MACROPHAGOUS CARNIVORY BY CILIATED LARVAE: DEVELOPMENT AND FEEDING OF AN UNKNOWN CARNIVOROUS CAPITELLA LARVA

by

SEBASTIAN BERGEN

A THESIS

Presented to the Department of Marine Biology
and the Robert D. Clark Honors College
in partial fulfillment of the requirements for the degree of
Bachelor of Science.

June 2022

An Abstract of the Thesis of

Sebastian Bergen for the degree of Bachelor of Science
in the Department of Marine Biology to be taken June 2022

Macrophagous Carnivory by Ciliated Larvae: Feeding and Development of an Unknown Carnivorous Capitella Larva

Approved: *George von Dassow, Ph.D.*
Primary Thesis Advisor

Long-standing tradition in larval biology has been to categorize invertebrate larval developmental strategies into two general modes: lecithotrophic versus planktotrophic development. Lecithotrophic larvae hatch from eggs containing all the nutritive materials required to form a juvenile, while planktotrophic larvae graze on unicellular algae as a requirement for metamorphosis into a juvenile. However, recent work, which includes studies on members of two large taxa of spiralian worms, polyclads and nemerteans, have revealed the widespread presence of larvae who consume animal prey instead of algae. We refer to this developmental mode as macrophagous carnivory. The intent of this thesis is to fully document larval development and prove that a carnivorous diet is required for the maturation of an unknown Capitellidae species found in the waters around Coos Bay. These larvae have a distinctive feeding behavior and morphologically develop on a solely carnivorous diet to settlement competency. These findings contribute to a growing body of evidence for macrophagous carnivory as a widespread larval strategy to extract resources from the plankton. Their developmental changes that occur from collection from plankton to post-settlement have been documented, though their identity was never uncovered, and their eggs and adult forms are current unknown, leaving their lifecycle incomplete.

Acknowledgements

I would like to thank Professor George von Dassow, my Primary Thesis Advisor, for both recommending this project to me, guiding me on the right course, and being there to help give me advice when I was having trouble. I am immensely grateful for his presence. I would also like to thank Professor Christina Ellison for allowing Daniel Tran and Kianna Manning to DNA barcode my samples since I was not able to do it myself. I would also like to thank Maya Watts for being my Primary Reader and taking the time out of her day to help, and for my CHC Representative Carol Paty for supervising the process. I would also like to thank Miriam Jordan for helping me with things on my Thesis and guiding me through my Thesis Prospectus and getting me prepared for everything.

I would like to thank my family for listening to me and motivating me to continue through this process, stay in the honors college, and finally complete my thesis. I would probably be far behind without all their support.

Finally, I wish to dedicate my Thesis to one of the kindest men alive, my Uncle Hans Helmut, who passed away recently due to COVID. His generosity and kindness shall never be forgotten.

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Introduction

Background

What are larvae?

Plankton are a diverse group of organisms, which include bacteria, viruses, protists, animals, and plants. They are adrift on the currents and are too small to reliably move themselves over significant distances (Brierley 2017). This is what separates what we call plankton from swimming organisms like fish.

Many ocean invertebrates possess a bi-phasic lifestyle that includes a free-swimming planktonic larval stage and an adult stage. These organisms hatch from eggs not as miniature adults, but as a distinct larval form. Although the larval biology community may debate on what exactly classifies as a larva, for this thesis, a larva is the life-stage of an organism's development when it utilizes a different environmental niche than the adult form, whether this be for transport or food intake (Haug 2020).

For example, *Strongylocentrotus purpuratus*, otherwise known as the purple sea urchin, has a larval stage that looks nothing like its adult counterpart. Instead, during the 'pluteus' stage, the urchin bears a resemblance to Sputnik, with thin rod-like arms that extend outwards into the water column. These arms are specialized structures which are used to collect unicellular algae from the planktonic environment. The juvenile grows as a small sack on the side of the larval stomach, until it eventually consumes the larval body and settles down to the benthic (seafloor) environment as a juvenile (Hinegardner 1969). The larva of *S. purpuratus* exemplifies the ideal representation of our 'larva' definition, since its pluteus form exploits a different environmental niche than the adult.

Traditionally, larval biologists classify larval development into two main modes: lecithotrophic vs planktotrophic development. Lecithotrophic developers develop from eggs provisioned with all the nutritive substance they need to metamorphose into an adult form, while planktotrophs graze in the plankton on unicellular algae to develop (Thorson 1950). Comparatively, lecithotrophic organisms invest more energy per egg and have larger and fewer eggs than planktotrophs of similar sizes and groups. Without a long pelagic life dependent on the availability of planktonic food, lecithotrophic larvae have the greatest chance to make it to adulthood. Meanwhile, planktotrophic organisms are the opposite. They invest relatively less energy per egg and produce more eggs than similar lecithotrophs, at the cost of a larger larval mortality rate and a dependence on a seasonal or unreliable planktonic food supply (Thorson 1950).

Although these developmental modes can be considered as either side of a developmental spectrum, the reproductive success vs energy invested is maximized only at the extremes, making intermediate development modes, like facultative planktotrophy, evolutionarily unstable (Vance 1973).

A different strategy: Macrophagous carnivory

Rumors and indirect observations of macrophagous carnivory have been present in literature since the 1920s, inspiring Johnson and Brink (1998) to summarize these findings. They conducted an experiment to see how likely this was to occur under natural plankton concentrations. During their experiment, they found no evidence of carnivory, suggesting instead that many previous findings may have been due to artificially high plankton concentrations present in nets while collecting. If

macrophagous carnivory did exist, it was not common at natural environmental conditions (Johnson & Brink 1998).

