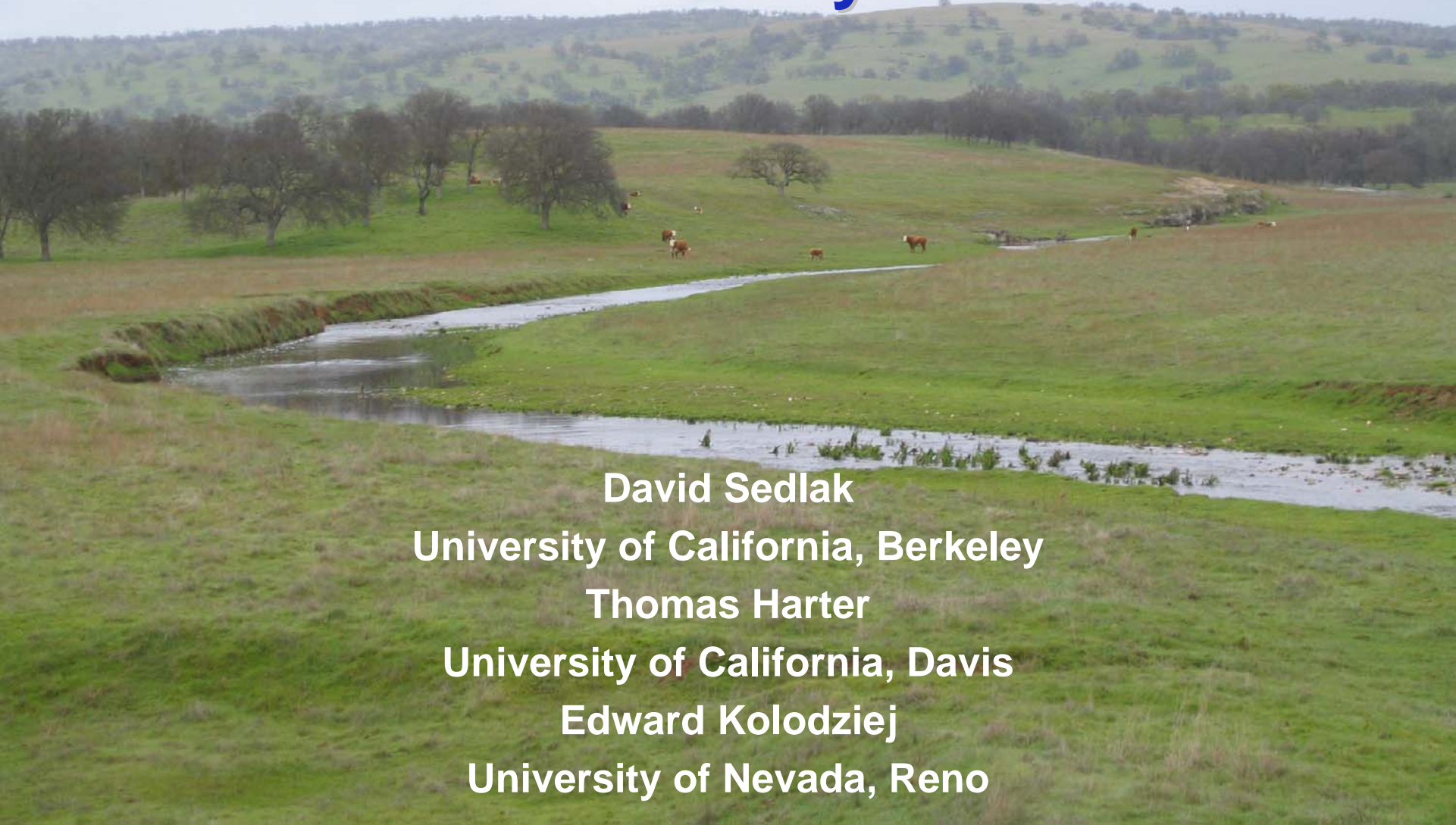


US EPA ARCHIVE DOCUMENT

Transport and Transformation of Natural and Synthetic Steroid Hormones at Beef Cattle and Dairy CAFOs



David Sedlak

University of California, Berkeley

Thomas Harter

University of California, Davis

Edward Kolodziej

University of Nevada, Reno

Outline

Previous Research

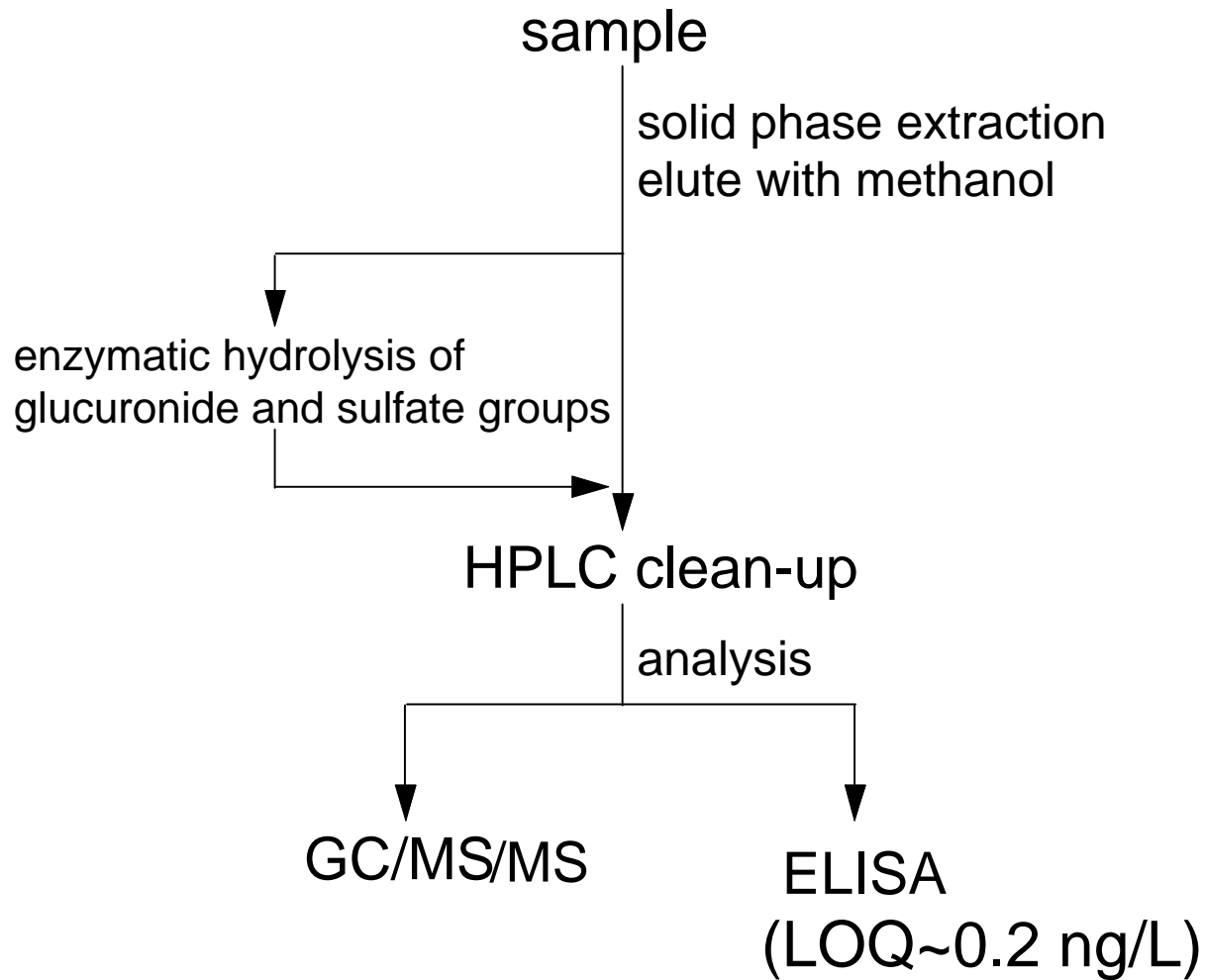
- Analytical methods
- Rangelands
- Dairies
- Regional scale

