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

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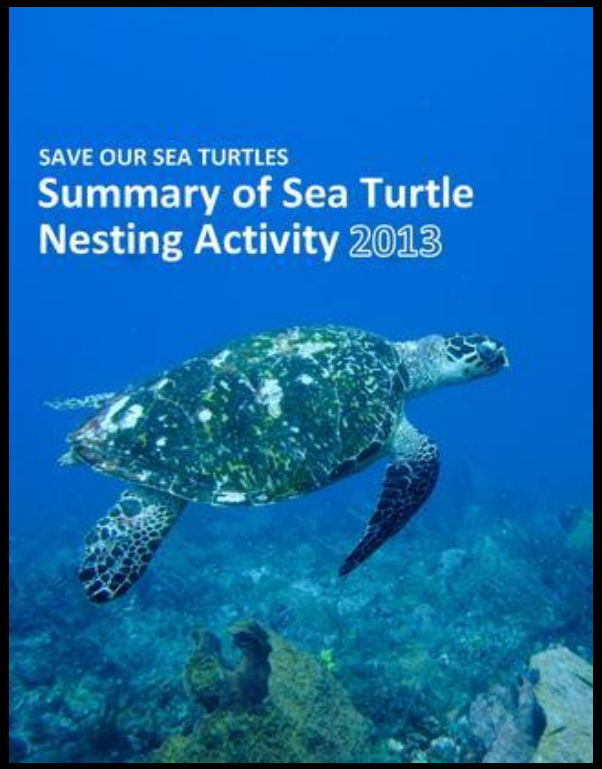
Background

- 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



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The Potential Problem(s) associated with Climate Change ???

Hatchling Impacts

↑ Acid Rain

↑ Egg Shell vulnerability

↓ Nesting events

? Gender

↑ Disease

↑ Sand Temperature

↑ Storms, Rough Seas

↑ Nesting Ground Loss
Sea Level Rise

EXTREME WEATHER

? Migration
nesting patterns

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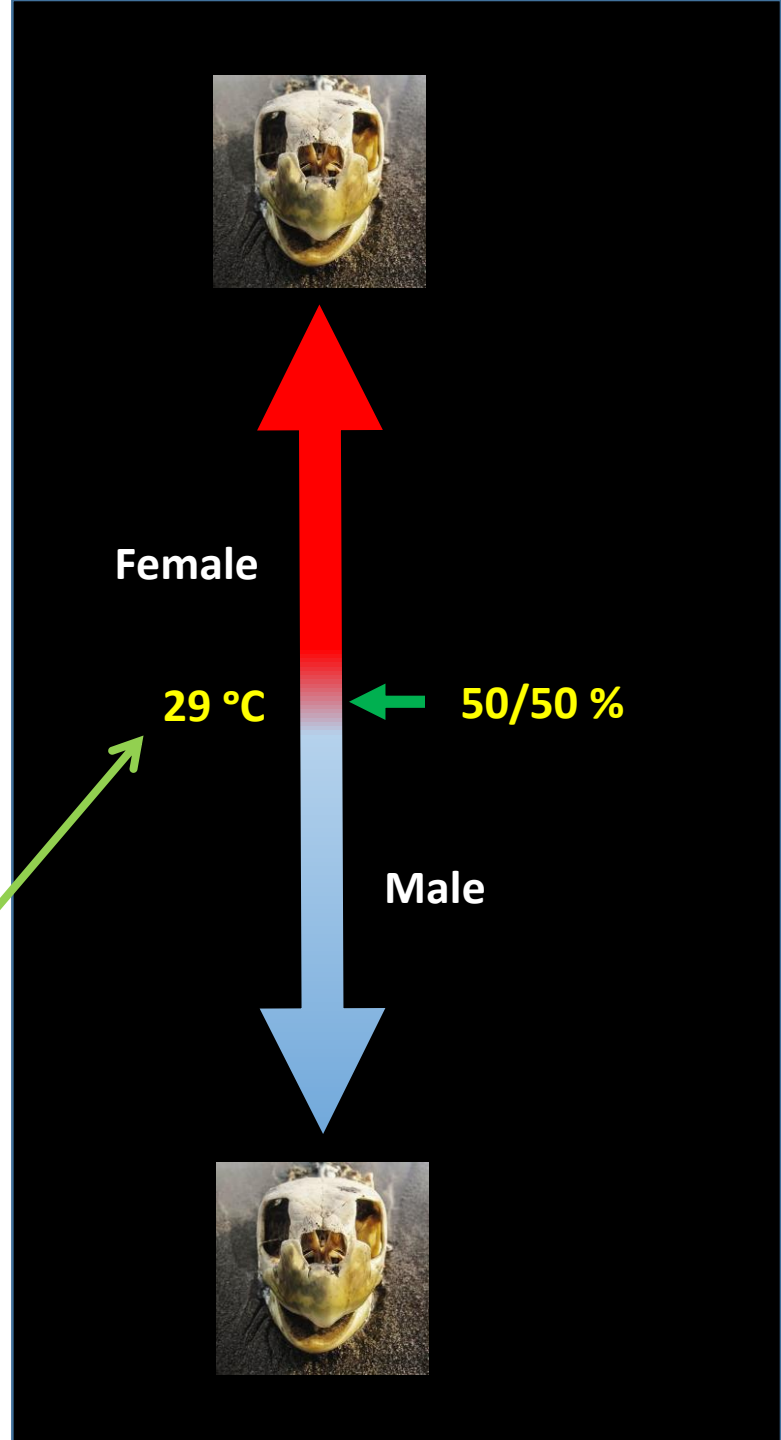
The Gender ‘Catch 22’

- 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 *(Mrosovsk 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?

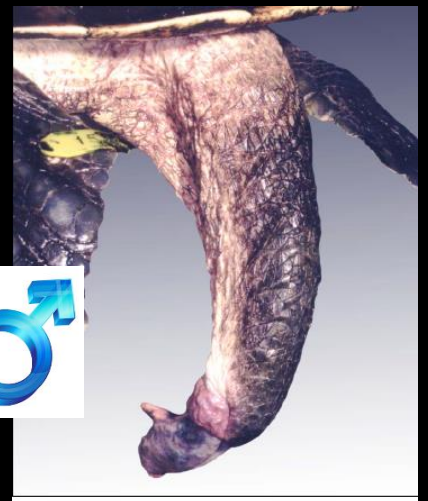
Intervention needs for management of nesting for increased hatchling survival?