Despite their dismissal, evidence for macrophagous carnivory feeding strategies continued to appear in literature. In his dissertation project, when Dunn tried to raise the supposedly lecithotrophic larvae of *Carcinonemertes errans* in the lab, he found that they could not be prompted to settle. However, those larvae that were caught in the plankton were larger and more well-developed than those raised in lab, and could be triggered to settle (Dunn 2011). Considering they had no feeding structures to capture algae (like mucous houses and ciliary band capture) (von Dassow et al. 2022) how they grew was a mystery.

Macrophagous carnivory was directly documented by George von Dassow in six species of hoplonemerteans, including *Carcinonemertes epialti*, solving the mystery (von Dassow et al. 2022) (**Figure 1**). Further studies conducted by von Dassow documented this behavior in multiple genera of polyclad flatworms (von Dassow & Mendes 2022) and paleonemertean larvae (von Dassow, unpublished data), which demonstrate that the behavior is widespread in worms, far more than what Johnson and Brink (1998) likely hypothesized.

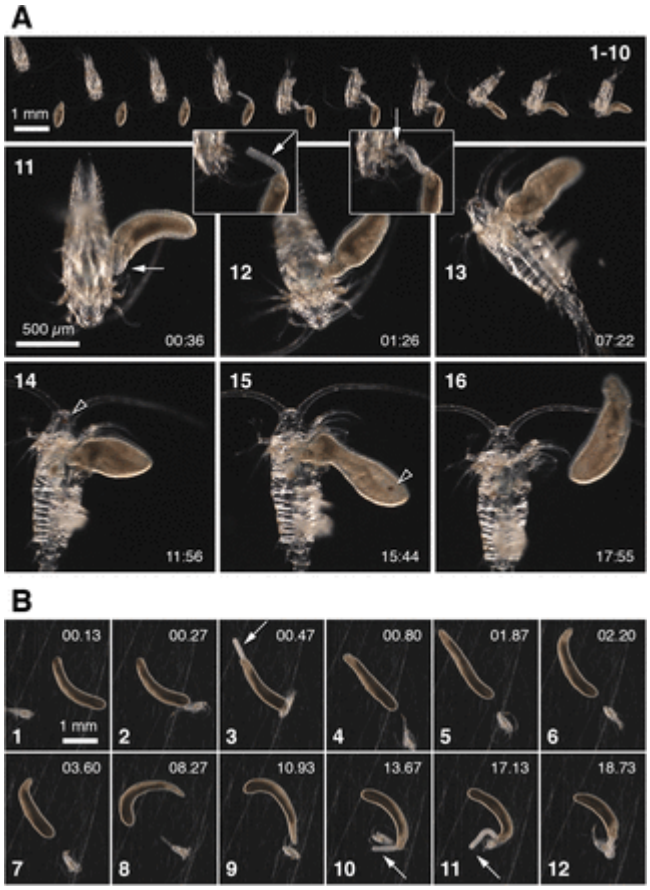

Figure 1: Predation on crustacean prey by hoplonemertean larvae

Credit to von Dassow et. al. 2022 for images. This image compilation reveals the sequence of events occurring in a normal prey capture by hoplonemertean larva. The worm uses its proboscis to grab and subdue their large prey, and then proceed to suck out the soft tissues inside.

Provisional identification

This experiment focuses on the larva of an unknown capitellid polychaete found in high concentrations off the waters of Coos Bay. It was classified as such based on its morphological similarity to other larvae in the genus *Capitella.*

Purpose

George von Dassow originally observed these capitellid larvae engaging in macrophagous carnivory in laboratory captivity. Video capture and photodocumentation

of this behavior formed the core of my project, but the scope was expanded to encompass the growth and development of the capitellid larvae in response to a solely carnivorous diet.

Relevance

Why this worm?

Primarily, this is a project of curiosity. I am studying the development of a worm larva possibly undocumented by science and getting to watch a distinct and fascinating feeding behavior unlike anything I have ever seen before.

However, the findings do have important scientific value beyond just my own interests. In determining that capitellid larvae do in fact require a carnivorous diet to grow and develop, I could contribute to the novel but growing body of work that demonstrates a widespread third major mode of development is present in larval development.

Furthermore, the documentation of larval development, settlement, and feeding mode of an unknown capitellid would provide contribution to our understanding of Capitellidae, as only three species have been well-documented in the group (Pernet et al. 2015). In addition, if the larvae are established as members of the cryptic *Capitella capitata* species complex (Grassle & Grassle 1976) their traits could either be matched to existing *C. capitata* members and identified (Blake 2009) or possibly add yet another member of the group to the complex, with either option contributing to our understanding of this group of organisms.

Materials and Methods

General

All organisms used in this experiment were collected with a 53-micron mesh net towed near the surface of the water around the Charleston Marine Boat basin. Capitellid larval specimens were collected in the Small Boat Basin, where they were abundant Winter and Spring 2020-2021.

The feeding and development of the unidentified capitellid larvae was determined through three experiments. The first two experiments, "Growth 1 and 2," were undertaken to observe the growth rate of larvae in response to consuming prey and to document the full feeding process from initial capture to engulfment via video recording. The third experiment, "Development", was undertaken to assess the morphological changes occurring in the larvae as they matured.

Growth and feeding

Feeding preference

A selection of wild-caught capitellid larvae were isolated from plankton tows, placed in an empty Syracuse dish, and then provided a selection of potential larval prey items found in the plankton that same day, which included barnacle nauplii, copepod nauplii (planktonic crustacean), ciliates (small protists, not larvae), veligers (mollusc larvae), crab zoea, and other polychaete worms.

Growth 1

Figure 2: Capitellid after collection from plankton

Microscope image of a freshly caught capitellid from the Development experiment "Fed" group.

During winter 2020, fresh plankton tows from the bay were sorted and condensed to locate the conspicuous larvae (**Figure 2**), which were isolated in a Syracuse dish via aspirator transfer. 20-30 individuals were selected to begin the initial culture, and then placed into a bowl half-filled with filtered seawater and left in a sea-table at temperatures around 12-13˚C for a few days.

