

A photograph of a large group of horseshoe crabs on a sandy beach. The crabs are dark brown and have a distinctive horseshoe-shaped carapace. They are clustered together, with some in the foreground and others further back near the water's edge. The water is shallow and has a white, foamy surf. The background shows a line of green vegetation under a clear sky.

# Do Female Horseshoe Crabs Gain from Mating with Multiple Males?

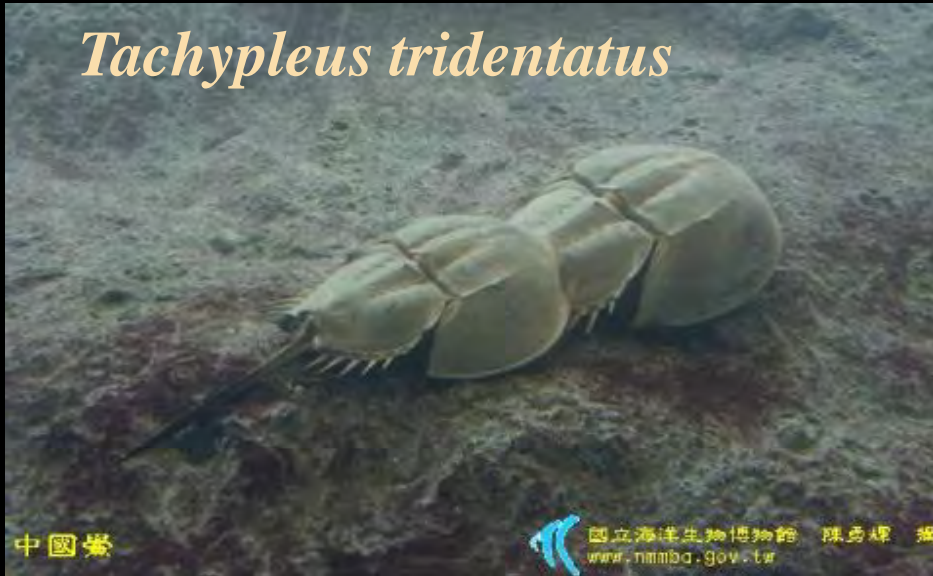
Sheri Johnson  
University of Otago, New Zealand  
&  
Jane Brockmann  
University of Florida, USA



# Horseshoe Crab Reproduction

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*Tachypleus tridentatus*



*Tachypleus gigas*



*Miami Limulus polyphemus*



*Carcinoscorpius rotundicauda*





# Mating System

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Operational Sex Ratio Is Usually Male Biased = competitive  
Males / 1F:

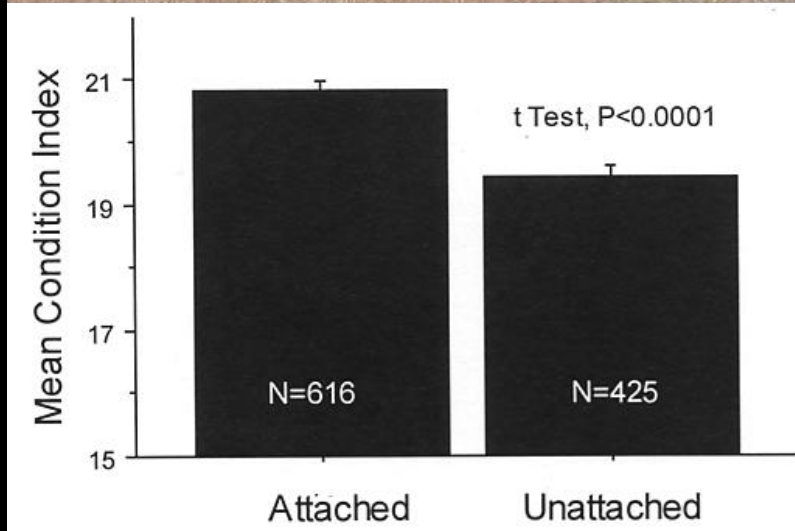
<i>Limulus</i> DE	1.9 – 5.9
FL SJB	1.4 – 2.5
FL SHK	1.1 – 6.0
MA	1.6 – 5.3
<i>T. gigas</i>	1.0 – 1.1
<i>T. tridentatus</i>	1.0
<i>C. rotundicauda</i>	1.1 – 1.4



Spawning *Limulus* at Raccoon Island, Georgia

(Data from Rudloe 1980; James-Pirri et al. 2005; Chatterji 1994; Botton et al. 1996)

