0.01%
	female		3	1.01%	0.52%
	male	Juvenile	2	0.05%	0.02%
	female		3	0.67%	0.16%
Experimental	male	Adult	15	0.06%	0.02%
	female		15	0.93%	0.74%
	male	Juvenile	10	0.03%	0.02%
	female		15	0.60%	0.29%

Table 4. Total number of bullfrogs caught and sampled and their respective sex based on gross morphology by collection period.

Sampling Period	Treatment	Number Caught	Number Sampled	Males	Females	Unknown
June - July	Reference	18	18	2	12	4
	Experimental	394	99	41	47	11
August	Reference	42	27	9	16	2
	Experimental	447	101	44	51	6
September	Reference	87	45	25	20	0
	Experimental	286	146*	71	75	2

* Pond R3 and W1 reported as 14 frogs captured but in both 15 were reported sexed.

REVIEWER'S COMMENTS:

This study represents a basic survey of a proposed study area. The limited number of water samples may not have provided sufficient characterization of the exposure potential to atrazine, particularly in reference sites. While an effort was made to characterize a limited number of herbicides, other pesticides apparently were not characterized. While the bullfrog appears to be clearly present, the relevancy of this species is questionable because no other studies looking at gonadal abnormalities are available on this species.

Weighing and measuring animals after cardiac puncture may influence the accuracy of these numbers. It is unclear why no mention is made of the number of adult animals where the sex could not be determined; in reference ponds the percentage was as high as 22%. Because the histology data have not been compiled, it is premature to conclude anything about the incidence of intersex in the bullfrog. Initial results, though, suggest that the field-collected bullfrog is not sensitive to atrazine.

CONCLUSIONS:

This is an interim report; however, based on the preliminary results, none of the indices measured (weight, length, GSI or the incidence of gross gonadal deformities) in the bullfrog indicate that variable exposure levels to atrazine and other triazine degradates are adversely affecting this species. While the bullfrog appears to be clearly present, the relevancy of this species is questionable because there isn't much information available on the bullfrog relative to some of the indices of interest, *i.e.*, steroid hormone levels, aromatase levels, background incidence, and types of gonadal abnormalities. Additional information is needed from the final report before a definitive conclusion can be reached for this study.

AVERAGE WEIGHT OF BULLFROGS BY TYPE (REFERENCE VS EXPERIMENTAL), SEX (MALE VS FEMALE) AND STAGE (ADULT VS JUVENILE)

Obs	TYPE	SEX	STAGE	_TYPE_	_FREQ_	WEIGHT	STD	CV
1	EXP	F	A	0	171	79.9756	91.079	113.884
2	EXP	M	A	0	156	80.4827	68.817	85.505
3	EXP	UNK	A	0	12	18.5758	15.421	83.015
4	REF	F	A	0	48	75.6256	104.667	138.402
5	REF	M	A	0	36	68.6925	71.508	104.099
6	REF	UNK	A	0	3	38.8833	57.196	147.097

AVERAGE LENGTH OF BULLFROGS BY TYPE (REFERENCE VS EXPERIMENTAL), SEX (MALE VS FEMALE) AND STAGE (ADULT VS JUVENILE)

Obs	TYPE	SEX	STAGE	_TYPE_	_FREQ_	LENGTH	STD	CV
1	EXP	F	A	0	171	85.7607	30.0012	34.9824
2	EXP	M	A	0	156	90.2558	29.2040	32.3569
3	EXP	UNK	A	0	12	57.2833	13.8312	24.1453
4	REF	F	A	0	48	76.9404	38.3845	49.8886
5	REF	M	A	0	36	80.2306	35.3826	44.1011
6	REF	UNK	A	0	3	63.6333	41.8914	65.8326

NONPARAMETRIC COMPARISON OF BULLFROG WEIGHT BETWEEN REFERENCE AND EXPERIMENTAL SITES 1418

---------- SEX=F STAGE=A ----------

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable WEIGHT
Classified by Variable TYPE

TYPE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
EXP	171	19714.50	18810.0	387.917074	115.289474
REF	48	4375.50	5280.0	387.917074	91.156250

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	4375.5000
Normal Approximation	
Z	-2.3304
One-Sided Pr < Z	0.0099
Two-Sided Pr > |Z|	0.0198
t Approximation	
One-Sided Pr < Z	0.0103
Two-Sided Pr > |Z|	0.0207

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	5.4367
DF	1
Pr > Chi-Square	0.0197

Median Scores (Number of Points Above Median) for Variable WEIGHT
Classified by Variable TYPE

TYPE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
EXP	171	90.0	85.109589	3.068004	0.526316
REF	48	19.0	23.890411	3.068004	0.395833

Average scores were used for ties.

