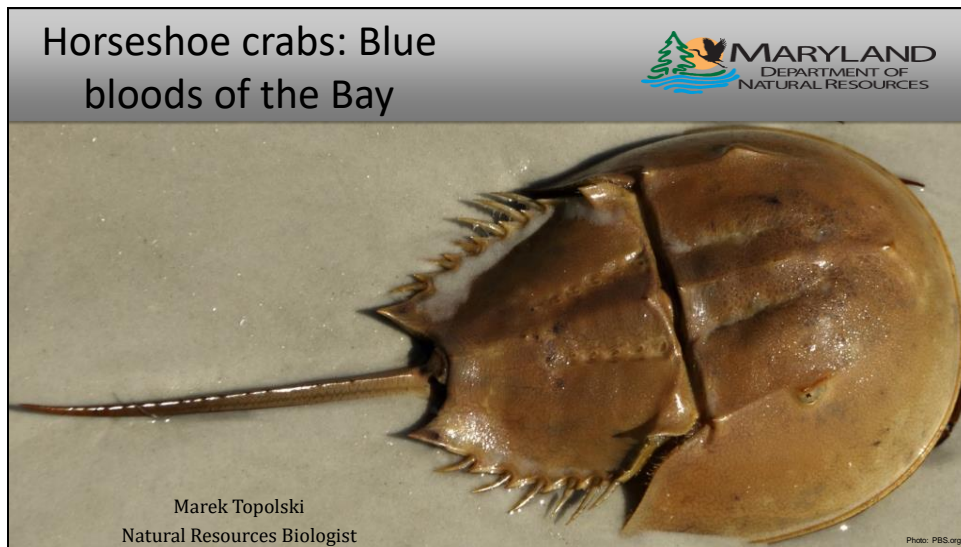


## Slide 1



Uniquely charismatic species  
Readily recognizable by the general public  
Their significance spans ecology, economy, and human health

## Slide 2

What is a horseshoe crab?

Maryland Department of Natural Resources

Arthropod Diversity

Horseshoe crabs are in a class by themselves: Merostomata

- Legs attached to mouth (crab legs attach to gills)
- Have chelicerae like spiders, scorpions, & ticks
  - small appendages for moving food into the mouth
- But no mandibles (mouth parts to grind food)
- No antennae
- Limulus ~445 million years
- Life span  $\geq 20$  years

Arthropod Diversity

Class Diplopoda, Class Chilopoda, Class Insecta, Order Decapoda, Order Isopoda, Order Amphipoda, Order Scorpiones, Order Aracari, Order Araneae, Order Pycnogonida, Class Merostomata

Subphylum Chelicerata, Subphylum Crustacea, Subphylum Hexapoda, Subphylum Chilopoda, Subphylum Diplopoda

Common ancestor

Arthropods with jaws, Arthropods with fangs or pinchers

Photo: matthewwells.com, Photo: FWC

So, what is a horseshoe crab?

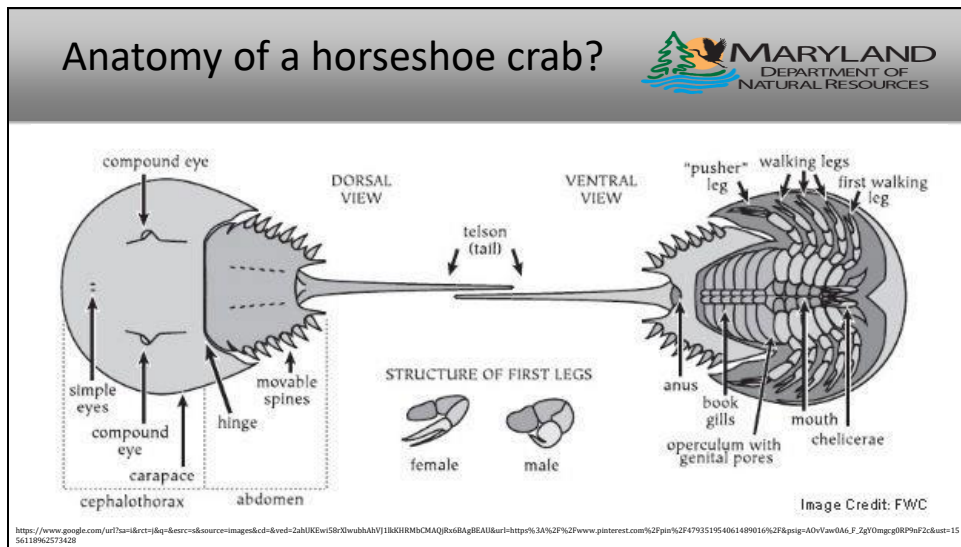
### [follow bullet points]

Unique among the arthropods

- due to the structure of mouth & legs

Ancient Class of arthropods

- Early forms evolved in Paleozoic Era, ~445 million years
- Predate dinosaurs & flowering plants
- Survived Paleozoic Cambrian & Permian [marine] extinctions & the Mesozoic Cretaceous [land & marine] extinction



### [use diorama & molt]

Heavily armored

Two body segments plus telson

- Cephalothorax / prosoma
- Abdomen / opisthosoma

Numerous eyes [will come back to]

First pair of claws are for feeding (not legs)

5 pairs of legs

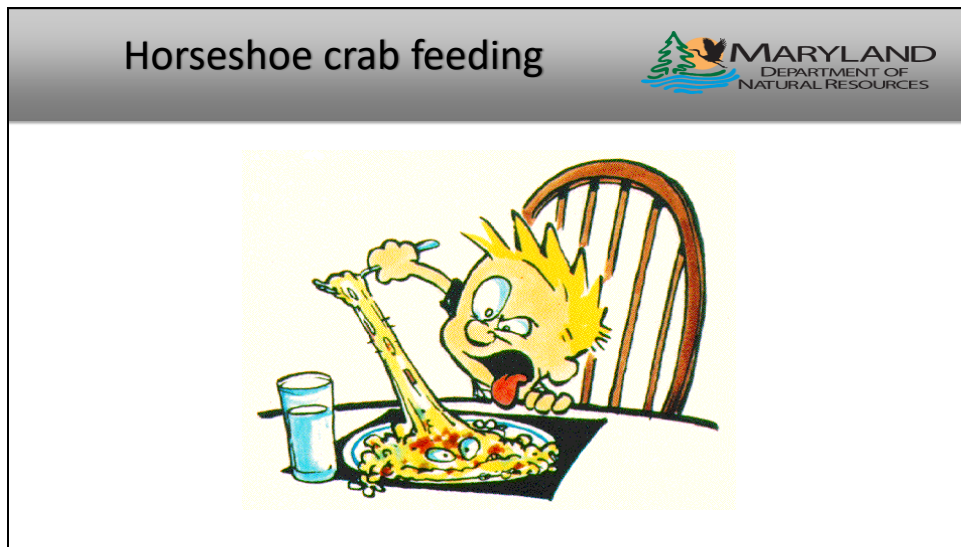
First walking leg is sexually dimorphic

Last pair of legs has modified claw (pusher)

