



Green Eggs and Sand: Spawning Behavior of Horseshoe Crabs

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Notes about this Presentation:

All figures are from photographs by Jane Brockmann except for the following:

Slide 10: from Mikkelson 1988

Slide 16. gull feasting on horseshoe crab is from www.dcwild.com

Slide 17. right photo from Shuster et al. 2003. *The American Horseshoe Crab*. Harvard University Press.

Slide 18. Photos courtesy of Skidaway Institute of Oceanography

Pickering Beach, June 2007



Horseshoe crabs migrate to Atlantic beaches in the spring where they spawn. They arrive on the high tide at the time of the new or full moons.

Fortescu, NJ in May 2011




New and full moon high tides are extra high (from 2 days before to 4 days after the new or full moon)

*Horseshoe crabs nest in the intertidal
i.e. where the beach has a slope*



In areas where high tides are smaller (<1.5 m), they nest at the top of the high tide line.



They nest
either during
the day or at
night often in
dense
aggregations



Pickering Beach, DE in May 2010



Making sense of horseshoe crab spawning



Pickering Beach, DE, June 2007

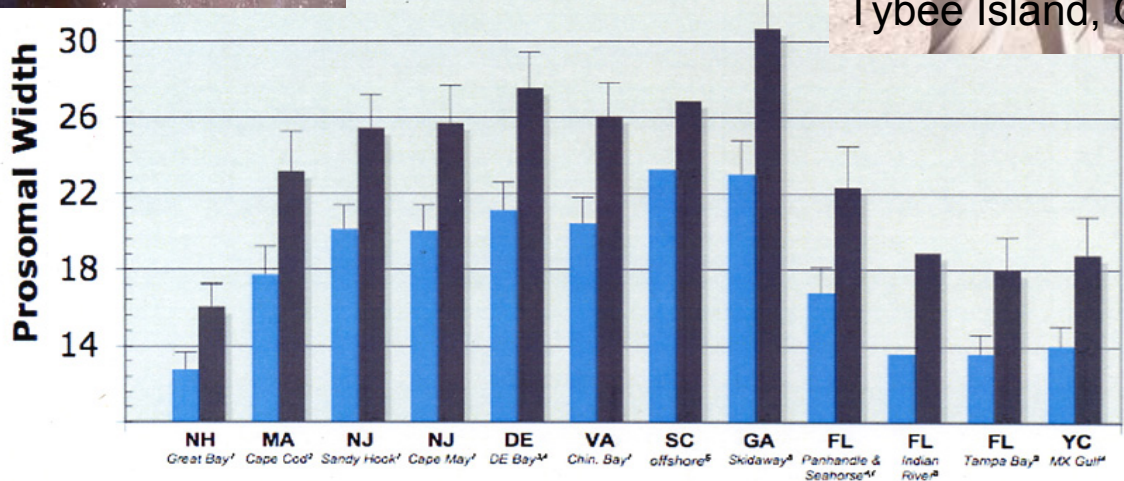


Yucatan, Mexico

Differences between Populations

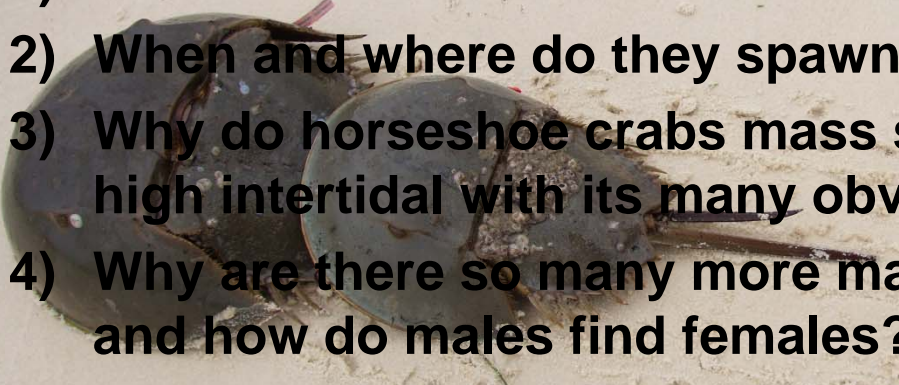


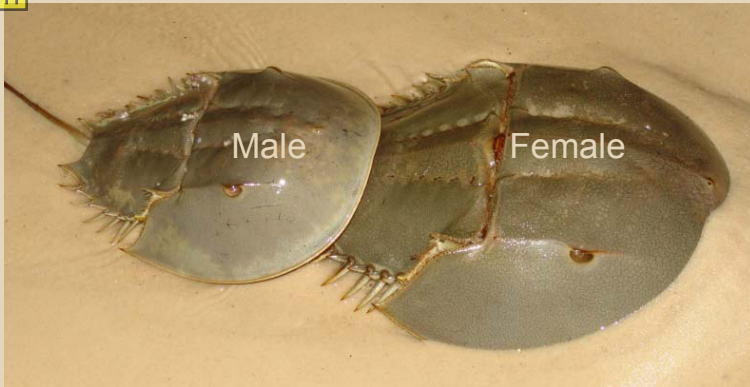
Tybee Island, GA





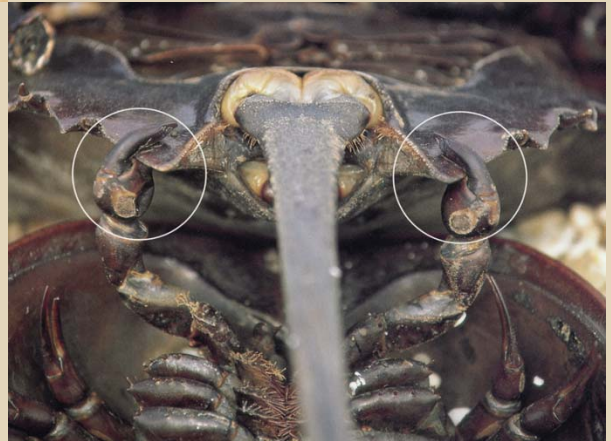
Questions about Spawning

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- 1) How do horseshoe crabs nest and spawn?
 - 2) When and where do they spawn?
 - 3) Why do horseshoe crabs mass spawn in the high intertidal with its many obvious costs?
 - 4) Why are there so many more males than females and how do males find females?
 - 5) What are the satellite males doing and do they differ from attached males?
 - 6) Is multiple mating costly or beneficial to females and why do some females mate multiply?