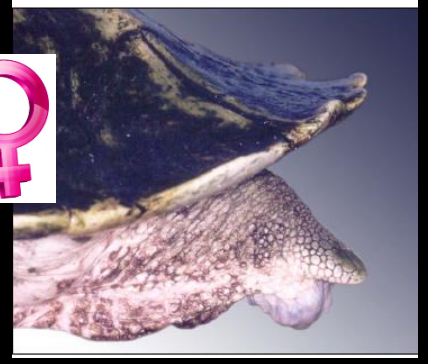
?

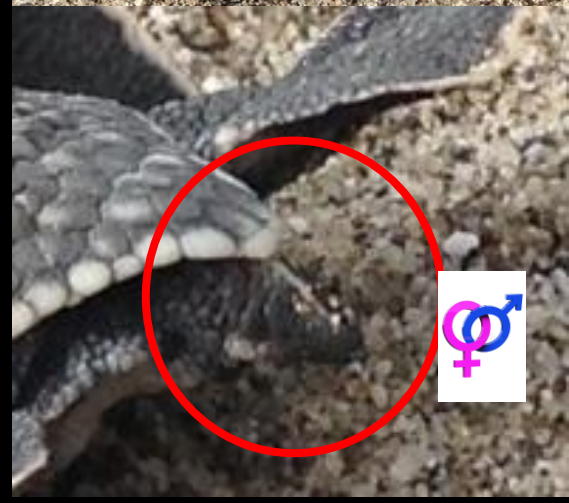
(Wyneken, n.d)

Sex ID – Phenotypic

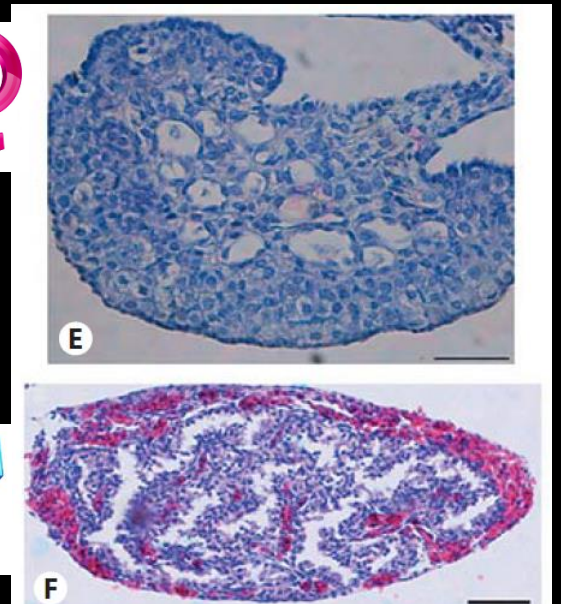
1. Length of tail
2. Position of cloaca
3. Gonadal isolation (incl. urogenital ridge)

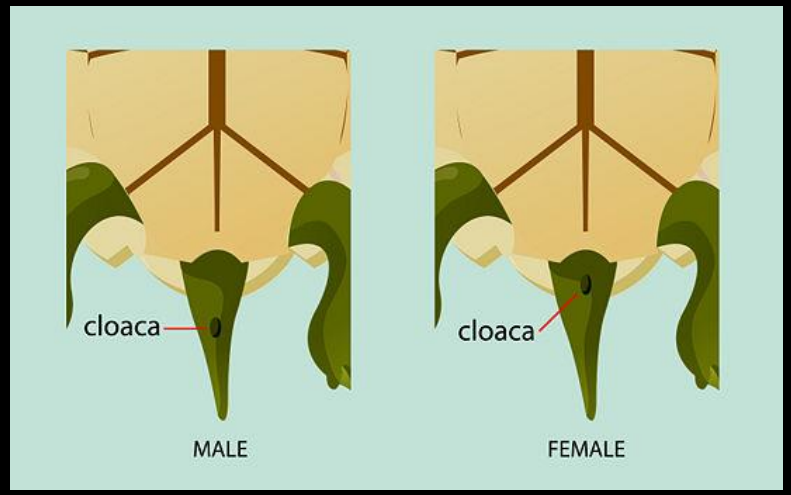






(Bieser & Wibbels 2014)



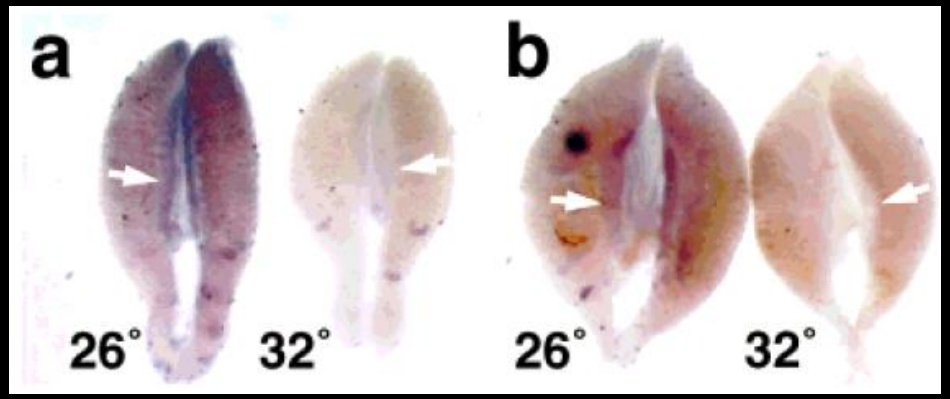


Sex ID - Genotypic

1. Blood

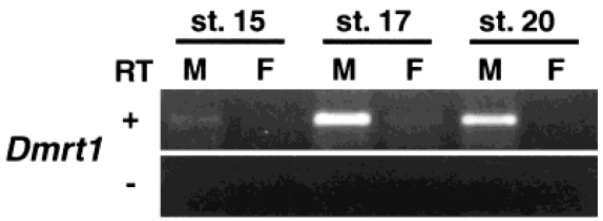
2. Probing of urogenital ridge
   - Time
   - Temp

3. Tissue (urogenital ridge)
   - mRNA signals
   - Gonadal hormones ID
     - ✓ *DMRT1*
     - ✓ *tSOX9*
     - ✓ *Dax1*