Median Two-Sample Test

Statistic	19.0000
Z	-1.5940
One-Sided Pr < Z	0.0555
Two-Sided Pr > |Z|	0.1109

Median One-Way Analysis

Chi-Square	2.5408
DF	1
Pr > Chi-Square	0.1109

NONPARAMETRIC COMPARISON OF BULLFROG WEIGHT BETWEEN REFERENCE AND EXPERIMENTAL SITES 1420

---------- SEX=M STAGE=A ----------

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable WEIGHT
Classified by Variable TYPE

TYPE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
EXP	156	15586.0	15054.0	300.539133	99.910256
REF	36	2942.0	3474.0	300.539133	81.722222

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	2942.0000
Normal Approximation	
Z	-1.7685
One-Sided Pr < Z	0.0385
Two-Sided Pr > |Z|	0.0770
t Approximation	
One-Sided Pr < Z	0.0393
Two-Sided Pr > |Z|	0.0786

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	3.1334
DF	1
Pr > Chi-Square	0.0767

Median Scores (Number of Points Above Median) for Variable WEIGHT
Classified by Variable TYPE

TYPE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
EXP	156	82.0	78.0	2.711233	0.525641
REF	36	14.0	18.0	2.711233	0.388889

Average scores were used for ties.

Median Two-Sample Test

Statistic	14.0000
Z	-1.4753
One-Sided Pr < Z	0.0701
Two-Sided Pr > |Z|	0.1401

Median One-Way Analysis

Chi-Square	2.1766
DF	1
Pr > Chi-Square	0.1401

NONPARAMETRIC COMPARISON OF BULLFROG WEIGHT BETWEEN REFERENCE AND EXPERIMENTAL SITES 1422

---------- SEX=UNK STAGE=A ----------

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable WEIGHT
Classified by Variable TYPE

TYPE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
EXP	12	102.0	96.0	6.922015	8.50
REF	3	18.0	24.0	6.922015	6.00

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	18.0000
Normal Approximation	
Z	-0.7946
One-Sided Pr < Z	0.2134
Two-Sided Pr > |Z|	0.4269
t Approximation	
One-Sided Pr < Z	0.2201
Two-Sided Pr > |Z|	0.4401

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	0.7513
DF	1
Pr > Chi-Square	0.3861

Median Scores (Number of Points Above Median) for Variable WEIGHT
Classified by Variable TYPE

TYPE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
EXP	12	6.0	5.60	0.80	0.500000
REF	3	1.0	1.40	0.80	0.333333

Average scores were used for ties.

Median Two-Sample Test

Statistic	1.0000
Z	-0.5000
One-Sided Pr < Z	0.3085
Two-Sided Pr > |Z|	0.6171

Median One-Way Analysis

Chi-Square	0.2500
DF	1
Pr > Chi-Square	0.6171

NONPARAMETRIC COMPARISON OF BULLFROG LENGTH BETWEEN REFERENCE AND EXPERIMENTAL SITES 1424

---------- SEX=F STAGE=A ----------

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable SVL
Classified by Variable TYPE

TYPE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
EXP	168	18998.0	18144.0	376.994577	113.083333
REF	47	4222.0	5076.0	376.994577	89.829787

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	4222.0000
Normal Approximation	
Z	-2.2640
One-Sided Pr < Z	0.0118
Two-Sided Pr > |Z|	0.0236
t Approximation	
One-Sided Pr < Z	0.0123
Two-Sided Pr > |Z|	0.0246

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	5.1315
DF	1
Pr > Chi-Square	0.0235

Median Scores (Number of Points Above Median) for Variable SVL
Classified by Variable TYPE

TYPE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
EXP	168	89.0	83.609302	3.037120	0.529762
REF	47	18.0	23.390698	3.037120	0.382979

Average scores were used for ties.