1. How do horseshoe crabs nest and spawn?

- Male clasps female's posterior spines with modified first pair of legs
- Pairs form offshore and migrate to shore
- Female digs into sand and lays eggs



“Primitive” and Unusual Reproduction



- Males and females extrude gametes into environment from paired gonopores that are under the operculum
- Only arthropod with broadcast spawning and *external* fertilization, i.e. with aquatic, free-swimming sperm; eggs are fertilized outside the female's body in the sand

Horseshoe Crab Nesting



We mark out the position of each egg batch with flags.

- Eggs laid in batches of 2-4,000 eggs each;
- Fertilization is external;
- Female visits beach several times during a week of high tides;
- Total of 60-80,000 eggs laid each year
- Eggs remain buried 10-20 cm in sand where they develop

2. When and where do they spawn?



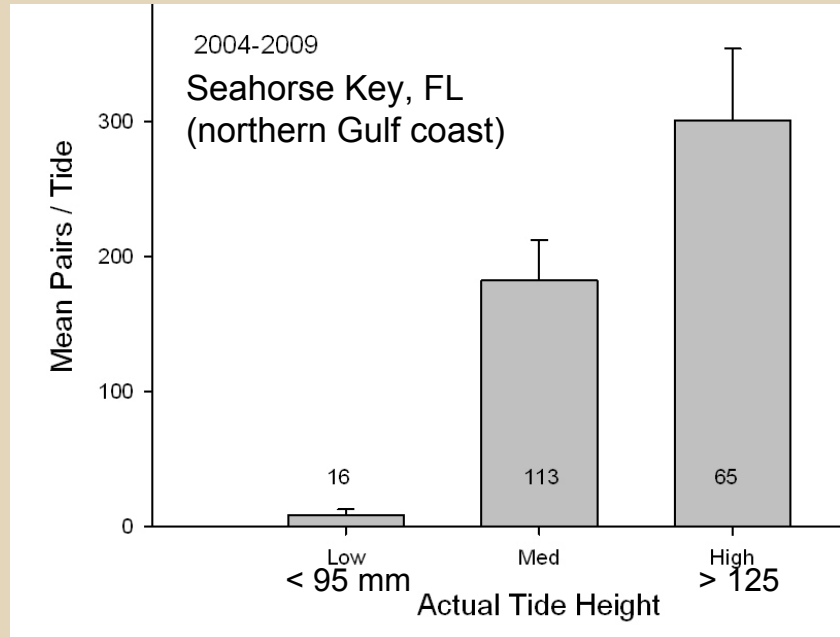
Pickering Beach 2010

- Mass spawning is synchronized with new and full moon high tides
- These are the highest tides in a month
- Nest at the top of the high tide line
- Both day and night high tides

Spawning affected by actual (not predicted) high tide height



Sheri Johnson taking a beach census at SHK, FL



From Brockmann & Johnson 2011

On higher energy beaches, fewer crabs nest when surf is higher



Horseshoe crabs roll in the surf at SHK, FL

Higher energy beaches have larger waves, coarser sand and steeper slopes.

High Intertidal Nesting is Costly

- Stranding
- Desiccation
- Predation
- Physiological stress
- UV damage
- Nesting competition



Limulus stranded along high tide line as the tide recedes (DE Bay)

Mass Spawning is Costly



Females dig up the eggs of other females as they nest at high densities



Eggs on the surface eaten by shorebirds!



Raccoon Island, GA, April 2006



Alligator in surf eating a horseshoe crab

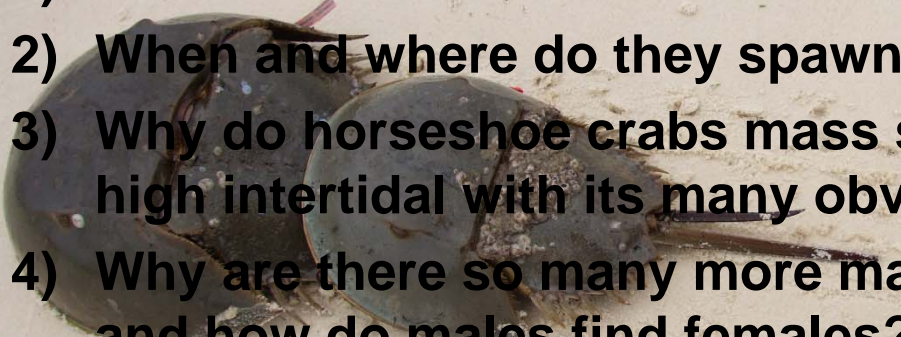
3. So why the high intertidal?