Hypotheses

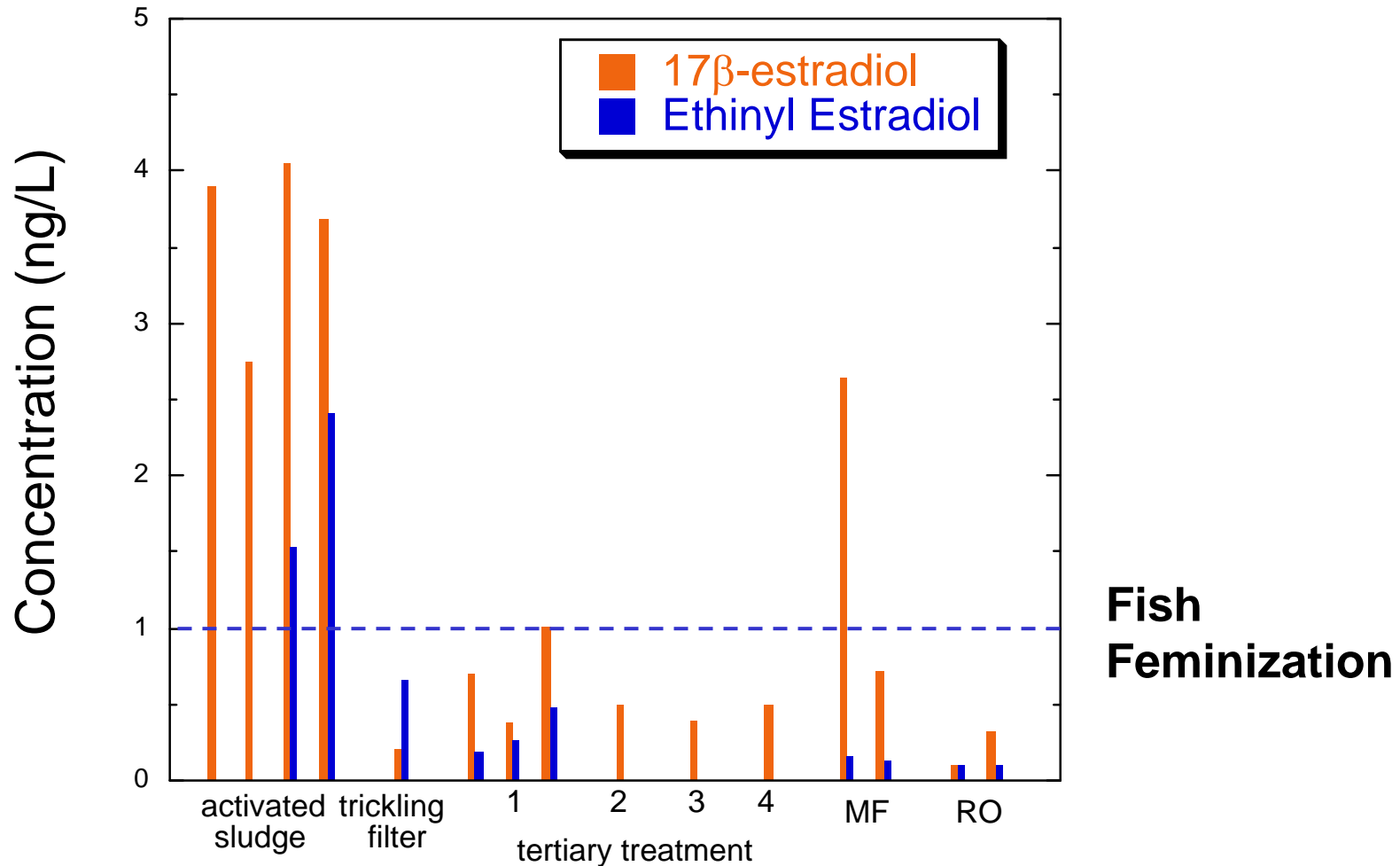
Proposed Research

Potential for Collaborations

Quantitative Analysis by ELISA



Hormones in Wastewater

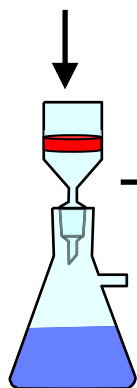


Current Approach



Water (4 L.)

Solid-phase
Extraction



C18 Disks

surrogates

10 mL MetOH

10 mL 40% MetOH

10 mL 75% MetOH

HFB derivatization

GC/MS/MS (LOQ~0.2 ng/L)

Exposures *in vitro/in vivo*
(Rainbow trout)

Estrogens:

17 α -estradiol

17 β -estradiol

Estrone

Estriol

Androgens

Testosterone

Androstenedione

Progestins

Progesterone

Medroxyprogesterone

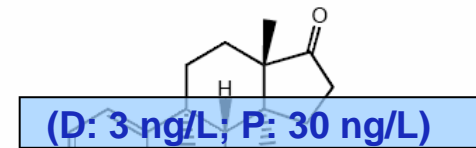
APEs

NP, NP1EO, NP2EO

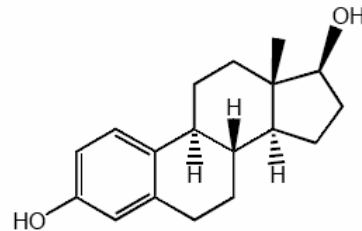
OP, OP1EO, OP2EO

Other Endpoints of Interest

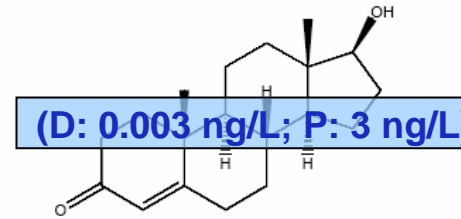
- Immune system
- Reproductive competition (Martinovic et al. 2007)
- Pheromonal effects (Kolodziej and Sedlak 2003)