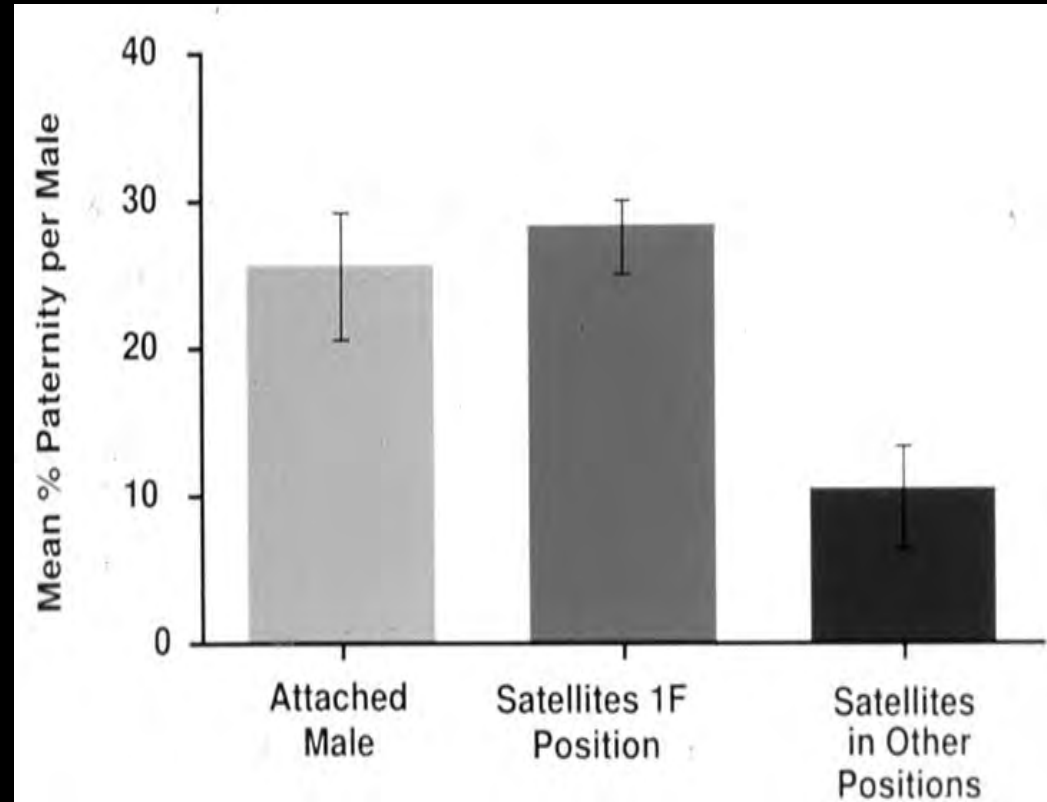
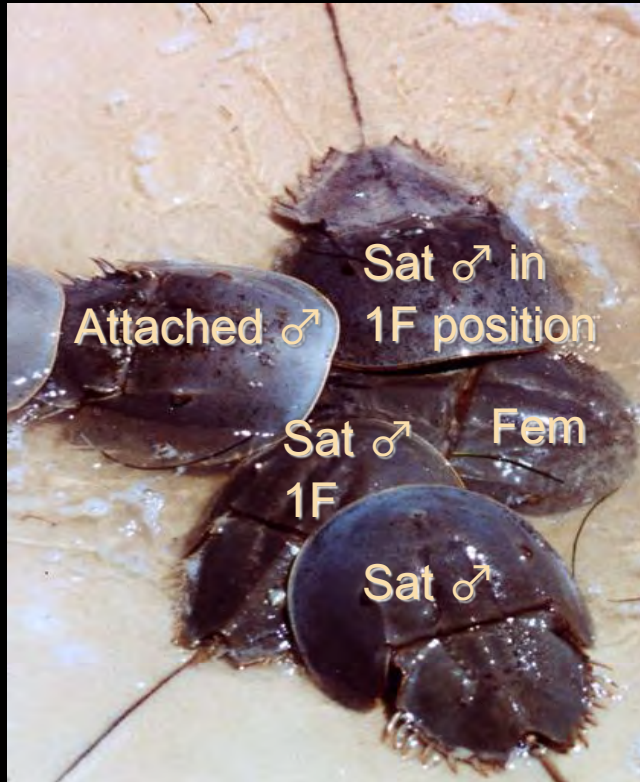
# Mating Affected by Male Condition



## Attached Males are

- Lighter in color
  - More slime
  - Less fouling
  - Carapace, eyes and spines in better condition
  - Younger
- than Unattached Males**

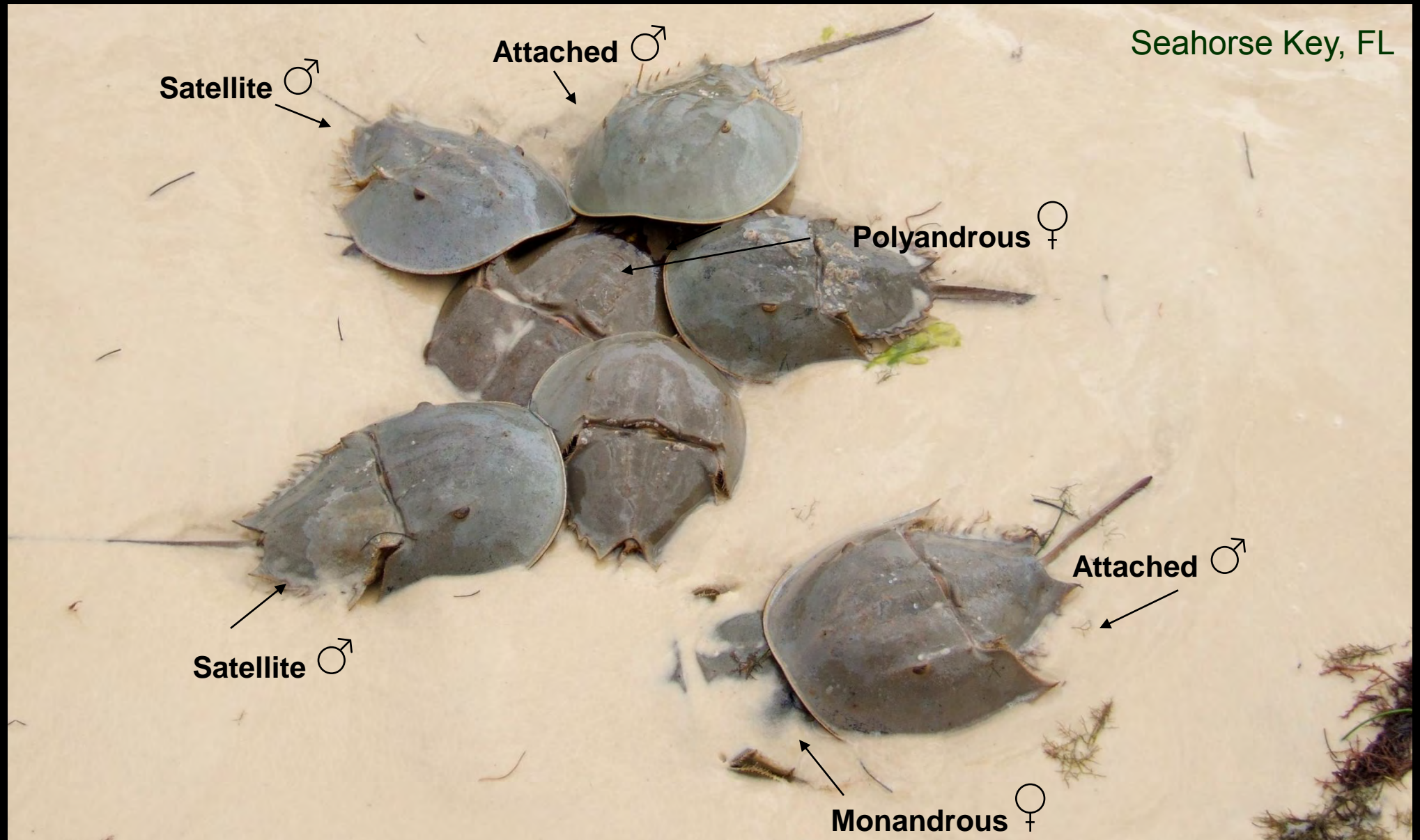
# Satellite Males Fertilize Eggs



(Brockmann et al. 1994)