- Eggs laid high on the beach have more oxygen, which is required for development
- Embryos are poisoned by hydrogen sulfide which is found in the lower parts of the beach

Synchronized spawning probably reduces predation on adults and larvae by swamping predators.



Questions about Spawning

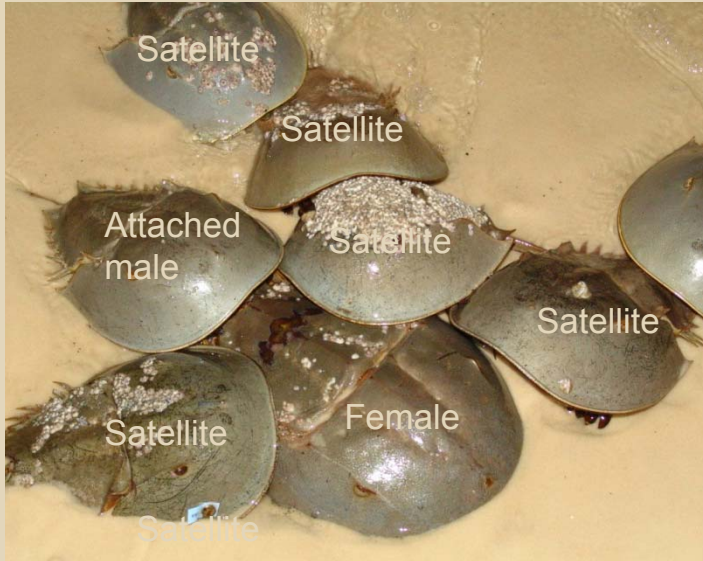
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 - 6) Is multiple mating costly or beneficial to females and why do some females mate multiply?

4. Why are there so many more males than females?



Females arrive paired with an attached male. After nesting has begun, they are joined by unpaired or “satellite” males.

Males return to the spawning beach repeatedly



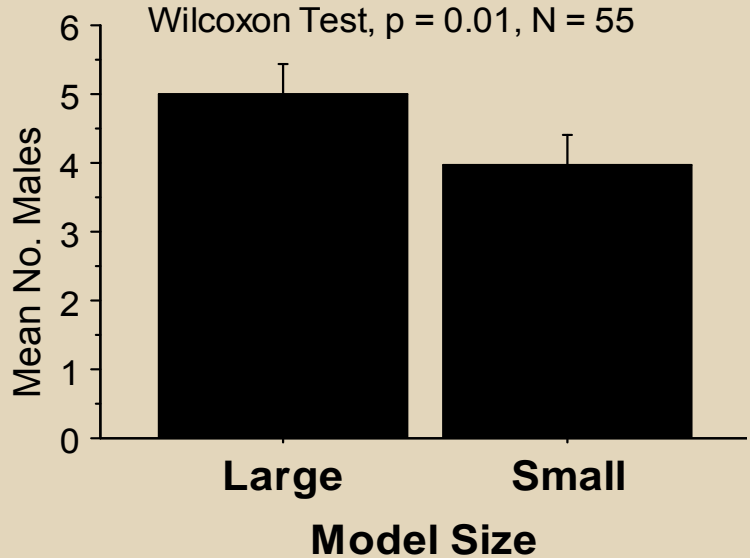
- Under natural conditions the population-wide sex ratio is equal.
- Females usually complete their nesting in 1-5 days and return to sea
- Males return to the beach repeatedly over the spawning season

How do males find females?

Visual and chemical cues

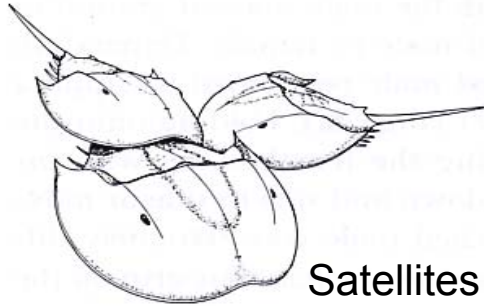


Unattached male approaches cement model of nesting pair.

