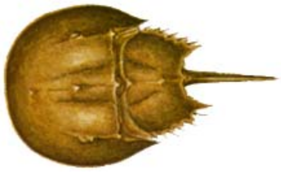


Exploring external anatomical features of the American Horseshoe Crab through observation of molt specimens

Developed by: **Gary Kreamer**, Delaware Division of Fish & Wildlife, Aquatic Resources Education Center, gary.kreamer@state.de.us

Activity overview: This activity utilizes molted (shed) exoskeletons of the American horseshoe crab, *Limulus polyphemus*, to engage students in identifying key structures in the external anatomy of the animal, and associating those structures with functions important to its way of life. An accompanying guidesheet and student worksheet are provided to direct and capture student observations. Instructions for preparation of specimens follows:

Prepping the molts: Upon drying, molts become brittle and easily broken, and this places limits on the handling required for close anatomical observation. To get around this, we discovered a way of pre-soaking molts in a 50/50 glycerin/water mixture that confers a great deal of flexibility and durability to the shells for repeated manipulation and observation over time. Note that there are two types of glycerin: vegetable-oil and petroleum-based. Both will work in making molts flexible. Vegetable oil-based glycerine is cheaper and easily accessible on line, but leaves a sticky residue. Though more expensive, petroleum-based glycerin yields a cleaner molt specimen - we found it for sale at http://www.tscpets.com/bci-1029.html for $35/gallon). Treating the molts after soaking with Lysol spray disinfectant is also recommended, as this helps keep them from getting moldy. Molts treated in this way have remained flexible and functional for use and reuse multiple times with groups of students for as long as two years.

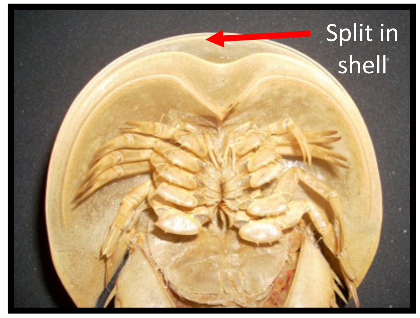
Specimen preparation and observation tools and tips: For presenting the lab, we put together molt study "kits" (one per team of students), each containing the following sets of materials:

Item	Description
molt specimen 1: top part of prosoma of large molt specimen	This can be a from a dry (not glycerine-soaked) damaged molt specimen; gently break off the prosoma from the rest at the hinge, then tear off the underside of the prosoma so that only the top part of the shell is left
penlight & hand-held magnifying glass	Use the penlight to shine light up through the compound eyes (from under the shell) while observing eye structure with the magnifier from above. Shining the penlight up through the median eyes also defines them better.
flexible metric ruler	can be used for aging any molt specimens according to prosomal width
molt specimens 2 & 3:	These specimens should be ones that are glycerine-soaked (for flexibility) and with operculum in good shape for observing the underside gonopores. If possible, provide a clear-cut example for each sex, labeling them A & B.
optional molt specimen (for bonus question on internal shell structures)	For this specimen, it is necessary to dissect the dorsal part of the molt shell from the ventral; use an exacto-knife or sharp scissors to cut around the rim of the shell until the top half can be separated from the bottom; these parts will be used to tackle the "Molting their guts out" bonus Q's.

More details on use of the kits for observation are provided in the accompanying guidesheet.

Horseshoe Crab Molt Anatomy Lab – basic structures

1. MEET YOUR MOLT: *To grow bigger, HSCs must shed their old shell and grow a new one underneath. They do this 16-18 times from the time they hatch out of the egg until adulthood.*
So how can we tell a molted (shed shell) from a dead HSCs?
HSC molts show a split along the front rim of the shell. This is where the old shell separated as the new shell beneath expanded. Find that split on your molt specimen.



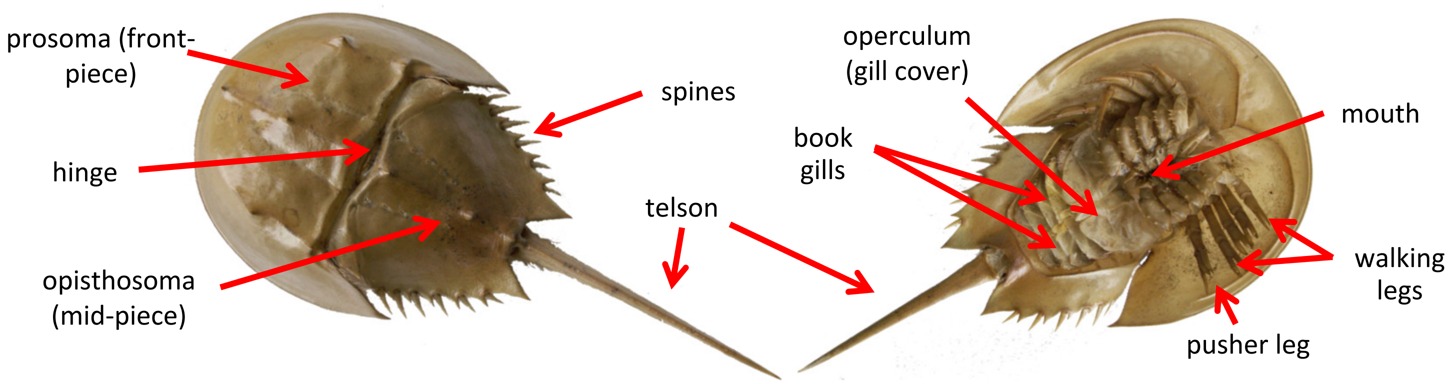
2. AGE YOUR MOLT: *The size of a molt can be used to estimate the age of the HSC at the time it molted. This is done by turning the molt over on its back and using a ruler to measure the distance (in cm) across the widest part of the shell.*