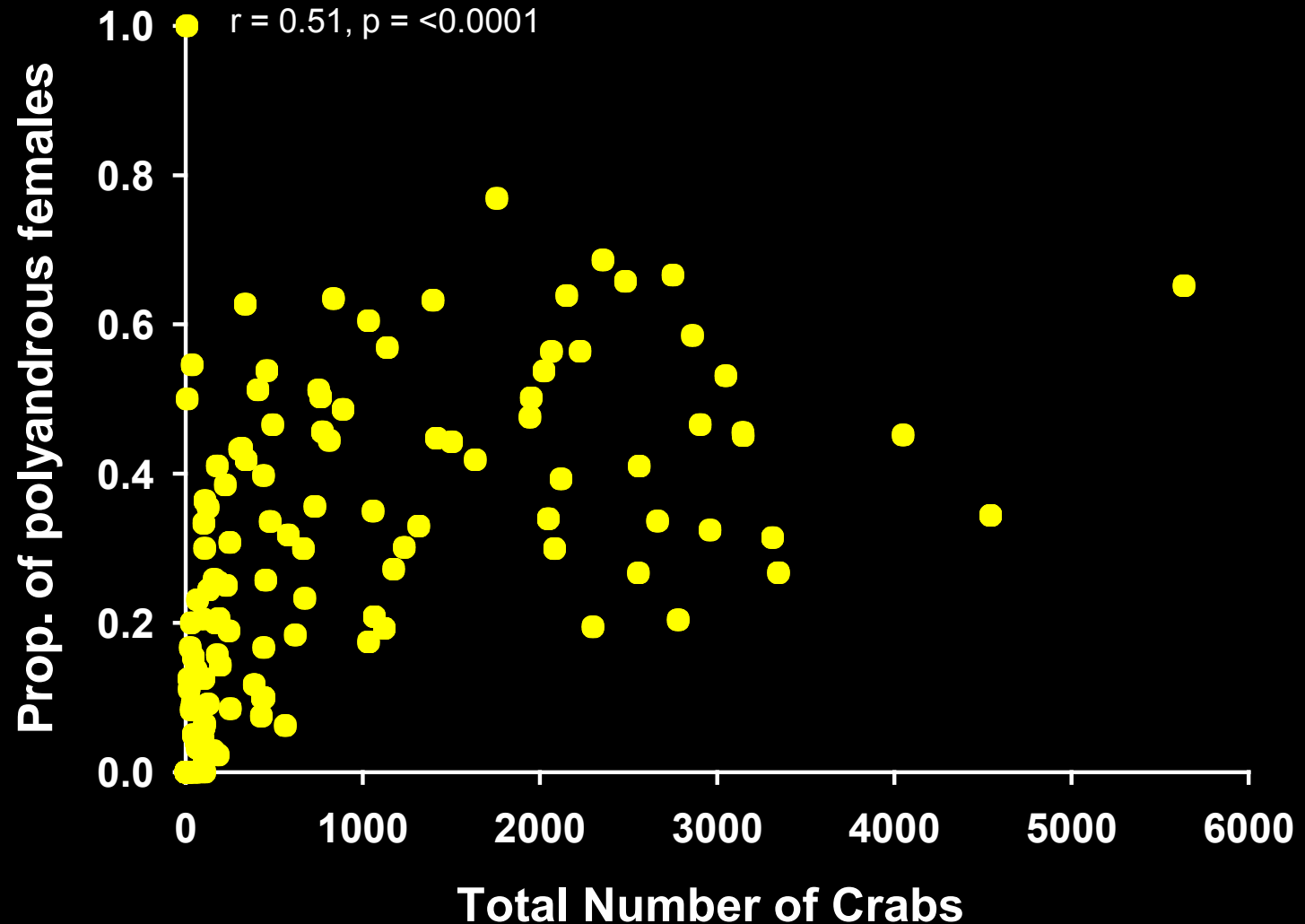
# Limulus Spawning



# Frequency of Multiple Mating in Limulus

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Seahorse Key, FL



# Consistency of Monandry/Polyandry in *Limulus*



Looking for marked  
*Limulus* at SHK, FL

Original status	% Return same status (1996)	% Return same status (2000)
Mono	69% (n=258)	82% (n=172)
Poly	54% (n=71)	33% (n=27)

1996:  $\chi^2 = 12.74$ ,  $p=0.0004$     2000:  $\chi^2 = 2.23$ ,  $p=0.135$



# Why Do Some Females Mate Multiply?



1. **Multiple mating (polyandry) is costly for females.** Results from male competition

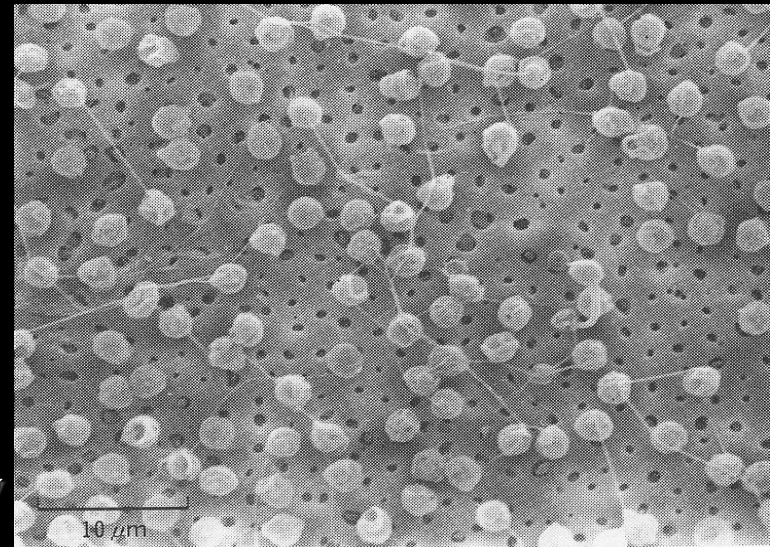
# Costs of Multiple Mating to Females

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Disturbance



Risk of polyspermy





# Why Do Some Females Mate Multiply?



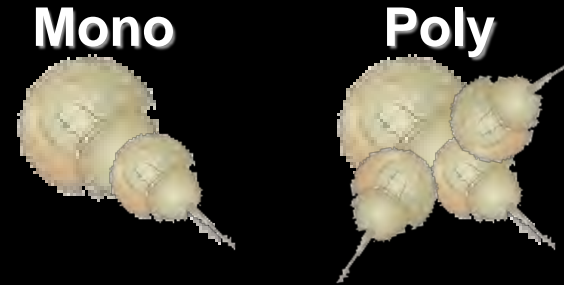
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- 2. Multiple mating is beneficial for females**
  1. Direct benefits (unlikely)
  2. Ensures fertilization
  3. Genetic benefits
    1. Genetic Diversity
    2. Male Quality (good genes)
    3. Compatibility

Horseshoe crab management:  
Are satellite males 'superfluous' ?