One to two times per week, the culture was removed, and ~20 individuals were selected at random, placing them in another Syracuse dish. Two similar-sized individuals were isolated via aspirator and placed under a coverslip with clay feet, compressed down just enough to restrain movement.

The prepared slide was then observed under a Leica DMi8 Microscope at x10 magnification, with the focus adjusted until the point that all sides of the larvae were maximally crisp. Photos were captured utilizing a Point Grey C-Mount x55 camera

connected to a computer running Streampix 6 x64 Edition, which is a program that allows the computer-based capture of video footage and images from the Leica DMi8 microscope. Two to three images of each specimen on the slide were taken and then the best ones saved for later analysis. Larvae were then retrieved from the slide and placed back into the main culture dish after photography.

Photographs were analyzed via ImageJ version 1.50e and calibrated to μm^2 using a stage micrometer. Sectional area was determined by using the polygon tool to outline the edges of the photographed specimen, and then the area of this polygon was taken via the program. The measurements from each session were summarized with mean, min, and max values, and saved to an Excel spreadsheet for later analysis of growth.

The process for recording feeding began with isolating preferred prey items, which included barnacle nauplii, copepod nauplii and ciliates, in a Syracuse dish.

The Leica S8APO microscope was modified with a 10x eyepiece-based smartphone adaptor and set up with my Pixel 3a phone for video capture. The prey dish was then poured into the original culture bowl to begin the feeding process, and the larvae were filmed to capture the full feeding process, from initiation to engulfment. For easier management of the footage, video was captured in 1–2-minute increments, with the length extended if the desired behavior was captured. Shorter videos could be deleted if nothing interesting occurred.

When the filming session was completed, the larval specimens were moved into a culture dish filled with fresh seawater, and then placed in the sea-table for a few days until the next feeding session. The same procedure was used every feeding session.

Growth 1 was maintained with the same feeding/imaging schedule until larval growth plateaued around 1-2 months. The remaining larvae were frozen in microcentrifuge tubes for later genetic analysis.

Growth 2

Growth 2 was undertaken in spring 2021 as a replicate of Growth 1. Initial culture establishment, culture care, growth imaging, and feeding processes were kept the same as above, though measurement and feeding sessions occurred on weekly intervals instead of 1-2 times per week. Footage was taken utilizing a similar procedure as Growth 1.

Development

The third experiment was conducted with the intent to observe morphological development. 20 capitellid larval specimens were separated into two groups of 10: "Fed" and "Starved." Water changes occurred on a weekly basis for both cultures, though their feeding treatment varied. "Fed" culture was fed after every water change, and the prey items were left in the bowl over the interval between water-changes so that every larva might have a chance to consume something. "Starved" culture was not fed for the 1 month duration of the experiment.

The imaging process for both cultures was similar to those for Growth 1 and 2, though both external (segmentation) and internal (organs, chaetae, etc...) features were observed and photographed. Similar sets of images from both "Fed" and "Starved" cultures were compared to note any developmental differences at the conclusion of the Development experiment.

Settlement

This experiment was conducted with the intent to trigger settlement and investigate morphological changes post-metamorphosis, utilizing the most mature-looking members of the "Fed" group.

Four selected larval specimens were imaged under the Leica DMi8 microscope, along with captured video that included various focal planes, movement, and behavior of the specimen. Three specimens, labeled A, B, and D, were placed in small vials filled with a thin layer of sediment from the Boat Basin, to evaluate whether settlement could be triggered. The fourth specimen, C, was placed into a vial with no sediment as a control variable.

After five days of observation, the vials were opened, and water/sediment was poured out into a petri-dish under the S8APO to locate juveniles.

Any located worms were removed from the petri dish and observed using the same procedure they would undergone before being placed in the vials, which created a consistent before and after comparison of the specimen's body plan.

Identification

DNA Analysis

Morphological species identification of Capitellidae and *Capitella* was difficult due to their cryptic nature, so two paths were taken to remedy the problem.

The first path was DNA barcoding the samples in **Table** 1.

Tube Name	What's in the tube	Collection info
CA	Unknown Capitella larva near pre-settlement	Grew them in lab until near-maturity
CC	Unknown Capitella larva near pre-settlement	Grew them in lab until near-maturity
CD	Unknown Capitella larva near pre-settlement	Grew them in lab until near-maturity
CE	Unknown Capitella larva near pre-settlement	Grew them in lab until near-maturity
CF	Unknown Capitella larva near pre-settlement	Grew them in lab until near-maturity
CG	Unknown Capitella larva near pre-settlement	Grew them in lab until near-maturity
CH	Unknown Capitella larva near pre-settlement	Grew them in lab until near-maturity
CI	Unknown Capitella larva near pre-settlement	Grew them in lab until near-maturity
CJ	Unknown Capitella larva near pre-settlement	Grew them in lab until near-maturity
CL	Unknown Capitella larva near pre-settlement	Grew them in lab until near-maturity
CM	Unknown Capitella larva near pre-settlement	Grew them in lab until near-maturity
CN	Unknown Capitella larva near pre-settlement	Grew them in lab until near-maturity
Settle B	Unknown Capitella larva post-settlement on boat basin mud	Grew in lab, then isolated out and placed on sediment to promote settlement. Worm collected by spreading sediment on petri dish and looking for a matured capitella
Adult 1	Adult Lecithotrophic Capitella, segment of posterios	Collected in black mud in the small boat basin, underneath rocks exposed at low tide. Sifted soil in a 0.5mm filter
Adult 2	Adult Lecithotrophic Capitella worm, segment of posterior	Collected in black mud in the small boat basin, underneath rocks exposed at low tide. Sifted soil in a 0.5mm filter
WB-1	Settled larvae of the Lecithotrophic Capitella worm	Mature larvae released from worm tube breakage, settled down and metamorphosed into these. Only one set of images found for these two samples, but they came from the same worm source
WB-2	Settled larvae of the Lecithotrophic Capitella worm	Mature larvae released from worm tube breakage, settled down and metamorphosed into these. Only one set of images found for these two samples, but they came from the same worm source