Median Two-Sample Test

Statistic	18.0000
Z	-1.7749
One-Sided Pr < Z	0.0380
Two-Sided Pr > |Z|	0.0759

Median One-Way Analysis

Chi-Square	3.1504
DF	1
Pr > Chi-Square	0.0759

NONPARAMETRIC COMPARISON OF BULLFROG LENGTH BETWEEN REFERENCE AND EXPERIMENTAL SITES 1426

---------- SEX=M STAGE=A ----------

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable SVL
Classified by Variable TYPE

TYPE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
EXP	156	15553.50	15054.0	300.536458	99.701923
REF	36	2974.50	3474.0	300.536458	82.625000

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	2974.5000
Normal Approximation	
Z	-1.6604
One-Sided Pr < Z	0.0484
Two-Sided Pr > |Z|	0.0968
t Approximation	
One-Sided Pr < Z	0.0492
Two-Sided Pr > |Z|	0.0985

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	2.7623
DF	1
Pr > Chi-Square	0.0965

Median Scores (Number of Points Above Median) for Variable SVL
Classified by Variable TYPE

TYPE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
EXP	156	81.0	78.0	2.711233	0.519231
REF	36	15.0	18.0	2.711233	0.416667

Average scores were used for ties.

Median Two-Sample Test

Statistic	15.0000
Z	-1.1065
One-Sided Pr < Z	0.1343
Two-Sided Pr > |Z|	0.2685

Median One-Way Analysis

Chi-Square	1.2244
DF	1
Pr > Chi-Square	0.2685

NONPARAMETRIC COMPARISON OF BULLFROG LENGTH BETWEEN REFERENCE AND EXPERIMENTAL SITES 1428

---------- SEX=UNK STAGE=A ----------

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable SVL
Classified by Variable TYPE

TYPE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
EXP	12	102.0	96.0	6.928203	8.50
REF	3	18.0	24.0	6.928203	6.00

Wilcoxon Two-Sample Test

Statistic	18.0000
Normal Approximation	
Z	-0.7939
One-Sided Pr < Z	0.2136
Two-Sided Pr > |Z|	0.4273
t Approximation	
One-Sided Pr < Z	0.2203
Two-Sided Pr > |Z|	0.4405

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	0.7500
DF	1
Pr > Chi-Square	0.3865

Median Scores (Number of Points Above Median) for Variable SVL
Classified by Variable TYPE

TYPE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
EXP	12	6.0	5.60	0.80	0.500000
REF	3	1.0	1.40	0.80	0.333333

Median Two-Sample Test

Statistic	1.0000
Z	-0.5000
One-Sided Pr < Z	0.3085
Two-Sided Pr > |Z|	0.6171

Median One-Way Analysis

Chi-Square	0.2500
DF	1
Pr > Chi-Square	0.6171

MEAN GONADOSOMATIC INDEX BY STUDY TYPE (REFERENCE VS EXPERIMENTAL), SEX (MALE VS FEMALE), A STAGE (ADULT VS JUVENILE)

Obs	TYPE	SEX	STAGE	_TYPE_	_FREQ_	GSI	STD	CV
1	EXP	F	A	0	15	0.92800	0.74356	80.1245
2	EXP	F	J	0	15	0.60133	0.29384	48.8645
3	EXP	M	A	0	15	0.06467	0.01552	24.0041
4	EXP	M	J	0	10	0.03500	0.01780	50.8432
5	REF	F	A	0	3	1.01000	0.52374	51.8551
6	REF	F	J	0	3	0.66667	0.15822	23.7329
7	REF	M	A	0	6	0.06833	0.01602	23.4451
8	REF	M	J	0	2	0.05000	0.02828	56.5685

NONPARAMETRIC COMPARISON OF GSI BETWEEN REFERENCE AND EXPERIMENTAL SITES 1431

---------- SEX=F STAGE=A ----------

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable GSI
Classified by Variable TYPE

TYPE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
EXP	15	138.0	142.50	8.432256	9.20
REF	3	33.0	28.50	8.432256	11.00

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	33.0000
Normal Approximation	
Z	0.4744
One-Sided Pr > Z	0.3176
Two-Sided Pr > |Z|	0.6352
t Approximation	
One-Sided Pr > Z	0.3206
Two-Sided Pr > |Z|	0.6413

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	0.2848
DF	1
Pr > Chi-Square	0.5936

Median Scores (Number of Points Above Median) for Variable GSI
Classified by Variable TYPE

TYPE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
EXP	15	7.0	7.50	0.813489	0.466667
REF	3	2.0	1.50	0.813489	0.666667

Average scores were used for ties.