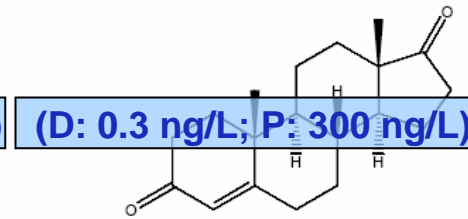
Estrone



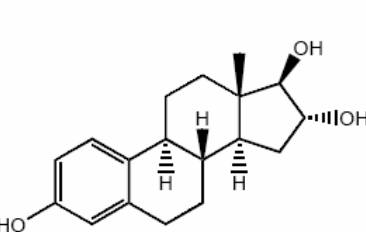
17β-Estradiol



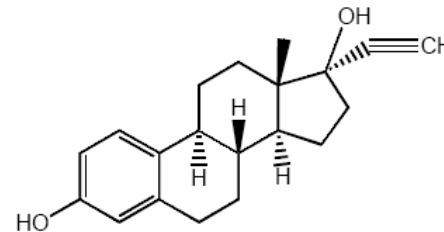
Testosterone



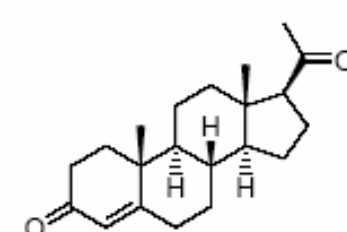
Androstenedione



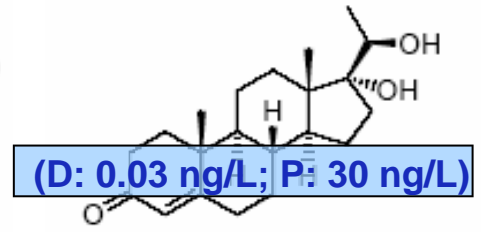
Estriol



Ethinyl-Estradiol



Progesterone

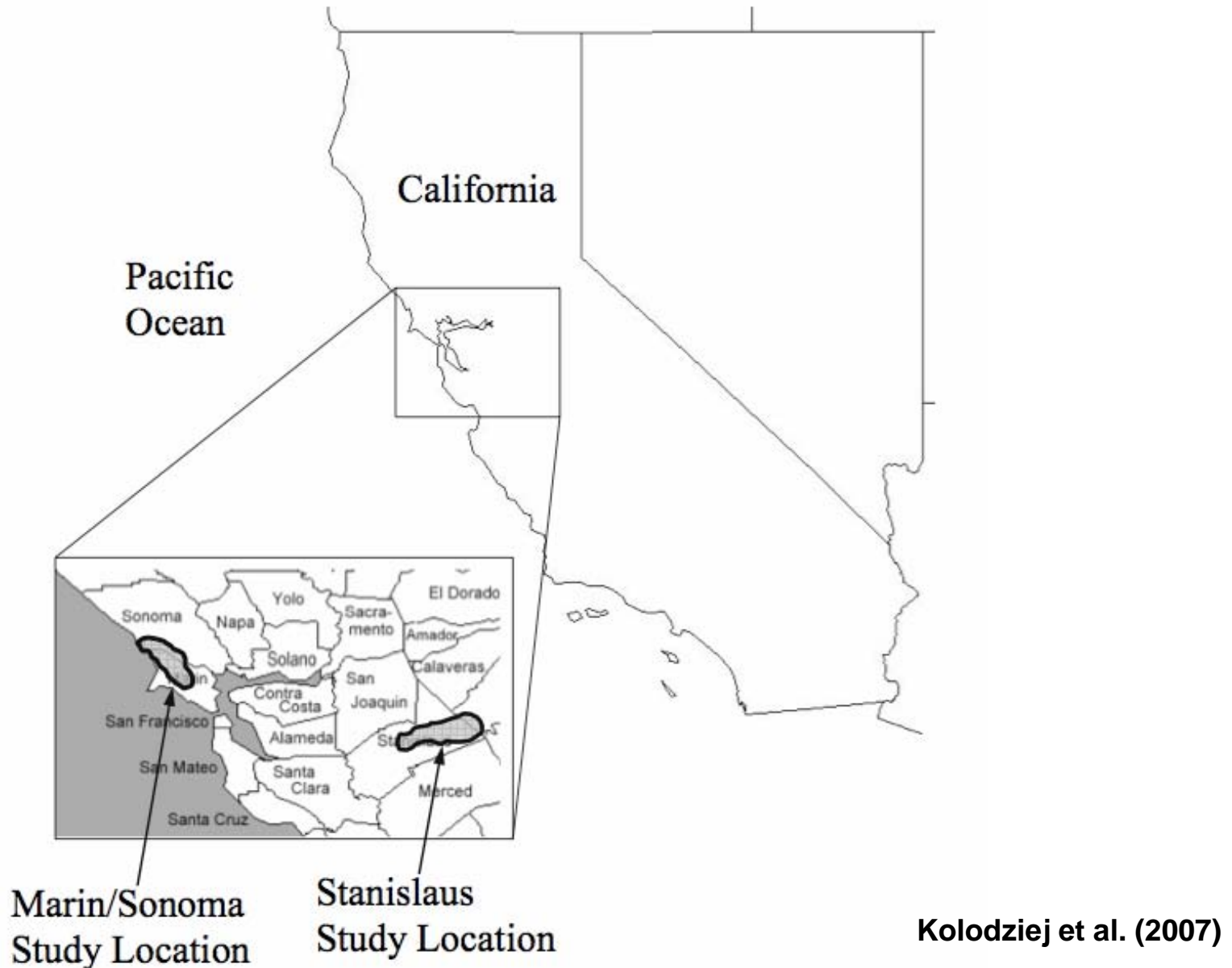


17α-20B-Dihydroxyprogesterone

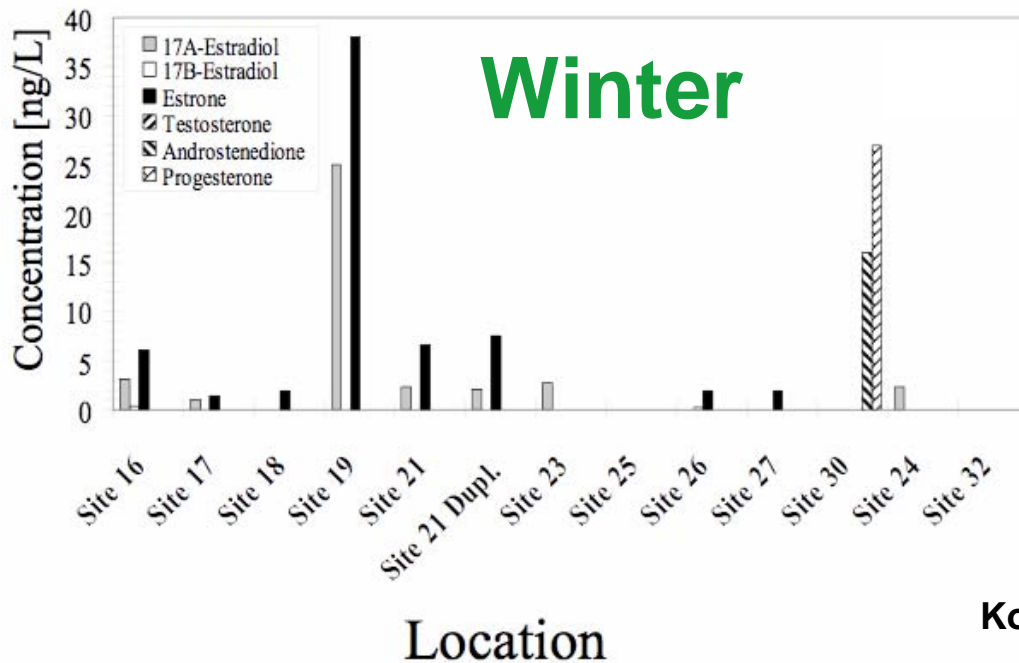
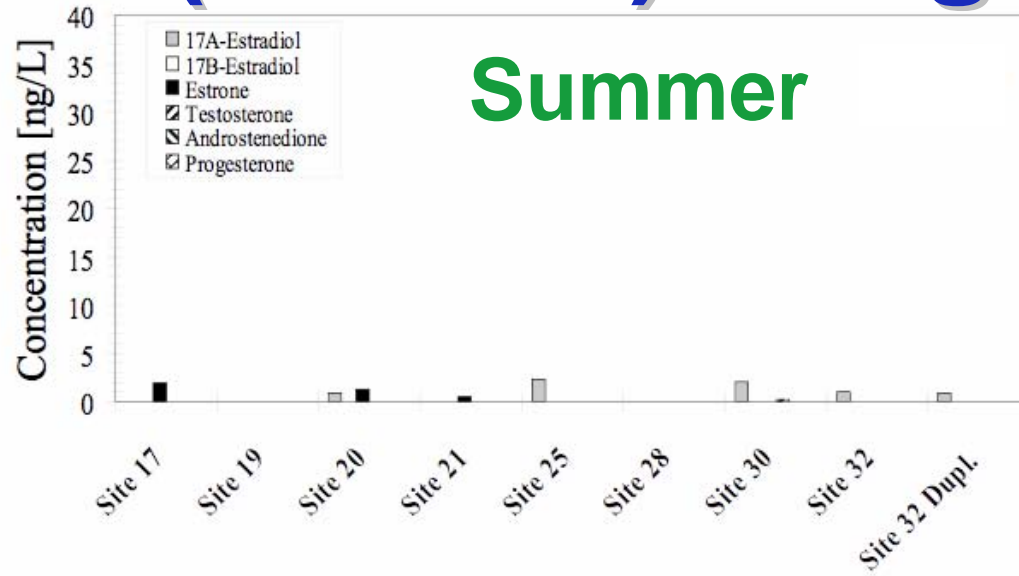
Grazing Rangelands