Book gill covers are modified abdominal appendages [will come back to]

Female ~1/3 larger than male

## Slide 4



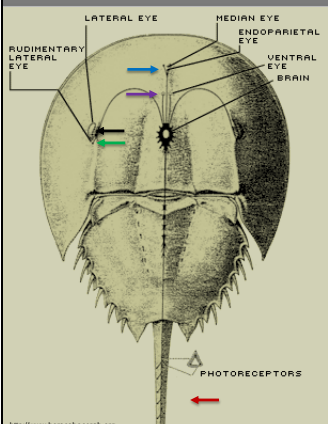
Video of how a horseshoe crab feeds [\[hyperlink\]](#)

Feed on invertebrates like marine worms & small molluscs and scavenge

Have a crop and gizzard

## Slide 5

### Light sensitivity



- Only Chelicerate to possess compound eyes (lateral eyes)
  - ~1,000 facets
  - Primarily for finding mates
- There are 5 additional eyes on the top of its shell: two median eyes, one endoparietal eye and two rudimentary lateral eyes
  - Median and endoparietal eyes sensitive to visible and ultraviolet light
    - Enhance telson's degree of adaptation to darkness based on amount of ultraviolet light
  - Rudimentary lateral eyes are photoreceptors (functional just before embryo hatches)
  - A "clock" in anterior brain controls sensitivity of lateral and median eyes
- Underside of crab has two ventral eyes located near the mouth
  - May help orient the animal when swimming
- Telson has series of light sensors along the top and side
  - Keeps brain synchronized with cycles of light and dark

<http://www.horseshoecrab.org>

### [follow the bullets]

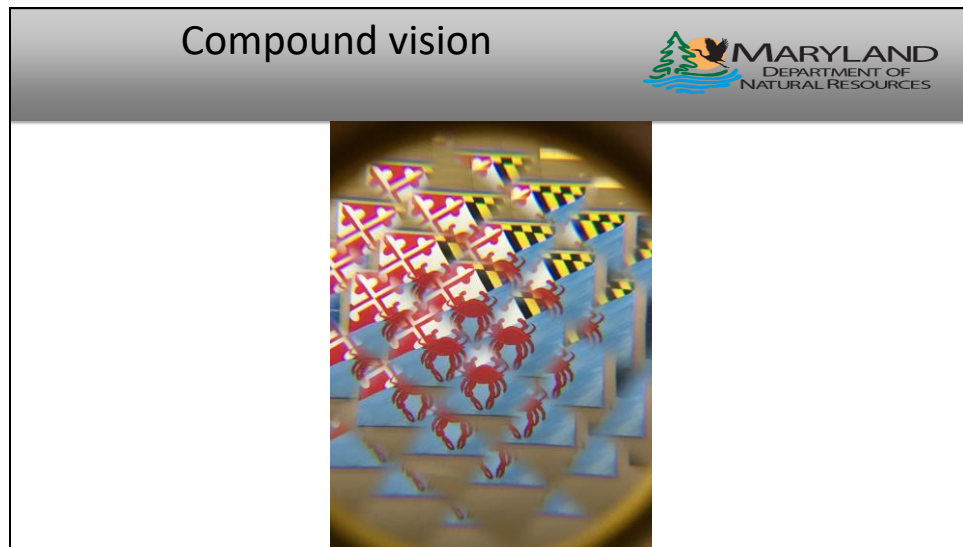
Complex array of light sensitive structures

Compound eyes – pass around the facet simulators

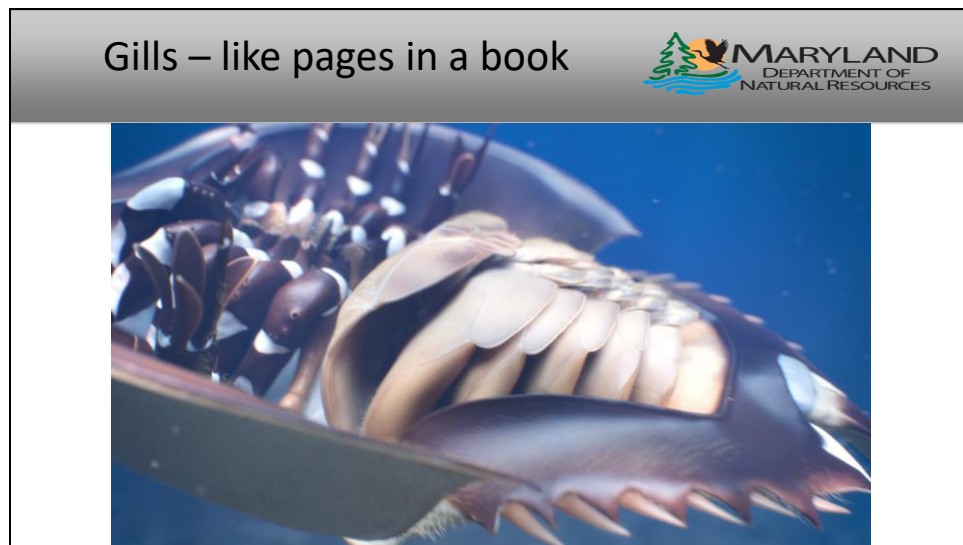
Facets are easily seen on the larger molt