Molt number	Year since hatching	Average Prosomal Width (cm)
6	1	1.7
7	1.5	2.2
8	2	3.0
9	2.5	4.1
10	3	4.9
11	4	6.3
12	5	7.7
13	6	9.1
14	7	10.3
15	8	11.5
16	9	13.4
17	10	16.0

*This measurement is called Prosomal Width (PW). The chart at left shows average PW of HSC molts from a population on Cape Cod.**
Using the chart at right and the ruler provided, determine the PW, molt number and estimated age of one of your molts. Record this data on your answer sheet..

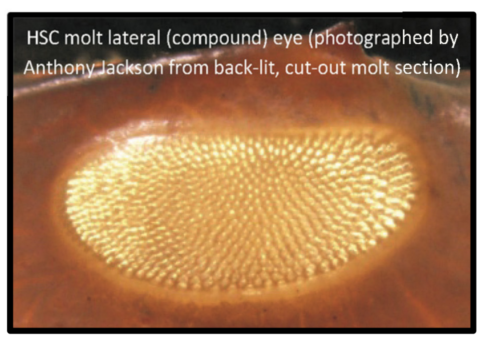
- Size with age of HSCs varies with geographic location. Data in chart from: RH Carmichael, D Rutecki and J Valiela. 2003. Marine Ecology Progress Series 246: 225-239.

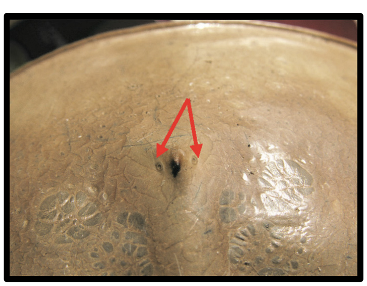
3. HSC ANATOMY BASICS: *The drawings below show the basic external HSC body plan.*



Find each of these structures on your molt and label them on your answer sheet drawings.

4. EYES: *Find the **lateral (compound) eyes** on your molt. Label them on your answer sheet drawing. These are like the eyes of a fly – each having hundreds of lenses that work together to help the HSC see. Use the penlight to shine light up through the eye while viewing with the hand lens.*
What do you observe about the design of the lateral eye?





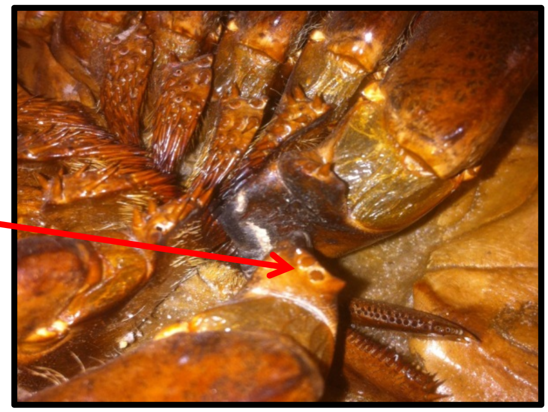
*Now locate and label the small pair of **median eyes** located next to the front-most spine on the top of your molt. Use the penlight to shine light up through these eyes. These eyes detect ultraviolet light reflected from the moon & stars. How could this help the HSC?*

Horseshoe Crab Molt Anatomy Lab – finer details

5. MOUTH: *One of the HSCs most unique features is its between-the-legs located mouth. Observe the many bristles on the leg bases that surround your molt's mouth. These are the* ***gnathobases****. Considering their location and structure, what purpose do you think they serve?*

Now find the small, leg-like ***chelicerae****. just above the mouth,and the two paddle-like* ***chilaria*** *at the lower end of the mouth. Both structures serve to direct food to the mouth. Label these parts on your answer sheet drawing.*