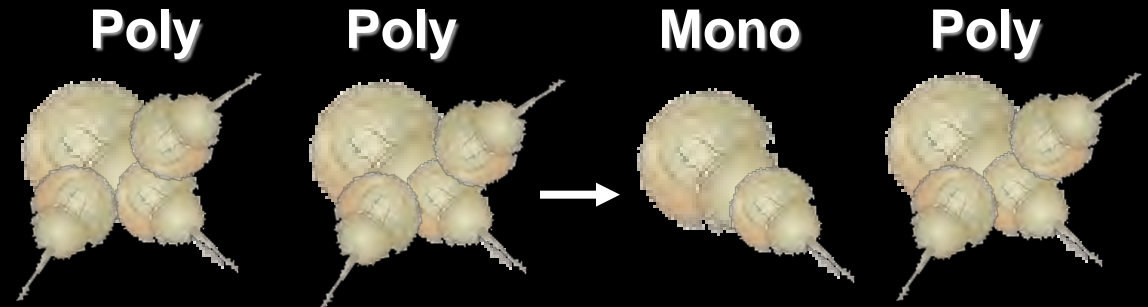
# Looking for Benefits/Costs to Polyandry

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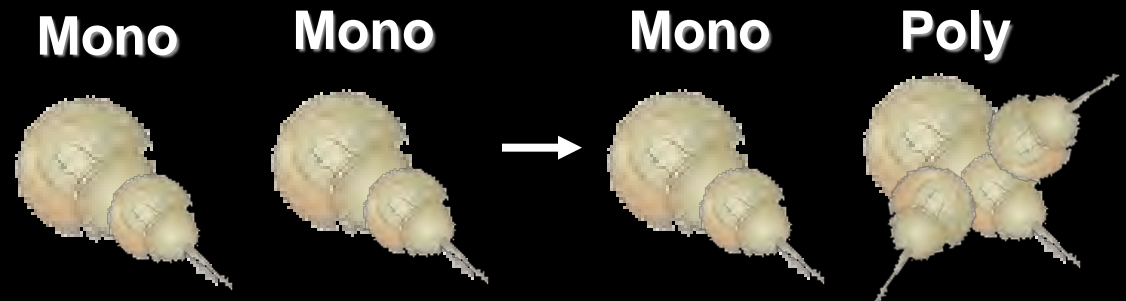
Exp. 1 Natural Variation



