

Light-Regulated Reproduction of Ctenophores **...and other misc**

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Invertebrate zoology 2025

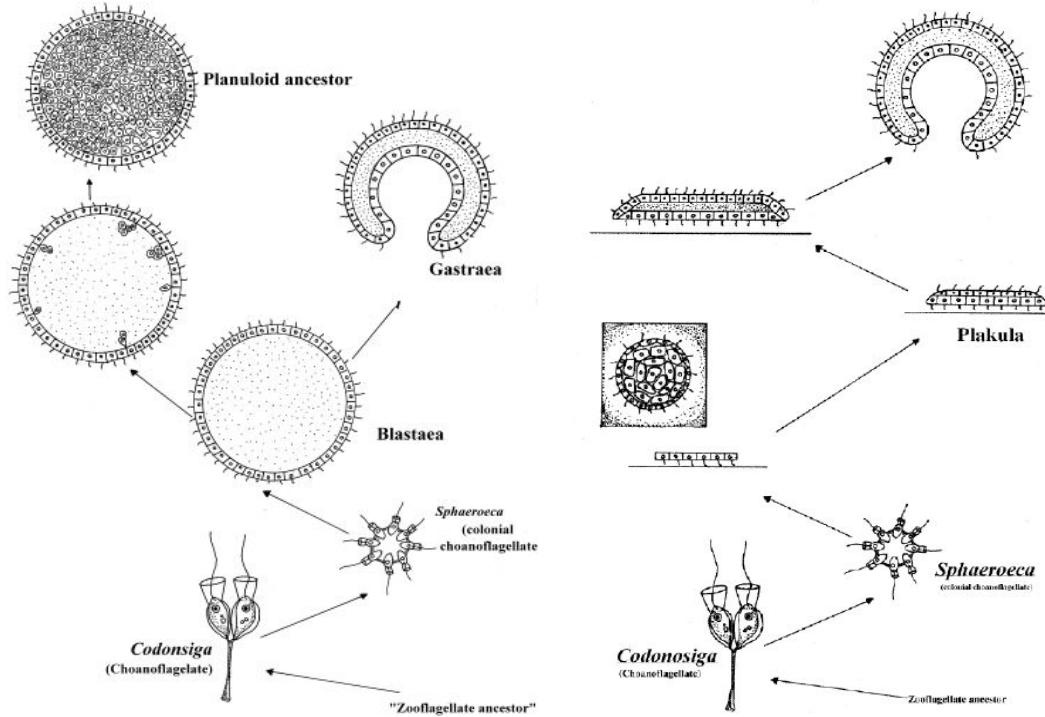
Table of contents

- Ctenophores as model for evolution questions
 - Development in ctenophore
 - Light regulated reproduction
 - Experiments and results
 - Bonus: can microCT scan water?

Urmetazoan - the earliest common ancestor of all animal

- First used in 20 century by Bütschli discussing common ancestor of all animals
- Several hypothesis:
 - Plakula Hypothesis
 - Gastraea hypothesis
 - Bilaterogastraea hypothesis...?

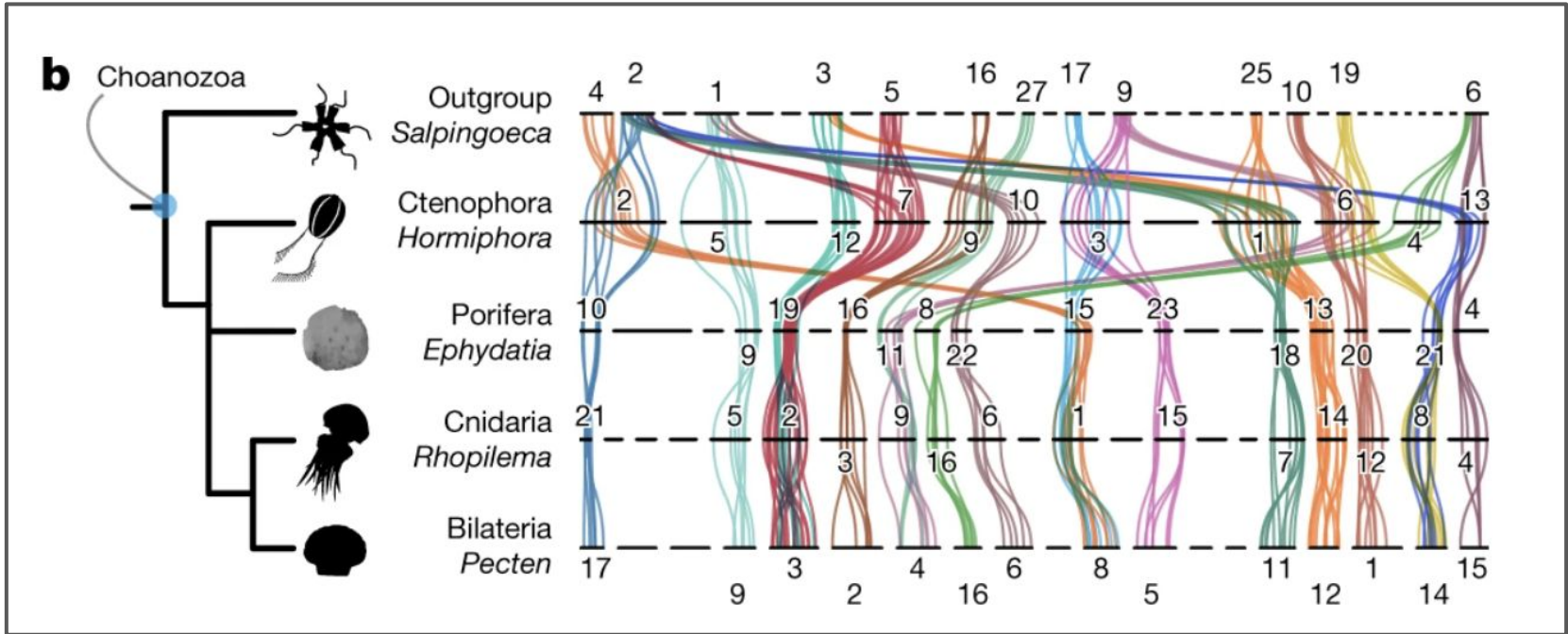
Urmetazoan - the earliest common ancestor of all animal



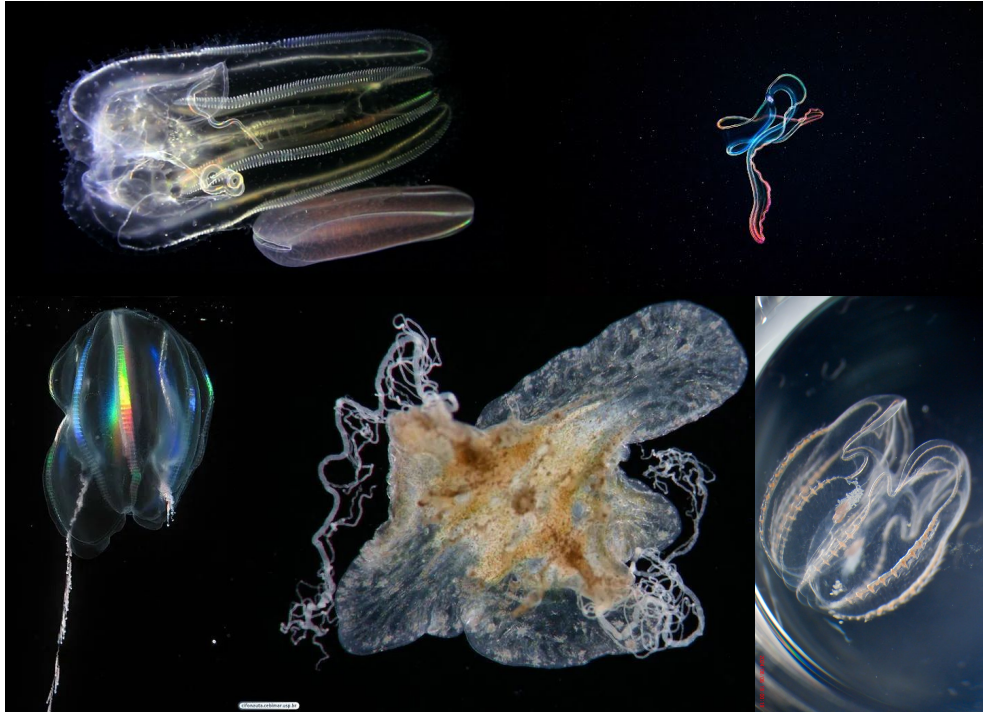
Urmetazoan - what we know (and know we don't know)