Table 1: Summary of Genetically Analyzed Samples

Basic summarization of samples placed into each PCR tube and frozen. This table includes information on how and where samples were collected

Samples CA-CN (**Table 1**) were the largest larvae at the conclusion of experiments Growth 1 and 2. To prep these larvae for DNA barcoding, they were starved for multiple days to remove any confounding prey material they may have contained after feeding. Settle B was the post-metamorphic juvenile of capitellid B from the Settlement experiment. Adults 1 and 2 were the severed pygidiums of large capitellid worms found in the Charleston Small Boat Basin mud, tentatively identified as *Capitella capitata* using the description by Heibert et. al. (2017). WB 1 and 2 were the larval specimens of broods found in the tubes of Adult 1 and 2.

Specimens were rinsed in purified seawater in preparation for DNA barcoding. The prepared specimen was placed into a clean microcentrifuge tube with a few microliters of seawater and then stored in a freezer at -80°C. This process was repeated for all other specimens.

Tran et. al. 2021 (**Attached**) barcoded the specimens.

Locating Adult Worms

Possible worm candidates were collected from the Charleston Small Boat Basin and the Portside Mudflats, resulting in two candidate worm types.

A large pink-red worm identified as *Capitella sp.* (and possible *Capitella capitata*) (Hiebert et al. 2017) was found while digging in mud at low tide in the Small Boat Basin. The other worm, a former *Capitella* member, *Mediomastus californiensis*, was a small red threadlike worm found in the high intertidal Charleston mudflats (Hiebert et al. 2017).

Collected worms were kept in separate bowls. The *Capitella sp.* individuals had brood tubes that could be broken open to release the larvae, while a fragment of their pygidiums was removed for DNA barcoding. Certain *M. californiensis* individuals were gravid with eggs, and minor incisions were made to allow the eggs to flow into the bowl.

Results

Feeding and Growth

Feeding preferences

The composition of plankton varied on a daily and seasonal basis, though the prominent component included ciliates, barnacle nauplii, and copepod nauplii. Capitellids consumed all three, but preferred copepod nauplii and ciliates, while avoiding crab zoea and other polychaete larvae. Algae was only consumed incidentally when cells stuck to the surface of a wrapped prey item, and veligers were too rare to collect any useful preference data on (save for one consumption event) (pers. observations).

While cannablism was not observed during the experiment, specimen numbers declined over the course of the experiment, suggesting cannibalism might occur.

Feeding behavior

Carnivorous behavior was observed and during development, from collection from plankton to the development of a kinked throat and large gut. During this time, the capitellids would sometimes target prey items larger than themselves, who frequently possessed a carapace with many protruding spines and setae, flailing sharp legs, and fast movements. Yet, the larvae were able to wrap up and engulf their prey (**Fig. 3**).

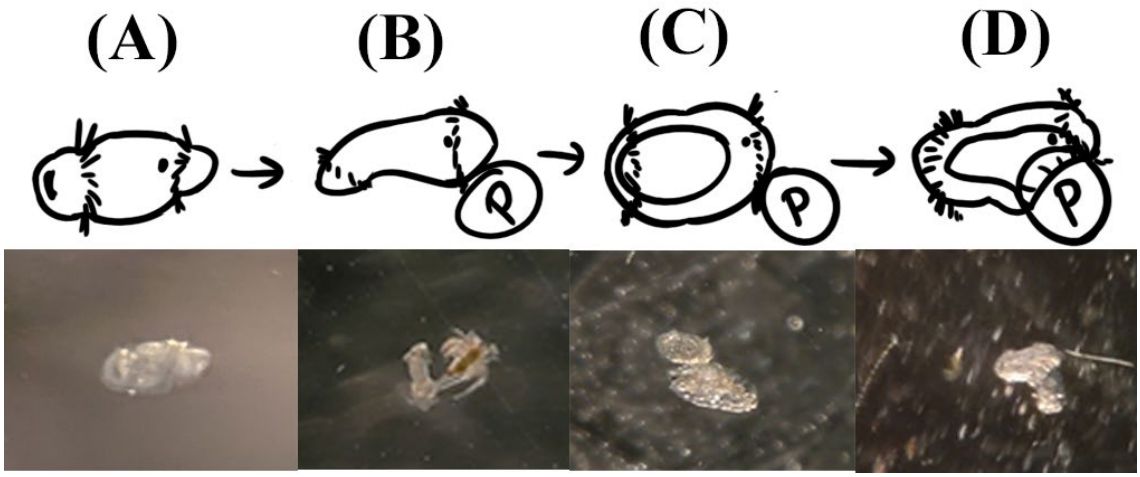

Figure 3: Process of feeding, both real images and idealized sketches.

Drawings display, in higher definition, the various stages of the feeding process. The particle (P) is being wrapped. (A) is the hunting larva, (B) is the swiftly initiating wrapping larva, (C) is the inflated larva late into the process and (D) is the final engulfment stage.

The attached video (**Video** 1) illustrates all stages in an ideal and complete feeding sequence that has been condensed into a reasonable timeframe.

When not feeding, larvae in the culture dish swim around at relatively swift speeds while making random turns and orientation changes (**Figure 3A**). On occasion, a collision triggers a feeding event. A behavior noted in **Video 1** and other footage was the steep orientation change of a capitellid to the direction of the selected prey item, possibly hinting at the capability to detect prey at a distance.