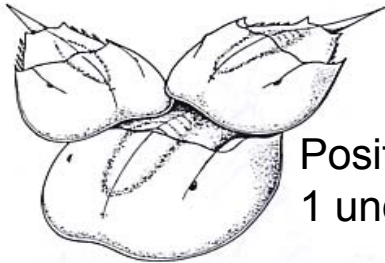
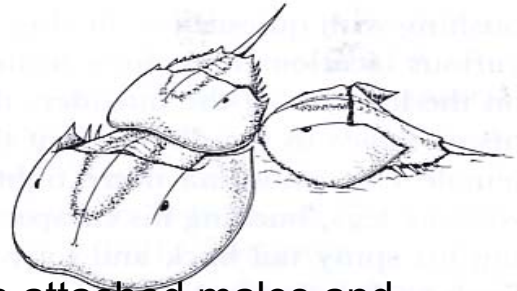


(Hassler & Brockmann 2001;
Schwab & Brockmann 2007)

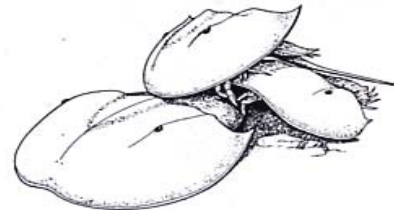
5. What are the Satellites Doing?



Satellites push on attached males and jockey for position



Position 1 under



But take-overs of attached males are rare

(Brockmann 1990)

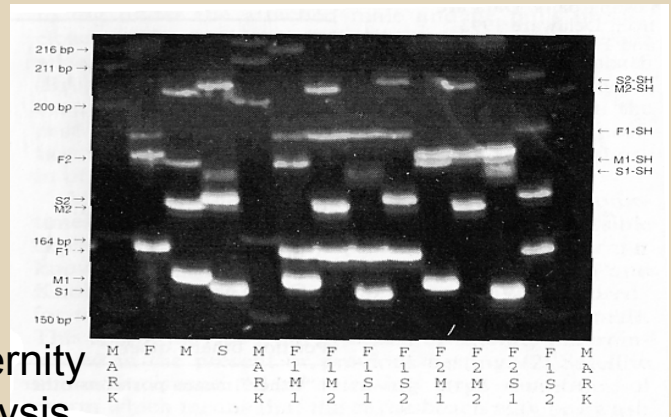
Are satellites fertilizing eggs?

