# Sex Ratio Assessment of Endangered Kemp's Ridley Sea Turtle Foraging Populations: Validation of a Testosterone ELISA for Juvenile Sex Determination

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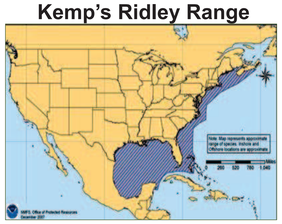
## Background and Introduction

Currently all species of sea turtles are listed as threatened or endangered with extinction under the U.S. Endangered Species Act. Due to their status, sea turtle conservation is a high priority for the U.S. National Marine Fisheries Service and U.S. Fish and Wildlife Service. The Kemp's ridley sea turtle *Lepidochelys kempii* is the smallest and perhaps the most critically endangered species of sea turtle.

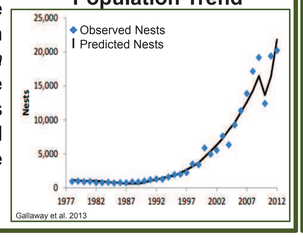
In order to effectively construct management approaches much information is needed for various sea turtle populations including demography, genetic origin, and critical habitat. One demographic piece of data that is lacking, is the sex ratio of turtle populations in foraging habitats. This data is integral to determining population abundance and ultimately informing management decisions. Because secondary sex characteristics (i.e. males have longer tails) are not evident until turtles start to reach sexual maturity, the sex of juvenile turtles cannot be easily determined externally. The least invasive way to determine the sex of juvenile turtles is through hormone analysis (testosterone) of the blood plasma. Radioimmunoassay (RIA) is the most commonly used method to determine hormone concentration in turtle plasma; we used a new technique, an enzyme-linked immunosorbent assays (ELISA), which more cost effective and user friendly than the RIA. The testosterone (T) ELISA has recently been validated for use with green sea turtle *Chelonia mydas* plasma but has yet to be validated for the other sea turtle species. The goal of this study was to validate the ELISA assay for Kemp's ridleys and subsequently compare the results of the same samples that were run on ELISA and RIA.