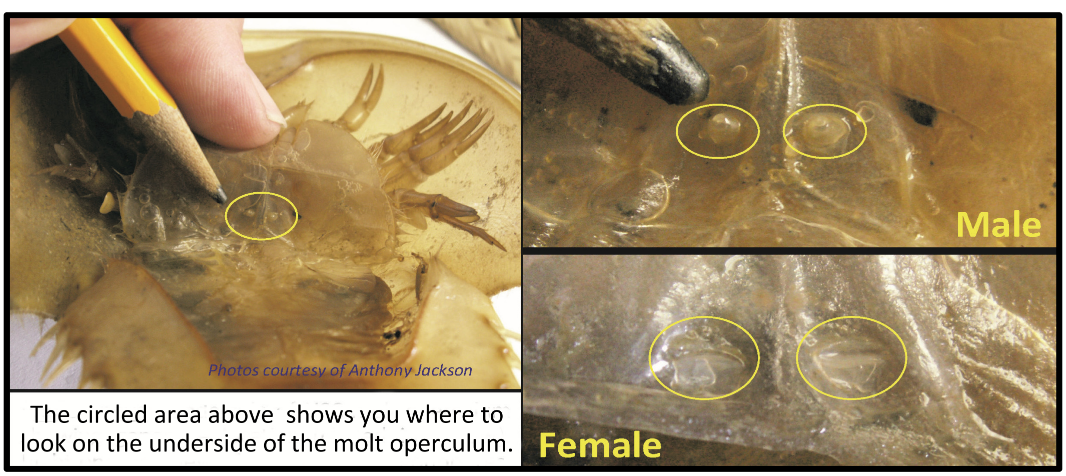
6. PUSHER LEGS: *Observe the hind legs of your molt. This is called the* ***pusher leg****. What do you see that's different about these legs (compared to the other legs)? Follow the pusher leg down to where it meets the mouth, and look for a strong spine there (in area at right arrow). These spines are used to open up clams for eating. How do you think the HSC does that?*



Find the part on your molt that's labeled ***F*** *in the photo at left. This is the* ***flabellum****. It holds hundreds of thousands of sensory receptors. Considering its location, what do you think the function of these sensors might be?*

7. SEXING YOUR MOLT: *The underside of the HSC operculum (gill cover) holds the pores through which eggs (in adult females) and sperm (in males) come out in spawning. Observing the shape and feel of these pores can tell us the sex of a molt. This is the only way of telling the boys from the girls in young HSCs.*

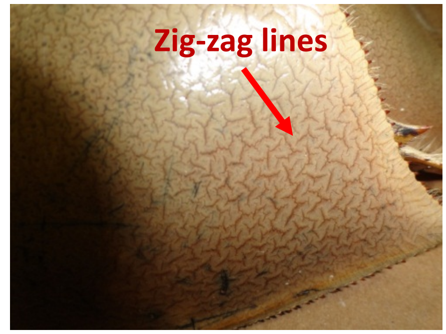
Find the ***operculum*** *(plate that covers gills) on your molt. Gently lift it up & run your fingers along its underside (in the area at the pencil tip in the photo at right) Can you tell which kind of HSC you have?*



The circled area above shows you where to look on the underside of the molt operculum.

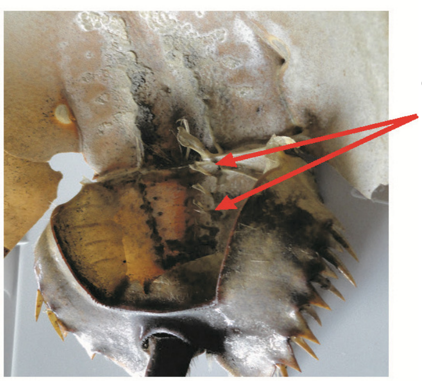
In male HSCs, the pores will stick out like little bumps and feel hard to the touch. When you run your hand along a female's operculum, it will feel soft and smooth.