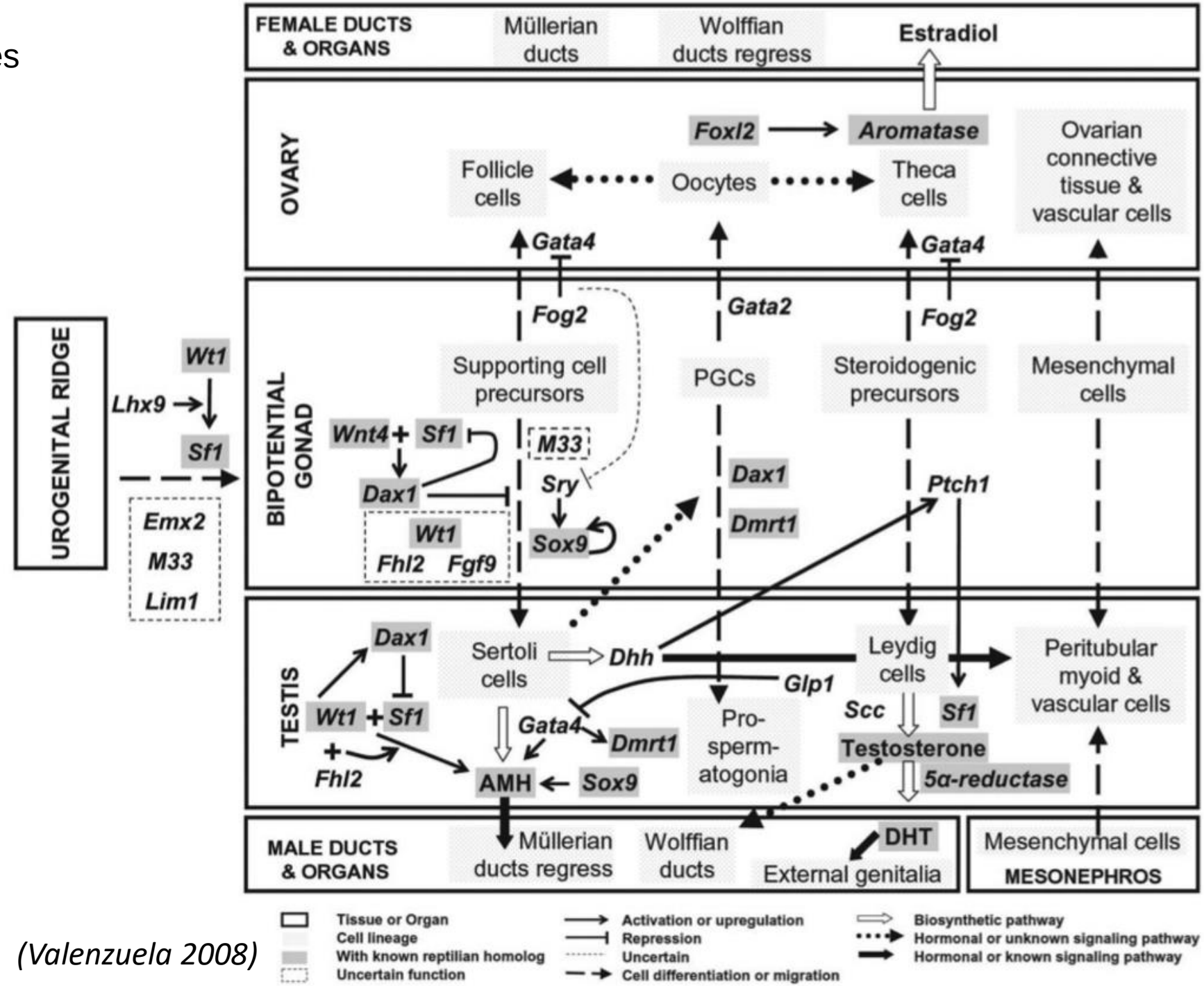


	TEMP.	EARLY		LATE		GONAD
Model 1:	26 deg.	gene X ON	→	HIGH Dmrt1	→	Testis
	32 deg.	gene X OFF		LOW Dmrt1		Ovary
Model 2:	26 deg.	Dmrt1 ON (LOW)	→	HIGH Dmrt1	→	Testis
	32 deg.	Dmrt1 OFF (LOW)		LOW Dmrt1		Ovary

Dmrt1 is conserved in vertebrates



Kettlewell et al. 2000



(Valenzuela 2008)