- Molts are fragile, but can be looked at afterward

Slide 6



Slide 7



CGI rendition of horseshoe crab

Book gill covers are modified abdominal appendages

- First is genital operculum
- 100"pages of lamellae per appendage

## Slide 8

# Morphology and behavior



0:00 / 2:11

<https://www.youtube.com/watch?v=wqeZ9aXFwv0>

I found a YouTube video of a small horseshoe crab in an aquarium [\[hyperlink\]](#)

It is useful to observe horseshoe crab behavior


- Swimming, Walking, Burrowing

This is not an endorsement of collecting horseshoe crabs for personal aquaria

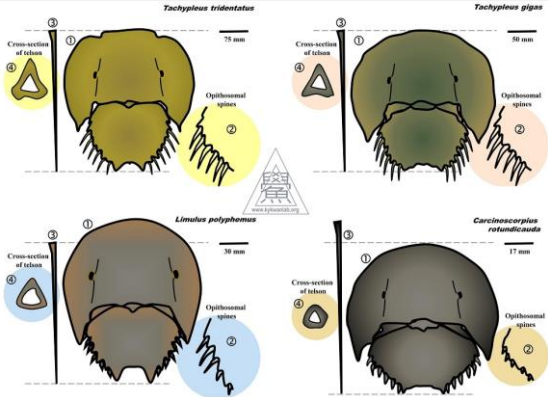
Also, the person had a sturgeon which is prohibited

## Slide 9

# Types of horseshoe crabs



MARYLAND  
DEPARTMENT OF  
NATURAL RESOURCES



*Tachyplesus tridentatus* 75 mm

*Tachyplesus gigas* 50 mm

*Limulus polyphemus* 38 mm

*Carcinocarpus rotundicauda* 17 mm

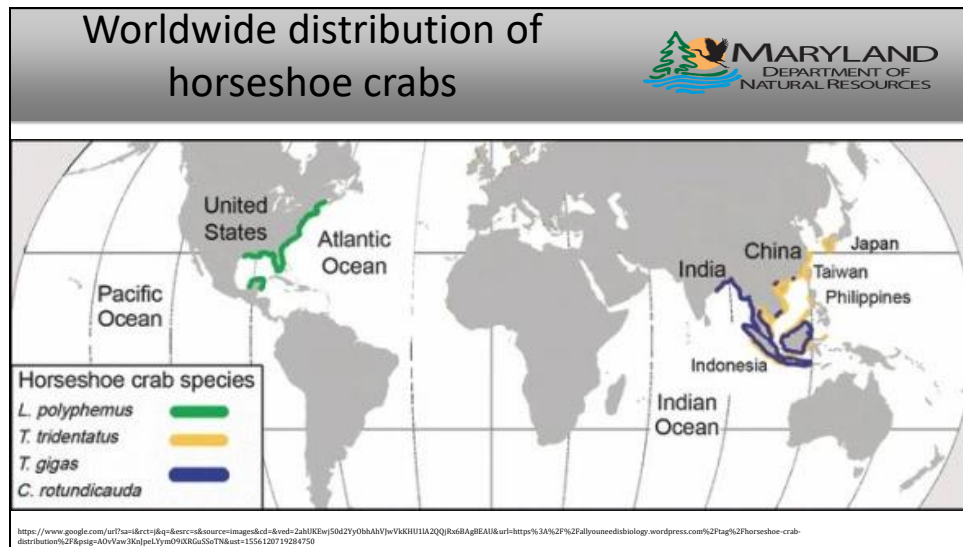
Each diagram includes a dorsal view of the carapace, a cross-section of the rostrum, and a detail of the opisthosomal spines.

<https://www.google.com/url?sa=i&ict=ik&q=icerc=ndsource=imaget&ved=2ah0KwJf84Cfye8bA9w1k0KVVMAQYQ8a6Ag8AUEAur&http=3A%2F%2Fkykor.anlab.org%2Fresources%2Fabout-buc%2Fkenned-40V9wE1FW0118r-F8G2Xa656898eater1556211646123017>

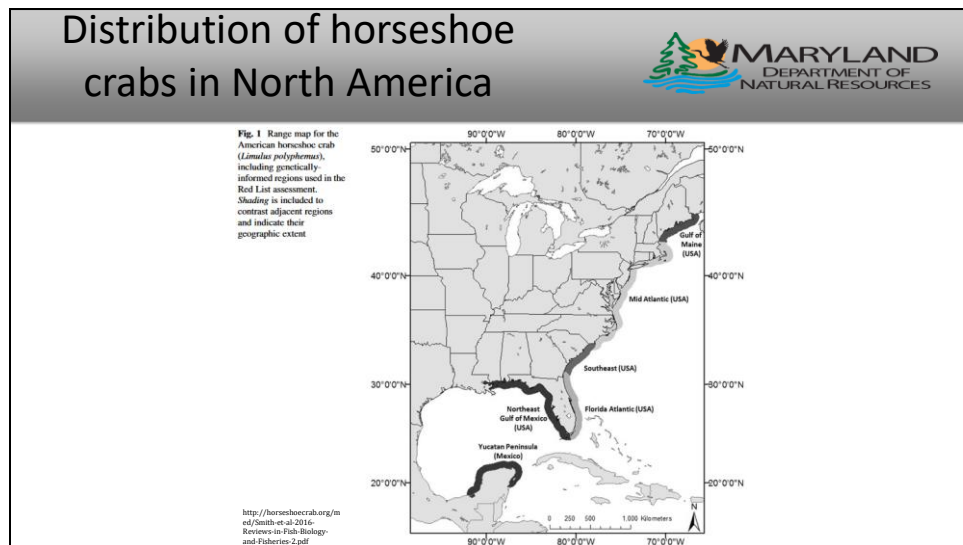
There are 4 species of horseshoe crab

Differentiate them fairly easily

- anterior margin of cephalothorax
- Opisthosomal spine configuration
- Length/width of cephalothorax and abdomen
- Telson length and shape (cross section)



