Preliminary sea turtle hatchling gender analysis in the face of Climate Change using TSOX9, DMRT1, β-ACTIN, and FOXL2 mRNA

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Background

• Primary sea turtle conservation focus
  • Nesting females
  • Census counts

• Deposited eggs
  • 55 – 75 day incubation
  • Assumed 50/50% male/female

• Paucity of information on
  • Hatchling success
  • Hatchling gender
  • Climate change
The Potential Problem(s) associated with Climate Change

- Nesting Ground Loss
- Sea Level Rise
- Acid Rain (EXTREME WEATHER)
- Storms, Rough Seas
- Nesting Ground Loss, Sea Level Rise
- Hatchling Impacts
- Egg Shell vulnerability
- Nesting events
- Disease
- Sand Temperature

Gender

Migration nesting patterns
The Gender ‘Catch 22’

- Gender either
  - X-Y chromosomes
  - Environmental sex determination
    - Temperature-dependent sex determination (TSD)

- TSD in Sea turtles
  - Pivotal temperature, 50/50%
    - Hawksbill 29.2 °C (Mrosouvsk et al. 1992)
    - Greens 29.2-29.3 (Godfrey and Mrosovsky 2006)
    - Loggerheads 29.3 °C (Mrosovsky et al., 2002)
    - Leatherbacks 29.5°C (Furler,2005)
    - Olive Ridley 30.5-32.5 °C (Wibbels et al 1998)
Our Questions

Given that air temperature is increasing....

1. Is gender 50/50 ratio at risk?
2. What is the potential effect at nest depth and grain size?

Intervention needs for management of nesting for increased hatchling survival?
Sex ID – Phenotypic

1. Length of tail
2. Position of cloaca
3. Gonadal isolation (incl. urogenital ridge)

(Wyneken, n.d.)
Sex ID - Genotypic

1. Blood

2. Probing of urogenital ridge
   - Time
   - Temp

3. Tissue (urogenital ridge)
   - mRNA signals
   - Gonadal hormones ID
     ✓ DMRT1
     ✓ tSOX9
     ✓ Dax1
Dmrt1 is conserved in vertebrates

Kettlewell et al. 2000

(Valenzuela 2008)
Preliminary Gender Study

• 56 tissue samples
  • 6 immature hatchling (IH)
  • 15 hatchling (H)
  • 9 Juvenile (J)
  • 26 confirmed females (NF)

- 5 Green
- 41 Hawksbill
- 10 no ID

• RNA extracted
  • (Qiagen Rneasy Kit)

• PCR
  • 4 target genes amplified with PCR
  • Visualized on 1.6% agarose gel
Genes of interest

(Valezuela 2008)
Preliminary Gender Study

(H/Ju)
GR  100% F
HB  85% F
UNK 71.4% F

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<th>Species</th>
<th>Type</th>
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<th>DMRT1</th>
<th>ACTIN</th>
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<th>Hatchling GENDER</th>
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Male 27%
NF 46%
IH 11%
J 16%
How does this relate to impacts of climate changes?

• Impact of air temperature on sand temperature?

• Role of beach characteristics?

• Darker sands warmer than lighter sands (Fadini et al. 2011)

**Methods**

- Hobo temps
- Shade v exposed
- Multiple depths
- Spatial v temporal

(Anon n.d.)
Air Temperatures

Varies greatly by site from national weather – important to **MEASURE TEMPERATURE AT NESTING SITE**
Sand Temperature

(Charles 2017)

- Temp at 80cm varies depending on proximity to foliage and water

Grande Riviere

Shade

• Temp at 80cm varies depending on proximity to foliage and water

Grande Riviere - Site 3

Grande Riviere - Site 4

Grande Riviere - Site 5
- TC has cool sand temp
  - More males?
  - Low hatchling success?

- LT and SP deeper sands warmer
  - LT = females or low hatchling success
  - LT & SP = males in shade? Females in sun?
Sand Grain Size

Course sand warmer than finer sands (Júnior and Castro 2003)

Helps explain why LT and SP warmer than TC

Methods
- ½” diam. corer
- Sieved per depth section (10 – 50cm)
- Weighed (g)
Preliminary Conclusions

• Trinidad and Tobago are already seeing higher % female hatchlings!
  • 83% of hatchlings tested female
  • Maybe linked to coarse sands
  • Possible to lose hatchlings due to excessive temps

• Beaches with very fine sands may produce more males

• Need to test
  • More Hatchlings and more species
  • Expand genes and mRNA analysis
  • Extend temperature experiment during active nesting season... *use nesting boxes?*
Thank You.....
Merci!

Any questions?