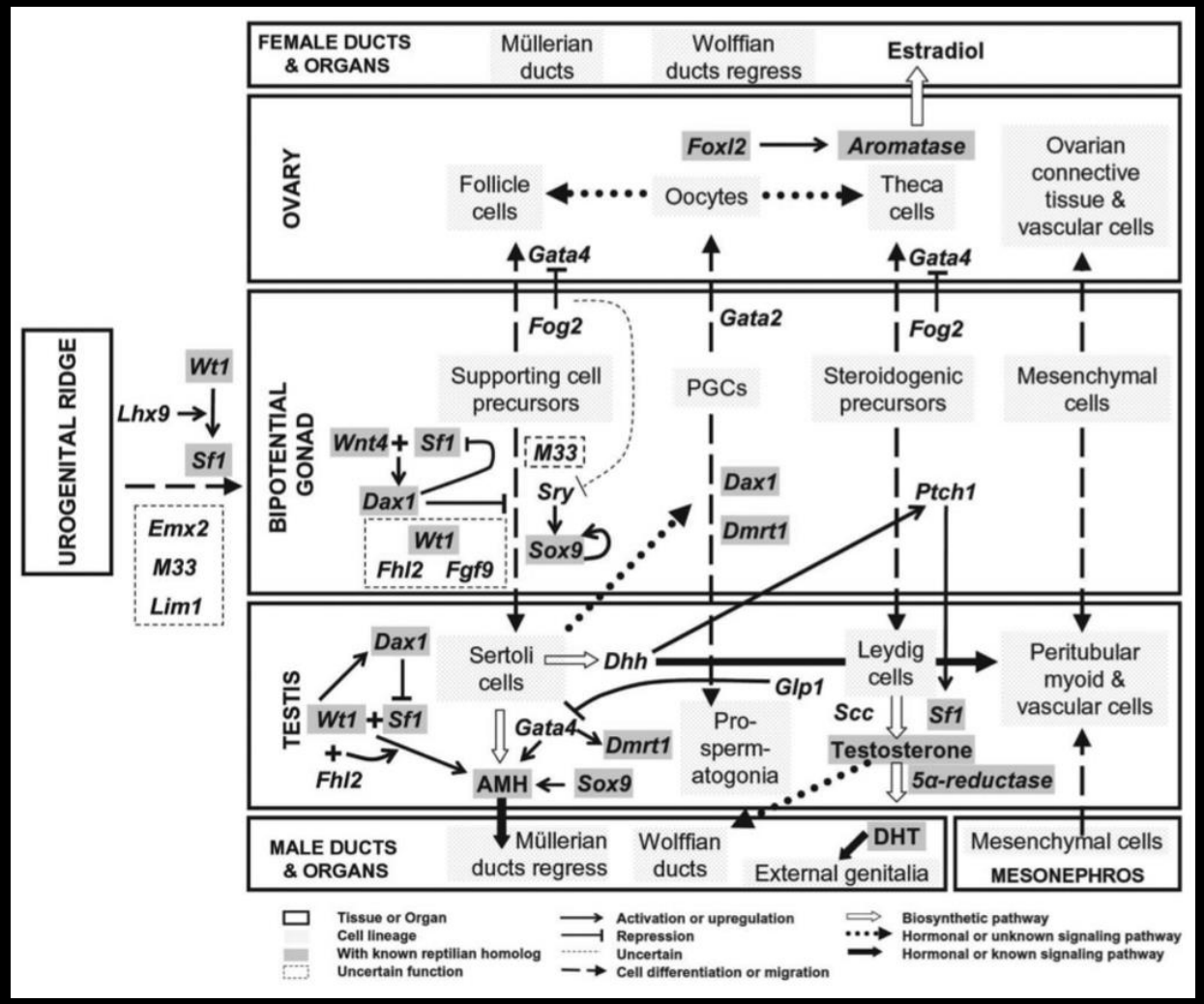
Preliminary Gender Study

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

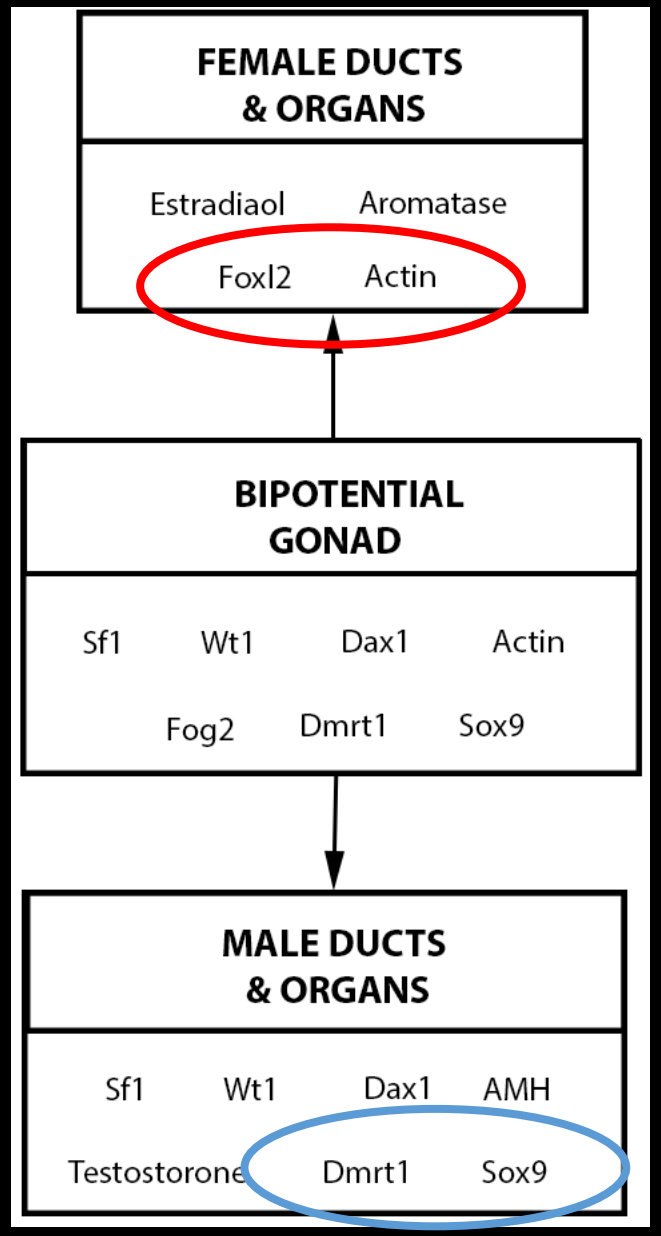
- 5 Green
- 41 Hawksbill
- 10 no ID

- **RNA extracted**
  - (Qiagen Rneasy Kit)
- **PCR**
  - 4 target genes amplified with PCR
  - Visualized on 1.6% agarose gel

Genes of interest

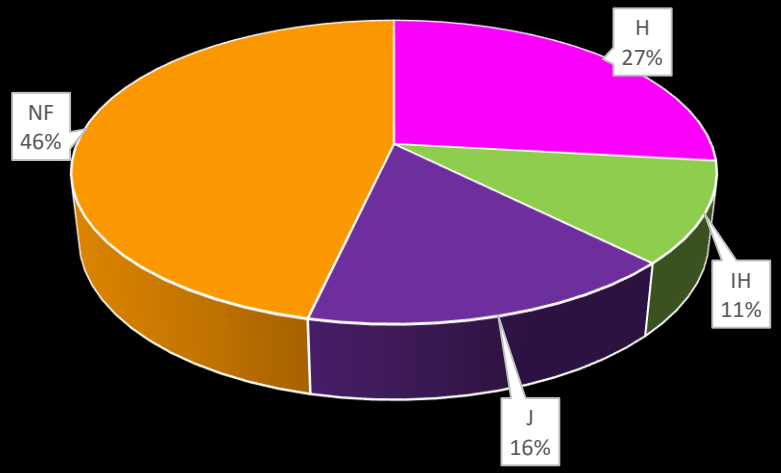


(Valenzuela 2008)