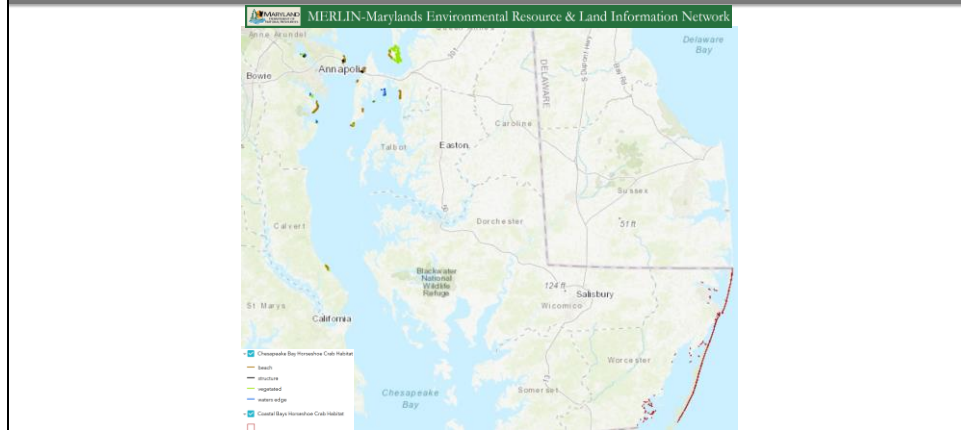
Distributed in IndoPacific and western Atlantic  
Only one species in western Atlantic



Several genetic populations of *Limulus polyphemus*  
Largest spawning population is in the Delaware Bay region  
- Notable spawning activity in Chesapeake Bay has been reported



## Distribution of horseshoe crab habitat in Maryland

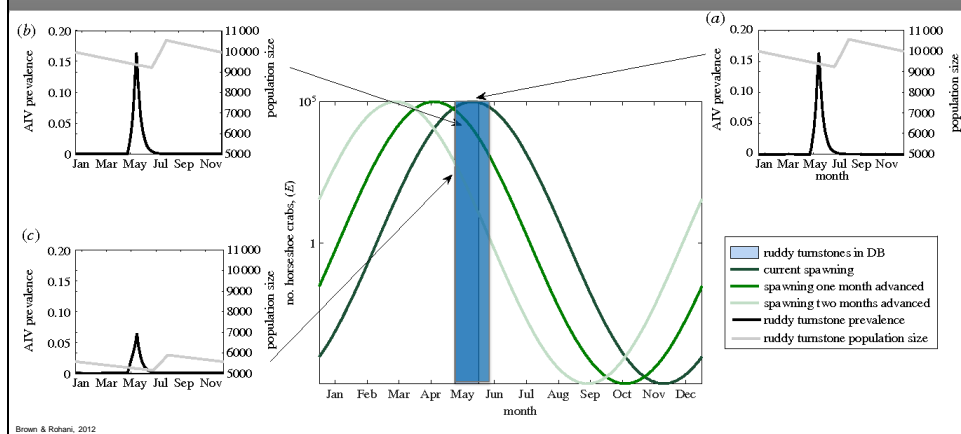


Horseshoe crab spawning habitat has been poorly characterized in Chesapeake Bay

Under-represents actual activity & habitat

- Problematic for effective management
- Limited resources and it is not a major priority

## When to spawn?



This is a model of Delaware Bay horseshoe crab spawning and there is some variability in timing

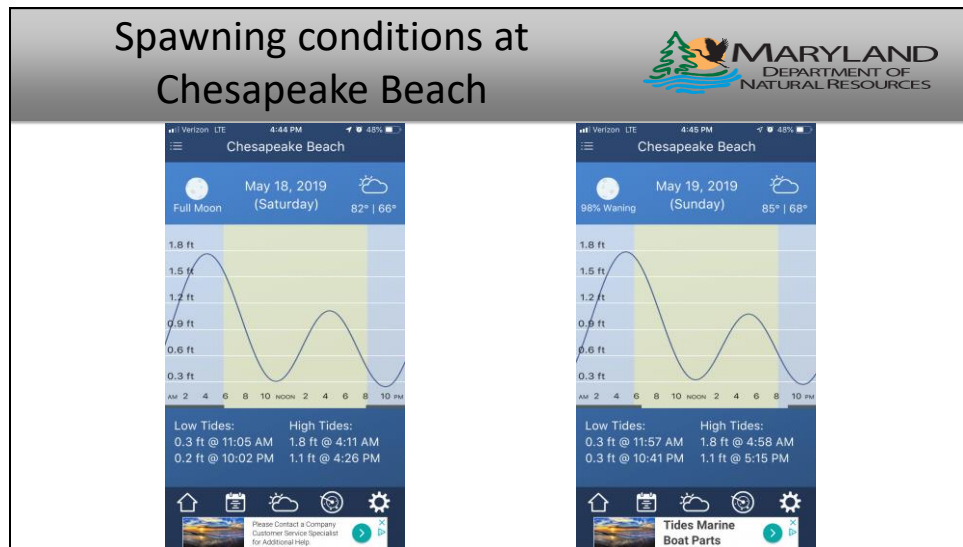
Water temperature  $\geq 58^{\circ}\text{F}$

Usually mid-May into June

## Hightide at night

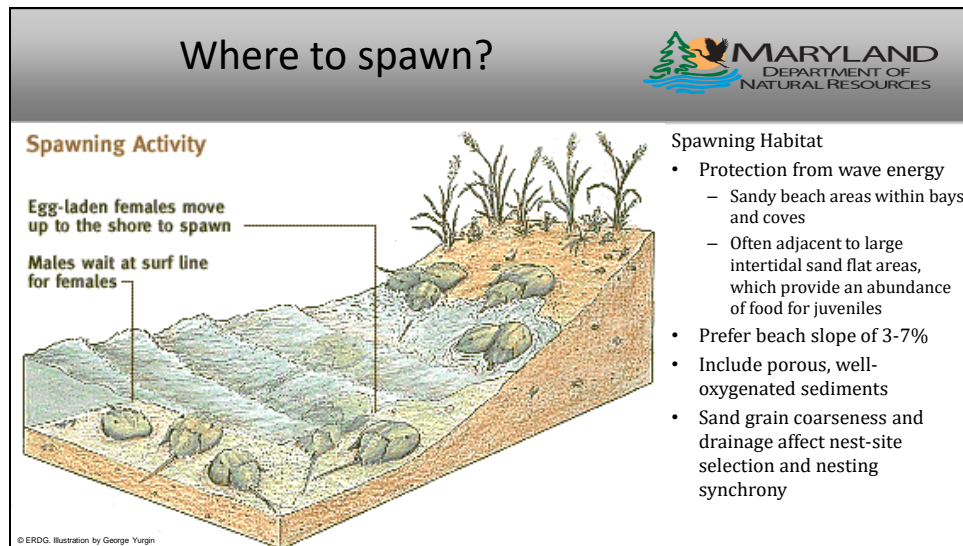
Preferably during new & full moons when spring tides occur, these are the highest tides

