

Sea turtle hatchling sex ratios determined via hormone assay: implications of climate change?

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Sea Turtle Species Listed Under the U.S Endangered Species Act (ESA)



Green-
Chelonia mydas



Hawksbill –
Eretmochelys imbricata



Loggerhead –
Caretta caretta



Leatherback –
Dermochelys coriacea



Kemp's Ridley –
Dermochelys coriacea



Olive Ridley –
Lepidochelys olivacea

Conservation Efforts

High Priority for U.S. National Marine Fisheries Service and U.S. Fish and Wildlife Service

Need to construct effective management approaches

- Critical Habitat
- Genetic Origin
- Demography

One critical piece of demographic data is the **Sex Ratio** of sea turtle populations

Sex Ratio data is important for determining sex-specific survival rates



Cannot use external morphology for sex determination of immature turtles

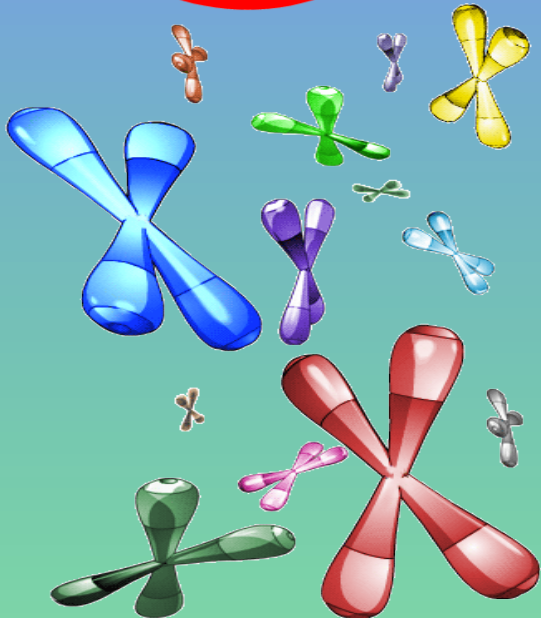
NMFS Research Permit #1591



Cannot use Genetics for sex determination of immature turtles

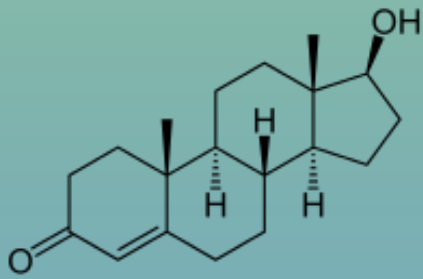
NO SEX CHROMOSOMES

Sea turtle sex is temperature dependent



Warmer = Female

Cooler = Male



Hormone Concentration

Then: Radioimmunoassay (RIA)

Now: Enzyme-linked Immunosorbent Assay (ELISA)

- Cheaper, quicker results, sensitive, and no radioactive materials

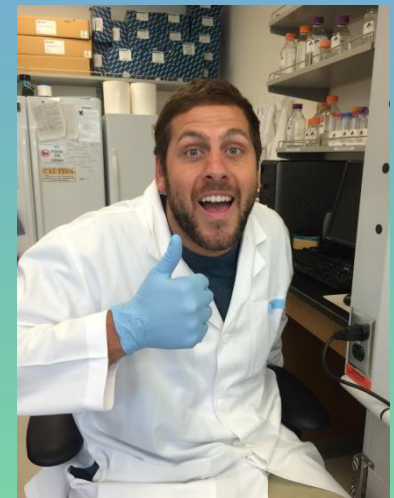


Lower Testosterone Concentration = Female



Higher Testosterone Concentration = Male

User Friendly



Past, Present, and Future?

- ENZO testosterone ELISA validated for use with the six species listed under the ESA
 - Effective at determining sex of immature sea turtles
 - Cross-lab analysis with RIA shows ELISA is as effective
- Global female bias

Immature Green Turtle Sex Ratio (F:M)	Location
0.96:1.0	Hawaii
1.40:1.0	Bahamas
2.0:1.0	Heron Island, Australia
3.26:1.0	Shoalwater Bay, Australia
3.5:1.0	San Diego Bay, California
4.0:1.0	Sabah, Malaysia
4.2:1.0	Clack Reef, Australia