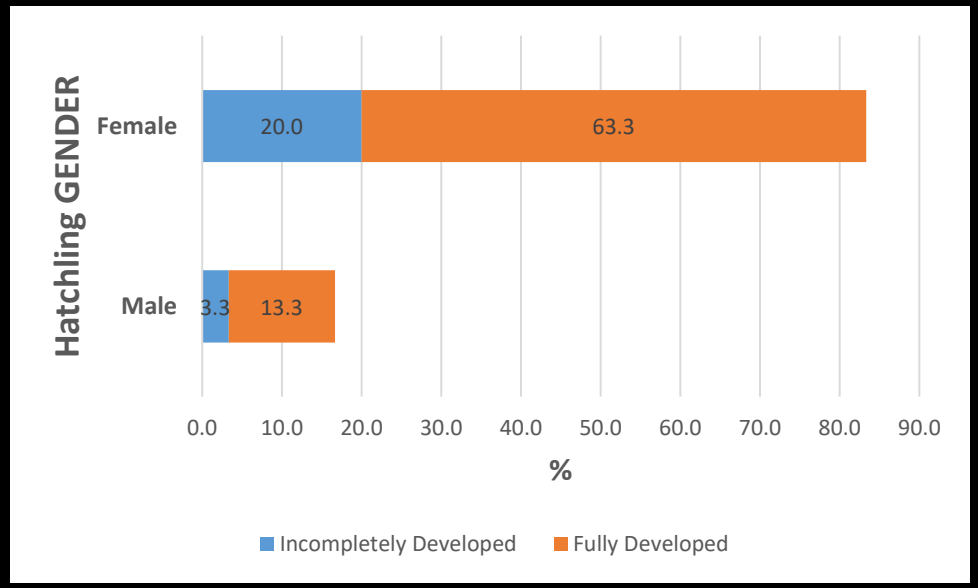
(Guppy 2018, Loffler et al., 2003)

Preliminary Gender Study



(H/Ju)

GR	100% F
HB	85% F
UNK	71.4 % F



Species	Type	TSOX9	DMRT1	ACTIN	FOXL2	Gender
HB	H			+	+	F
HB	H	+			+	IF
HB	H			+	+	F
HB	H			++	+	F
HB	H	+			+	IF
HB	H	+			++	F
HB	H			+	+	F
HB	H?		++	+	+	M
UNK	H?	++	++	+	+	M
UNK	H?		++	+	+	M
UNK	H?	+		+	++	F
UNK	H?	+		+	++	F
UNK	H?			+	++	F
UNK	H?			+	++	F
UNK	H?	†		+	++	F
HB	IH	+			+	IF
HB	IH	+	+	+		IM
HB	IH	+	+			M
HB	IH	+		+		IF
HB	IH			+		IF
HB	IH	+			+	IF
GN	JU	+		+	++	F
GN	JU			+	++	F
GN	JU	†		+	++	F
HB	JU	+		+	++	F
HB	JU	+		+	++	F
HB	JU	+		+	++	F
HB	JU	+		+	++	F
HB	JU	†		+	++	F
HB	JU	†		+	++	F

How does this relate to impacts of climate changes?

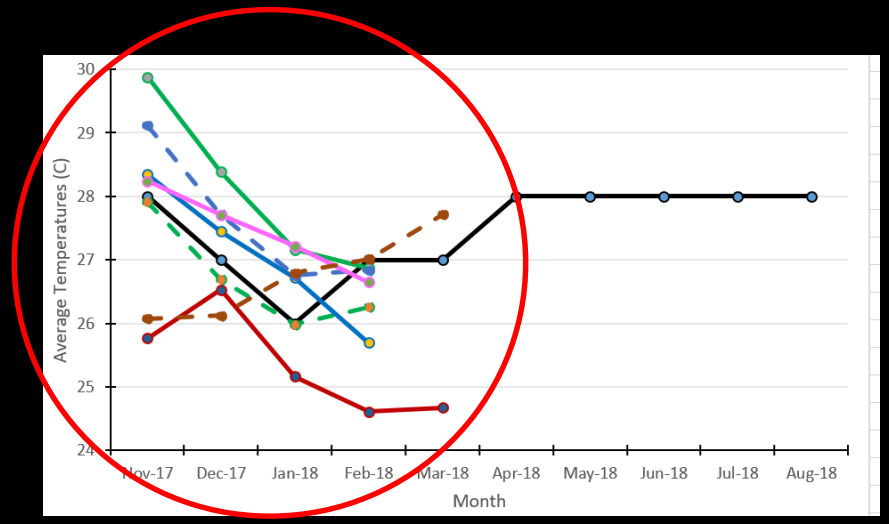
- Impact of air temperature on sand temperature?
- Role of beach characteristics?
- Darker sands warmer than lighter sands *(Fadini et al. 2011)*

Methods

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

(Anon n.d.)