- Developmental toolkits
 - homologous regulatory pathways
- Functional proteins
 - Opsin

Ctenophores are one of the earliest diverged animals

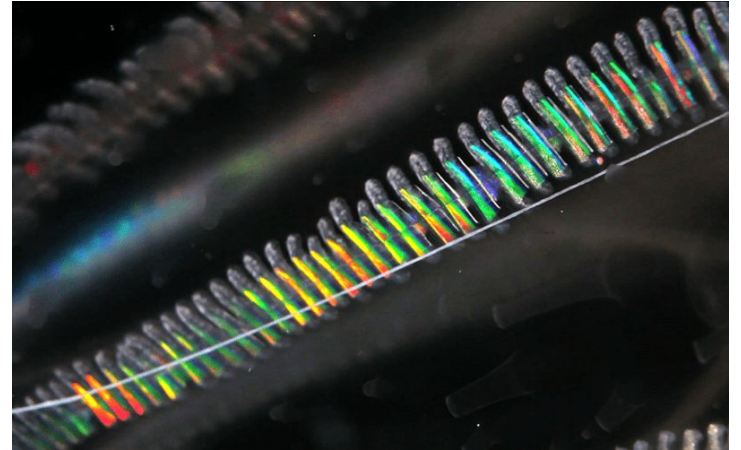


Ctenophores diversity



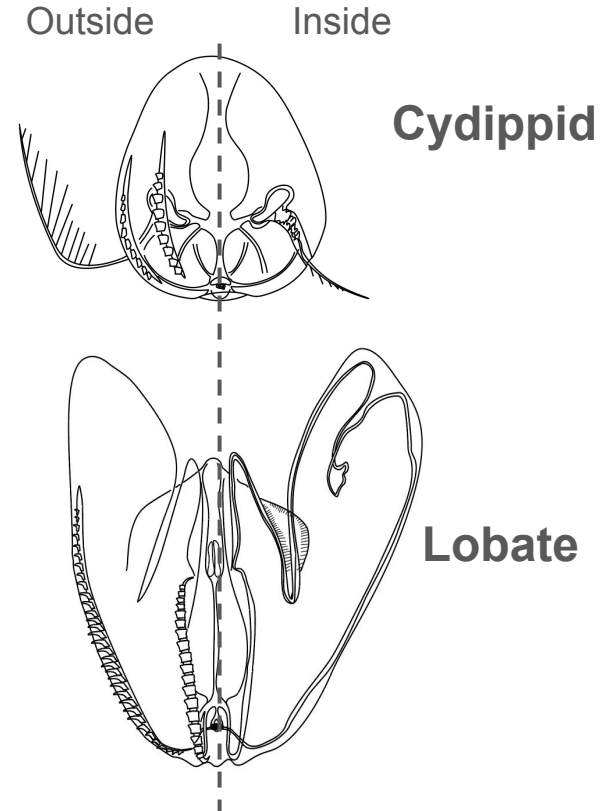
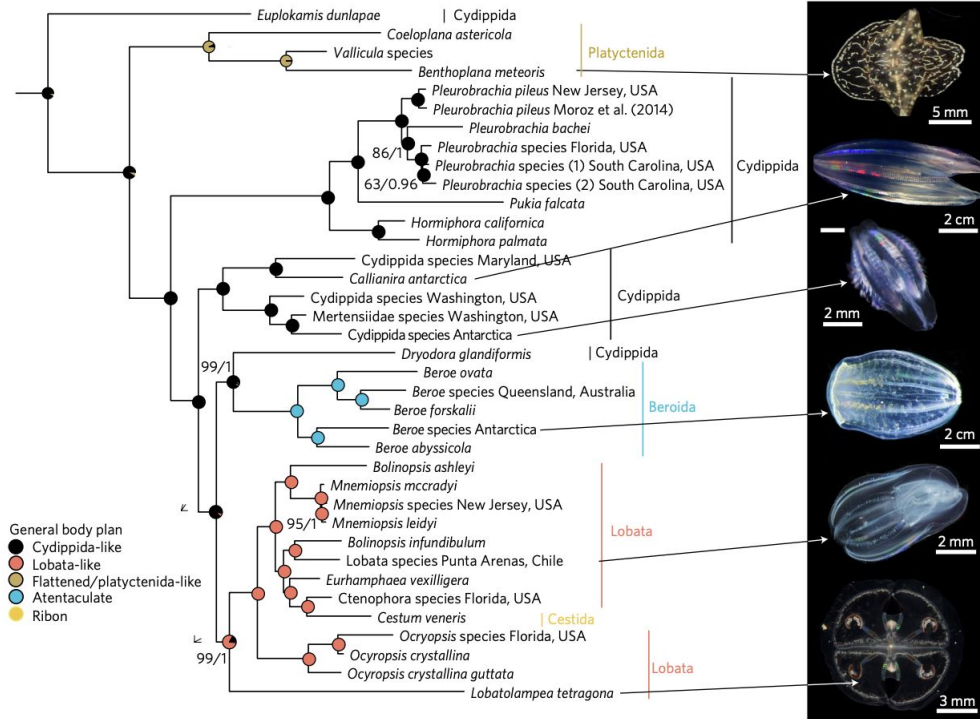
From left to right, up to down: C & N..Sardet, Alexander Semenov, Kevin Raskoff, Alvaro E. Migotto and Otto M. P. Oliveira

Cteno - “comb”