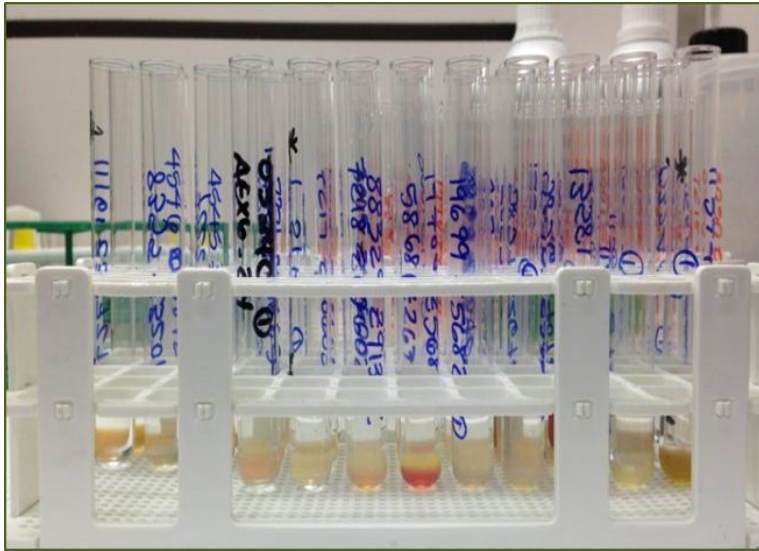
My Project

1. Validate testosterone (T) and estradiol (E) ELISA for use with loggerhead sea turtle plasma
2. Measure the concentration of both T and E in hatchling plasma samples
3. Analyze the E:T ratios and assign sex to loggerhead hatchlings
4. Compare assigned sex to known sex



Methods

Day 1- Hormone Extraction



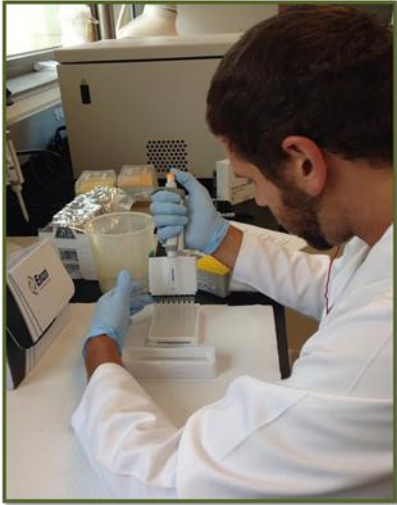
Followed D. W. Owen's lab extraction methodology

(Wibbles et al. 1987)

1. 50 uL plasma
2. Ether added to plasma
3. Frozen with liquid nitrogen
4. Ether layer decanted
5. Dried down
6. Re-suspended with acetone
7. Dried down overnight

Methods

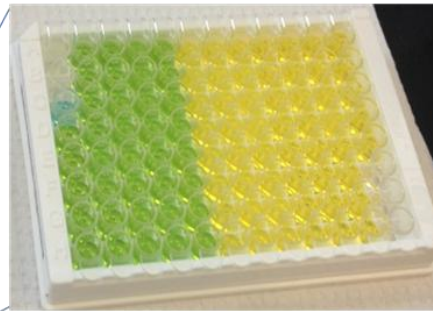
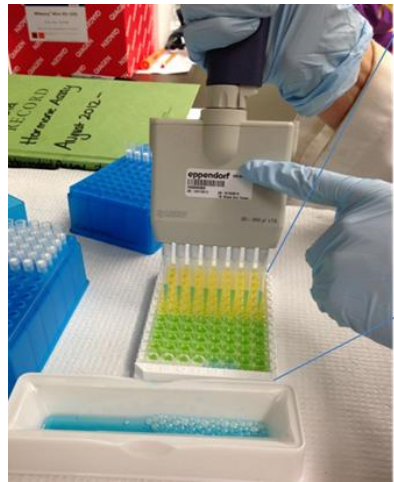
Day 2- Hormone Assays