Exp. 2 Satellites Removed

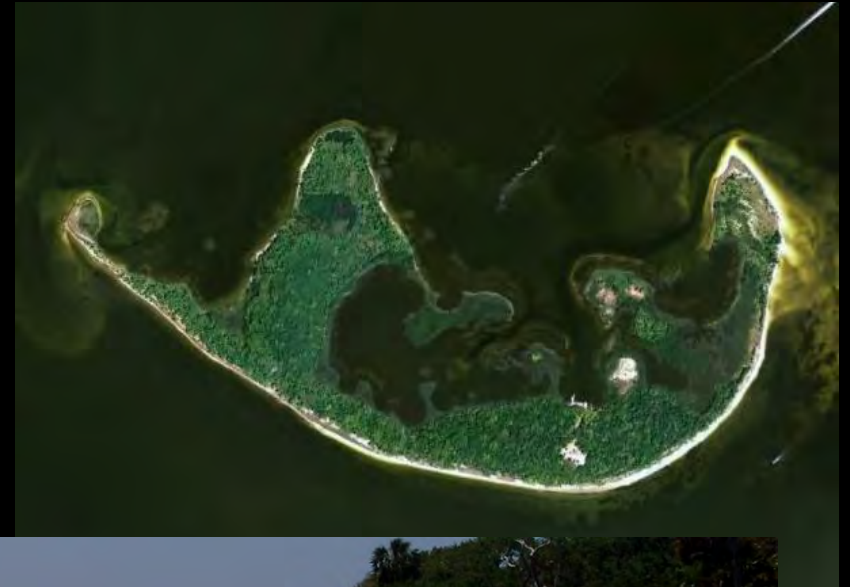


Exp. 3 Satellites Added





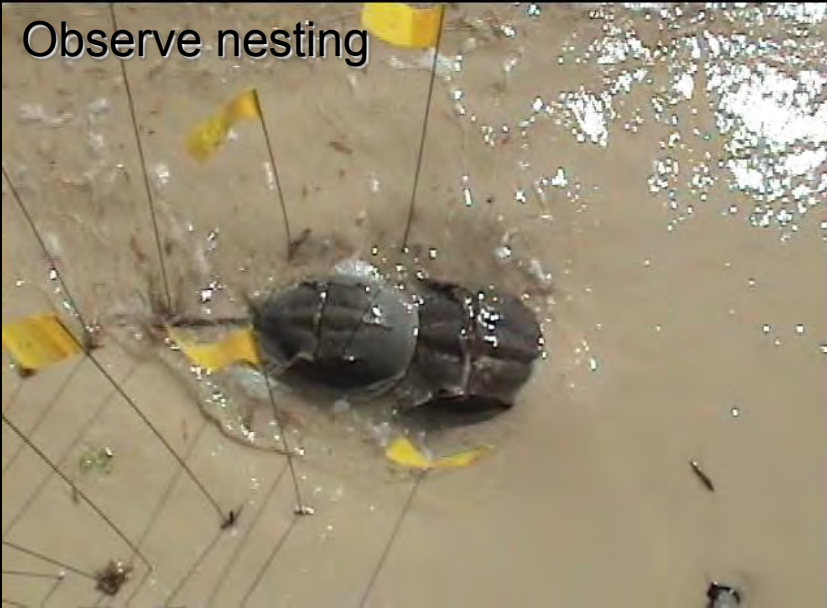
# Field Site - Seahorse Key, Florida





# Field Methods

Observe nesting



Measure



Collect eggs from marked nests



Rear larvae

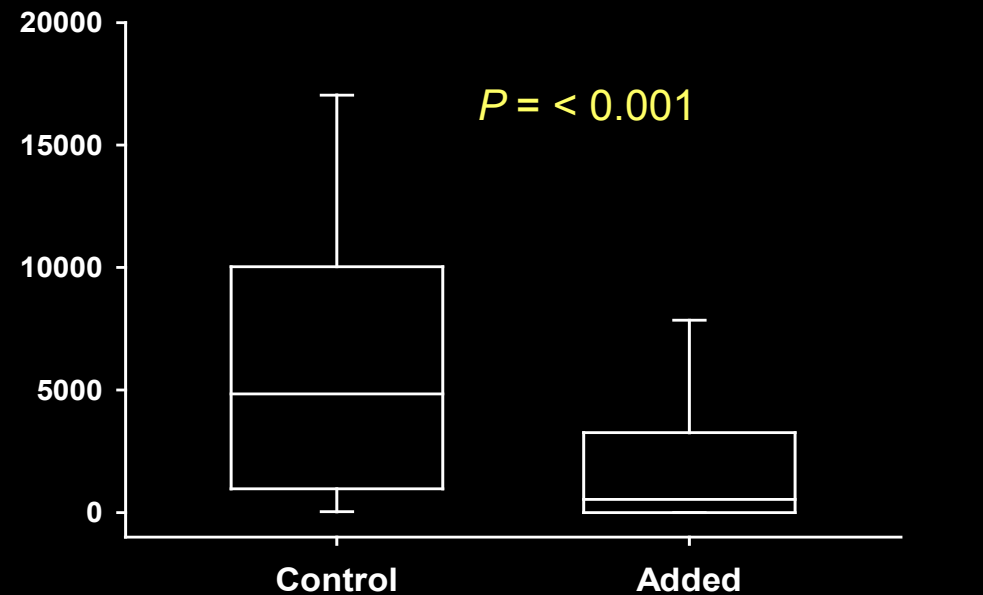
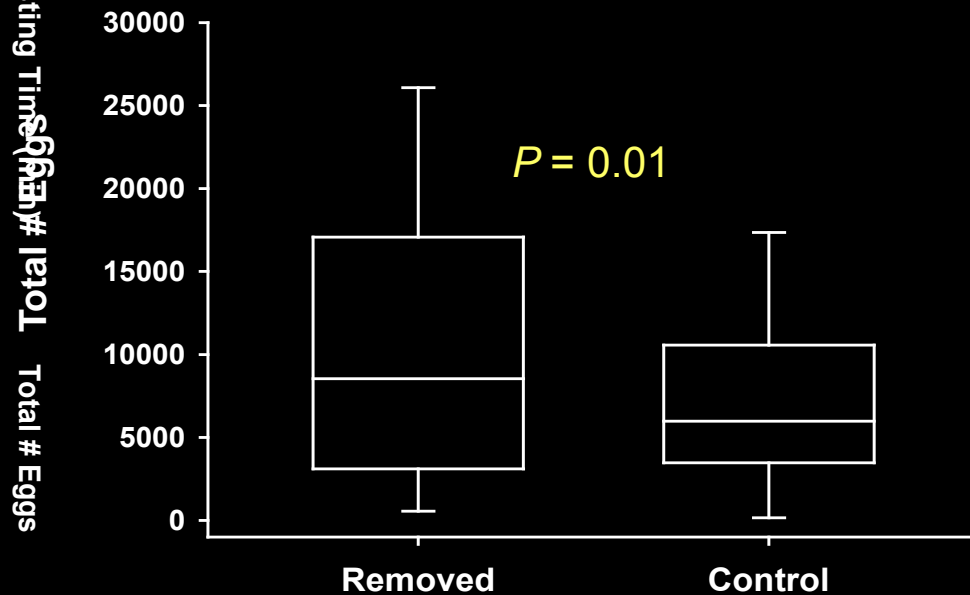
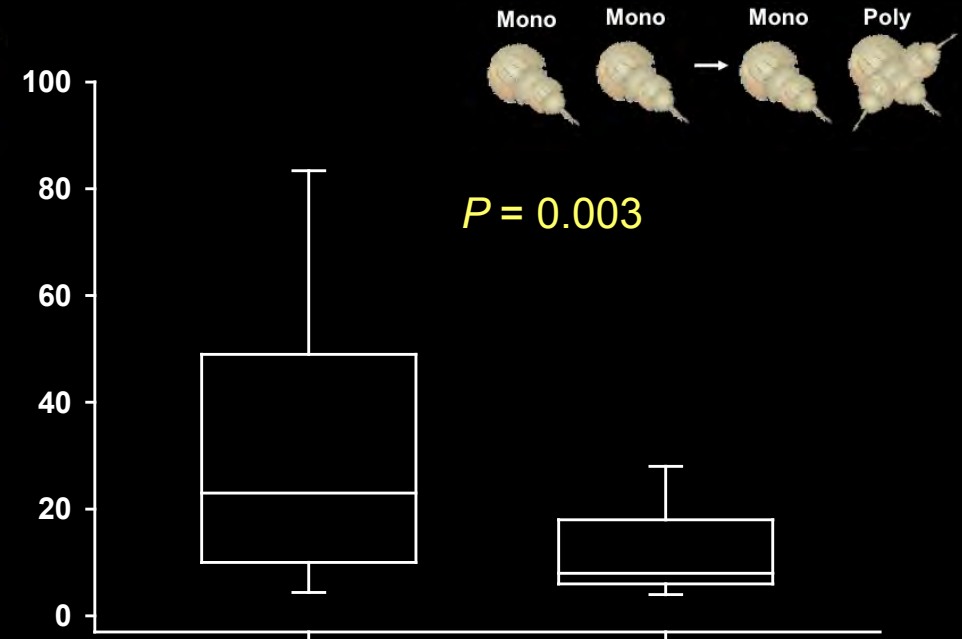
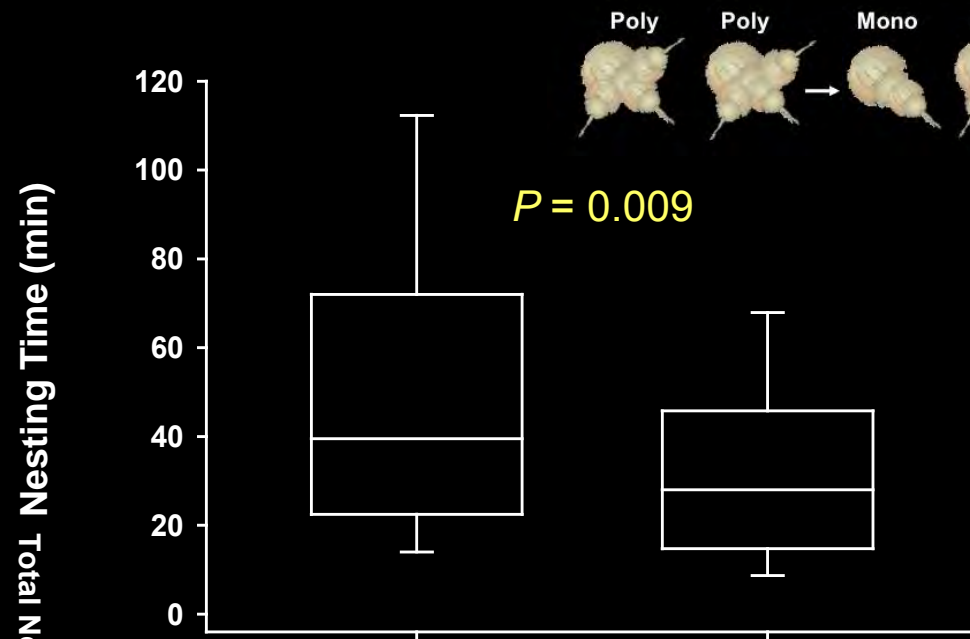