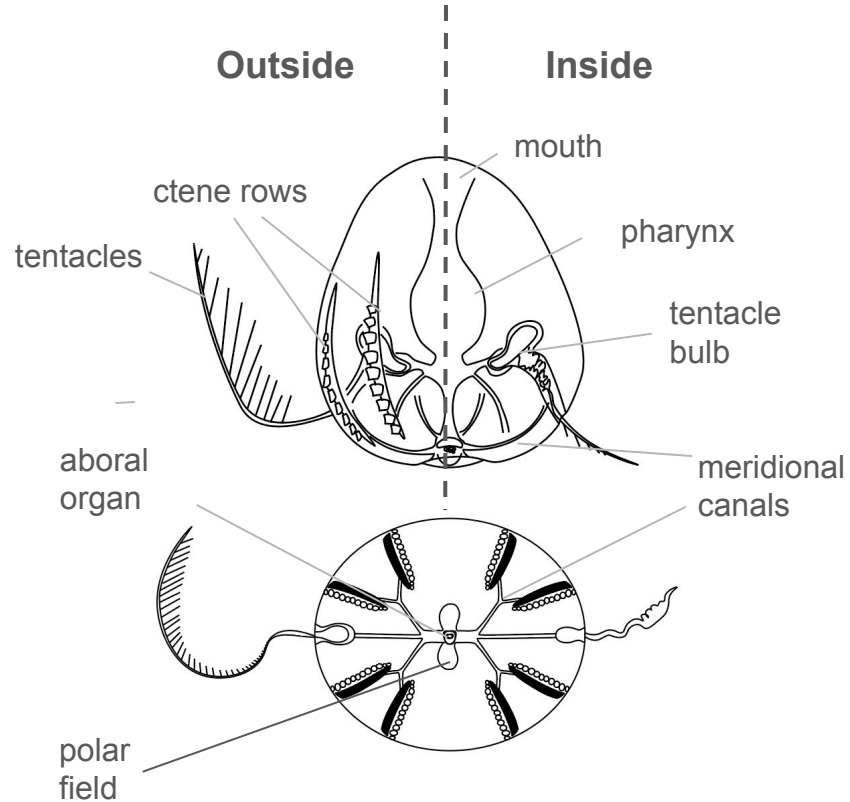
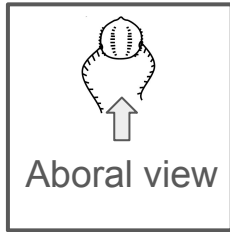
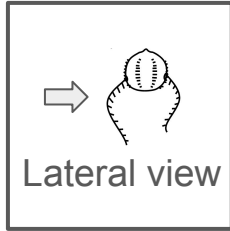


From C.Sardet plankton chronicles

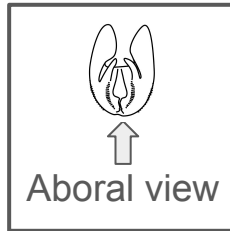
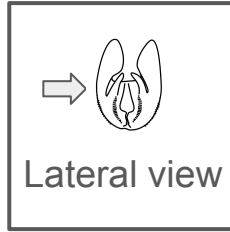
Common body plans in Ctenophora



Ctenophora body plan: cydippid

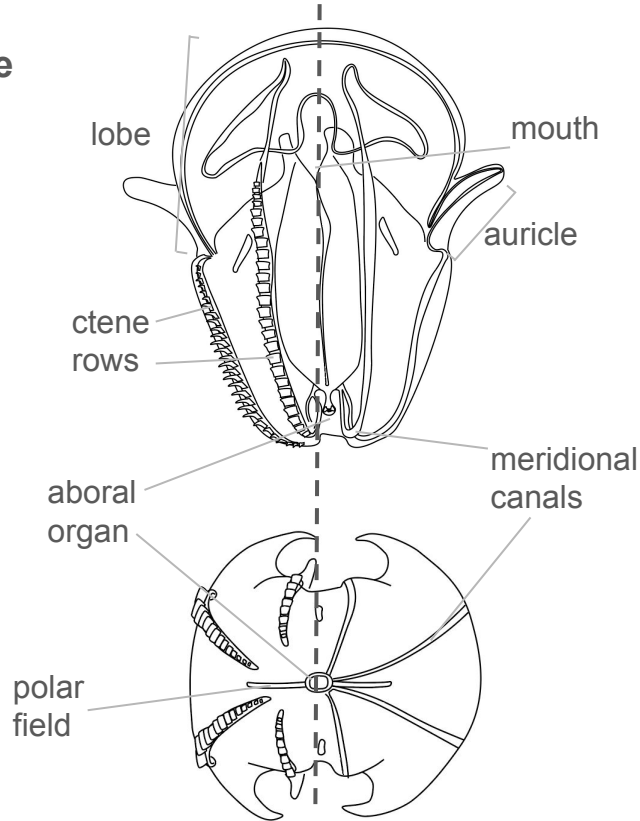


Ctenophora body plan: lobate

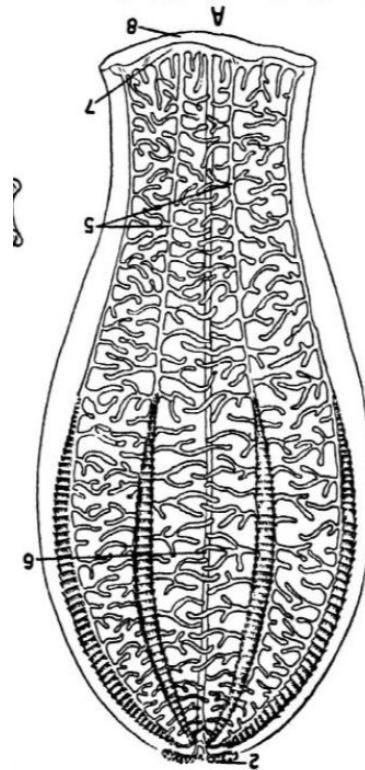
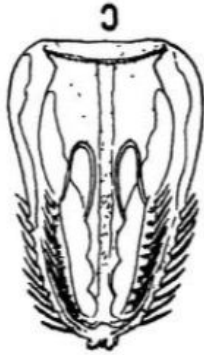