**Kemp's Ridley Arribada**


**Kemp's Ridley Range**



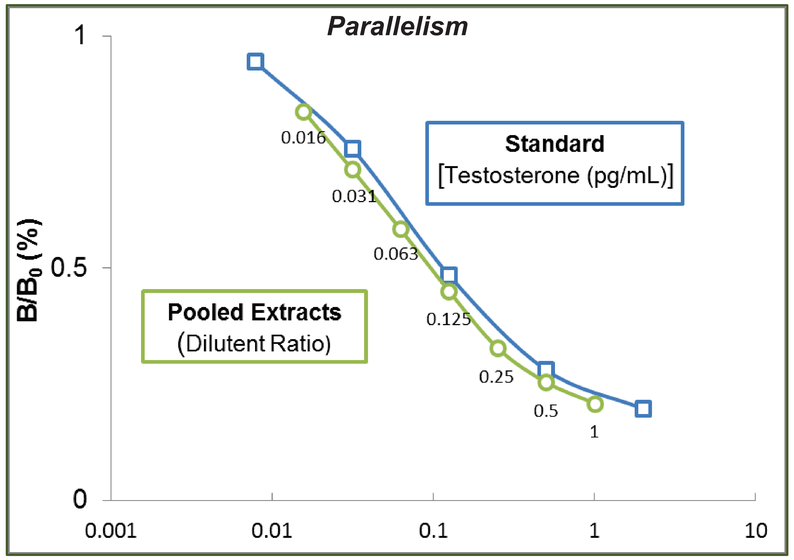
**Kemp's Ridley Population Trend**




Gallaway et al. 2013

## Results 1 – Assay Validation

**Testosterone assay measured the same antigen in the standard controls and plasma extracts**




***Quality Control***

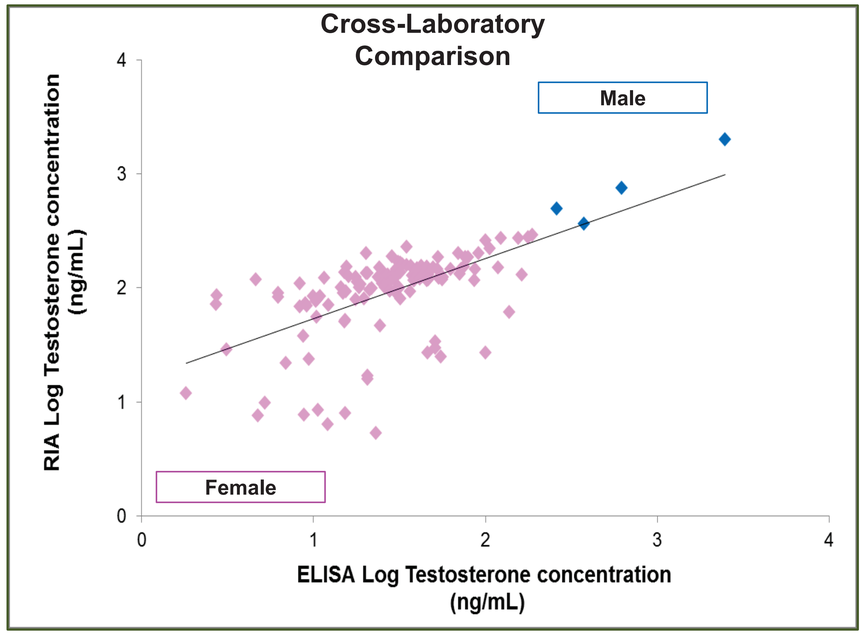
**Extraction Efficiency** = 99.6%

**Inter-Assay Variation** = 10.6%

**Intra-Assay Variation** = 9.5%

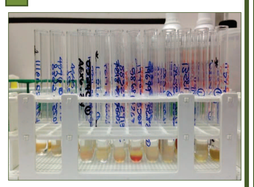
## Results 2 – ELISA and RIA Comparison

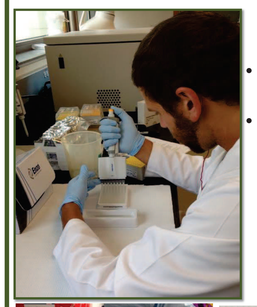
**Good correspondence between the two assays**




## Methods

- ~140 Kemp's ridley plasma samples were sent to us from the NOAA Southeast Fisheries Science Center



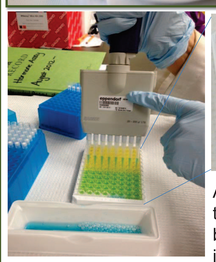


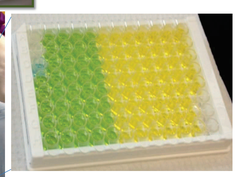
### Extraction

- Hormones were extracted and isolated from blood plasma following previously published method by Wibbels *et al.* (1987)
- Ether added to plasma → frozen with liquid nitrogen → ether layer decanted → dried down → re-suspended with acetone → dried down overnight

### Testosterone ELISA

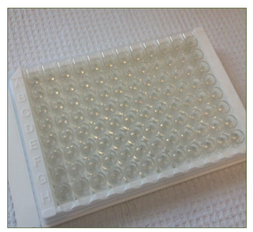
- We quantified testosterone concentration via a colorimetric competitive enzyme immunoassay (ELISA)





Addition of Blue Conjugate to Yellow Antibody between first and second incubation periods

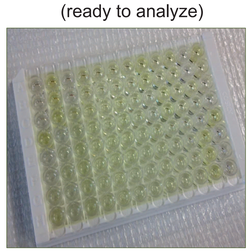
Before Final Incubation



Reaction Occurs

1 hour incubation period 37ºC

After Final Incubation (ready to analyze)



## Discussion / Conclusion

- The parallelism test demonstrated excellent correspondence indicating that the assay was detecting the testosterone in the plasma
- The intra- and inter-assay variations were also low, showing that I had good precision when pipetting the same sample on a single plate multiple times and the assay consistently provided very similar results for samples ran on multiple assays
- The cross laboratory comparison showed that the ELISA method is just as effective for determining sex as the Radioimmunoassay (RIA), however, the correspondence between assays at the lower concentrations (<50 pg/mL) was not as good. The variation between the two techniques could be attributed to a few factors:
  - First, they are entirely different assays being run by different lab technicians
  - Second, plasma samples are not from the same tube, but rather duplicate samples
  - Finally, the majority of the samples were collected over 10 years ago and it is possible the hormones degraded slightly


Juvenile green turtle (size of an adult Kemp's ridley)


Photographing an adult green turtle's (Donna) plastron


## Acknowledgements

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