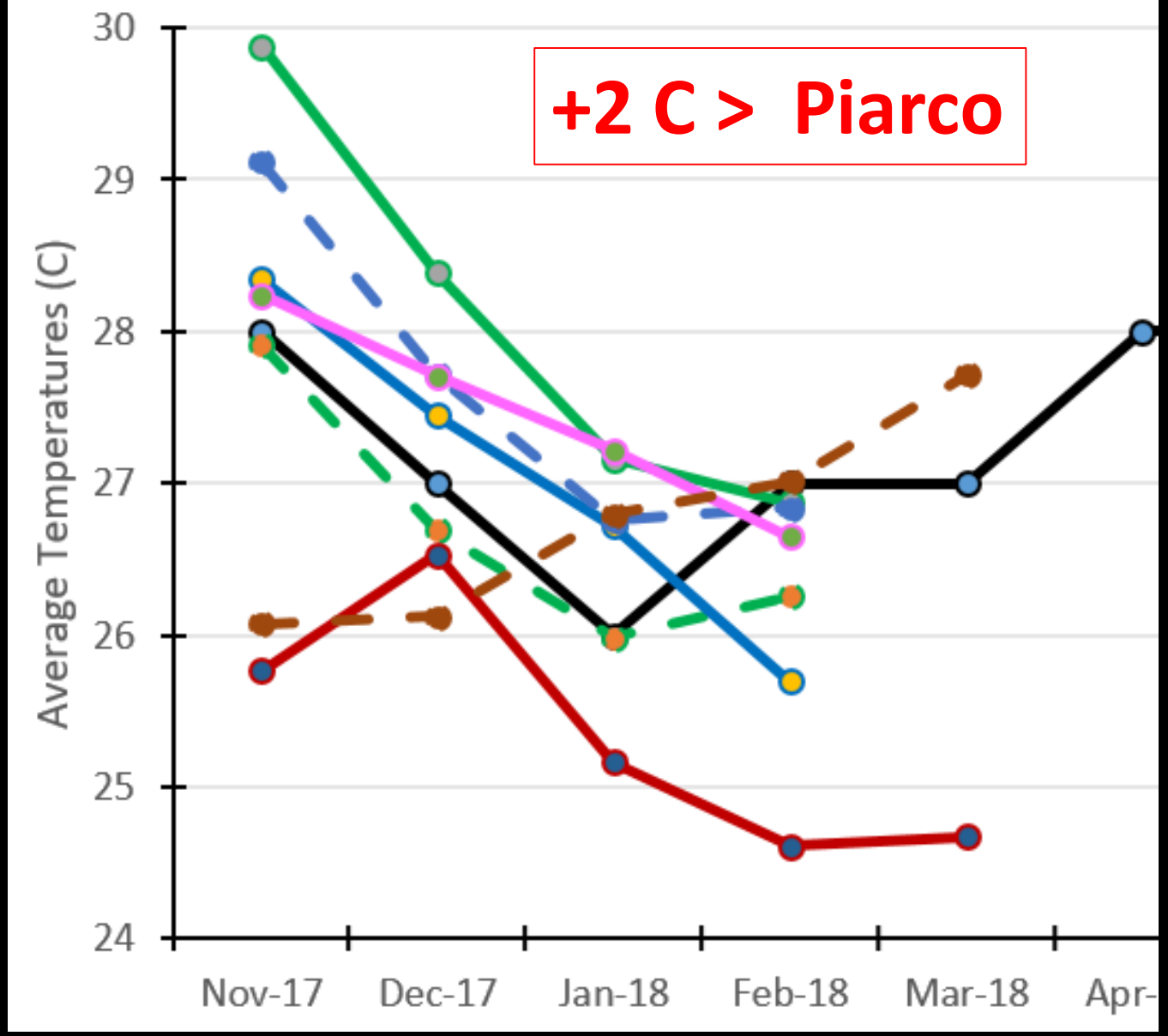
Air Temperatures



Piarco LTs Lte SPs

SPe TCs GRs Gre

4 sites



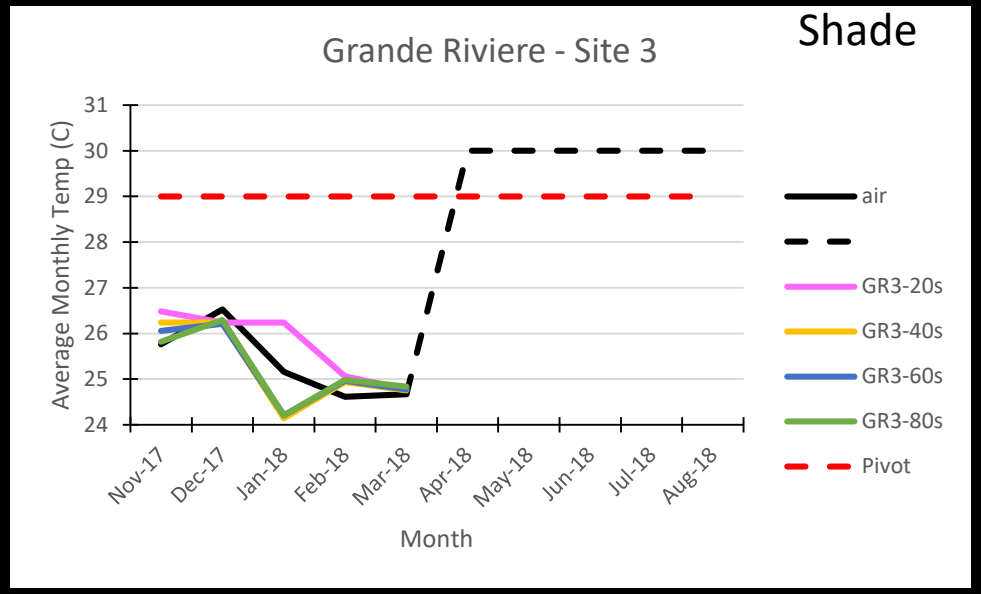
Varies greatly by site from national weather – important to **MEASURE TEMPERATURE AT NESTING SITE**