Outside

Inside

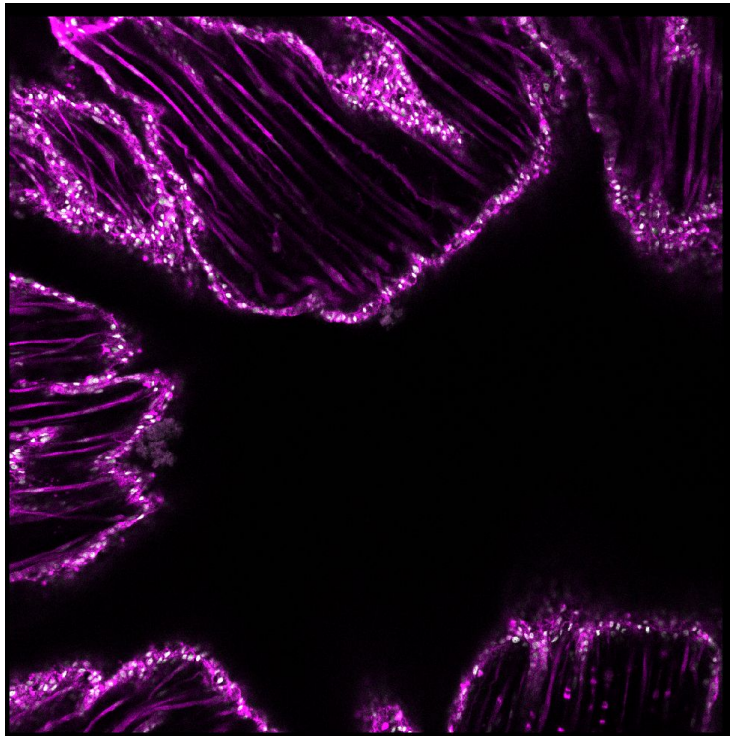


Ctenophora body plan: beroe



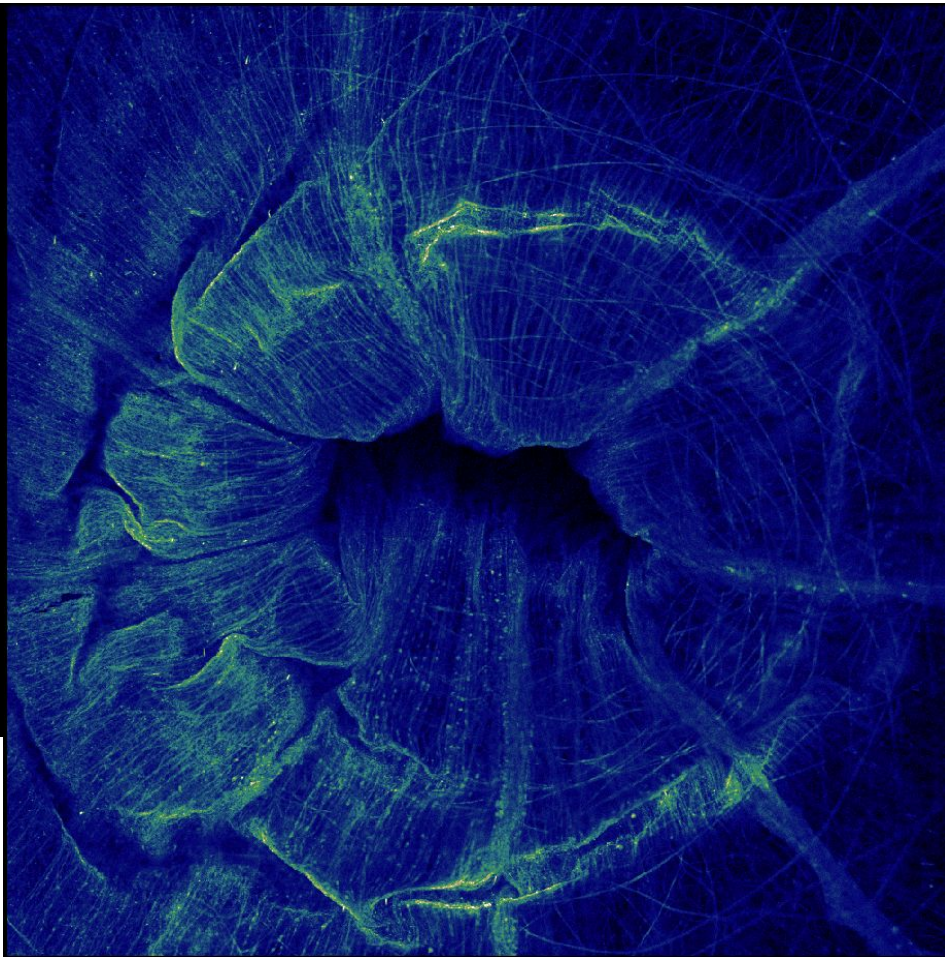
Hyman, 1940

Morphology

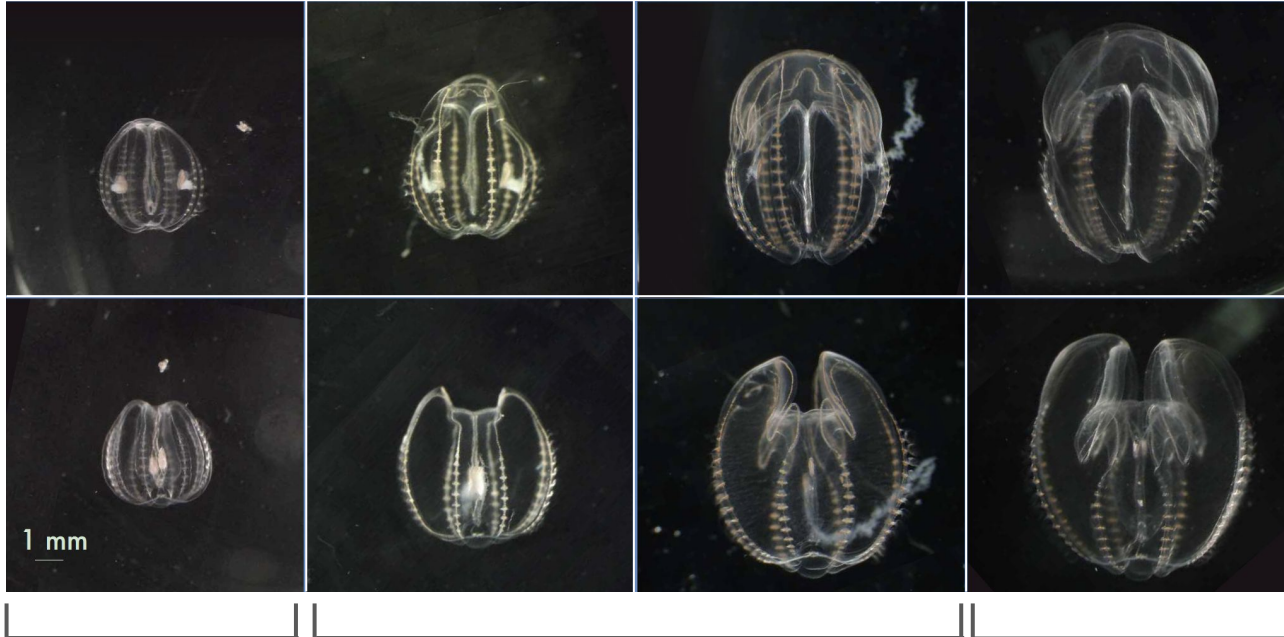


Left - Confocal fluorescence images - phalloidin (purple)
sytoxgreen (bright green) 100x