Median Two-Sample Test

Statistic	2.0000
Z	0.6146
One-Sided Pr > Z	0.2694
Two-Sided Pr > |Z|	0.5388

Median One-Way Analysis

Chi-Square	0.3778
DF	1
Pr > Chi-Square	0.5388

NONPARAMETRIC COMPARISON OF GSI BETWEEN REFERENCE AND EXPERIMENTAL SITES 1433

---------- SEX=F STAGE=J ----------

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable GSI
Classified by Variable TYPE

TYPE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
EXP	15	135.50	142.50	8.436615	9.033333
REF	3	35.50	28.50	8.436615	11.833333

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	35.5000
Normal Approximation	
Z	0.7705
One-Sided Pr > Z	0.2205
Two-Sided Pr > |Z|	0.4410
t Approximation	
One-Sided Pr > Z	0.2258
Two-Sided Pr > |Z|	0.4516

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	0.6884
DF	1
Pr > Chi-Square	0.4067

Median Scores (Number of Points Above Median) for Variable GSI
Classified by Variable TYPE

TYPE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
EXP	15	7.0	7.50	0.813489	0.466667
REF	3	2.0	1.50	0.813489	0.666667

Average scores were used for ties.

Median Two-Sample Test

Statistic	2.0000
Z	0.6146
One-Sided Pr > Z	0.2694
Two-Sided Pr > |Z|	0.5388

Median One-Way Analysis

Chi-Square	0.3778
DF	1
Pr > Chi-Square	0.5388

NONPARAMETRIC COMPARISON OF GSI BETWEEN REFERENCE AND EXPERIMENTAL SITES 1435

---------- SEX=M STAGE=A ----------

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable GSI
Classified by Variable TYPE

TYPE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
EXP	15	155.50	165.0	12.575486	10.366667
REF	6	75.50	66.0	12.575486	12.583333

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	75.5000
Normal Approximation	
Z	0.7157
One-Sided Pr > Z	0.2371
Two-Sided Pr > |Z|	0.4742
t Approximation	
One-Sided Pr > Z	0.2412
Two-Sided Pr > |Z|	0.4825

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	0.5707
DF	1
Pr > Chi-Square	0.4500

Median Scores (Number of Points Above Median) for Variable GSI
Classified by Variable TYPE

TYPE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
EXP	15	5.666667	7.142857	0.971534	0.377778
REF	6	4.333333	2.857143	0.971534	0.722222

Average scores were used for ties.

Median Two-Sample Test

Statistic	4.3333
Z	1.5194
One-Sided Pr > Z	0.0643
Two-Sided Pr > |Z|	0.1287

Median One-Way Analysis

Chi-Square	2.3087
DF	1
Pr > Chi-Square	0.1287

NONPARAMETRIC COMPARISON OF GSI BETWEEN REFERENCE AND EXPERIMENTAL SITES 1437

---------- SEX=M STAGE=J ----------

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable GSI
Classified by Variable TYPE

TYPE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
EXP	10	60.50	65.0	4.522670	6.050
REF	2	17.50	13.0	4.522670	8.750

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	17.5000
Normal Approximation	
Z	0.8844
One-Sided Pr > Z	0.1882
Two-Sided Pr > |Z|	0.3765
t Approximation	
One-Sided Pr > Z	0.1977
Two-Sided Pr > |Z|	0.3954

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	0.9900
DF	1
Pr > Chi-Square	0.3197

Median Scores (Number of Points Above Median) for Variable GSI
Classified by Variable TYPE

TYPE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
EXP	10	4.666667	5.0	0.594588	0.466667
REF	2	1.333333	1.0	0.594588	0.666667

Average scores were used for ties.

Median Two-Sample Test

Statistic	1.3333
Z	0.5606
One-Sided Pr > Z	0.2875
Two-Sided Pr > |Z|	0.5751

Median One-Way Analysis

Chi-Square	0.3143
DF	1
Pr > Chi-Square	0.5751