Horseshoe Crab Molt Anatomy Lab – Bonus challenge



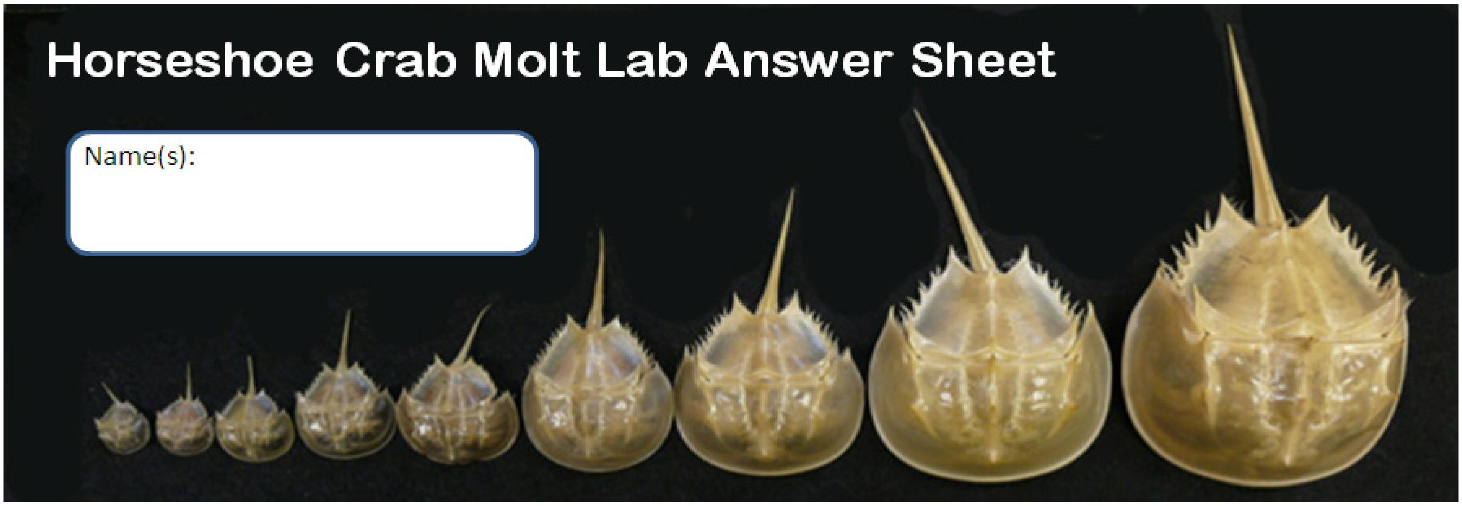
MORE on MOLTING: *Replacing a shell isn't easy. Using its old shell as a mold, the HSC grows a larger one underneath. This new shell is soft and wrinkled. As it takes in water and expands, the new shell pushes against the old, causing it to split around the front. The crab crawls out leaving its old shell behind. The photo at right shows a series of fine zig-zag lines that can be found on the side of an HSC shell. Use the hand lens to look for these lines on the front side of your molt. What do you think caused them?*

MOLTING THEIR GUTS OUT: *When HSCs molt, along with forming new external shell parts, they also re-form certain chitinous parts of their digestive system, including the lining of the esophagus, crop-gizzard and rectum. Observe the molt specimen in your kit that has the top shell separated from the bottom so you can see what its under-side looks like (as in photo at right). Use that photo to help you find the structure pointed to on your molt. Thinking about where this structure is located (including what sits above it), what do you think it might be used for?*



Now look at the bottom part of the underside of the top of your dissected molt (as shown in photo at left). Notice the deeply vaulted space inside the bottom of the shell. If you look carefully inside that space, you'll see two lines (one on each side, running from the hinge down) each with 6 thin protruding structures (see arrows at left). Using the underside of one of your whole molts as reference, figure out what key HSC body parts lie above these ridges, and describe how they could help those body parts work.

Horseshoe Crab Molt Lab Answer Sheet



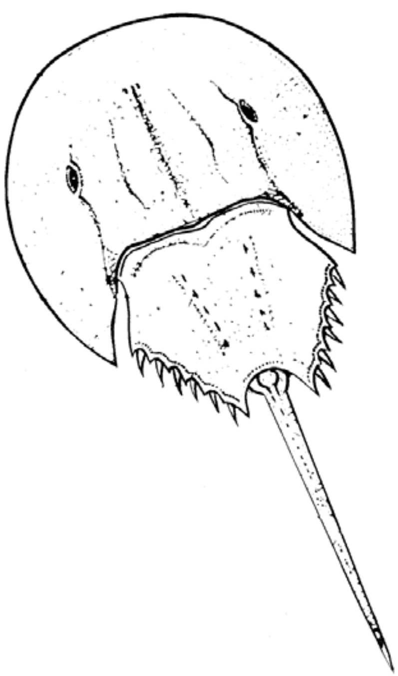
Use this sheet to answer the questions on the big Horseshoe Crab Molt Anatomy Lab instruction sheets.

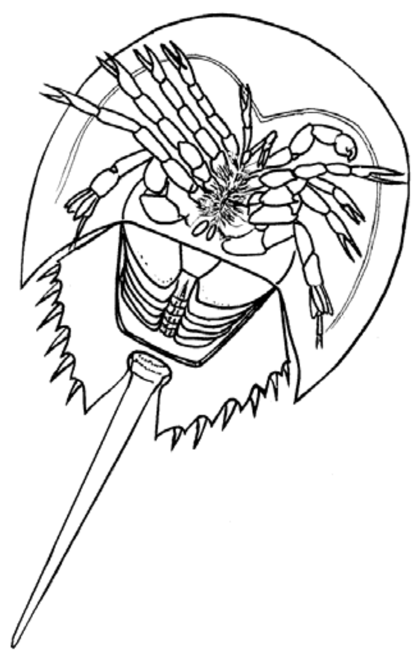
1. **MEET YOUR MOLT:** If you found an HSC shell on the beach, how would you know if it's a molt?

2. **AGE YOUR MOLT:** Write down the data in the spaces below pertaining to the aging of your molt.