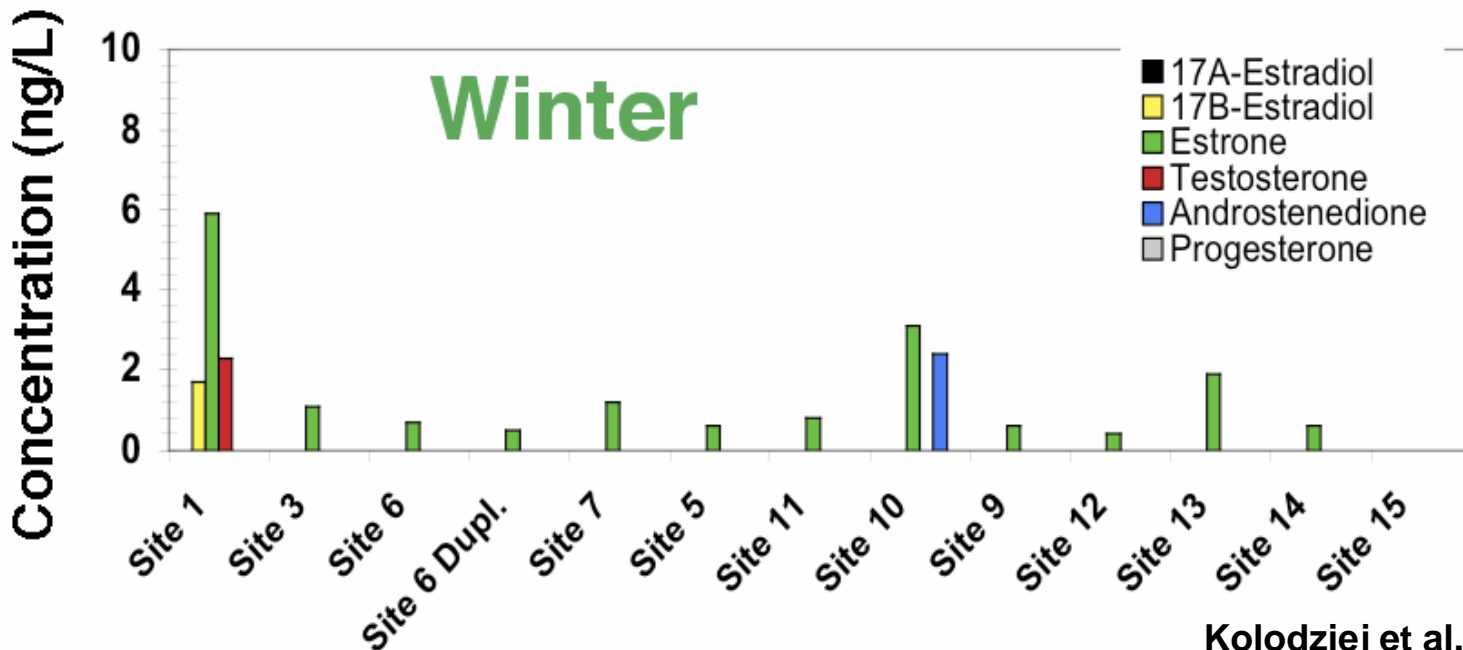
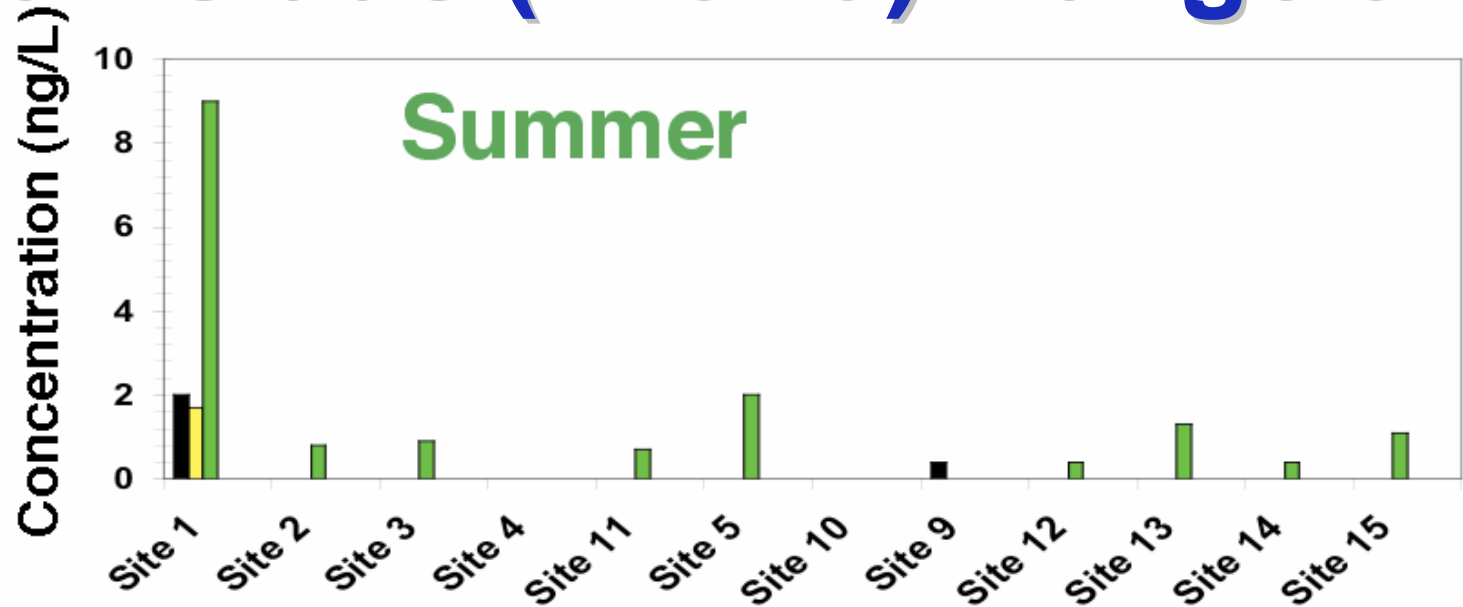
Grazing Rangelands