# Satellites Removed

/

# Satellites Added



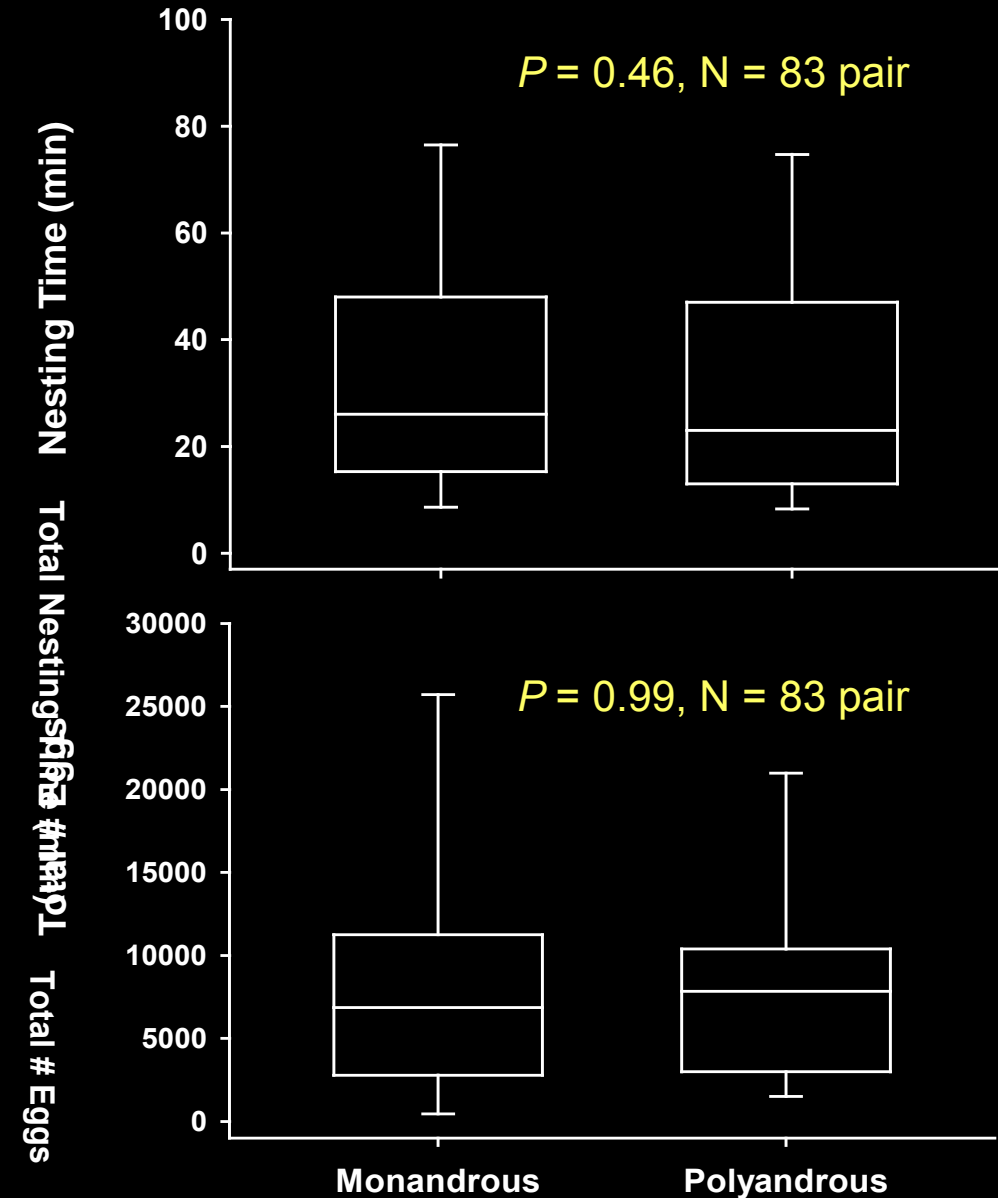
# Resistance to Polyandry in *Limulus*

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# Do Females Benefit from Mating Multiply?