Marking nests with flags



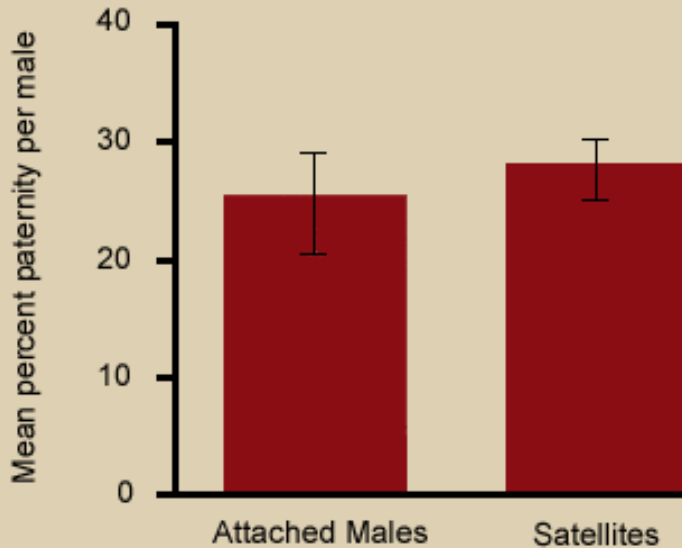
Digging up eggs

Rearing eggs

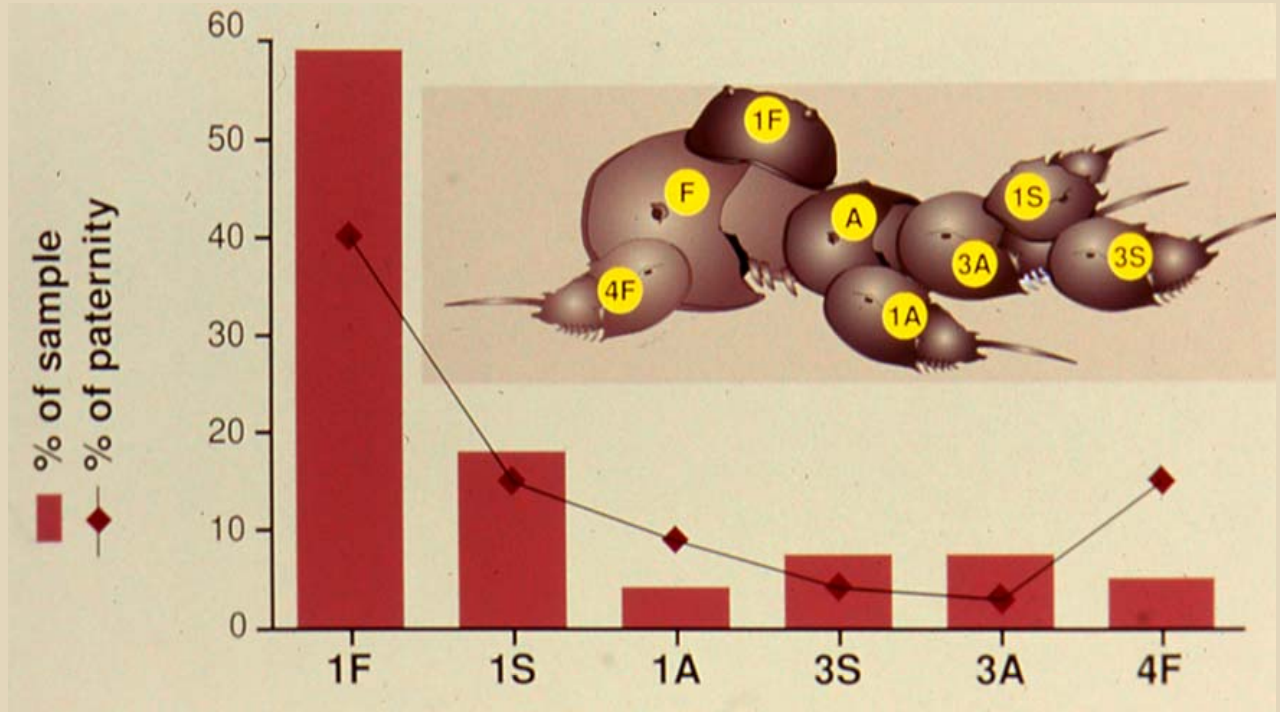


Paternity analysis

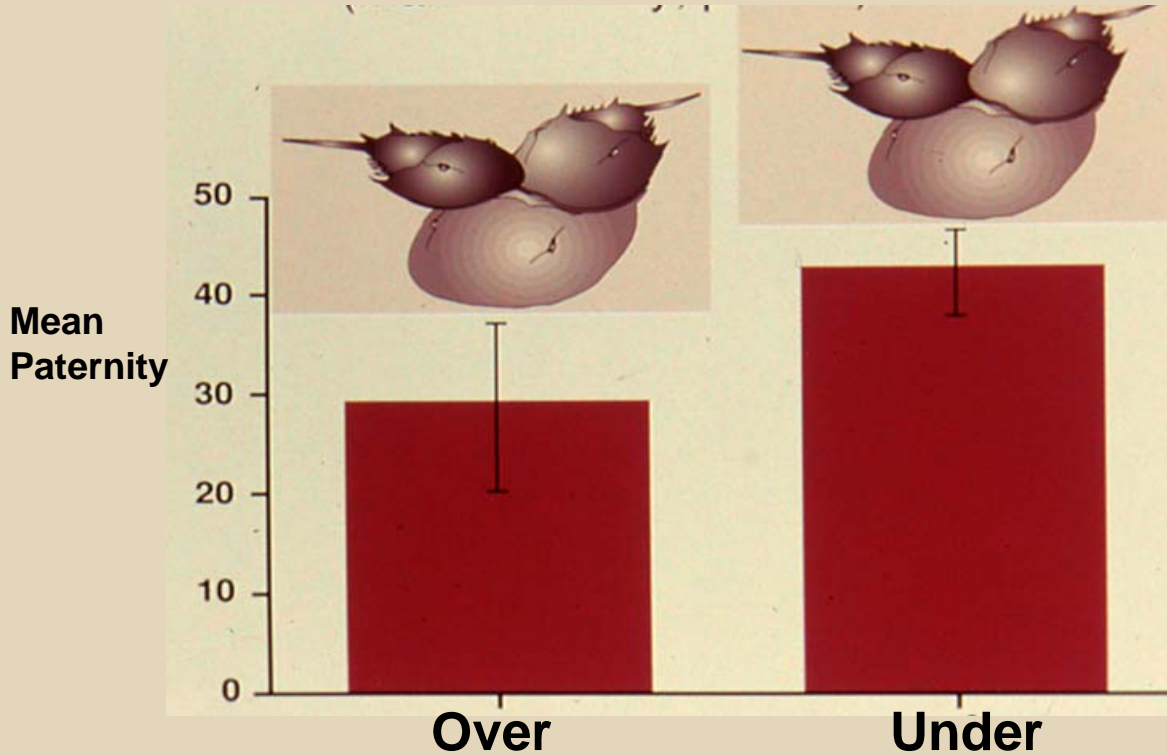
Satellite males fertilize as many eggs as attached males!



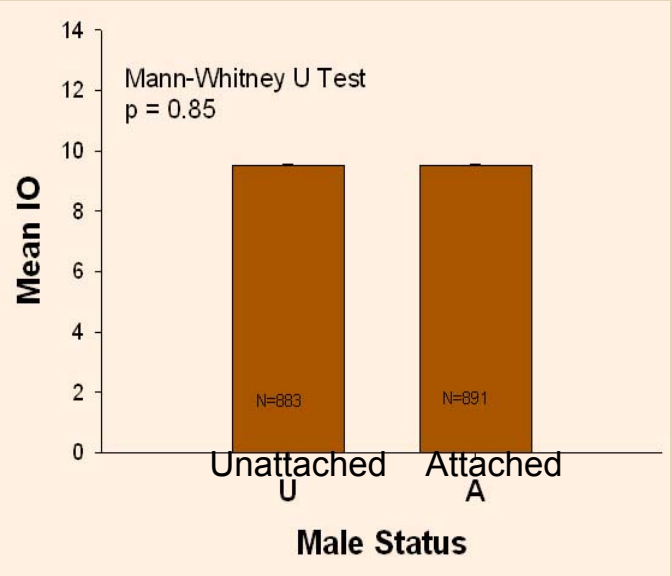
Satellite location affects paternity



Satellite behavior affects paternity



Do satellite and attached males differ? Does size affect male tactic?