Larvae targeted the broad dorsal carapace of their prey and excreted a substance that allowed them to attach to their prey. **Video 1** demonstrates this substance's existence when the larva attempts to pull away from its prey but seems to be restrained by an invisible tether. When the prey was attacked, it attempted to escape. Copepod nauplii were observed jumping around the bowl, while barnacle nauplii flailed their legs over their dorsal carapace to try to dislodge the attached capitellid. This prey escape

response behavior was only successful in some instances. During a moment of rest, the capitellid began to rapidly orbit around its target, restraining its struggling prey while the prey flailed about to escape (**Figure 3B**). Usually, this struggling just further restrained the prey, their legs sticking to the substance the capitellid laid down. Within 5-10 seconds, the prey was incapacitated and twitched weakly.

During the initial wrapping process, other organisms were able to stick to the surface. Anything from algal particles to barnacle nauplii could be caught up in the sticky substance. The capitellid could moderately adapt in this scenario, but too many stuck organisms would cause the capitellid to abandon its meal.

By this point, the capitellid switched from orbiting the incapacitated prey to spinning the prey in its mouth while moving around the bowl. Capitellids were able to move relatively unencumbered even while carrying larger prey items, though they swam at a more sedate pace. When this stage was reached, the capitellid rarely gave up its prey and proceeded to completely consume its prey.

While swimming, the capitellid continued to inflate its rudimentary gut lumen with water, attaining a more rounded balloon-like shape in preparation for engulfment (**Figure 3C**). When sufficient expansion occurred, the capitellid latched on their still-living prey and began to extend their mouthparts over the prey body, slowly engulfing it lengthwise while swimming in a slow barrel-roll motion over the course of 30-45 seconds (**Figure 3D, Video 3**). In **Video 1** the larva engulfs a prey item stuck to the bottom of the bowl, but this is likely a lab artifact and not what occurs in nature.

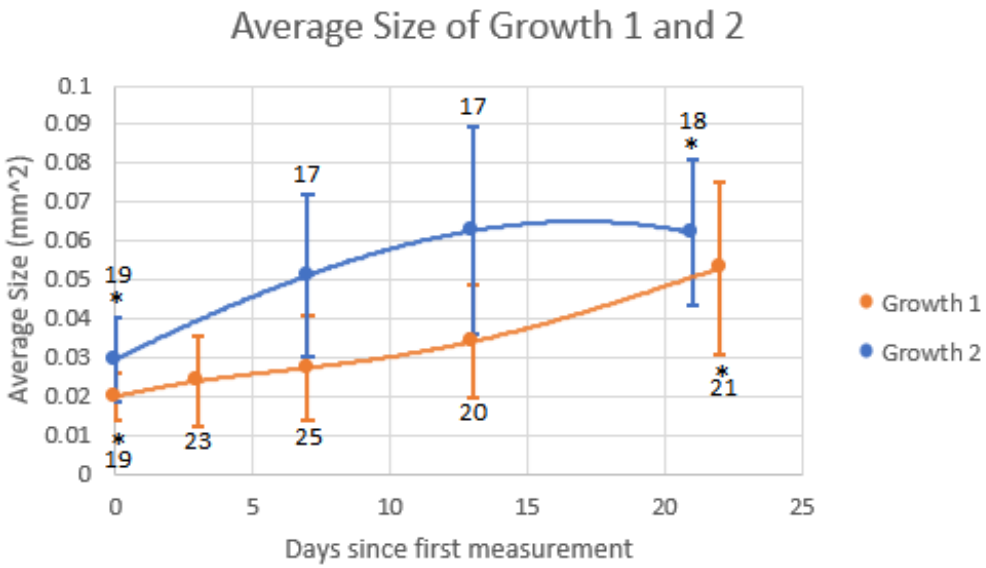

Figure 4: Combined growth vs time graphs of "Growth 1" and "Growth 2"

Trendlines on this graph represent each Growth Experiment. Error bars represent the SDs of the SA at each point. The number of specimens measured during each measurement session is shown below Growth 1 trendline and above Growth 2 trendline. The (*) symbol indicates statistically significant measurements. Total number of specimens declined in both experiments.

Growth 1

The change in average size over the course of the Growth 1 experiment was illustrated in **Figure 4**. The average sectional size of freshly caught larvae in culture was $2.0x10^4$ μm^2 with the largest individual being $2.9x10^4$ μm^2 in sectional area (SA) and the smallest being $1.1x10^4$ μm^2. The experiment ran for 22 days, before growth plateaued and the experiment was concluded. At the end of this period, the average culture size was $5.3x10^4$ μm^2 with the largest individual observed displaying an impressive SA of $9.7x10^4$ μm^2 and the smallest $2.3x10^4$ μm^2. This equates to an approximate 4-fold increase in volume.

Growth 2

Change in size over the Growth 2 experiment was illustrated in **Figure 4**. Initial average sectional area was $3.0x10^4$ μm^2 with the largest individual having a SA of $5.3x10^4$ μm^2 and the smallest having a SA of $1.3x10^4$ μm^2. The experiment ran for 21 days, after which growth plateaued and the experiment was concluded. At the end of the experiment, the culture of Growth 2 had an average SA of $6.2x10^4$ μm^2, with the largest individual having a SA of $9.2x10^4$ μm^2 and the smallest being $2.3x10^4$ μm^2, representing yet another 4-fold volume increase. All sectional areas plateaued at a point not much different from Growth 1.

Development

Starved

During the experiment, "Starved" culture specimens withered away and vanished without developing any visible morphological changes. Segmentation never developed, and they rapidly became too small to be seen effectively even with maximum magnification (x45).

Fed

"Fed" group specimens grew steadily through the experiment, developing visible morphological changes both externally and internally (**Figure 5-12**). They became metamorphically competent and stopped feeding once organs developed.

Morphological development

Larvae collected from the plankton had a small dome-shaped head anterior to a prototroch (head-region ciliary band), a cylindrical body, a telotroch (rear-region ciliary band), a short bulbous posterior (rear), and neurotroch (small anterior-posterior oriented ciliary band) (**Figure 5**). They had a bumpy surface with no visible segmentation and a ciliated orifice located near the prototroch (**Figure 6**).