- Poly females with satellites removed: lay more eggs
- Mono females with satellites added: lay very few eggs

➤ **Costly**

- Mono & poly do equally well

➤ **Indirect benefit?**

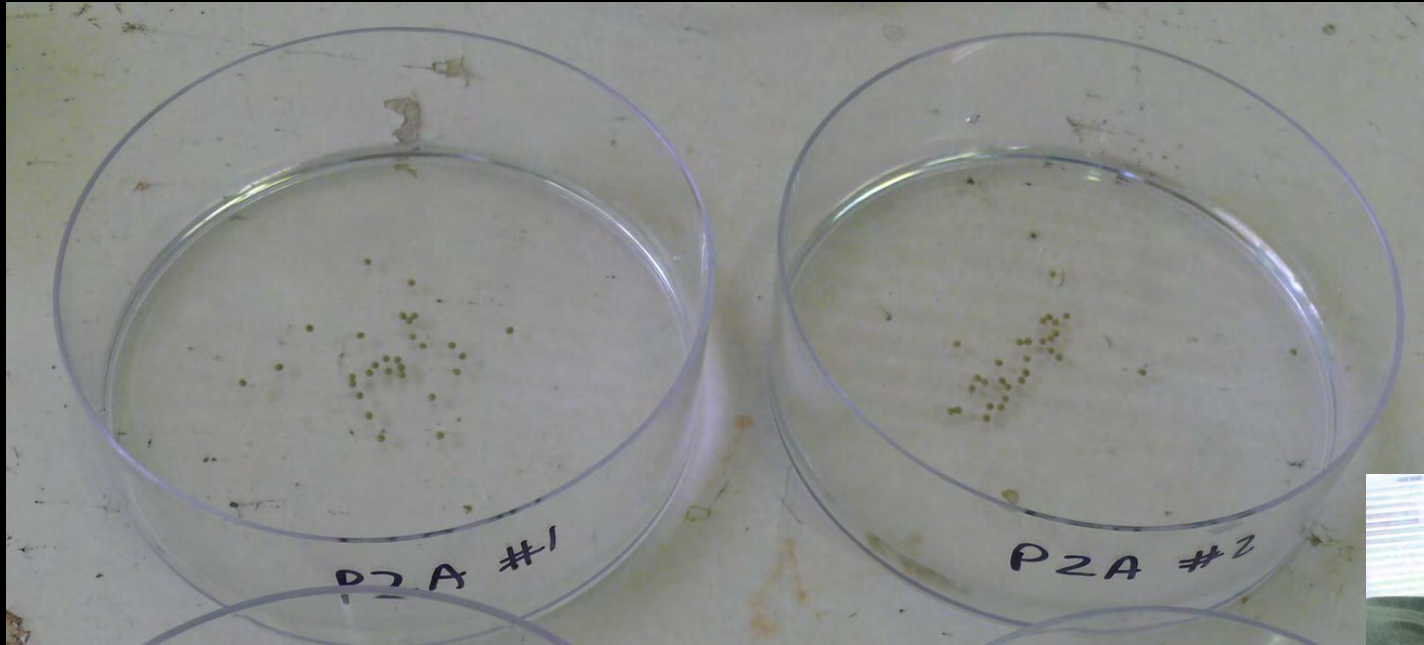
# ***In vitro*** Fertilization Assays

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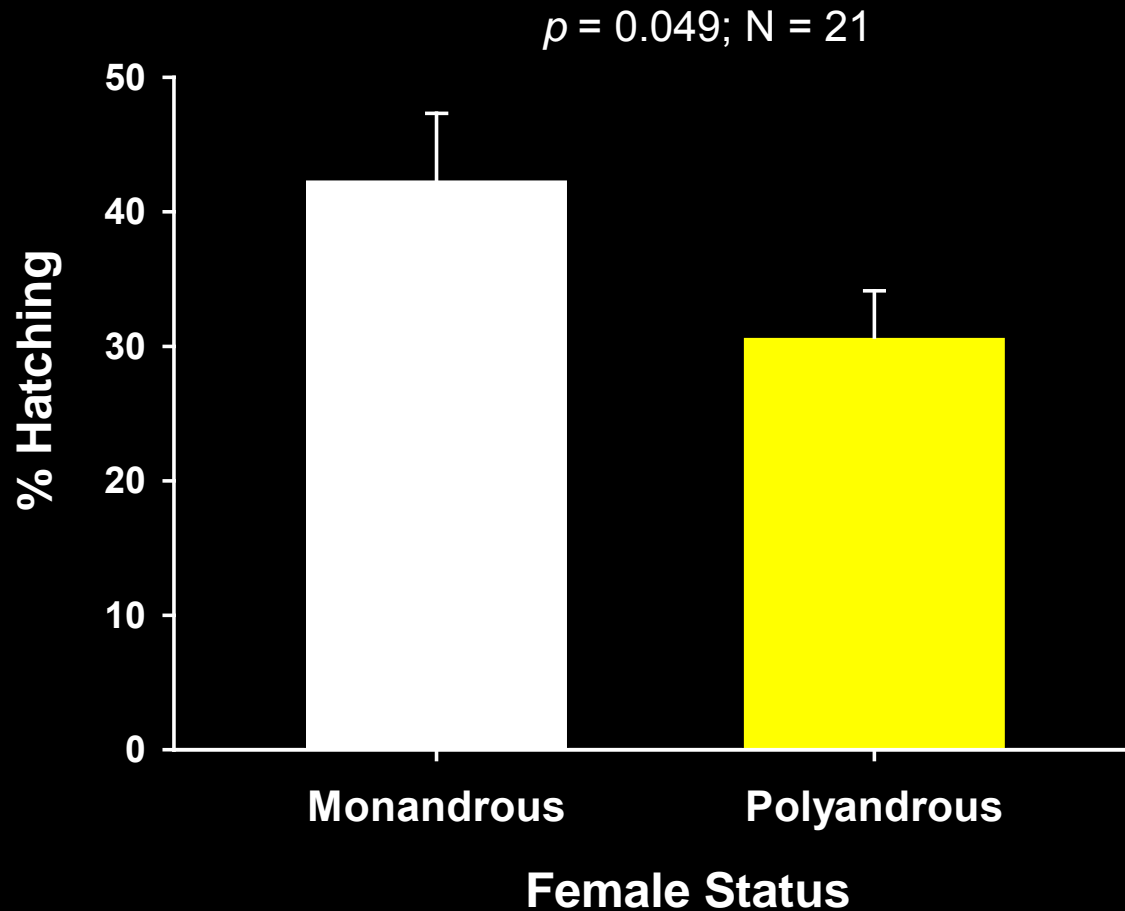