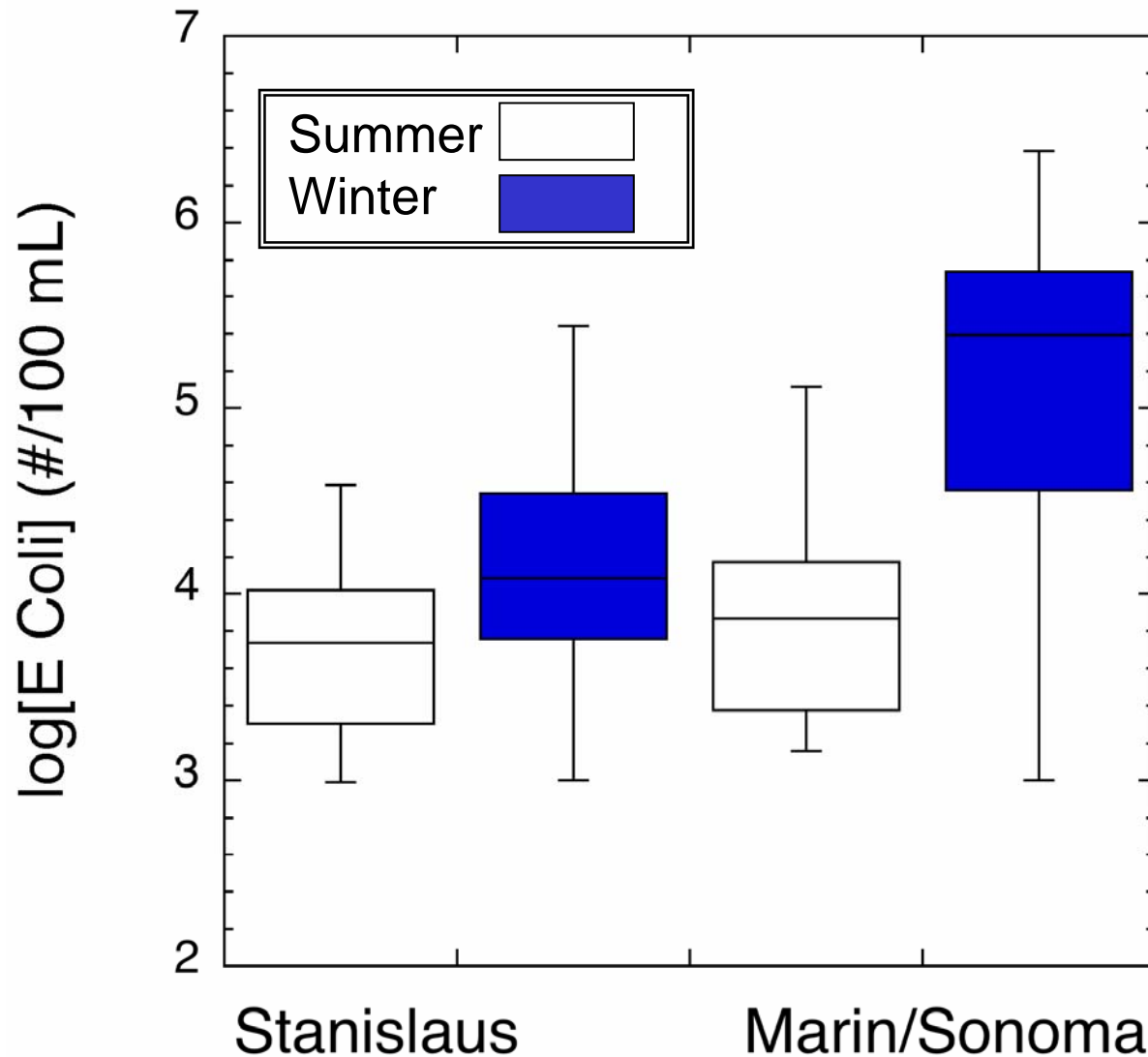
Marin (Coastal) Rangeland



Stanislaus (Inland) Rangeland



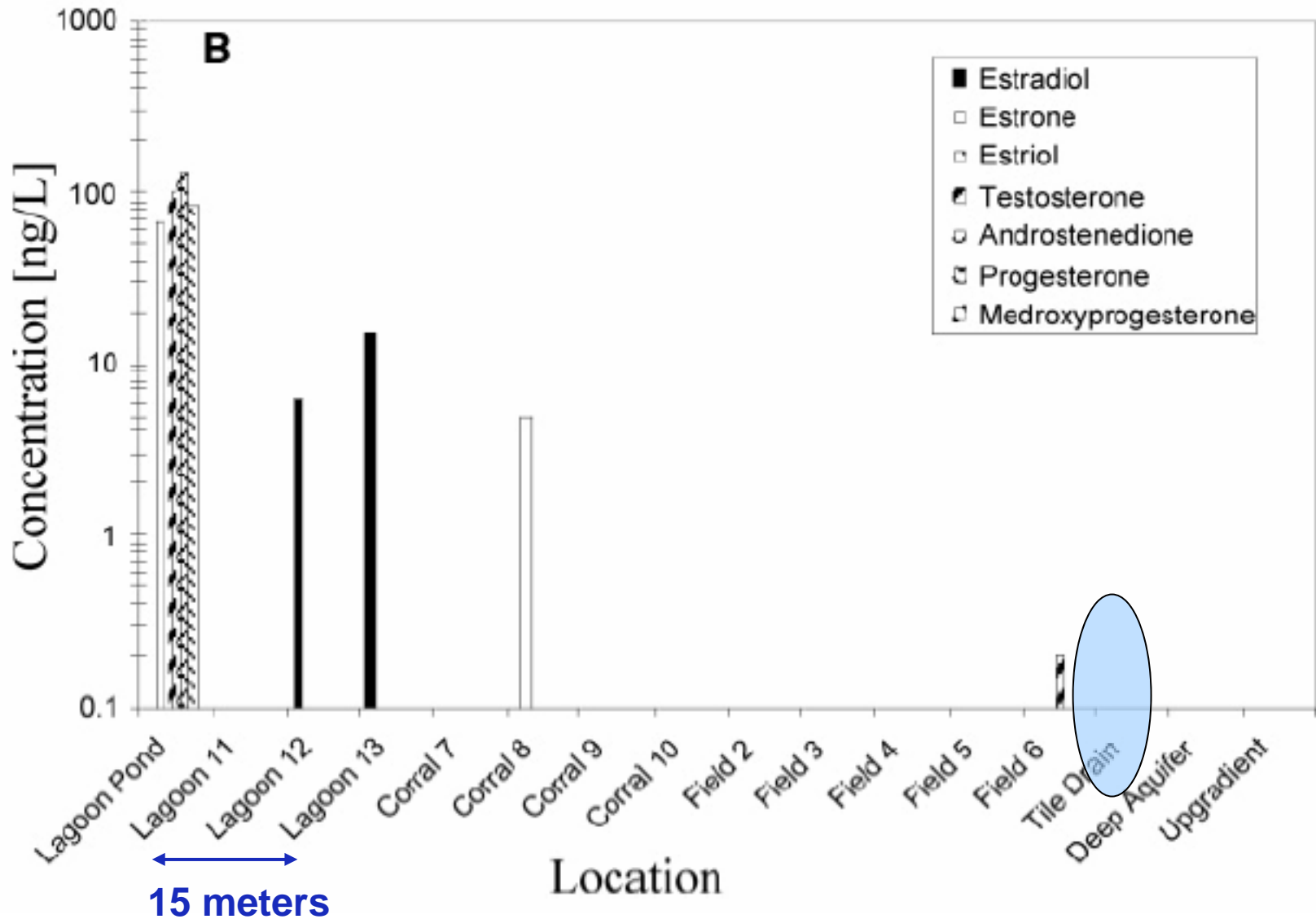
E. Coli as Indicator of Direct Discharge



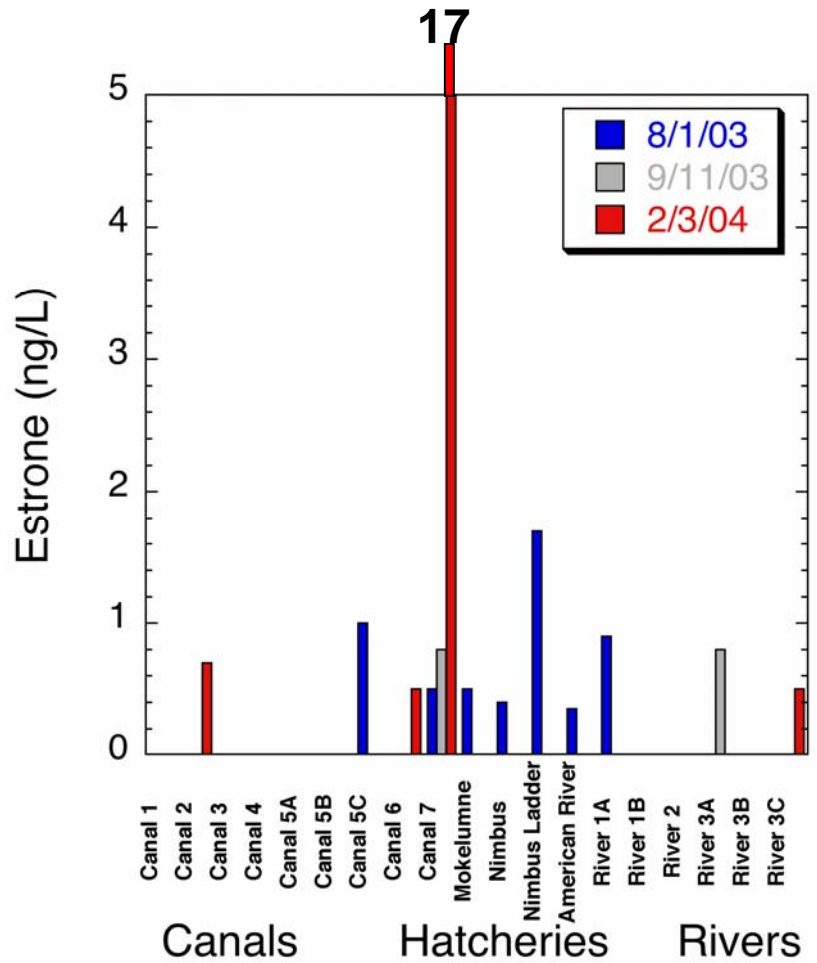
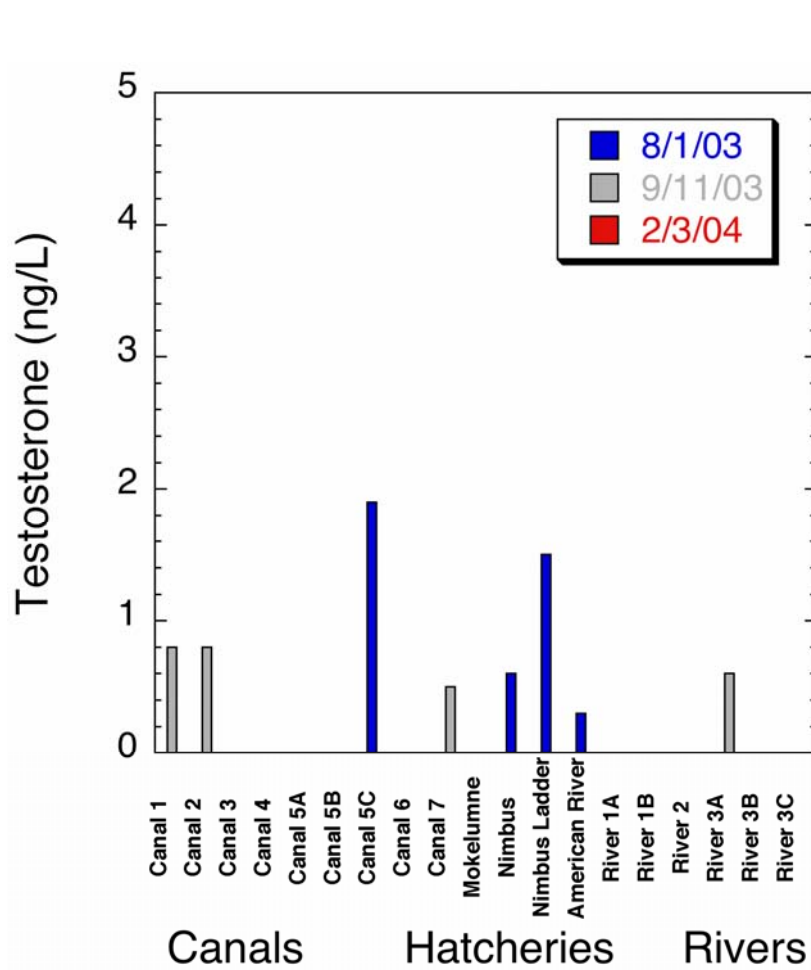
Dairy Watersheds