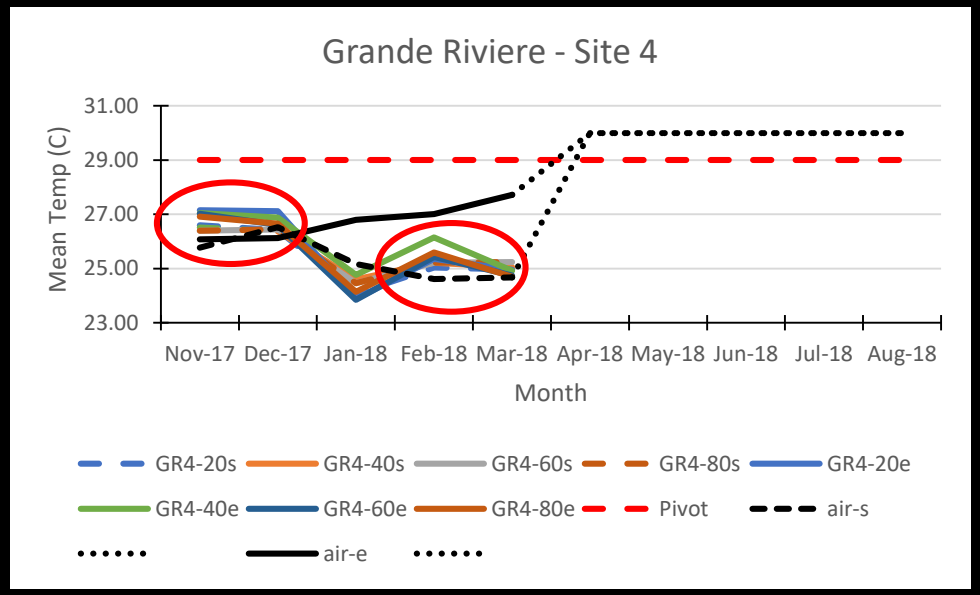
Grande Riviere

Sand Temperature

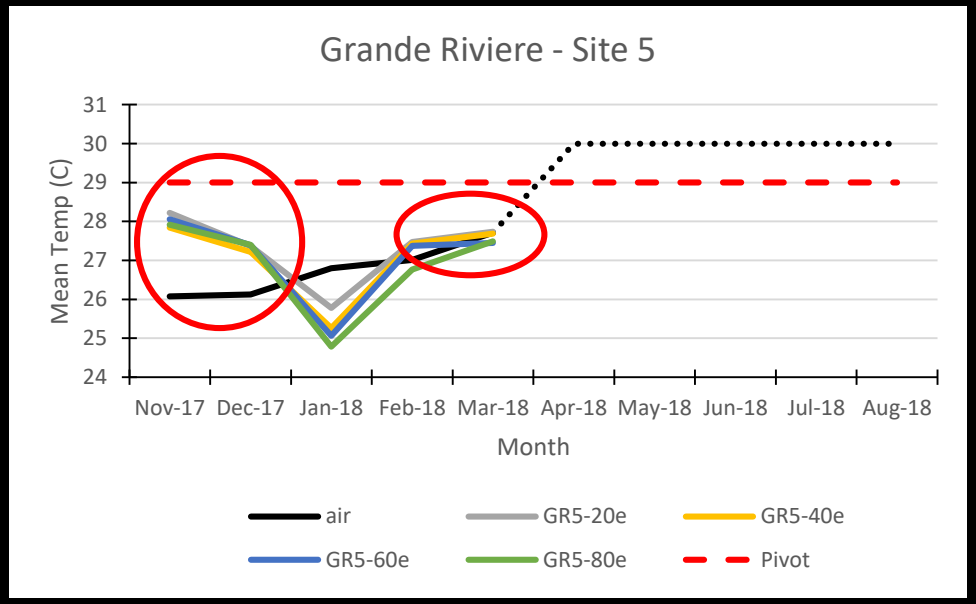
(Charles 2017)