Right - Confocal fluorescence images - all channels
in blue palette 25x



Lobata undergoes dramatic morphological changes throughout its life

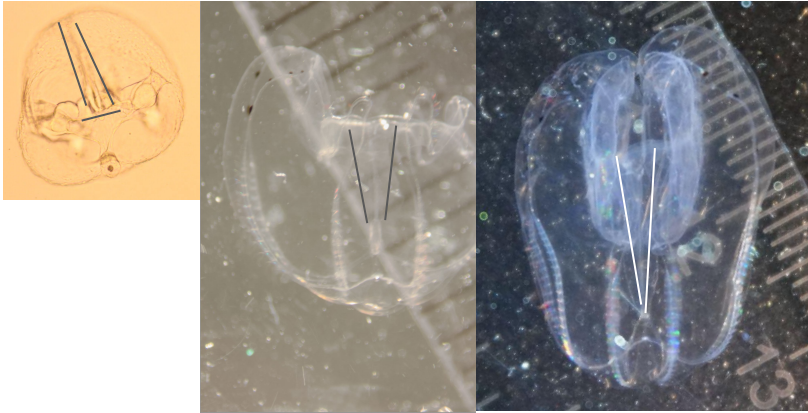


Cydippid

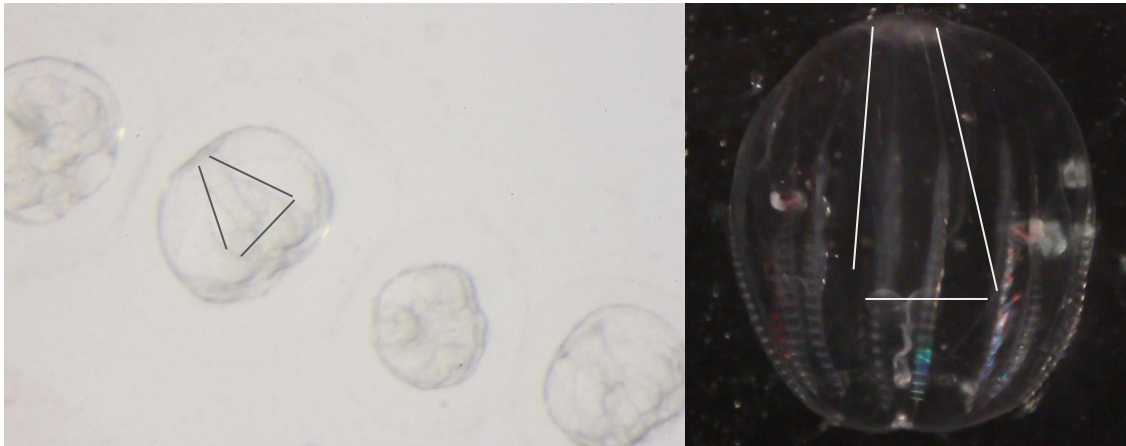
Transition

Lobate

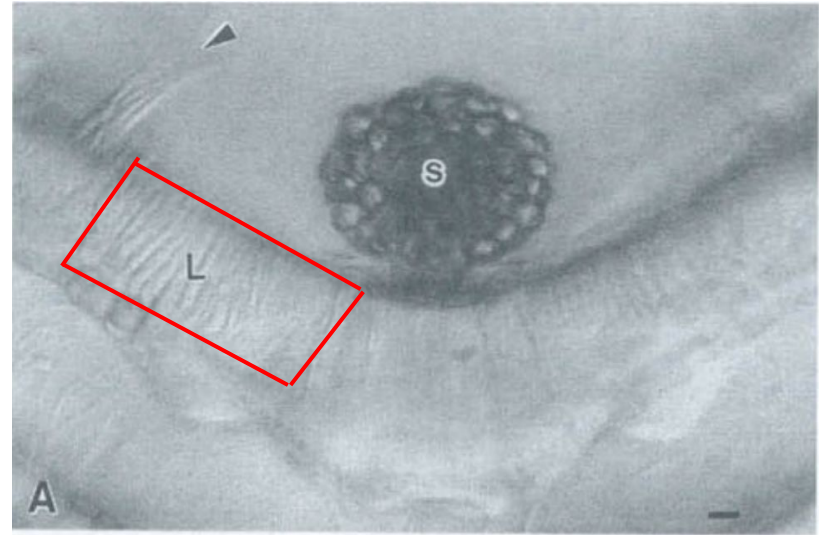
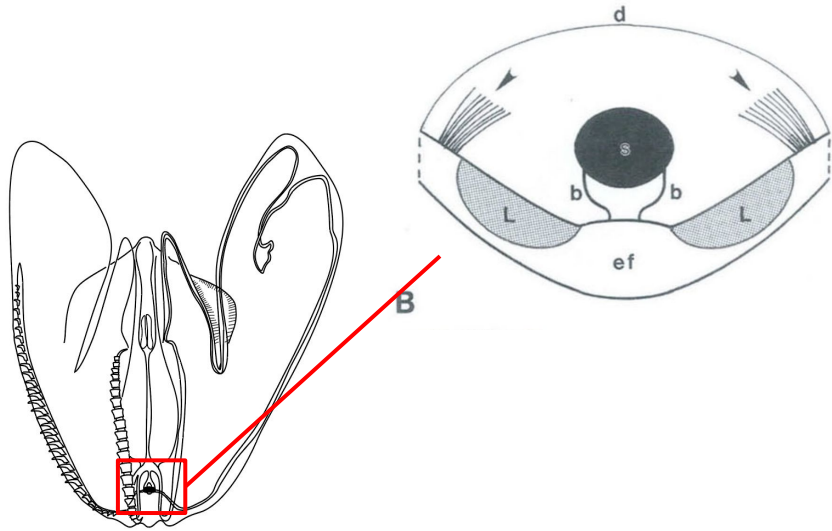
Morphology



Allometric development
- Work for future!



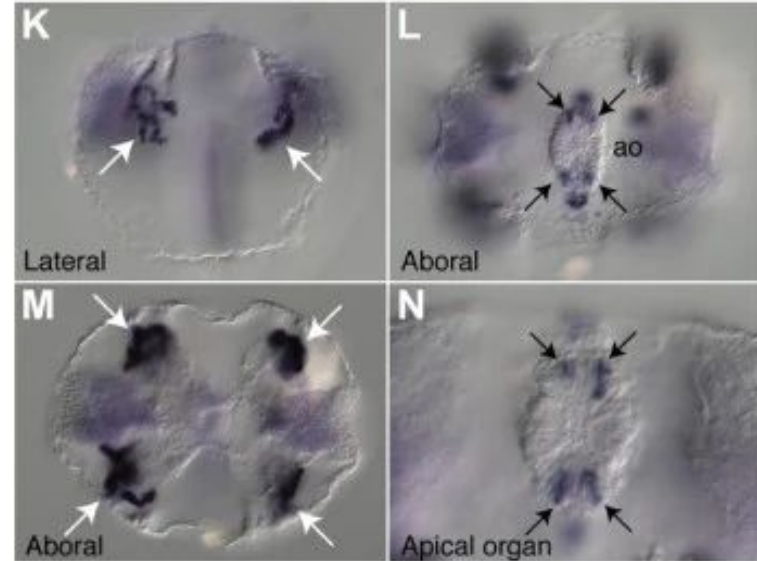
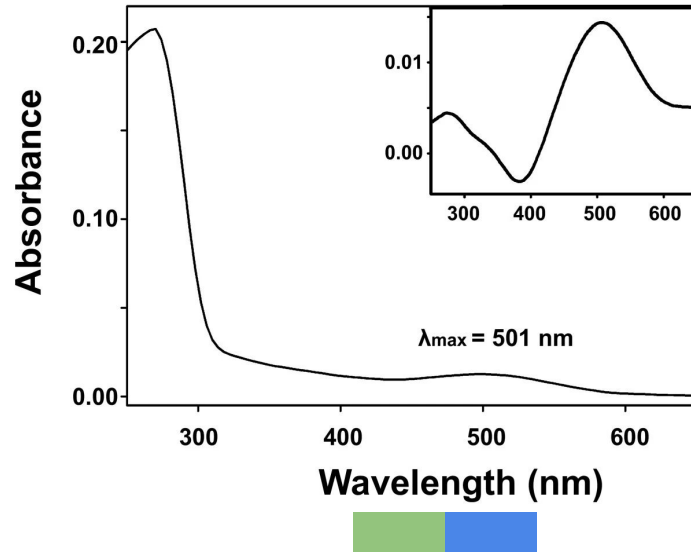
How does Ctenophore sense light?