Besides the rudimentary gut, small larvae did not have visible organs, had no internal segmentation, and lacked chaetae. Other than two eyespots, located posterior to the prototroch and on either side of the sagittal plane (left and right), their interior seemed to be mostly lipid droplets and pigment granules (**Figure 5**).

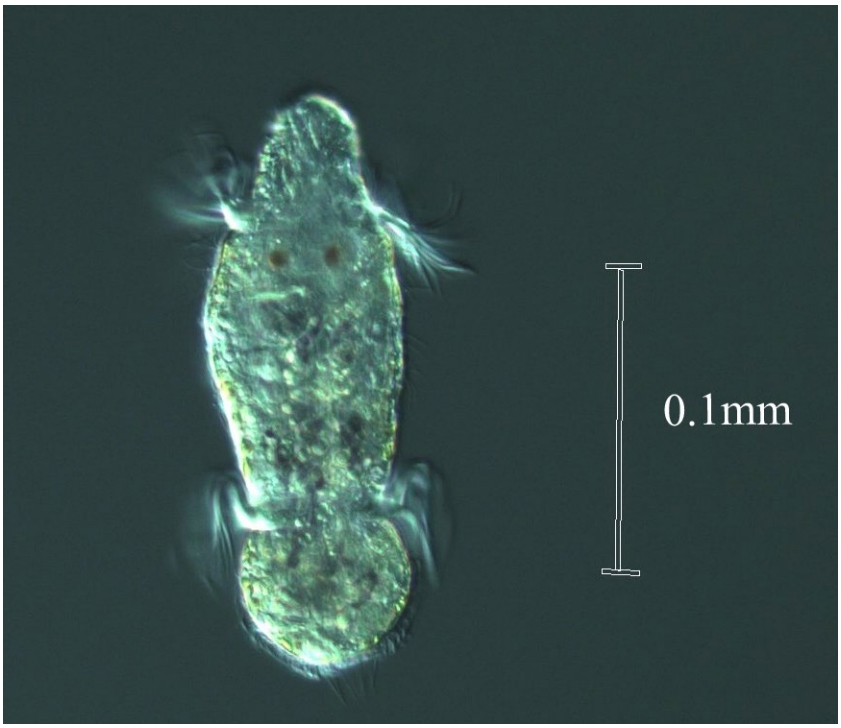

Figure 5: Young capitellid larva

Larvae look like this when they are captured from the plankton. Note ciliary bands and cilia, eyespots, pigment granules, bubbles, and a lack of overt segmentation or visible organs. A rudimentary gut is present, but not visible in this image.

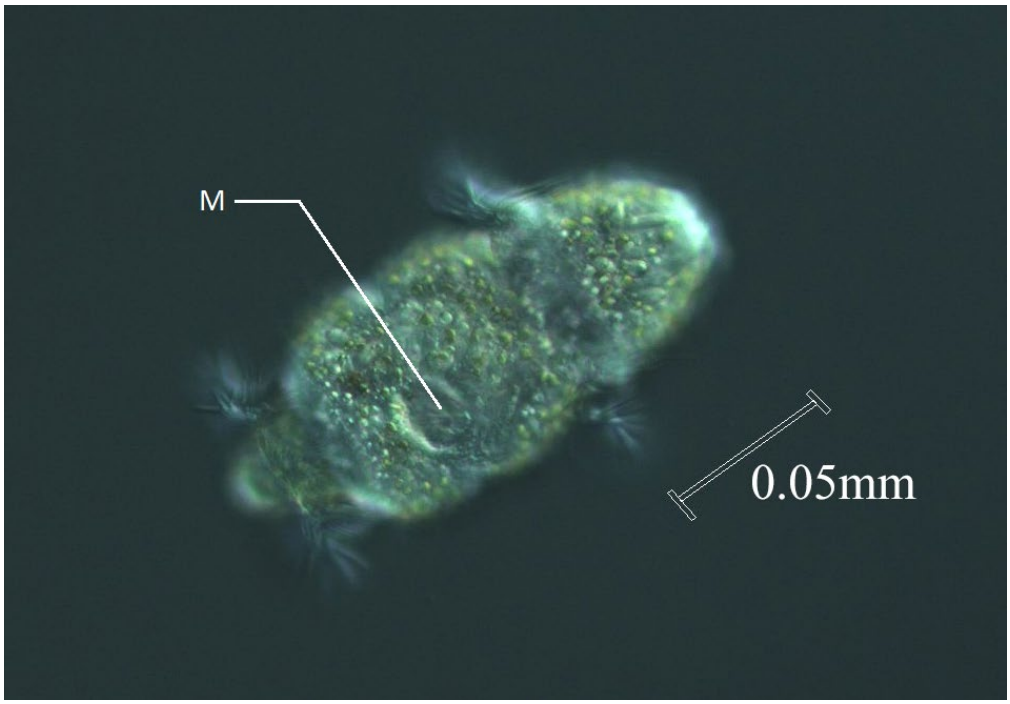

Figure 6: Young capitellid larva, external morphology

In this image a plankton-collected specimen shows an unsegmented, bumpy surface and a noticeable ciliated mouth (M).

By 8 days, internal and external morphology grew more discrete. Their midsections had extended, forming a more cylindrical shape, and segmentation had begun to develop on their surface (**Figure 7**). The pygidium developed a black-pigmented blotch, forming into either a bar-like or circular shape (**Figure 7**). Highly visible internal morphology developed, with the formation of a developing gut (**Figure 8B**), the formation of internal segmentation (**Figure 8A,B**), and primordial chaetae (**Figure 8B**).