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





$X_{s,e}$

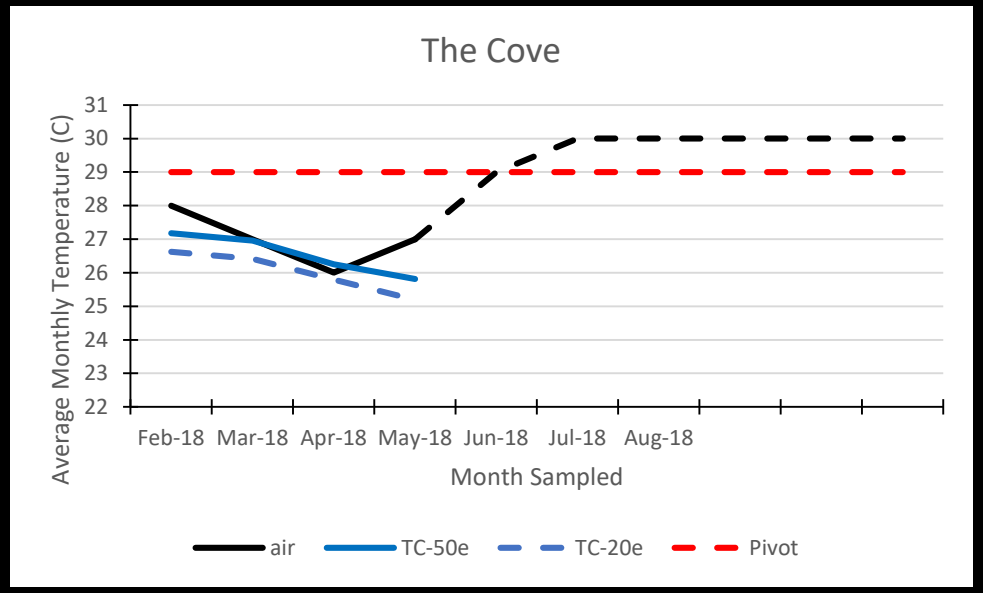


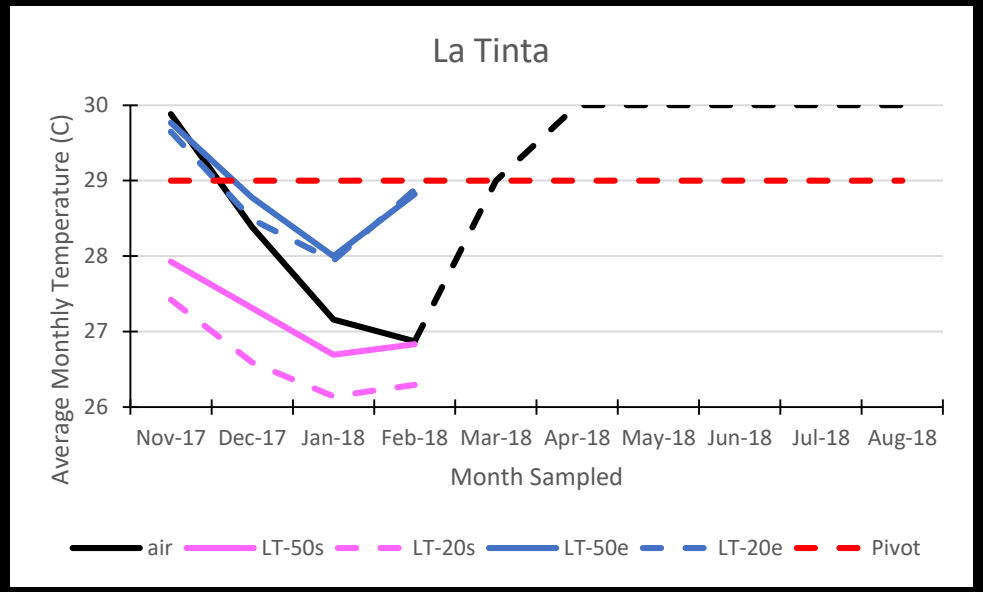
X

Chacachacare

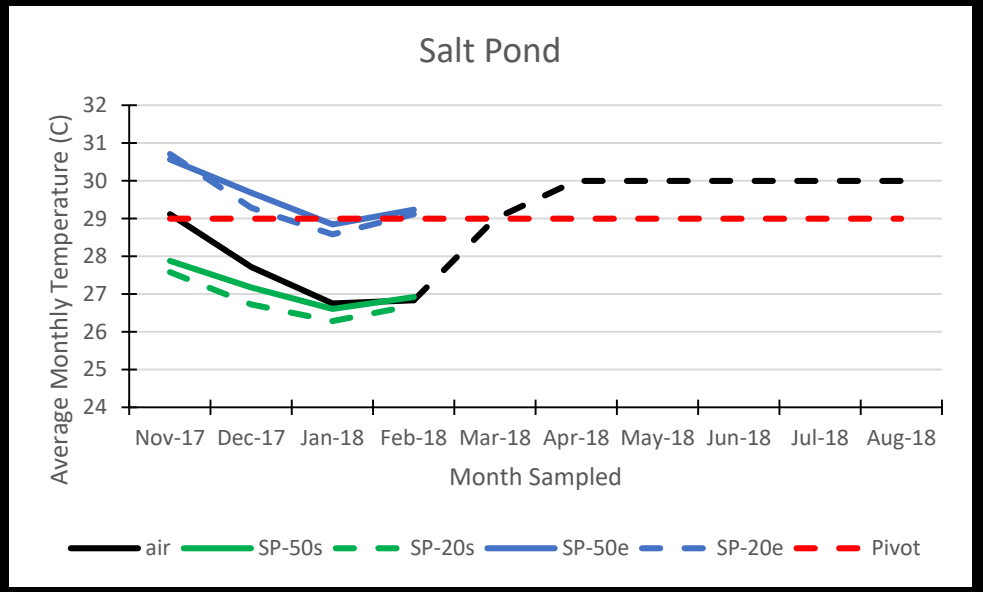
Sand Temperature

(Gittens 2018)





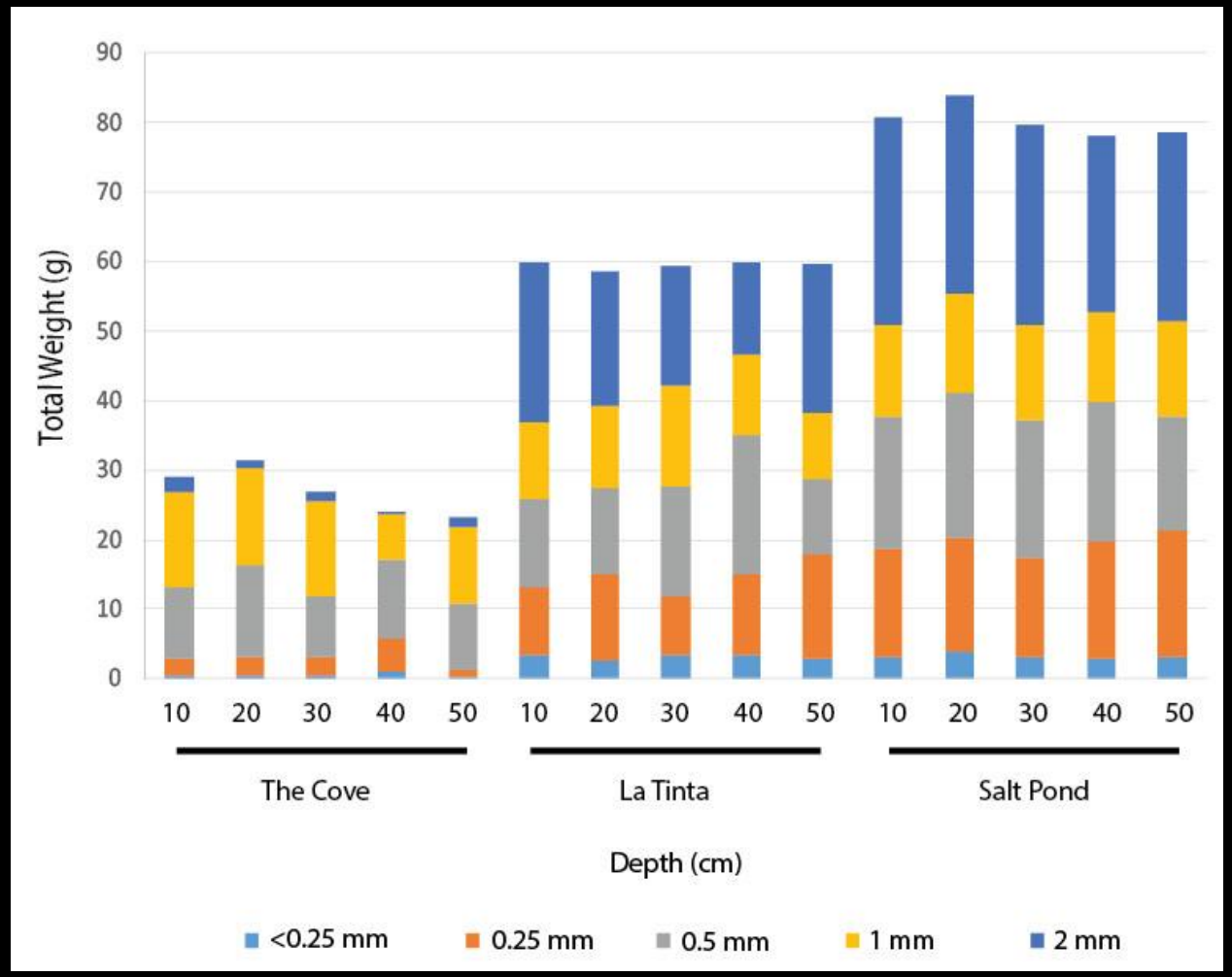
Xe



Xe

- 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

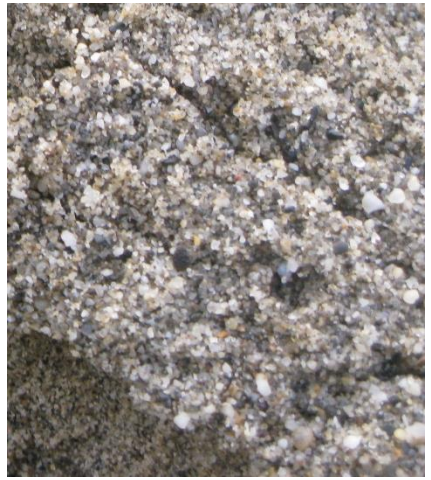


Course sand warmer than finer sands *(Júnior and Castro 2003)*

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?*

(Anon n.d.)

Thank You…..
Merci!

Any questions?

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