Tamm, *Biol. Bull.* 2016

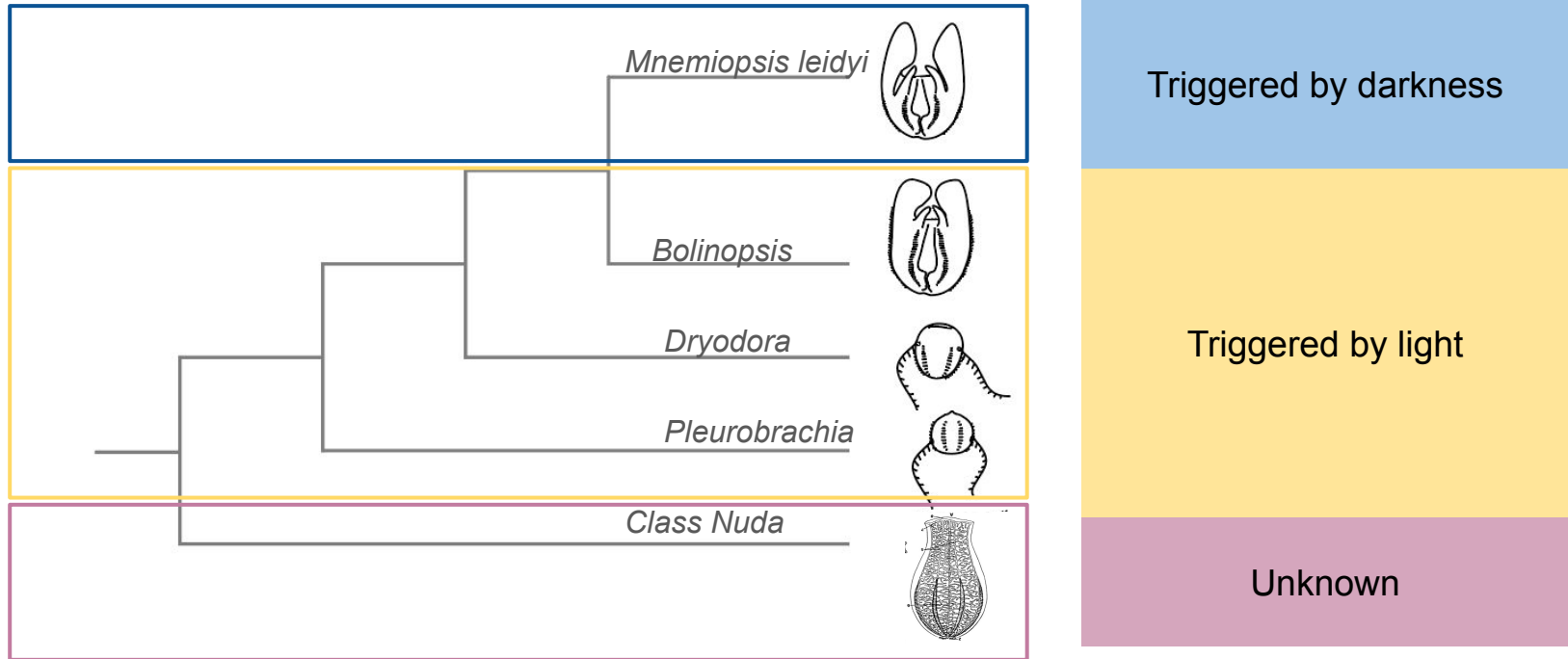
Lamellate bodies (in group)

How does Ctenophore sense light?



Schnitzler, C. E. et al, BMC Biology, 2012

Light as a spawning cue for multiple ctenophore species



Light regulated reproduction

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<input checked="" type="checkbox"/>	opsin 1 [Beroe ovata]	Beroe ovata	536	536	94%	0.0	76.38%	354	XCV16117.1
<input checked="" type="checkbox"/>	opsin-1 [Pleurobrachia bachei]	Pleurobrachia bachei	510	510	91%	0.0	74.84%	314	AGE89244.1
<input checked="" type="checkbox"/>	opsin-2 [Pleurobrachia bachei]	Pleurobrachia bachei	182	182	86%	9e-54	34.34%	416	AGE89245.1
<input checked="" type="checkbox"/>	rhodopsin-like isoform X2 [Bolinopsis microptera]	Bolinopsis microptera	140	218	83%	7e-38	38.50%	422	XP_063688749.1
<input checked="" type="checkbox"/>	rhodopsin-like isoform X1 [Bolinopsis microptera]	Bolinopsis microptera	140	219	85%	8e-38	38.50%	442	XP_063688748.1
<input checked="" type="checkbox"/>	opsin 2 [Beroe ovata]	Beroe ovata	138	220	79%	2e-37	39.13%	401	XCV16118.1
<input checked="" type="checkbox"/>	melatonin receptor type 1A-like [Bolinopsis microptera]	Bolinopsis microptera	52.8	52.8	63%	1e-07	22.47%	372	XP_063683486.1
<input checked="" type="checkbox"/>	opsin-VA-like isoform X4 [Bolinopsis microptera]	Bolinopsis microptera	49.3	49.3	86%	2e-06	24.13%	513	XP_063692074.1
<input checked="" type="checkbox"/>	opsin-VA-like isoform X3 [Bolinopsis microptera]	Bolinopsis microptera	48.5	48.5	86%	4e-06	24.06%	570	XP_063692073.1
<input checked="" type="checkbox"/>	opsin-VA-like isoform X1 [Bolinopsis microptera]	Bolinopsis microptera	48.1	48.1	86%	5e-06	24.06%	578	XP_063692071.1
<input checked="" type="checkbox"/>	opsin-VA-like isoform X2 [Bolinopsis microptera]	Bolinopsis microptera	48.1	48.1	86%	6e-06	24.06%	577	XP_063692072.1



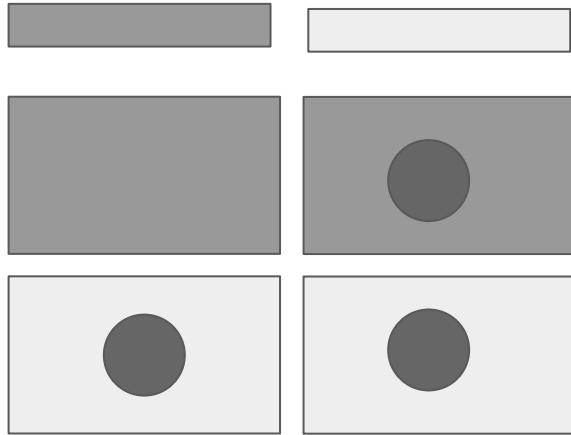
Experiment setup



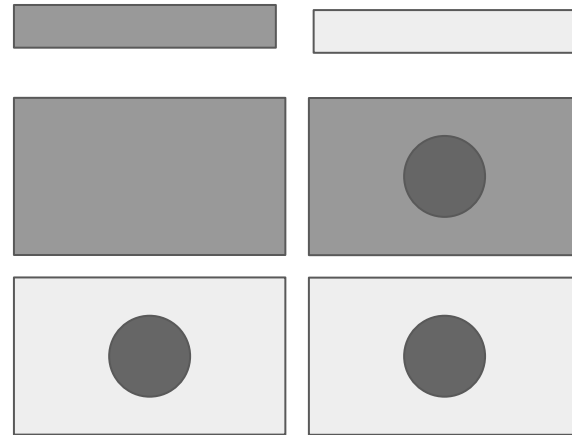
- Animals were collected before / 2 hours after sunset
- Put animal in glass jars, one jar under headlight and one jar in dark under tinfoils, observe whether embryo present after 12 hours
- Species: *Bolinopsis microptera*, *Pleurobrachia bachei*, N=24

Qualitative results

Bolinopsis



Pleurobrachia



Qualitative results

Beroe abyssicola (n = 2)