# *In vitro* Fertilization Assays



# Is There Evidence for Benefits?

**Do monandrous females have higher success with their attached males?**





# Why Do Some Females Mate Multiply?



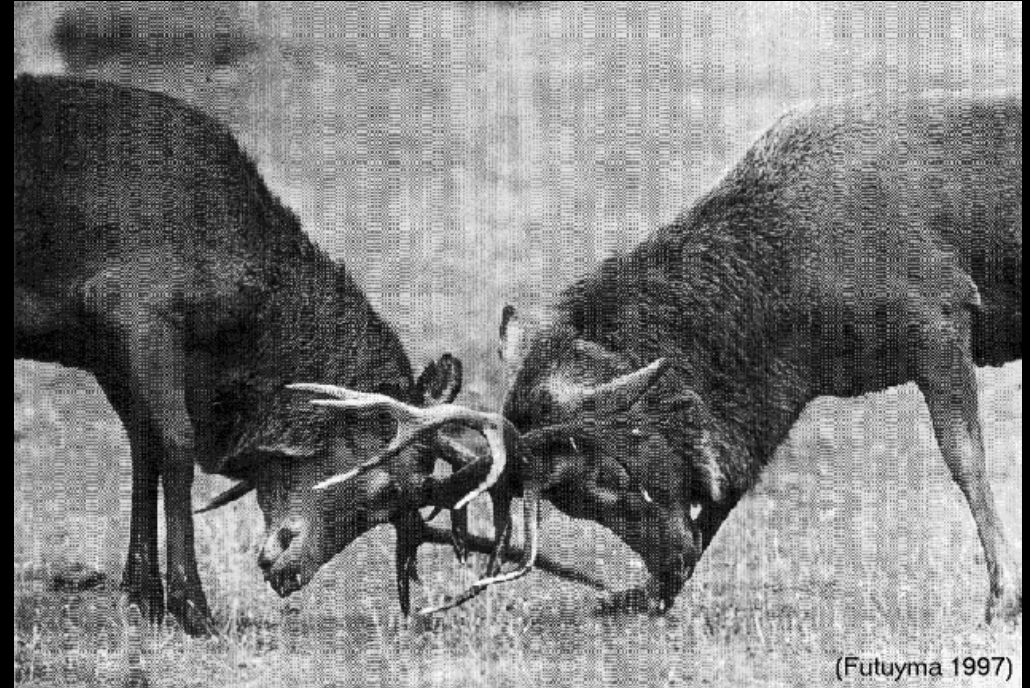
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Horseshoe crab management:  
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# Good Genes

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# Compatibility

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## Mate choice to ensure:

- prevention of inbreeding or selfing
- increased heterozygosity
- MHC compatibility
- avoid selfish genetic elements [i.e., transposable elements, segregation distorters, inherited bacterial symbionts (e.g., *Wolbachia*)].



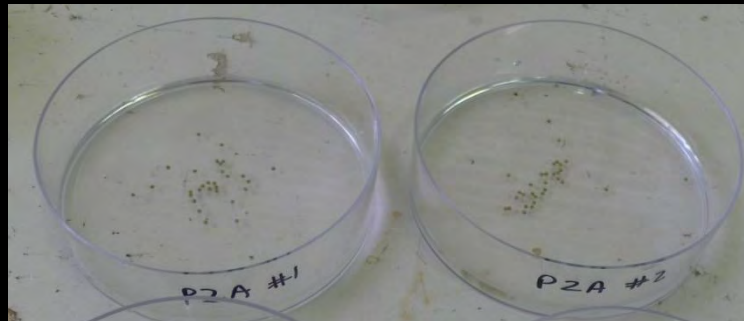
# Is There Evidence for Genetic Benefits?

North Carolina Design II (4 x 4)

	Male 1	Male 2	Male 3	Male 4
Female 1	$2(m_1 \times f_1)$	$2(m_2 \times f_1)$	$2(m_3 \times f_1)$	$2(m_4 \times f_1)$
Female 2	$2(m_1 \times f_2)$	$2(m_2 \times f_2)$	$2(m_3 \times f_2)$	$2(m_4 \times f_2)$
Female 3	$2(m_1 \times f_3)$	$2(m_2 \times f_3)$	$2(m_3 \times f_3)$	$2(m_4 \times f_3)$
Female 4	$2(m_1 \times f_4)$	$2(m_2 \times f_4)$	$2(m_3 \times f_4)$	$2(m_4 \times f_4)$

N = 11 sets (176 replicated families)

~30 eggs/dish;  $10^4$  sperm  $\text{ml}^{-1}$





# Evidence for Genetic Compatibility

	<i>df</i>	<i>% Hatched</i>	<i>% Metamorphosed @ 45 d.</i>	<i>Juvenile Size</i>
Male	33	3.33 (0.052)	5.71 (0.043)	3.86 (0.134)
Female	33	54.8 (<0.001)	30.1 (<0.001)	20.1 (<0.001)
Male x Female	96	7.03 (0.026)	14.3 (0.004)	13.6 (0.050)
Residual	176	34.7	49.6	62.4

Mean ± SE       $\longrightarrow$       42.8 ± 1.42      6.18 ± 0.71      5.05 ± 0.015

# Summary & Conclusions

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- Multiple mating costly in terms of nesting success; BUT natural monandrous and polyandrous females do equally well.
  - suggests there is some compensating benefit
- Hatching success higher for mono females with attached males than poly females with attached males.
  - suggests differences in genetic quality (good genes or compatibility) between the attached males
- Differences in offspring success, resulting from partial incompatibilities, provide a selective advantage to females that engage in polyandry.
  - This has the potential to offset the costs of mating multiply

Are Monandry & Polyandry Alternative Reproductive Tactics?



# Why Do Some Females Mate Multiply?



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# Implications for for Management & Conservation

What does this mean for the asian species?

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Estuaries and Coasts

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# **A Long-Term Study of Spawning Activity in a Florida Gulf Coast Population of Horseshoe Crabs (*Limulus polyphemus*)**

**H. Jane Brockmann • Sheri L. Johnson**

# Acknowledgements

~30 field assistants, undergraduates, graduate students, and high school students contributed to the collection of these data

## Facilities

Seahorse Key Marine Lab  
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## UF Faculty

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Jamie Gillooly

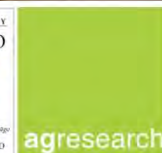
## Permits

US Fish & Wildlife  
(Cedar Key National  
Wildlife Refuge)

## Otago Faculty

Neil Gemmell  
Shinichi Nakagawa

Florida Fish & Wildlife  
Conservation  
Commission



CENTRE FOR  
REPRODUCTION  
AND GENOMICS