## Slide 14



I looked up the tidal forecast for this weekend at Chesapeake Beach for planning purposes

## Slide 15



Beaches can be very small

Avoid high peat content

Do not have beach fidelity each year of spawning

Waves less than 1 ft to avoid being flipped



Slide 16



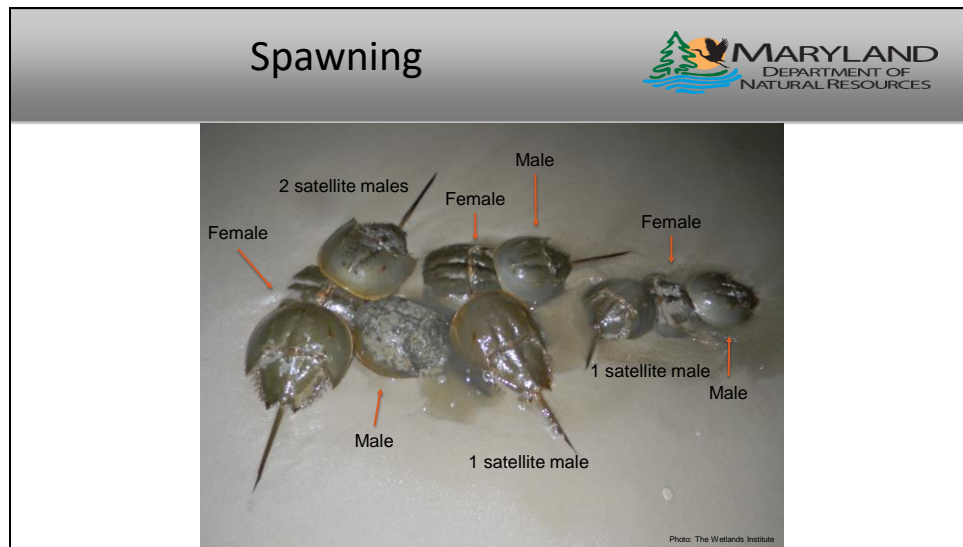
A little blurry, but we can see the male first walking leg hooked onto the female abdomen

Slide 17



Eggs are stored/develop throughout the cephalothorax

## Slide 18



Males hitch a ride from the shallows to where the female will lay eggs

- They are not spawning
- National Geographic video at minute 1:07 [[hyperlink](#)]

Satellite males congregate around female in an effort to also fertilize eggs

## Slide 19



Here is a buried female laying eggs

Eggs are deposited 6-10 inches below surface just above high tide line

This female has been tagged – white disk

Slide 20



Eggs are green  
They somewhat blend in

Slide 21



Hatching takes two weeks to several months depending on conditions  
The warmer the water and the higher the salinity, the more rapid the development  
No distinct telson at hatch  
Telson is prominent after first molt

[illegible]

17 molts during first 9-11 years to reach full size and sexual maturity

- Then one molt per year


## Exit the front of the shell

- Many crustaceans exit through the back

Tissue expands with water prior to hardening of shell

If a horseshoe crab shell has a split along the front, it is a molt

# Juvenile & adult habitat



## Nursery Habitat

- Shoalwater and shallow water areas of bays (such as Delaware Bay and Chesapeake Bay) are essential nursery areas.
- 99% of juveniles found in waters >5 parts per thousand
- Juveniles usually spend first two years on intertidal sand flats
- After two years juveniles migrate to deeper bay and ocean waters until sexually mature

## Adult Habitat

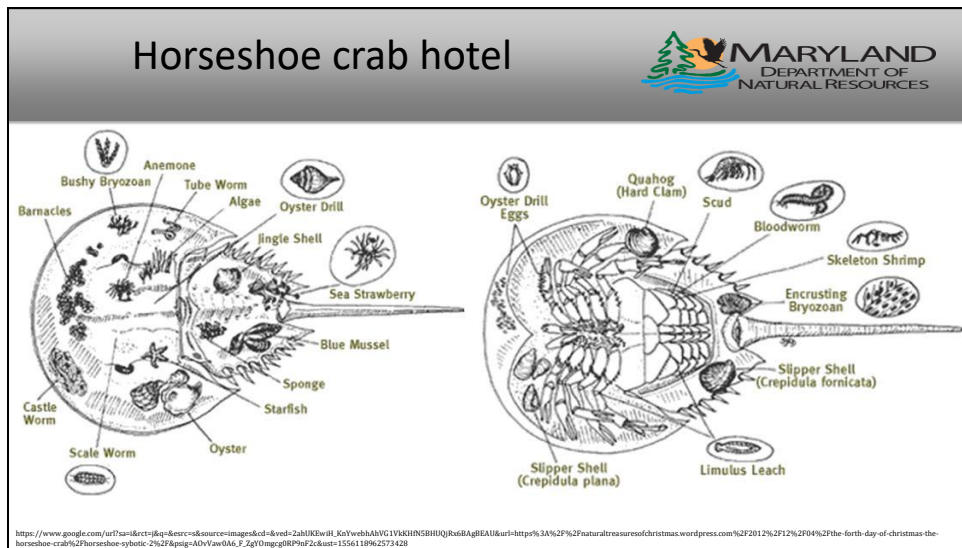
- Specific requirements are not known
- Have been found at depths > 200 meters (656 feet)
- May prefer depths < 30 meters (98 feet)
- During the spawning season, adults typically inhabit bay areas adjacent to spawning beaches
- In the fall, adults may remain in bay areas or migrate into the Atlantic Ocean
- May overwinter on the continental shelf