- Likely not have a fully developed gonad at the time of experiment

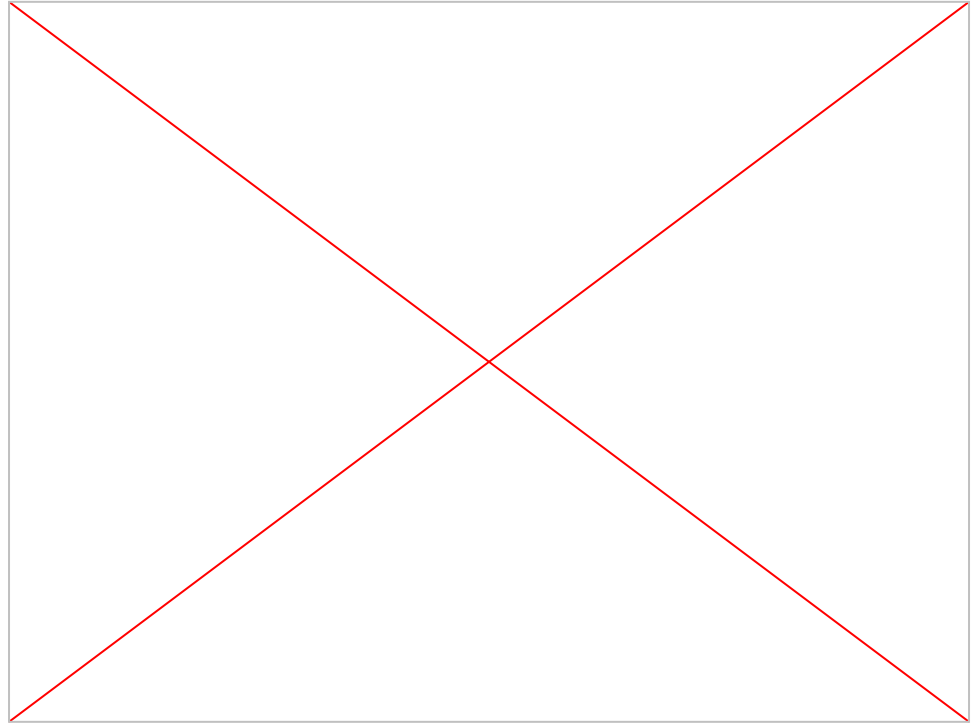
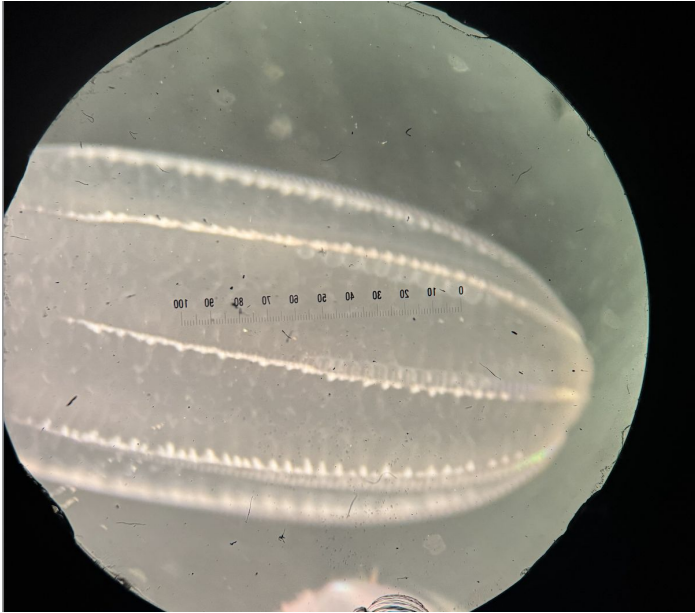
Qualitative result: interpretation

- Gradual transition from light to dark is needed for dark inhibition of spawning - potentially circadian rhythm? (although they do not have necessary genetic component for circadian rhythm)
- Light activation / inhibition of spawning seems to not associated with phylogenetic relationship

Qualitative result: error (and why it's not quantitative)

- Diet heavily affect their reproductive output
- And It's difficult to keep them alive for over 2 days (through beakers on float table)
- Small experiments group (first trial $n = 7$, second trial $n = 4$)
- Headlight is not very consistent

***Beroe* did spawn... under a cocktail of stress**



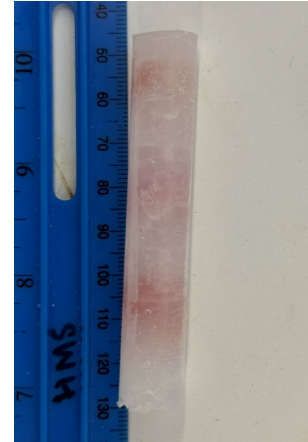
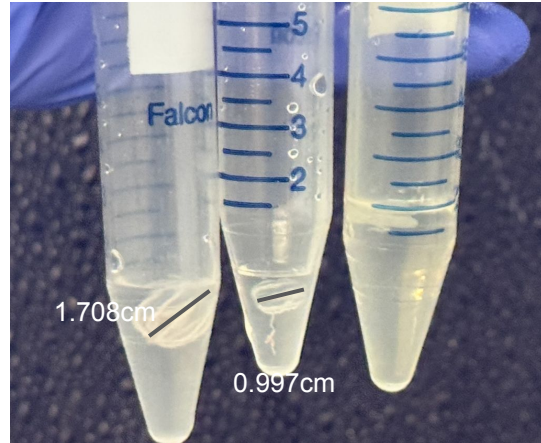
Can you scan 100% water?

Can you scan 99% water?

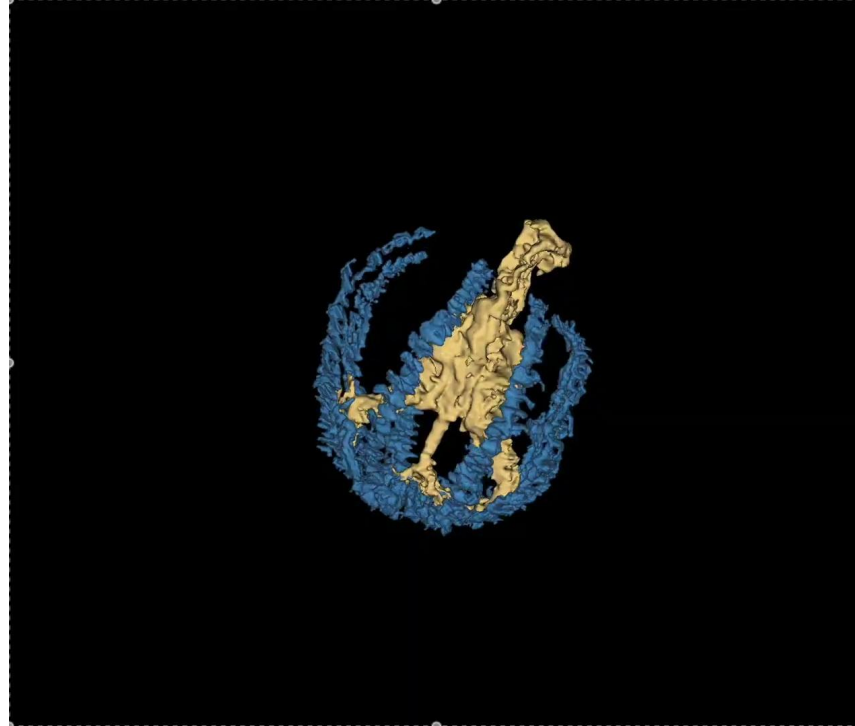
YES!