   _____ prosomal width (cm) _____ molt number _____ age (years)

3. **HSC ANATOMY BASICS:** Label all of the HSC body parts that you can in the drawings below.





4. **EYES:** Label the eyes in the drawing above. What did you observe about the HSC's lateral eyes?

   How do you think the ability of the median eyes to detect ultraviolet light could help the HSC?

5. **MOUTH:** Label the HSC mouth structure in the drawing at right corresponding to what you observed in your molt. Considering the location of the **gnathobases** in relation to the mouth, what purpose do you think they serve?

6. **PUSHER LEGS:** How does the pusher leg appear different than the other (walking) legs of the HSC?

How do you think the HSC uses the strong spine on the base of its pusher leg for eating clams?

Given their location, what do you think the function might be of the sensors on the HSC's flabellum?

7. **SEXING YOUR MOLT:** Use what you learned to identify the sex of the molt specimens in your kit.

sex of specimen A: ___________ Why you think so: _____________________________________

sex of specimen B: ___________ Why you think so: _____________________________________

sex of mystery molt specimen: ____

Bonus #1 **MORE on MOLTING:** What do you think caused those zig-zag lines on the molt shell?

Bonus #2 **MOLTING THEIR GUTS OUT:** To what HSC part does the pointed-to structure connect with? Given its placement, what would you think its purpose is?

What key HSC body parts would be located above the 6 ridges that are pointed to in the photo at the very bottom of the bonus sheet? How do you think these ridges help those body parts work?

Bonus #3 **SIMPLY IMPOSSIBLE:** Look carefully at the two drawings on the front side of this sheet. There is something wrong about one part of those drawings. Tell what that is and why you'd never find an HSC like that in the wild.

Horseshoe Crab Molt Lab Answer Sheet

Name(s):

Use this sheet to answer the questions on the big Horseshoe Crab Molt Anatomy Lab instruction sheets.

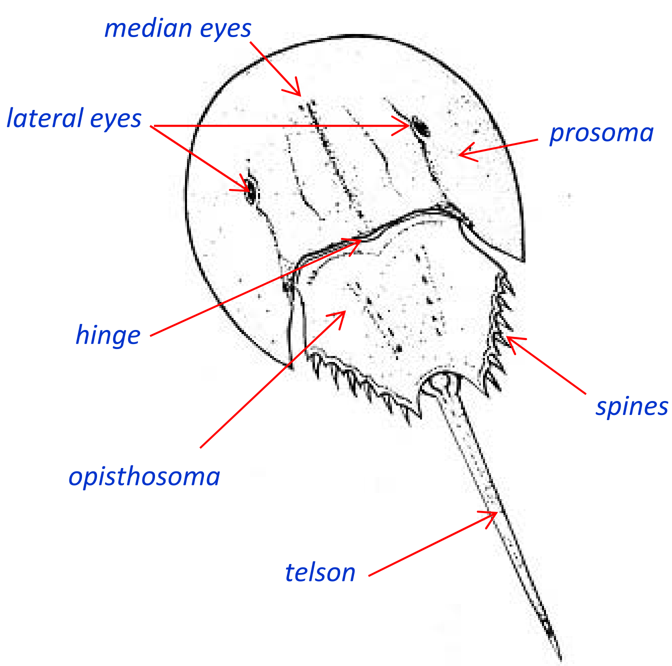
1. **MEET YOUR MOLT:** If you found an HSC shell on the beach, how would you know if it's a molt?

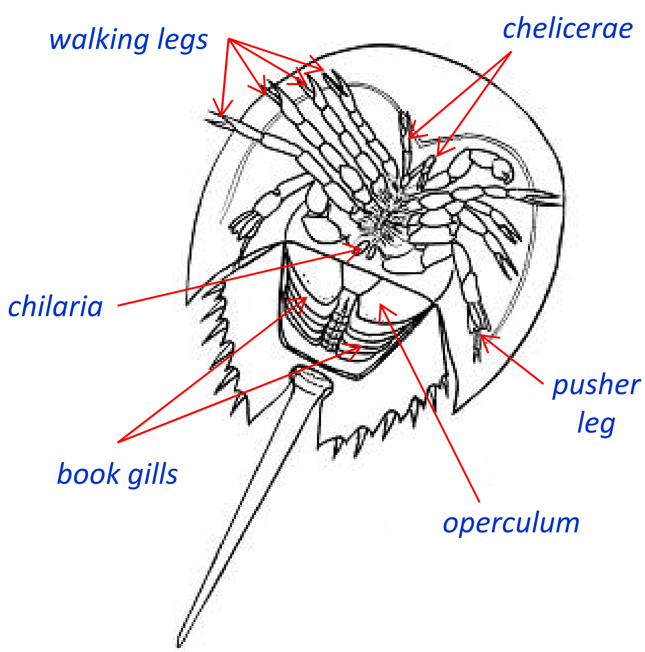
   Look for a split along the front rim of the shell. Also wouldn't smell bad (like a dead crab)

2. **AGE YOUR MOLT:** Write down the data in the spaces below pertaining to the aging of your molt.