During this time, larval behavior had begun to change. In cultured bowls, they continued to swim normally, but when they were placed under tight coverslips, they began to move via peristaltic contractions (compressing and extending in waves). In

fresh plankton-caught specimens, such behavior was never observed, and they would continue with swimming motions even while restrained.

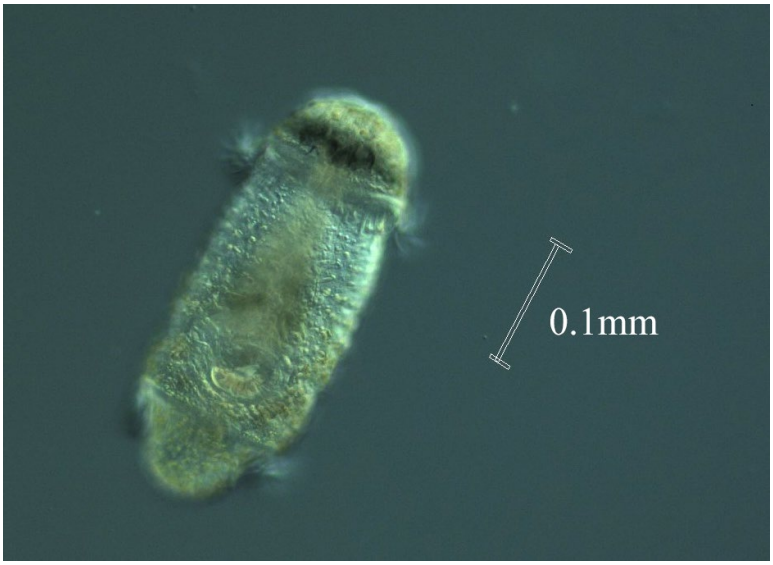

Figure 7: Fed larva after 8-12 days, external morphology

Roughly 9 segments are visible on the surface of this larva. Accurately identifying all segments on any larva was a difficult affair due to focusing differences.

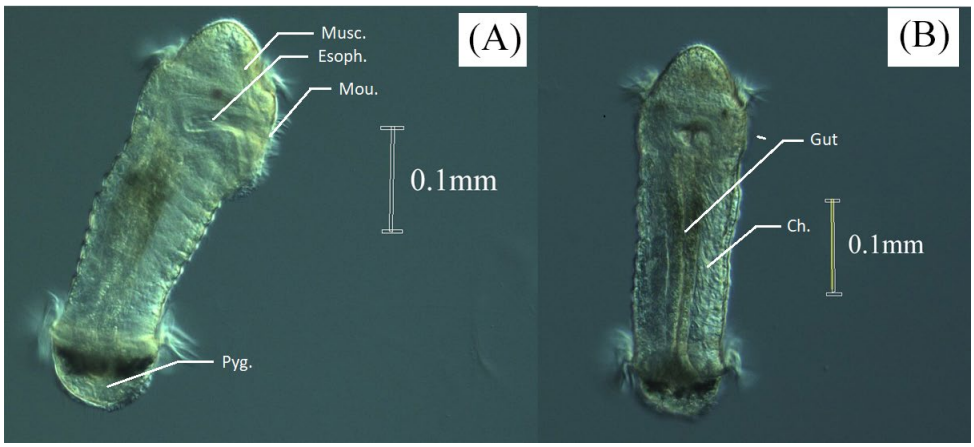

Figure 8: Fed Larvae after 8-12 days, internal morphology

Development of easily observable discrete morphological development. Image (A) shows a side-view of the mouth (Mou.) and throat, displaying the musculature (Musc.) that stretches into the anterior region and down below the mouth, along with a visible esophagus (Esoph.). The dark pigment is also visible in the pygidium (Pyg.) Image (B) is a dorsal-ventral view showing the initial formation of chaetae (Ch.), segments, elongated/cylindrical body, and the developing gut (Gut).

After 14-18 days of development, further morphological changes occurred. Larvae during this stage were significantly larger than their earlier-stage counterparts. The bar of discrete internal flesh had been replaced by a visibly differentiated digestive system, complete with a kinked throat and large stomach region (**Figure 9A,B**). Small tufts of cilia on the pygidium were spotted, although their current function and earlier presence was unknown (**Figure 10A**). Their chaetae were fully developed, and the larvae extended and retracted them at will (**Figure 9C**).

Previous peristaltic movements under coverslips became far more pronounced, and they extended and retracted their chaetae as they squirmed underneath the coverslip. However, they still swam normally when given the opportunity.