Ctenophore microCT scan: experiment

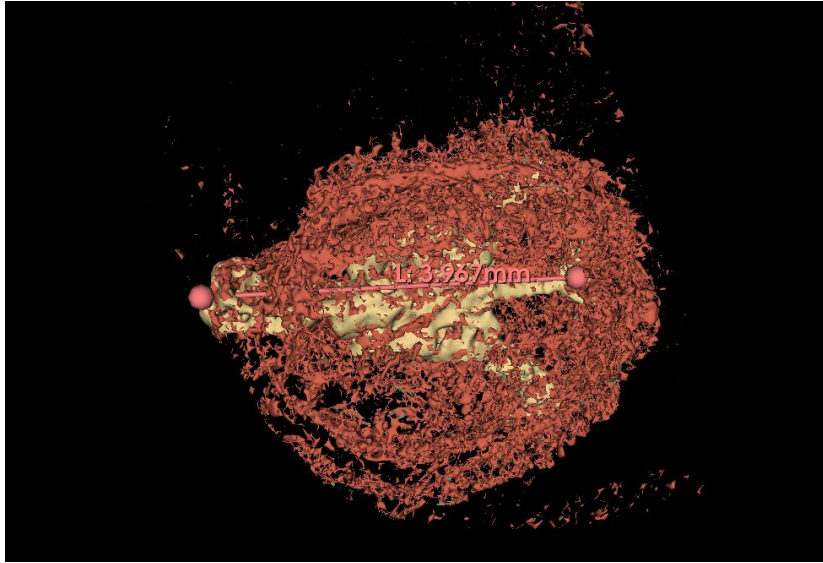
- Fix *Pleurobrachia* in rainX (seawater to rainX: 1:0.6) for 1 hour in room temperature
- Dye *Pleurobrachia* with 100-200 uL diluted lugol with buffer for 15-20 mins
- Fix (in a physical way) *Pleurobrachia* in paraffin
- Scan



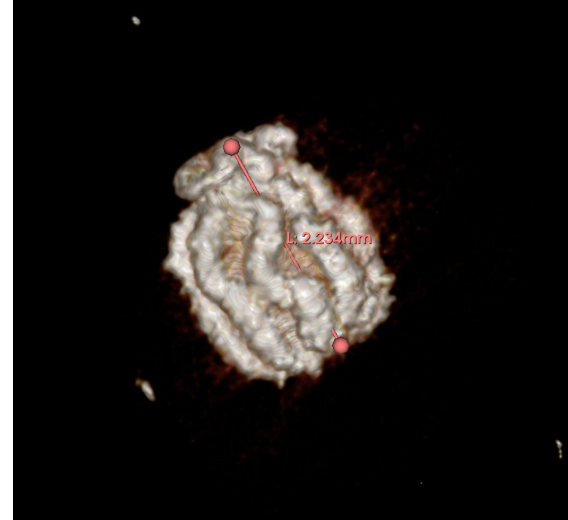
Ctenophore microCT scan: result



Ctenophore microCT scan: result



Shrink by 430%



Shrink by 446%

Current conclusion

- Light regulated reproduction is likely associated with other downstream regulatory pathway
- Ctenophore inner structure can be detected in microCT using iodine dye apart from shrinkage in size and minor deformation in general shape

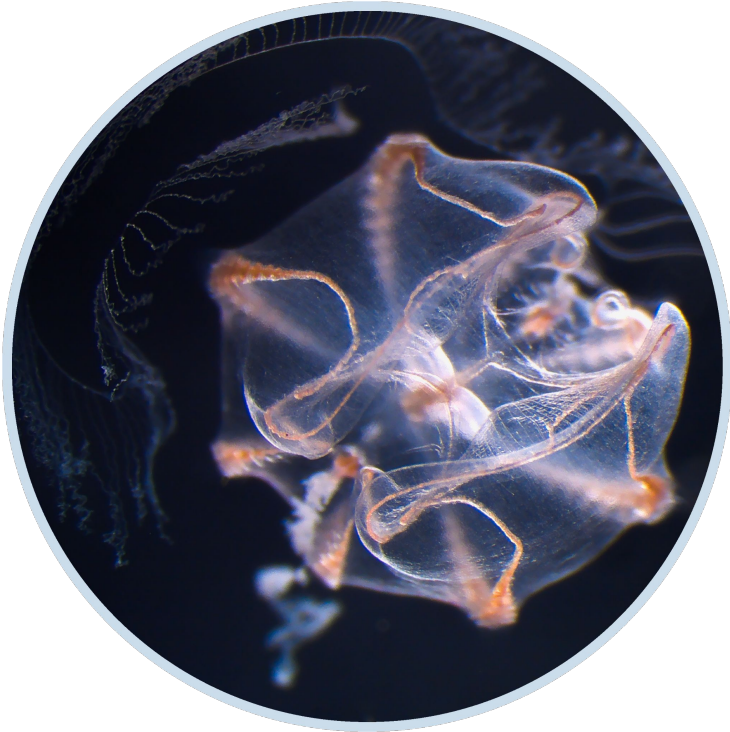
Next step & Ongoing projects

- Refine protocol of MicroCT scanning ctenophore (and potentially try on more marine gelatinous animals)
- Morphometric on shape change during development for cydippid and lobate Ctenophore
- Try to spawn *Beroe abyssi* again at home and potentially cell fate study

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Acknowledgement



Dr. Rebecca Varney
Jorge Merchán
Dr. Megan Schwartz
Dr. Eric Edsinger

Everyone who shouted positively at me
when they found ctenophore

Every cooperative or non cooperative
ctenophores

<https://lifehistorylab.com/>

I worked on ctenophores in the Salish Sea, and I managed to explore several preliminary tests on them.

1. Light-regulated spawning in Ctenophore

Light is one of the most common environmental factors affecting animal behaviors and life cycles. Ctenophora, as one of the earliest branched lineages among metazoans¹, can provide insight on how light-regulated behaviors have evolved in animals. *Mnemiopsis leidyi*, an Atlantic coast ctenophore, requires 3-4 hours of darkness to spawn². *Bolinopsis microptera* and *Pleurobrachia bachei* suggest they need to be exposed under bright light. This requirement is opposite of *M.leidyi*, despite *B.infudibulum* is phylogenetically closely related to *M.leidyi*. However, there is no experiment that clearly determines whether the spawning happens in a dark environment or light environment³. Furthermore, it is not clear whether it's the dark to light transition or only the light cue triggers the spawning. I hypothesize that strong light triggers spawning in *Bolinopsis*, *Pleurobrachia*, and another species *Beroe abyssicola* regardless of day-night cycle.

I conducted preliminary experiments on *B.microptera* and *P.bachei*. They were collected after sunset (*B.microptera* n = 9, N = 18, *P.bachei* n = 5, N= 10) and before sunset (*B.microptera* n = 4, N = 8, *P.bachei* n = 4, N= 8). The number of animals in each group changes due to availability at the FHL dock. The experiment group was exposed to headlight for 12 hours, and the control group was kept under cover of tin foil in darkness for 12 hours. There is no *B.abysicola* around the dock now, so all the animals used (n=2, N=4) come from kreisel maintained by Dr. Eric Edsinger. Each group was put under light for 2 hours. The results show that all *B.microptera* and *P.bachei* collected after sunset spawned under light and did not spawn in dark. *B.microptera* and *P.bachei* collected after sunset spawned in both groups. No *Beroe* spawned. The results indicate that light activation of spawning seems to not be associated with phylogenetic relationship, and gradual transition from light to dark is needed for dark inhibition of spawning.

2. Ctenophore imaging methods

I developed a protocol to stain ctenophores for microCT scanning collaborating with Dr. Rebecca Varney and Jorge Merchán. The *Pleurobrachia bachei* was fixed in Rain X for one hour at room temperature⁴. 1.5% lugol iodine solution was added to the sample for contrast staining. From the microCT scan results, shrinkage in size has been observed, but the morphology, including internal structure remains intact.

References

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4. Mitchell, D. G., Edgar, A. & Martindale, M. Q. Improved histological fixation of gelatinous marine invertebrates. *Front. Zool.* **18**, 1–13 (2021).