   Answers will vary prosomal width (cm) _____ molt number _____ age (years)

3. **HSC ANATOMY BASICS:** Label all of the HSC body parts that you can in the drawings below.





4. **EYES:** Label the eyes in the drawing above. What did you observe about the HSC's lateral eyes?

   Students should note the compound nature of the HSC eye and the hundreds of facets in each. More detailed descriptions of individual facet structure may be offered, pending magnification.

   How do you think the ability of the median eyes to detect ultraviolet light could help the HSC?

   Anything that suggests helping its night vision is good. The sensitivity of the median eyes to UV light helps the HSC gauge how much light is available to adjust compound eye sensitivity to it.

5. **MOUTH:** Label the HSC mouth structure in the drawing at right corresponding to what you observed in your molt. Considering the location of the **gnathobases** in relation to the mouth, what purpose do you think they serve?

Hopefully, students can infer - by their surrounding-the-mouth location and their spiny/bristly structure, that the gnathobases serve some food manipulation (teeth-like) function.

6. **PUSHER LEGS:** How does the pusher leg appear different than the other (walking) legs of the HSC?

Students should note the leaf-like, splayed appendages at the end of the pusher leg (in contrast to the long scissor-like claws at the end of each walking leg).

How do you think the HSC uses the strong spine on the base of its pusher leg for eating clams?

The correct answer relates to rubbing the muscle that opens & closes the clam shell against the spines to weaken it (causing shell to open). but anything that suggests opening the shell is fine.

Given their location, what do you think the function might be of the sensors on the HSC's flabellum?

The proximity to the book gills, as well as the notched space between the back of the prosoma & front of opisthosoma, suggests a role in sensing the water passing through and over those areas.

7. **SEXING YOUR MOLT:** Use what you learned to identify the sex of the molt specimens in your kit.

sex of specimen A: *female* Why you think so: *gonopores are flat & soft (not hard & pointy)*

sex of specimen B: *male* Why you think so: *gonopores are hard, pointy & cone-shaped*

sex of mystery molt specimen: *Optional (answers will vary)*

Bonus #1 **MORE on MOLTING:** What do you think caused those zig-zag lines on the molt shell?

These are fold lines from molting. The new shell (as it forms) is soft and wrinkled, and as it takes in water and expands, those wrinkles unfold, leaving behind the network of fine stretch lines.

Bonus #2 **MOLTING THEIR GUTS OUT:** To what HSC part does the pointed-to structure connect with? Given its placement, what would you think its purpose is?

Hopefully, students can trace the connection between the pointed to sac-like structure (actually the crop-gizzard) and the mouth below it, and thus liken it to a stomach or other digestive organ.

What key HSC body parts would be located above the 6 ridges that are pointed to in the photo at the very bottom of the bonus sheet? How do you think these ridges help those body parts work?

Students should notice that the ridges of chitin line up with, and associate with, the book gills. If they can realize their role as points of attachment for muscles working the gills, awesome!

Bonus #3 **SIMPLY IMPOSSIBLE:** Look carefully at the two drawings on the front side of this sheet. There is something wrong about one part of those drawings. Tell what that is and why you'd never find an HSC like that in the wild.

The ventral view shows one front walking leg claw for a male and the other a female.

If you ever find an HSC like this, be sure to send us a picture!