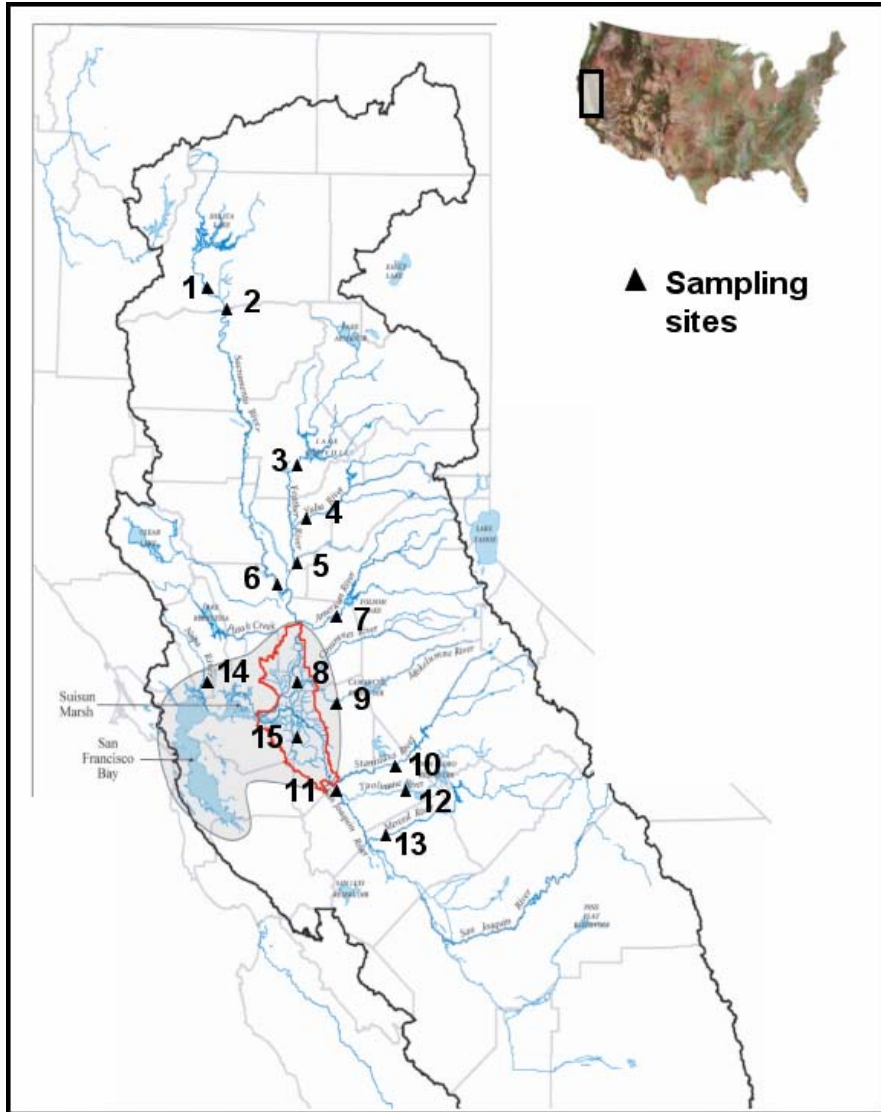
Attenuation in Groundwater



Dairy Canals and Local Rivers



Regional View (Current Project)



-Co-PI: Dan Schlenk (UCR)

-Sacramento River

-San Joaquin River

Winter/summer 2006/7

Water Extracts (~100 samples)

-Steroids

-APEs

-Bioassays

Bioassays



Water (4 L.)

Solid-phase
Extraction



C18 Disks

surrogates

10 mL MetOH

10 mL 40% MetOH

10 mL 75% MetOH

HFB derivatization

GC/MS/MS

Exposures *in vitro/in vivo*
(Rainbow trout)

Extract exposures

In vitro hepatocytes
exposure

In vivo whole fish
exposure



Primary Hepatocytes
isolation and culture



Intraperitoneal
extract injection (x2)

Extract added in the
media (0.6% v/v)

Incubation
24 h

Total mRNA extract

VTG mRNA
determination

Incubation
7 days

Plasma collection

VTG protein levels
determination

Hypotheses

- **Steroids are attenuated during groundwater infiltration.**
- **Surface runoff (wet/dry) is important to steroid transport.**
- **Synthetic steroids are more persistent than endogenous steroids.**
- **Common water quality parameters may provide insight into steroid hormone fate and transport.**

Method Development

- **Synthetic Steroids by GC/MS/MS**

<i>Synthetic Steroid</i>	<i>Type</i>	<i>Derivative</i>	<i>Endogenous Steroid</i>	<i>Type</i>	<i>Derivative</i>
17 α -Trenbolone	androgen	MSTFA-I ₂ ^a	17 α -Estradiol	estrogen	HFB ^c
17 β -Trenbolone	androgen	MSTFA-I ₂ ^a	17 β -Estradiol	estrogen	HFB ^c
Trendione	androgen	MSTFA-I ₂ ^a	Estrone	estrogen	HFB ^c
Melengestrol	progestin	MSTFA-I ₂ ^a	Estriol	estrogen	HFB ^c
Zeranol	estrogen	MSTFA-I ₂ (HFB) ^b	Testosterone	androgen	HFB ^c
β -Zearalanol	estrogen	MSTFA-I ₂ (HFB) ^b	Androstenedione	androgen	HFB ^c
Zearalanone	estrogen	MSTFA-I ₂ (HFB) ^b	Progesterone	progestin	HFB ^c

