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)

- **Threatened/Endangered**: Green - *Chelonia mydas*
- **Endangered**: Hawksbill – *Eretmochelys imbricata*
- **Threatened**: Loggerhead – *Caretta caretta*
- **Endangered**: Leatherback – *Dermochelys coriacea*
- **Endangered**: Kemp’s Ridley – *Dermochelys coriacea*
- **Threatened**: 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
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

<table>
<thead>
<tr>
<th>Immature Green Turtle Sex Ratio (F:M)</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.96:1.0</td>
<td>Hawaii</td>
</tr>
<tr>
<td>1.40:1.0</td>
<td>Bahamas</td>
</tr>
<tr>
<td>2.0:1.0</td>
<td>Heron Island, Australia</td>
</tr>
<tr>
<td>3.26:1.0</td>
<td>Shoalwater Bay, Australia</td>
</tr>
<tr>
<td>3.5:1.0</td>
<td>San Diego Bay, California</td>
</tr>
<tr>
<td>4.0:1.0</td>
<td>Sabah, Malaysia</td>
</tr>
<tr>
<td>4.2:1.0</td>
<td>Clack Reef, Australia</td>
</tr>
</tbody>
</table>
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

Credit: Blair Witherington, FL FWC
Methods

Day 1- Hormone Extraction

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

Followed D. W. Owen’s lab extraction methodology
(Wibbles et al. 1987)
Methods
Day 2- Hormone Assays

ENZO High Sensitivity Testosterone

ARBOR ASSAYS Estradiol

Quantify hormone concentration via a colorimetric competitive enzyme immunoassay

Final Plate Color Reaction

ENZO Testosterone
Parallelism/linearity test demonstrated that the assay detects hormones in plasma samples.
## Results – Assigned Sex

<table>
<thead>
<tr>
<th>Assigned Sex</th>
<th>n</th>
<th>Estradiol Mean ± STD Range (pg/mL)</th>
<th>Testosterone Mean ± STD Range (pg/mL)</th>
<th>E:T Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>25</td>
<td>9.1 ± 19.9 (n = 1)</td>
<td>28.4 ± 19.9 3.1 – 88.8</td>
<td>0.7 (n = 1)</td>
</tr>
<tr>
<td>Females</td>
<td>20</td>
<td>39.2 ± 20.1 13.4 – 80.0</td>
<td>20.2 ± 8.2 9.5 – 34.2</td>
<td>1.6 (n = 13)</td>
</tr>
<tr>
<td>Unknown</td>
<td>13</td>
<td>Not Detectable</td>
<td>Not Detectable</td>
<td>-</td>
</tr>
</tbody>
</table>
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?