**[follow the slides]**





Between molts, the shell becomes a complex mobile reef



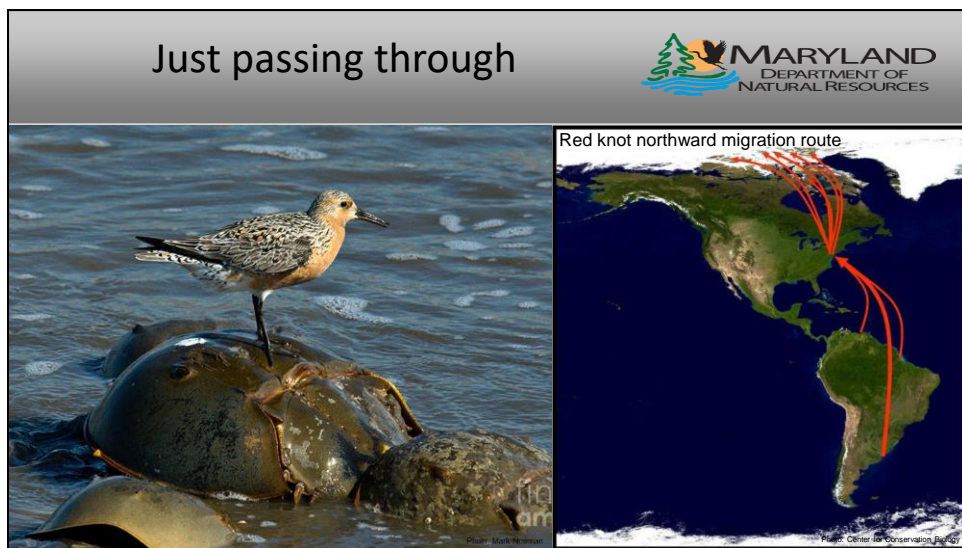
[point out the different types of potential encrusting and parasitic organisms]

Slide 26



Migratory shore birds are a primary predator of horseshoe crab eggs  
Red knot will double body weight  
They are an important food resource without which successful migration would not be possible

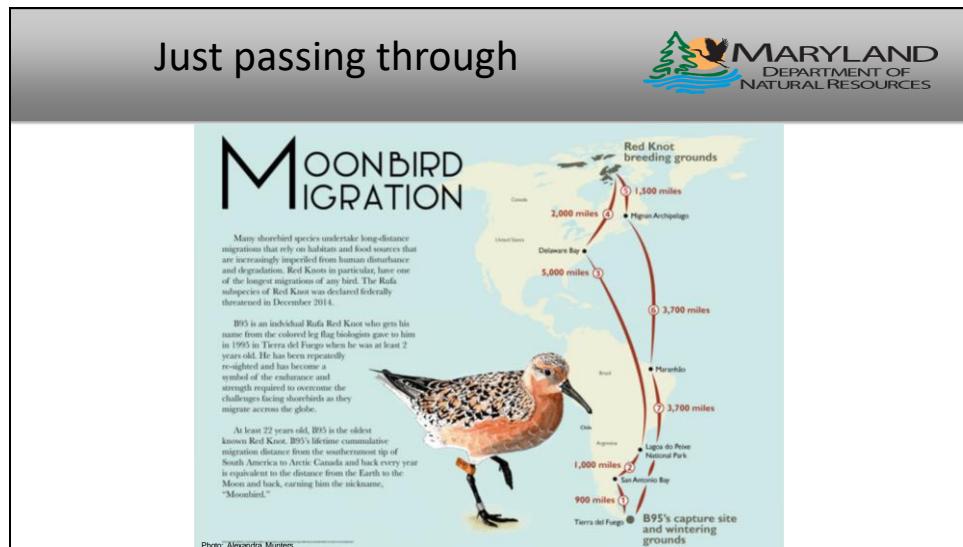
Slide 27



Migration can be up to ~9,300 miles  
Do it in ~1,500 mile stages



Slide 28

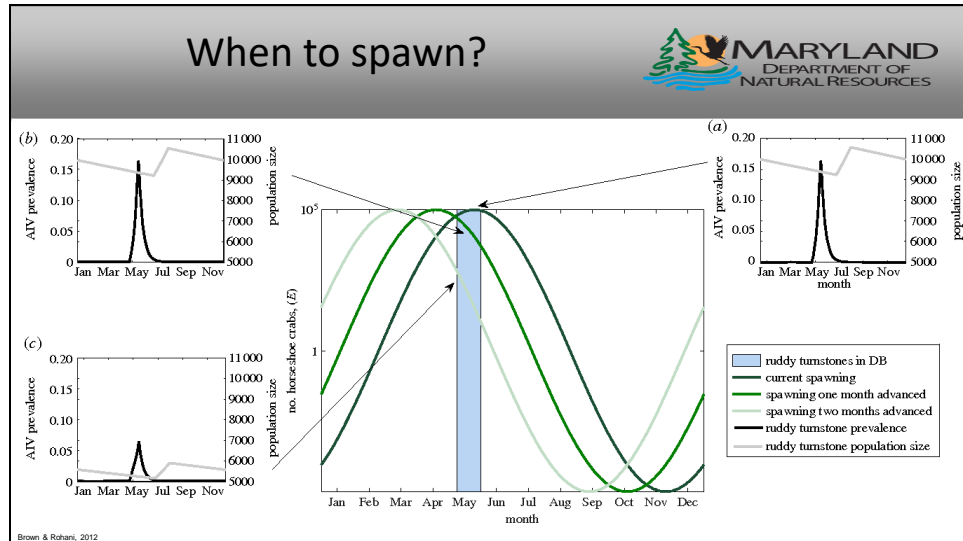


Band number B95

Tagged in 1995 in Tierra del Fuego, Argentina/Chile at age 2-3

Flown distance to moon and back, hence the name "Moonbird"

Slide 29



We saw this graph before

It is important because it indicates how bird migration is timed to horseshoe crab spawning

If horseshoe crab spawning season shifts to earlier in the year, eggs are not available to migrating birds

Why a shift, climate change

- Waters warm sooner so spawning is sooner
- Would bird migration also shift to earlier, that is not certain