Horseshoe crabs molt for the last time when they become sexually mature

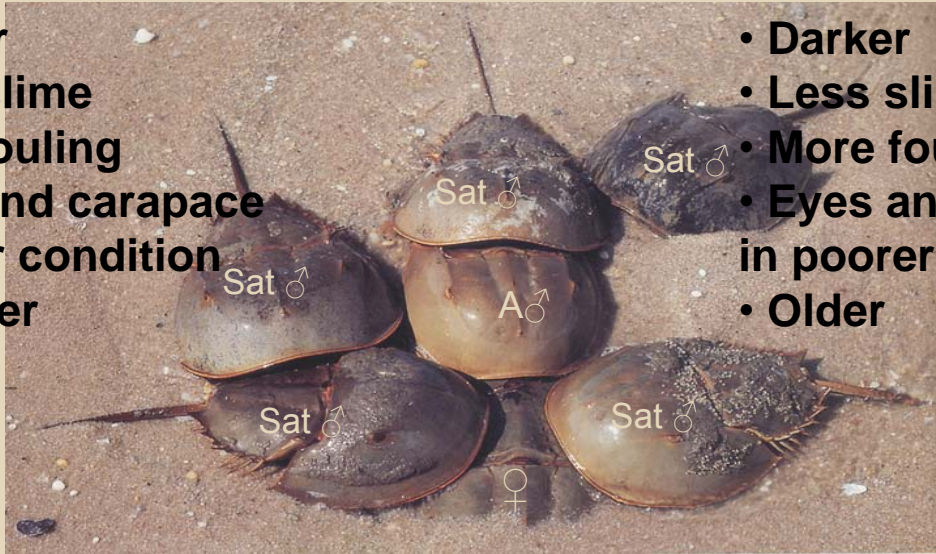
Male mating tactics are correlated with condition and age

Attached Males

- Lighter
- More slime
- Less fouling
- Eyes and carapace in better condition
- Younger

Satellite Males

- Darker
- Less slime
- More fouling
- Eyes and carapace in poorer condition
- Older



Attached and satellite males do *not* differ in size

Younger males are better at finding females

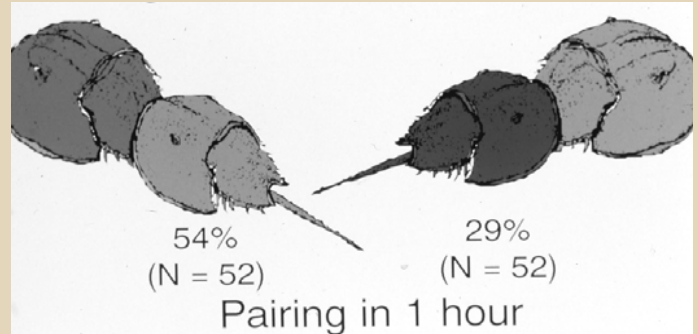


Experimental Manipulation

- Attached males detached, marked, returned to sea
- Satellite males marked, returned to sea
- Next day: younger (former attached) males were significantly more likely to return with a female than older males

(Brockmann and Penn 1992)

Younger males pair more quickly and are more likely to remain paired



(Brockmann and Penn 1992)

Younger males behave differently when forced to be satellites



Former satellite (darker) males more likely to return than former attached (lighter) males when none can attach (claws are covered).

(Brockmann 2002)

Condition-dependent Mating Tactics

Attached Males

- Lighter
- More slime
- Less fouling
- Eyes and Carapace in better condition
- Younger
- attach more quickly
- stay attached better
- more active
- seek unattached females at sea

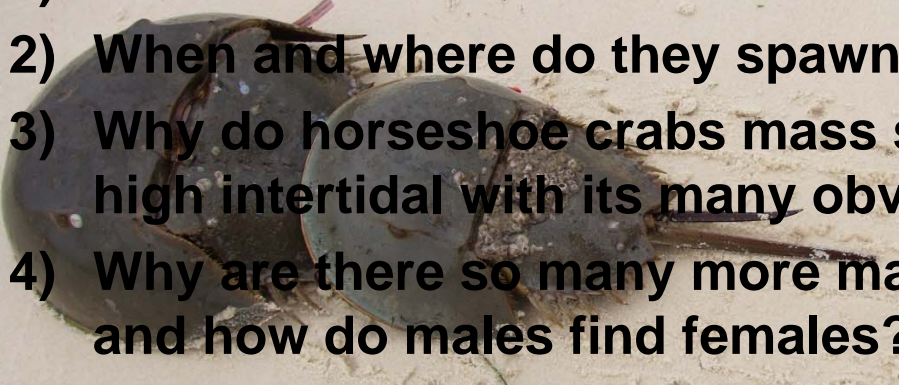



Satellite Males

- Darker
- Less slime
- More fouling
- Eyes and Carapace in poorer condition
- Older
- attach slowly
- let go easily
- less active
- seek pairs on shore



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We've considered this system
from the male's perspective,
what about the females?



Two Female Mating Tactics



Are monandrous and polyandrous females different?



Monandrous females are

- Slightly smaller
- Lay fewer eggs/clutch
- Lay fewer eggs/nest
- Consistently monandrous

Condition-dependent female mating tactics

Why do some females mate multiply?



Is multiple mating beneficial or costly to female horseshoe crabs?