Field Sites

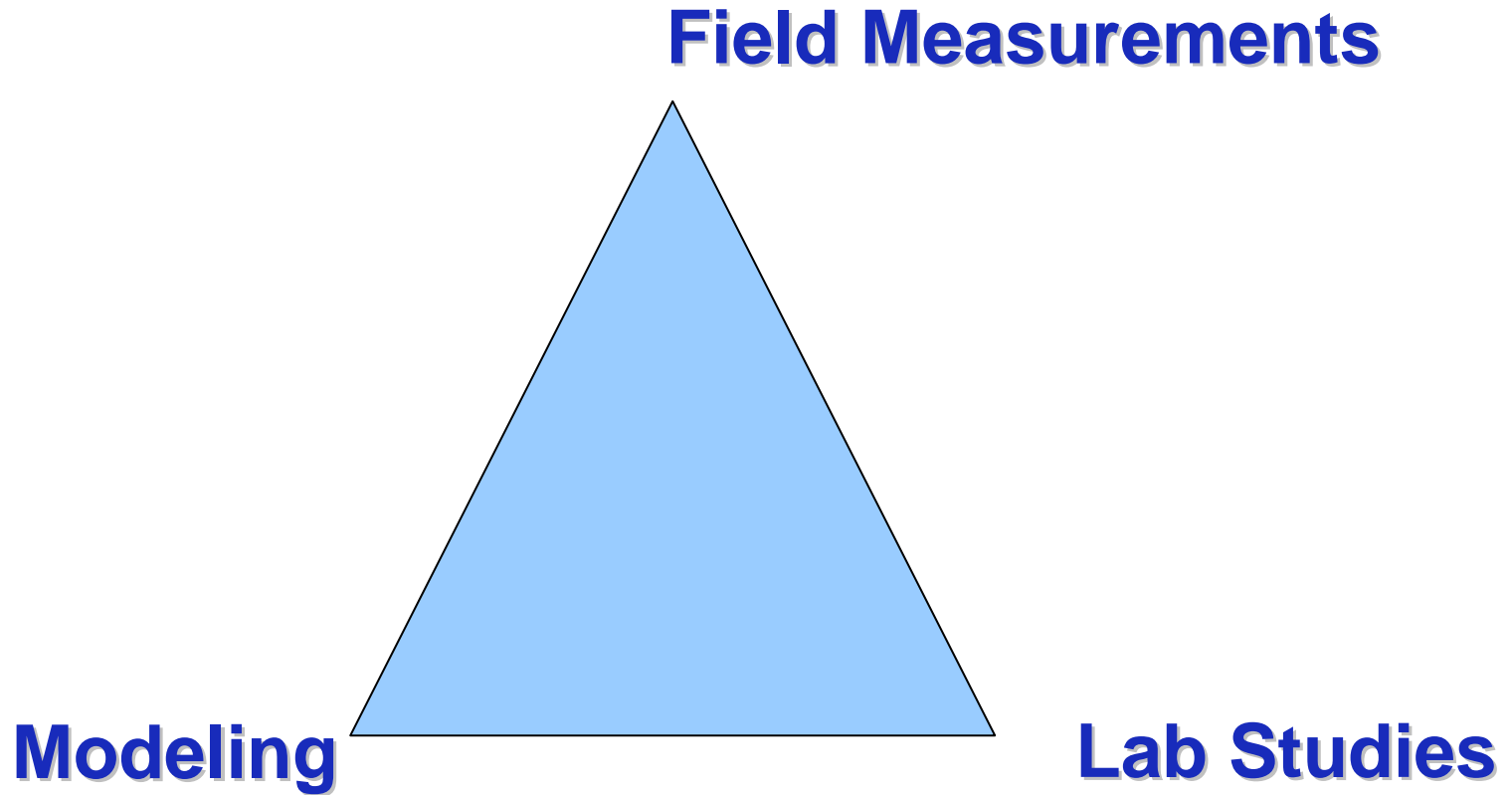
- **UC Davis Research Feedlot**
 - **Runoff studies: 20-30 animals treated with**
 - **Trenbolone & estradiol**
 - **Melengestrol acetate**
 - **Zeranol**
 - **Control**
 - **Soil attenuation**



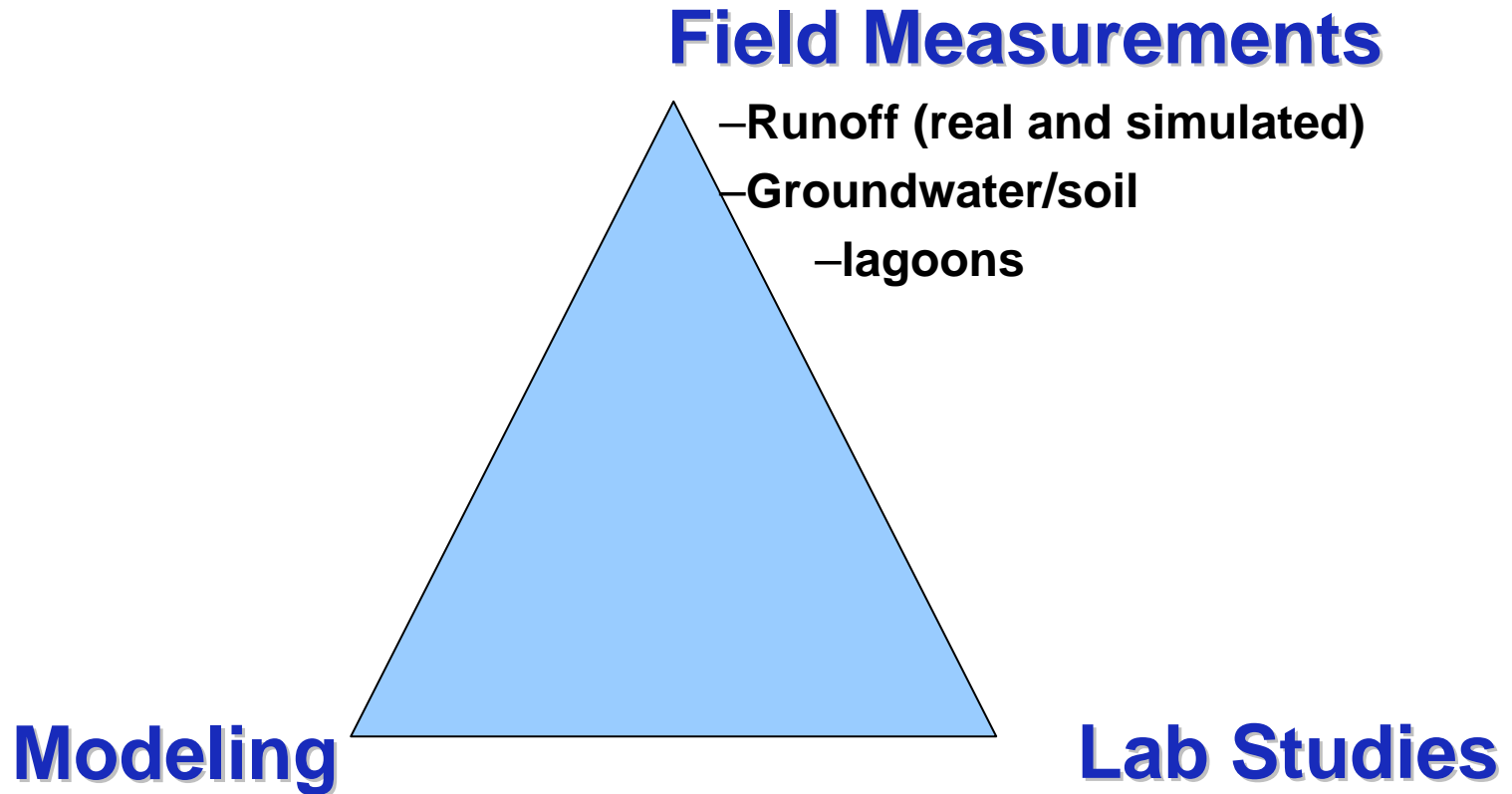
Field Sites

- **UC Davis Research Feedlot**
 - **Runoff studies: 20-30 animals treated with**
 - **Trenbolone & estradiol**
 - **Melengestrol acetate**
 - **Zeranol**
 - **Control**
 - **Soil attenuation**
- **4-6 California CAFOs (UC Cooperative Extension)**
- **2 Colorado CAFOs (Jessica Davis, Colorado State)**
- **Iowa CAFO (Robert Burns, Iowa State)**
- **California Dairies (Western United Dairymen)**