## Commercial uses



### Fertilizer



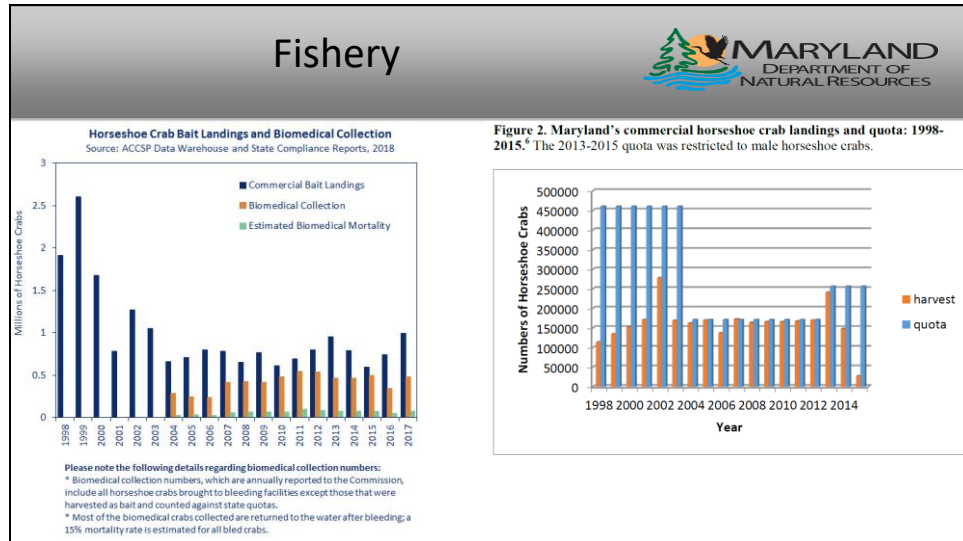
Photo: Delaware Public Archives

### Bait for whelk & eel traps



Photo: <http://jimbodouglass.blogspot.com/2014/03/films-i-love-horseshoe.html>

Humans have a history of utilizing horseshoe crab  
 Historical use was fertilizer  
 Modern use is for bait




Horseshoe crab harvest and utilization have been monitored and managed for several decades  
 Majority of recent landings have been for bait

- Landings are tightly regulated
- Harvest has dipped below quota in recent years

Collection for biomedical use for the past ~25 years

## Slide 32

### Fishery management



**Quota**  
The annual total allowable landings of male horseshoe crabs for the commercial fishery is 255,980. There is no female harvest permitted.

**Season**

- May 1, 2019 through July 7, 2019:
  - A person may catch or land horseshoe crabs outside of 1 mile of the Atlantic coast.
  - A person may catch or land horseshoe crabs in Maryland's coastal bays and their tidal tributaries.
  - A person may not catch or land horseshoe crabs within 1 mile of the Atlantic Coast, or the Chesapeake Bay and its tidal tributaries.
- July 8, 2019 through November 30, 2019: A person may catch or land horseshoe crabs from the tidal waters of the State.
- December 1, 2019 through April 30, 2020: A person may not catch or land horseshoe crabs in Maryland.

**Catch Limits**

- An individual may not land more than 25 male horseshoe crabs unless they are in possession of a valid horseshoe crab landing permit.
- May 1, 2019 through July 7, 2019: A permittee may not land more than 150 male horseshoe crabs per day.
- July 8, 2019 through November 30, 2019: A permittee may not land more male horseshoe crabs than the amount specified on their permit.

All other rules remain the same (Code of Maryland Regulations 08.02.10.01).

Harvest is managed with annual quota, catch limits, and landing permits

No female harvest permitted

Regulations designed to reduce pressure on spawning individuals – females in particular (includes pre-spawning individuals)

- Bird nesting beaches are often used and also protected areas

## Slide 33

### Horseshoe crab bleeding





- Hemocyanin – blue blood
  - Copper based
  - Helps survive hypoxia
- Up to \$15,000 per quart
  - *The Atlantic*, 2018

<https://geneticliteracyproject.org/2018/05/21/the-end-of-using-horseshoe-crabs-for-drug-testing/>


**[pass around the vial of blood]**

Blood is blue because it is copper based

- Mammalian blood is iron based

Makes it easier to survive hypoxia

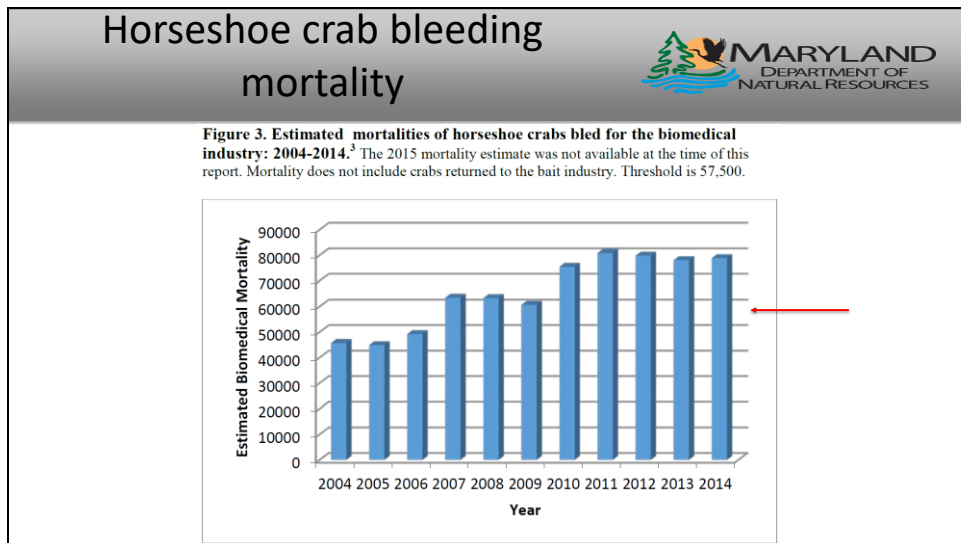
## Horseshoe crab blood use



- Humans can become sick if exposed to bacterial endotoxin
  - Endotoxins are a type of fever-causing agents
  - Is a component of the exterior cell wall of Gram-negative bacteria
  - Especially dangerous if sufficient amount of endotoxin enters blood stream or spinal fluid (such as from an injectable drug)
  - Symptoms can include fever, shock, organ failure, and even death
  - All injectable drugs and medical devices that come in contact with blood stream or spinal fluid are tested for endotoxin
- Horseshoe crab has an immune and blood coagulation system that protects it against infection
  - Inside blood cell (amebocyte) are proteins that cause blood to clot around the injury and bacteria
  - Research showed that their blood is very sensitive to endotoxin (1960s)
  - Blood gels & clots when it comes in contact with endotoxin
  - *Limulus* amebocyte lysate (**LAL**) test developed and was commercialized in the United States in the 1970s. (similar test, **TAL**, from *Tachypleus tridentatus*)
  - A version of the LAL test for diagnosis of invasive fungal infections is being developed