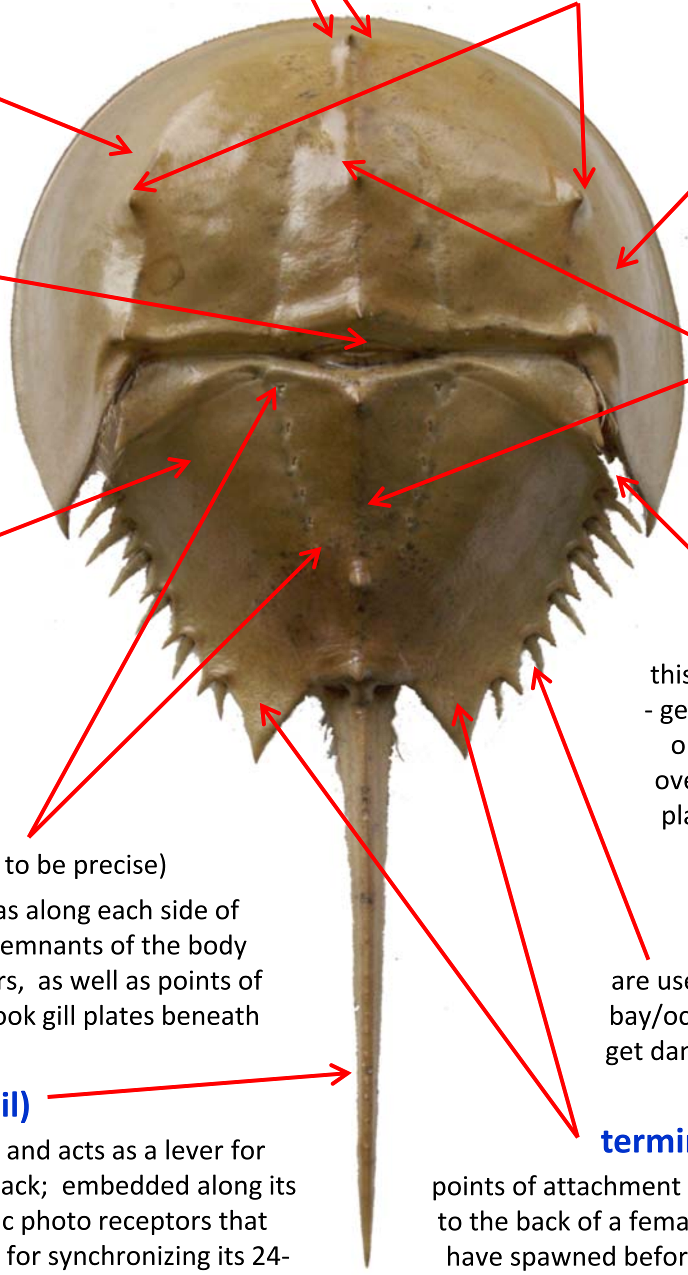
Limulus polyphemus anatomy guide - dorsal features

median (simple) eyes

sit on each side of the front-most spine of the HSC shell; these eyes detect ultraviolet light reflected from the moon and stars - information the HSC brain uses to instruct the compound eyes to adjust their sensitivity to light up to a million times at night, enabling them to see and find their mates as well on a dark new moon night as in bright daylight

lateral (compound) eyes

like the eyes of a fly, contain hundreds of image-forming facets, providing a wide field of visual data for the HSC to assess its surroundings - which the HSC brain interprets into one somewhat fuzzy (compared to what we see) view of the world; the seeing cells inside the HSC eye are 100x larger than those of humans, making it a great model for studying how vision works (3 Nobel prizes have been awarded for research done on HSC eyes)



prosoma

large front circular shield of the HSC three-part body plan; provides strength & protection to all the body parts below it.

carapace

term applied to the entire shell, comprised of a material called **chitin;** the surface of the HSC carapace hold over a million touch-sensitive receptors

hinge

strong muscles across this area enable HSC to flex its opisthosoma up to 90° when disturbed; also the area where a needle is inserted for biomedical bleeding

heart sinus area

this raised area of the HSC shell encompasses the heart sinus, which holds about 30% of the HSC's blood volume, including that which is taken during biomedical bleeding

opisthosoma

also called the mid-piece, this vital body section holds and protects the gills underneath and also is the part of the body that flexes when disturbed

channel

this space is where currents of water - generated by beating of the gills and operculum - are brought into and over the gills; it's also the preferred place for satellite males to position over females in spawning

pits (**entapophseal pits** to be precise)

there are 6 of these hollow areas along each side of the opisthosoma, representing remnants of the body segments of ancient HSC ancestors, as well as points of attachment to the shell for the book gill plates beneath

moveable spines

are used for "feeling" its way along the bay/ocean bottom; some of these may get damaged or broken in older animals

telson (tail)

serves as a rudder in swimming, and acts as a lever for righting itself when flipped on its back; embedded along its length are a series of microscopic photo receptors that transmit signals to the HSC's brain for synchronizing its 24-hour biological clock to night/day cycles; the observable side-to-side sweeping motion of the telson probably serves to keep sediments away from fouling its sensors

terminal projections

points of attachment where male clasper claws lock on to the back of a female during spawning; females that have spawned before show blackened "mating scar" markings in these areas (and on opisthosoma above it) where the rubbing of male claspers and shell have eroded the outer surface of the carapace

Developed by Gary Kreamer (2014), Delaware Aquatic Resources Education Program; gary.kreamer@state.de.us
Special thanks to Dr. Carl N. Shuster Jr. & Dr. Jane Brockmann, for their helpful review and contributions to this material.