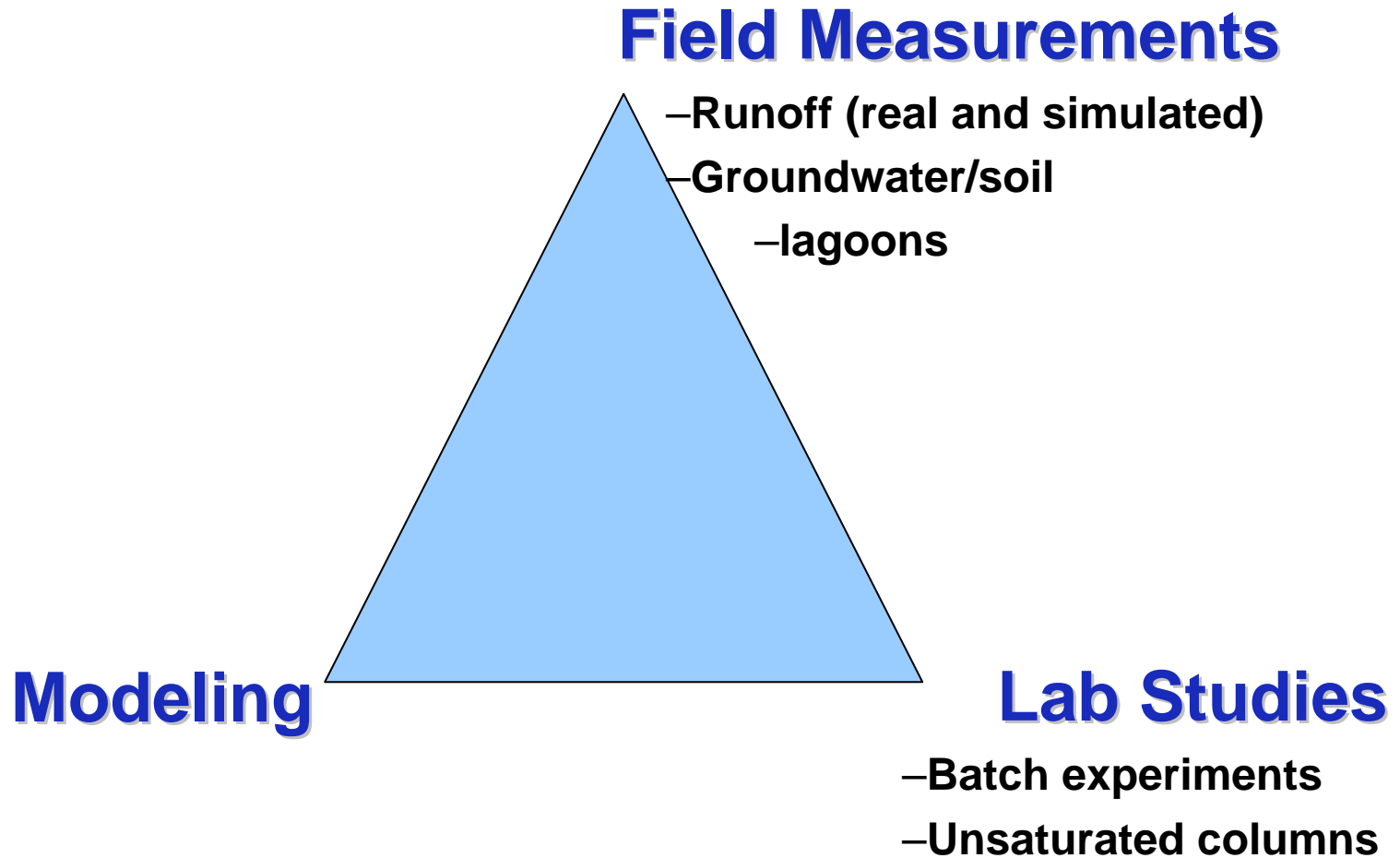
General Approach



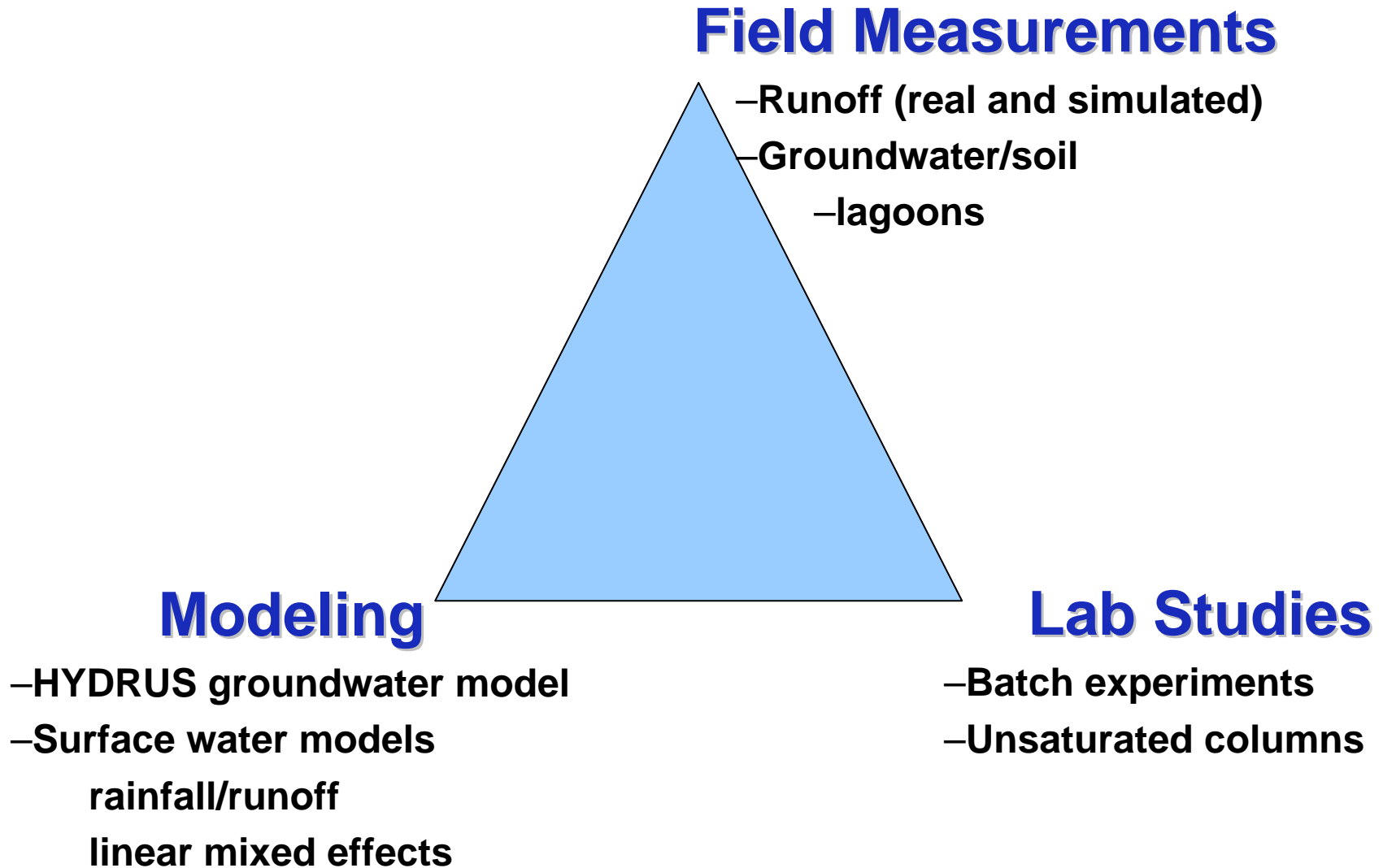
General Approach



General Approach



General Approach



Potential Collaborations

- **Analytical method development/testing**
- **Bioassays**
- **Model applications/water quality parameters**

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