Multiple Mating is Costly

1. reduces nesting success
2. reduces developmental success

Multiple Mating is Beneficial

1. increases fertilization success
2. improves mate quality
3. improves mate compatibility

Are satellite males costly or beneficial: field experiments

1. Satellites Removed: **Poly** → Mon (Exp Rem)
→ Poly (Control)
2. Satellites Added: **Mon** → Poly (Exp Add)
→ Mon (Control)



Measured:

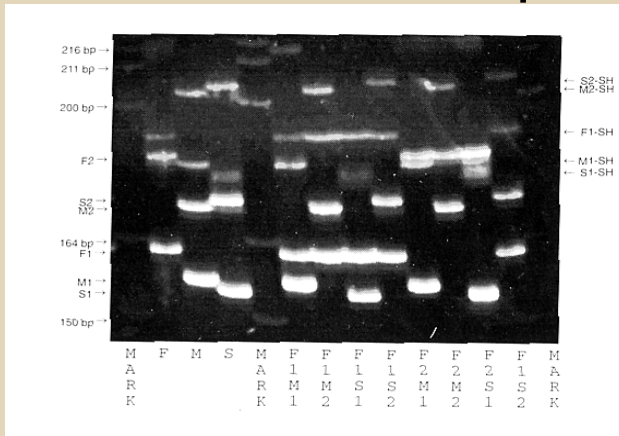
- Nesting
- Fertilization
- Developmental Success

Are satellites costly to females?



- Nesting success: Polyandrous females with satellites removed had *higher* nesting success (more clutches; stayed on beach longer), so multiple mating is costly
- Fertilization success: no difference
- Developmental success: no difference (surprising because females attract these males)

Paternity analyses reveal that polyandrous females with no satellites *nonetheless use* satellite sperm, so this experiment did not measure developmental rates successfully.

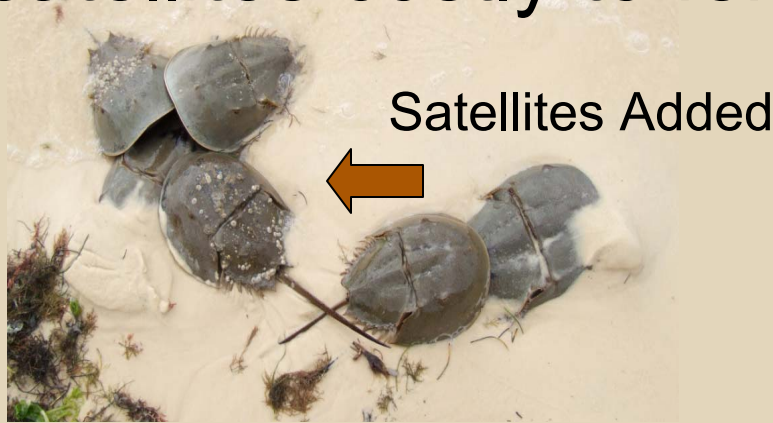


Paternity analysis using microsatellites



Fertilization is external

Are satellites costly to females?



- Nesting success: Monandrous females with satellites added had *lower* nesting success (fewer clutches; left quickly), so multiple mating is costly
- Fertilization success: no difference
- Developmental success: no difference (expect multiple mating to be costly for development)

But many monandrous females leave the beach rather than nest with satellite males.

Their satellites drop off as the female enters the water and she then returns to the beach and re-nests.



So in this experiment we were measuring developmental success of only those females that chose to remain long enough to lay eggs, which was very few.

In Vitro Fertilization



Eggs and sperm are obtained by electro-ovulation and ejaculation; we then fertilize 30 eggs with a known quantity of sperm. We compare developmental success of monandrous and polyandrous females mated with their attached male and a satellite male.

(Dan Sasson & Sheri Johnson, in prep.)

In vitro Fertilization Results

Monandrous ♀



attached
male
sperm



satellite
male
sperm

Developmental success
equal for attached and
satellite males

Polyandrous ♀



attached
male
sperm



satellite
male
sperm

Developmental success
higher with satellite male
than with attached male

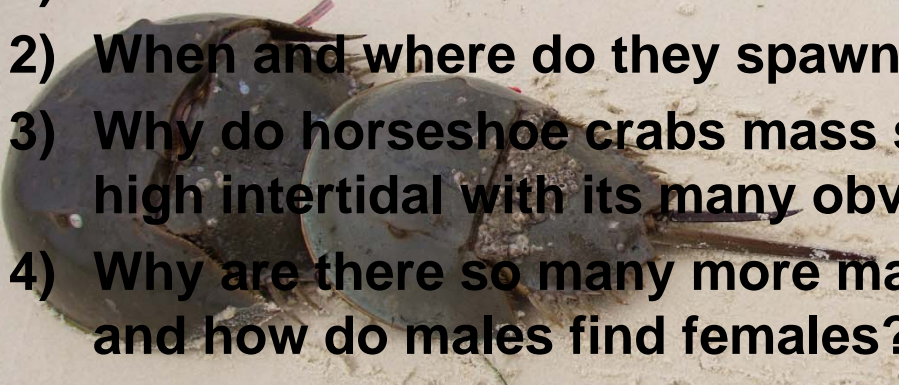


Monandrous and polyandrous females differ in the benefits and costs associated with having multiple mates