<http://www.horseshoecrab.org>


[follow the bullets]



Estimates of bleeding mortality vary, but 15% is assumed  
 Management problem, total bleeding mortality often exceeds the threshold

## Slide 36

### Horseshoe crab bleeding mortality



- Best practices to reduce mortality
  - Keep crabs cool and moist (transport & facility)
  - Do not bleed injured crabs
  - Separate unbled from bled to avoid rebleeding
  - Use of sterile needle
  - Allow blood to flow or drip into the container until the flow stops naturally
  - Bleeding horseshoe crabs to death is not an acceptable practice in the U.S.
  - Return to ocean waters from harvest site within 24 hours
  - Mark prior to return to avoid a second bleeding within a year
  - Bleed horseshoe crabs caught for use as bait
  - Return to the ocean at their harvest site.
- Alternatives are being developed that will reduce the use of horseshoe crab blood

[follow the bullets]

## Slide 37

### Reduction of LAL extraction



BROWSEPUBLISH

OPEN ACCESS

PERSPECTIVE

## Saving the horseshoe crab: A synthetic alternative to horseshoe crab blood for endotoxin detection

Tom Maloney , Ryan Phelan, Naira Simmons

Version 2  Published: October 12, 2018 • <https://doi.org/10.1371/journal.pbio.2006607>

Research is being conducted to develop a synthetic version of LAL  
Possibility to reduce LAL use by 90%

Slide 38



Spawning challenges in the Chesapeake Bay are largely due to habitat alteration and loss  
Here are aerial images of the shoreline in this area

Slide 39



This is an example of shoreline hardening and loss of sandy beach



Slide 40



The contrast in spawning success and survival for repeat spawning is stark  
Return to water and “Just flip ‘em”

Slide 41



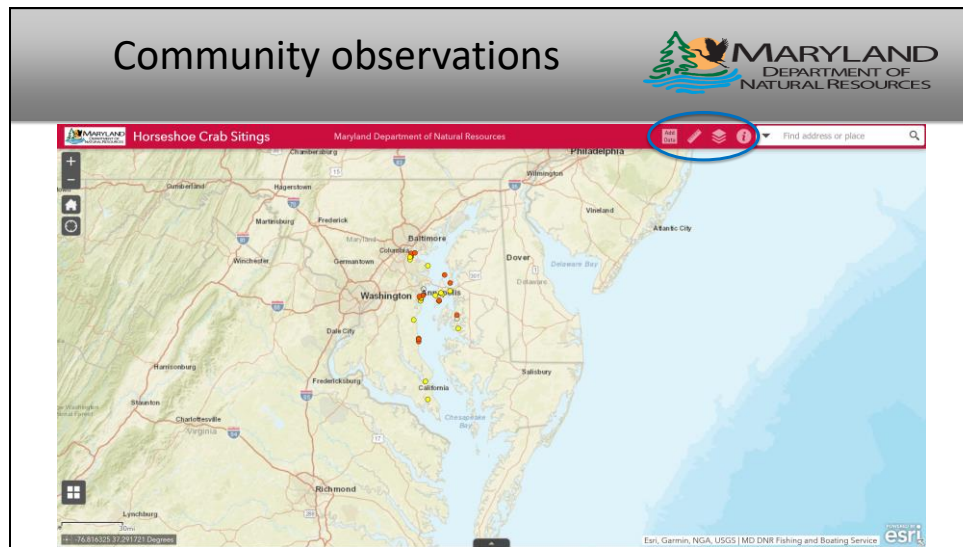
In the absence of resources and a survey, we rely on public reports of horseshoe crab activity

- Location
- Spawning or not
- Abundance
- Habitat characteristics
- Photos - sometimes

How to report

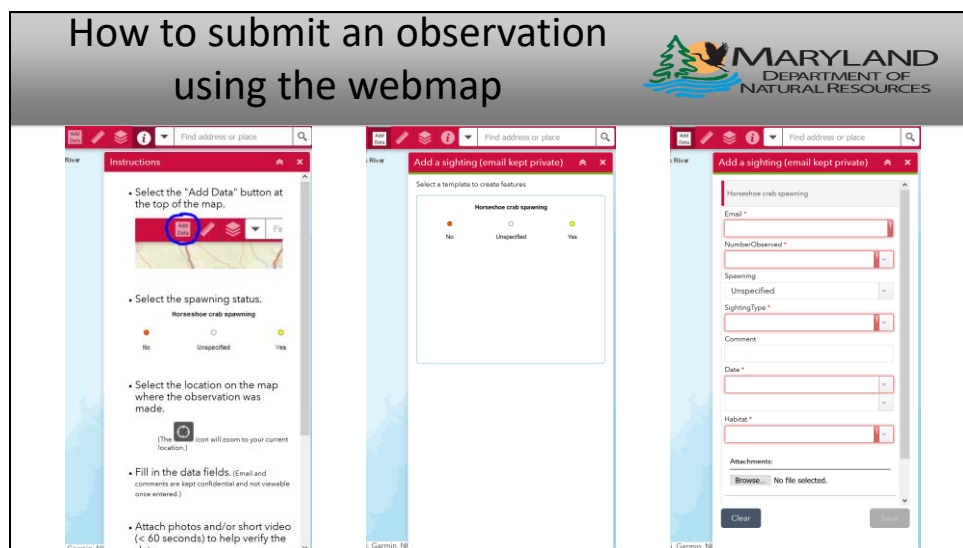
- Phone
- Email
- Webmap (DNR website & mobile app)

Slide 42



Webmap is mobile friendly [[hyperlink](#)]  
[walk through how it works]

Slide 43



Click the observation location on the map  
Select the type of horseshoe crab activity (spawning, no spawning, not sure)  
Fill in the sighting form and save  
Observation will not be visible on the map until it has been confirmed by DNR