ENZO High Sensitivity Testosterone

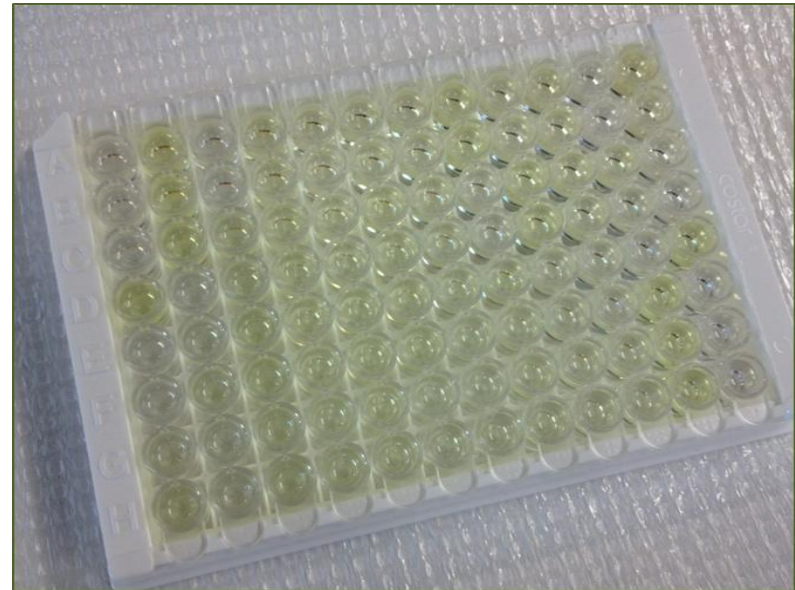
ARBOR ASSAYS Estradiol

Quantify hormone concentration via a colorimetric competitive enzyme immunoassay



ENZO Testosterone

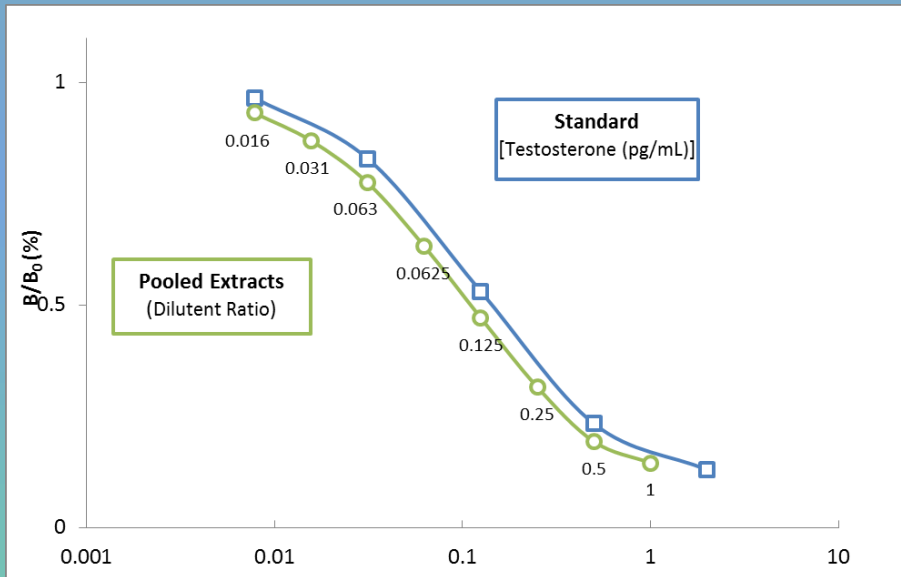
Final Plate Color Reaction



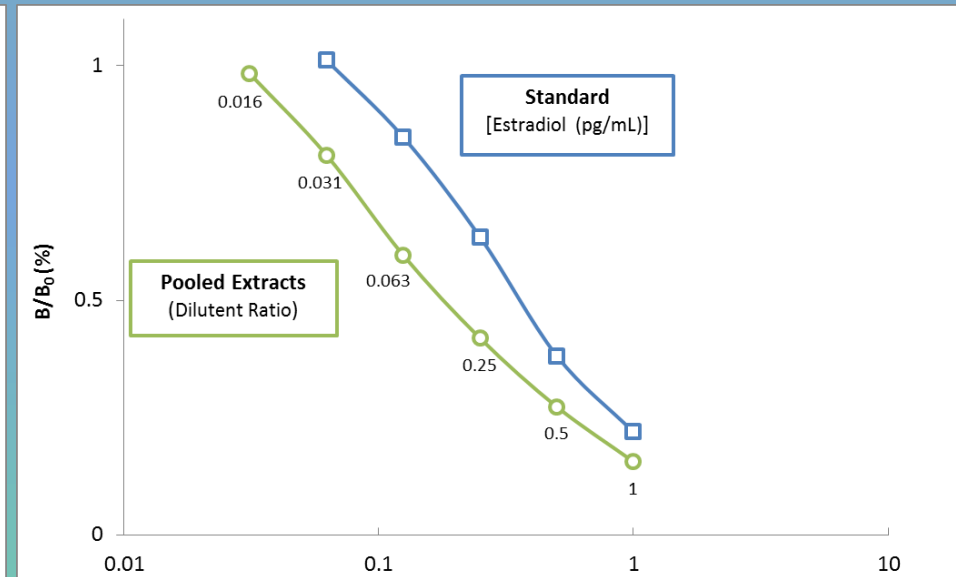
Results - Assay Validations

Parallelism/linearity test demonstrated that the assay detects hormones in plasma samples

ENZO High Sensitivity Testosterone



ARBOR ASSAYS Estradiol



Results – Assigned Sex

	n	Estradiol Mean ± STD Range (pg/mL)	Testosterone Mean ± STD Range (pg/mL)	E:T Ratio
Males	25	9.1 (n = 1)	28.4 ± 19.9 3.1 – 88.8	0.7 (n = 1)
Females	20	39.2 ± 20.1 13.4 – 80.0	20.2 ± 8.2 9.5 – 34.2	1.6 (n = 13)
Unknown	13	Not Detectable	Not Detectable	-

Results- Comparison to known Sexes

- Data sent to collaborator.... We are awaiting confirmation of predicted sexes

Acknowledgements



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More Neat Pics



Questions?