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Making sense of horseshoe crab spawning behavior



My *Limulus* Classroom



Measurements



Population Surveys



Measuring nests & numbers of eggs



Observing behavior & conducting manipulations



Studying juvenile behavior & life-history patterns

Science Field Projects

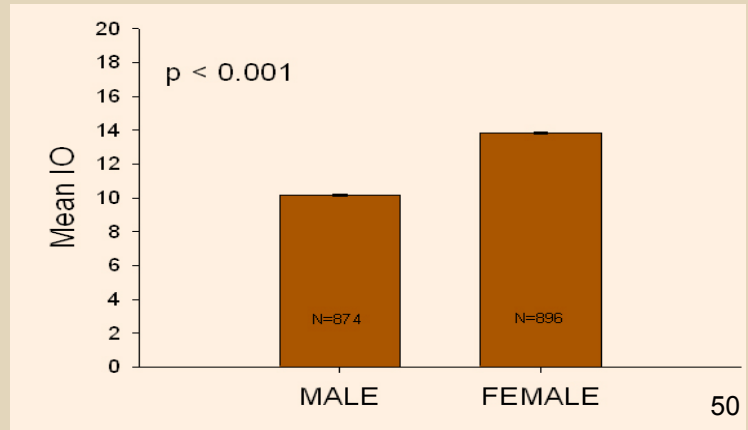
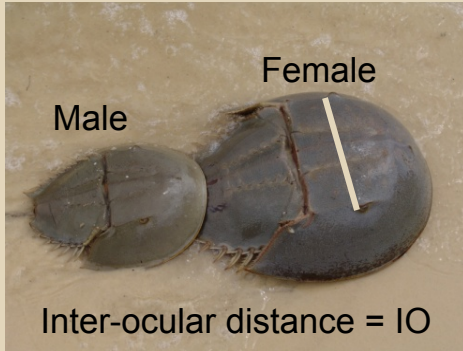
Hypothesis testing

Measurement

Observation

Data collection and analysis

Data presentation



Horseshoe Crab Spawning Survey Methods



Pickering Beach Survey May 2010



Field-based projects

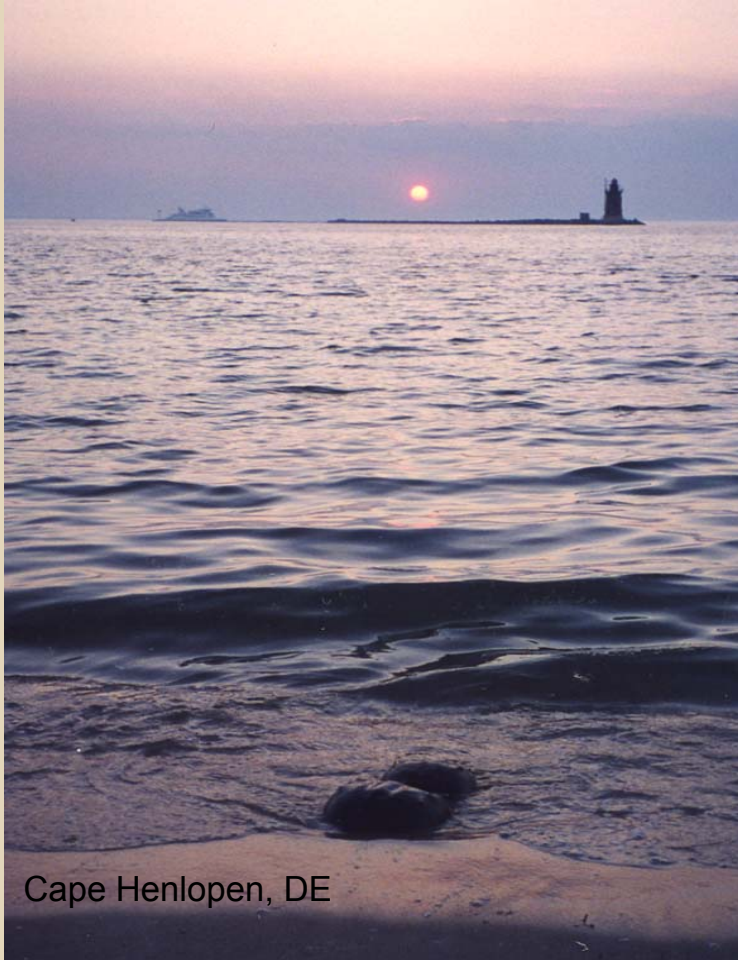
- Encourages students to observe, to ask questions,
- to learn about the process of science; and that
- science includes field-based, whole organism biology.
- Students learn to take measurements and that there are different kinds of measurements and data;
- learn how to compile, analyze and present data;
- learn how to discover new information and how to identify reliable sources of information.

And it's a day at the beach they'll never forget!

When considering harvest management:



- Horseshoe crabs take 10 yrs to mature;
- breed once per yr for about 8 yrs;
- many factors limit breeding (eg. temperature, wind);
- have low fecundity (60K eggs/yr) and
- most eggs & juveniles die before maturing.
- The contribution of satellite males is not known



Cape Henlopen, DE



My research is
funded by the
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Science Foundation

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