Limulus polyphemus anatomy guide - ventral features

chelicerae

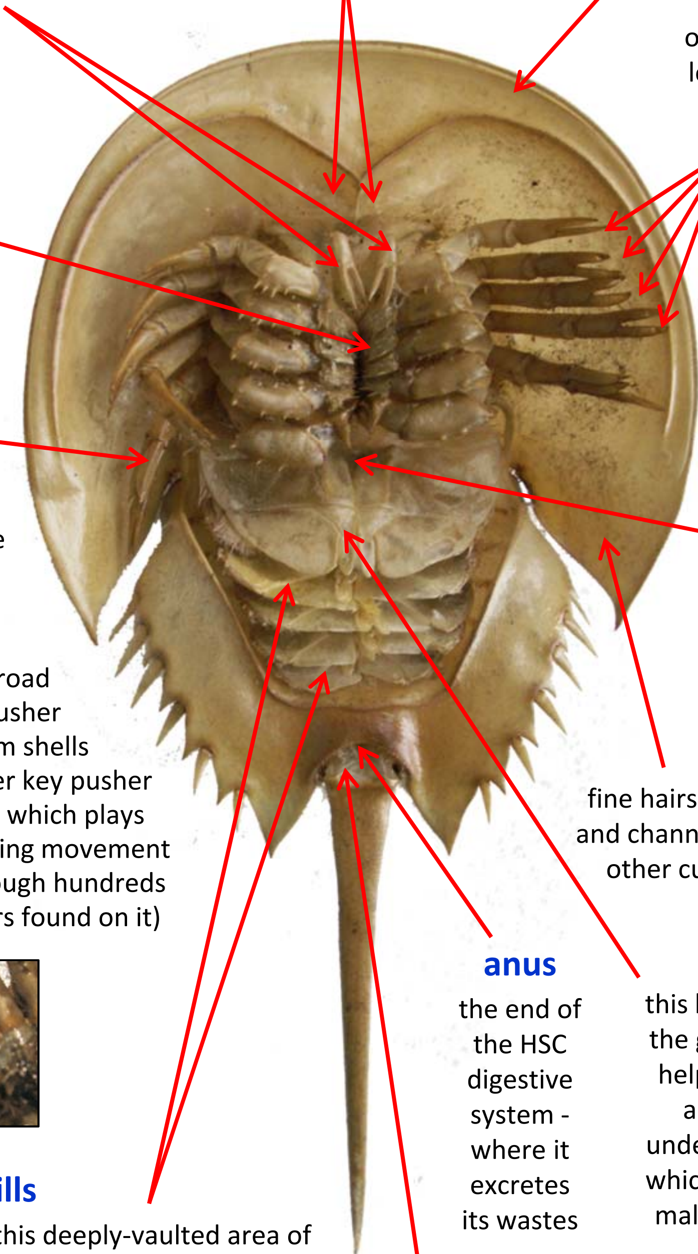
pair of small feeding claws located just above the mouth, which serve to direct food into the mouth; one of the features that makes the HSC more like a spider than a true (crustacean) crab

ventral eyes

microscopic photoreceptors embedded in the HSC shell in this area provide light information to the HSC when it's flipped over on its back

flange (molting suture)

front rim of the HSC shell, where the shell splits in molting (typically appears yellow prior to molting)



mouth

the HSC's unusual mouth sits between its legs; at the base of those legs surrounding the mouth are bunches of bristle-like spines, which the HSC uses to soften the food as it works it into its mouth

walking legs

on each side of the HSC are 4 sets of legs; in addition to aiding in moving and feeding, the tips of these legs are loaded with chemosensory receptors, enabling the HSC to "smell with its feet"; in adult male *Limulus*, the claws on the front pair of legs are modified into special structures used for clasping to a spawning female

male clasper claw

pusher leg

among the neatest functional features of the HSC anatomy are the hind legs, ending in spatulate appendages, which they use for digging, pushing off the bottom (in scuttling) and (in females) for molding eggs into clusters; the broad strong spine at the base of the pusher is put to good use in opening clam shells (a favored food of HSCs); another key pusher leg structure is the **flabellum (F)**, which plays a key role in directing and assessing movement of water over the book gills (through hundreds of thousands of sensory receptors found on it)

chilaria

paired paddle-shaped structures in this area function in pushing food forward towards the mouth.

sensory hairs

fine hairs along the surface of the carapace and channel help the HSC sense currents and other cues from the water around them

anus

the end of the HSC digestive system - where it excretes its wastes

operculum

this large strong flap of tissue protects the gills; it also works with the gills in helping propel the HSC in swimming and scuttling movements; on its underside are the gonopores, through which eggs (in females) and sperm (in males) are released during spawning

book gills

5 pairs of plate-like gills occupy this deeply-vaulted area of the HSC abdomen, with each plate bearing numerous thin sheets of tissue (appearing like pages in a book), allowing optimal surface area for respiration; in addition to their role in gas exchange, the book gill surfaces are loaded with sensory receptors for assessing the condition of the water; rapid flapping of the gills and operculum help propel the HSC in swimming & scuttling movements

tail muscle

a large muscle connects the telson to the rest of the shell; it is critical to an HSC's ability to right itself when flipped over; <u>NEVER</u> pick up a live HSC by its telson, as this can damage the muscle and hinder its survival; Note: the anus of the HSC is located just above this muscle

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Special thanks to Dr. Carl N. Shuster Jr. & Dr. Jane Brockmann, for their helpful review and contributions to this material.