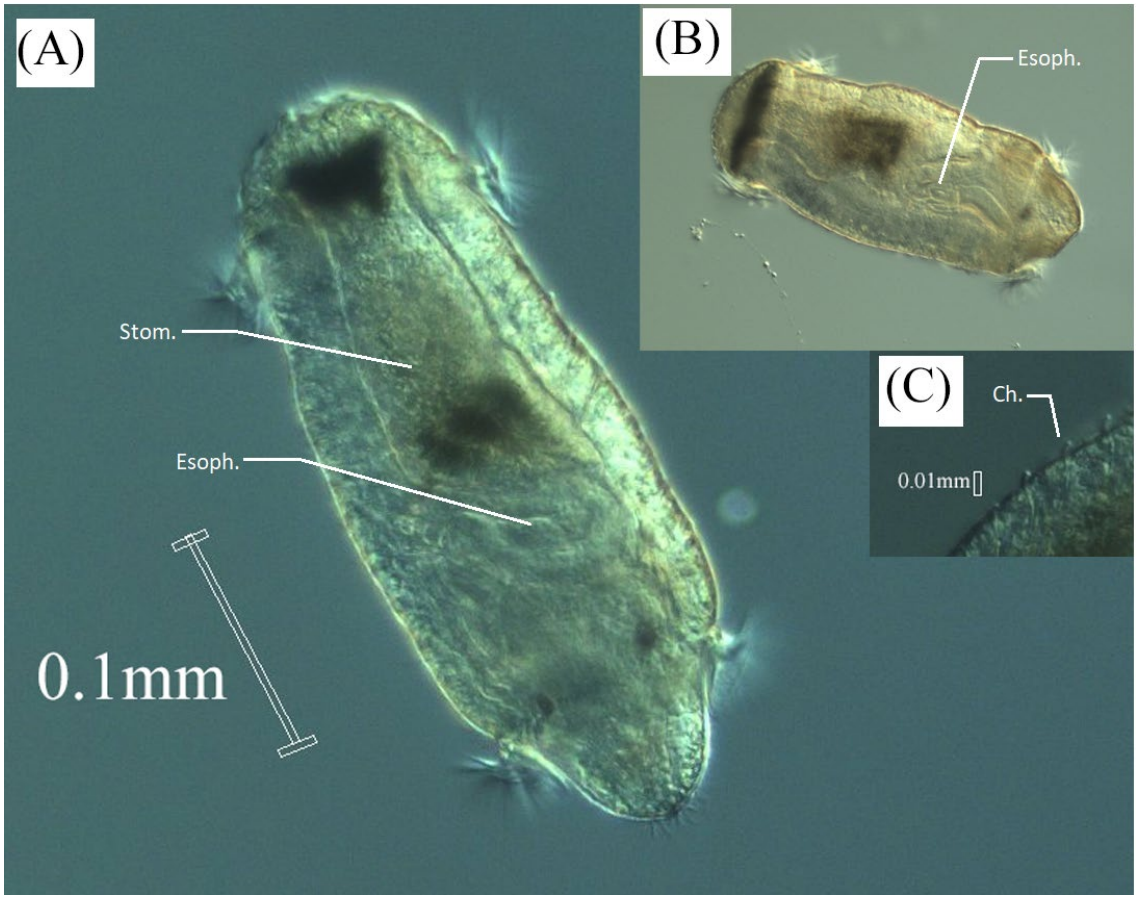

Figure 9: Composition photo of highly developed capitellidae larvae

(A) Shows the formation of visible and discernable organs, like the stomach (Stom.) and Esophagus (Esoph.), not present in the previous stages. (B) Shows a side view of a capitellid larva from Growth 1 after 20 days of development. The curved throat (Esoph.) is more visible in this image. (C) Shows a closeup view of a mature Fed capitellid larva, showing the developed chaetae (Ch.).

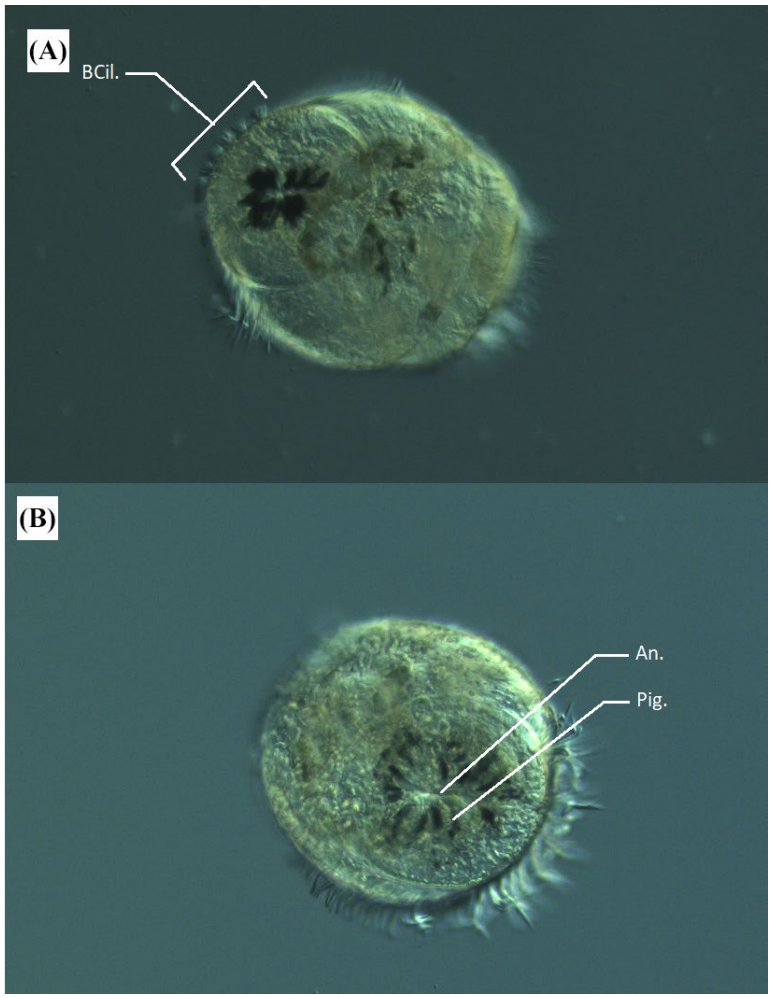

Figure 10: Posterior view of an older larva from Fed group

A posterior view of a capitellid larvae that shows a radial arrangement of the pigment granules (Pig.) which look like a bar from the usual side view.(A) Not only shows this radial arrangement but also highlights 6 tufts of brush-like cilia (BCil.). (B) Shows the radial arrangement but also the anus (An.).

Settlement

Figure 11: Capitellid C larva before settlement experiment

This image shows some internal and external morphology of the metamorphically competent settlement control capitellid C. Internal organs, eyespots, chaetae, and a dark pigment blotch in the pygidium can be seen.

During settlement, the control capitellid C never settled, remaining morphologically identical to when the experiment began (**Figure 11**). Capitellid A and D vanished from the water column and were not located in sediment. Only the juvenile of capitellid B was found post-settlement. Capitellid B was isolated for observation using the Leica DMi8, resulting in **Figure 12** and **Video 2** (**Attached**).

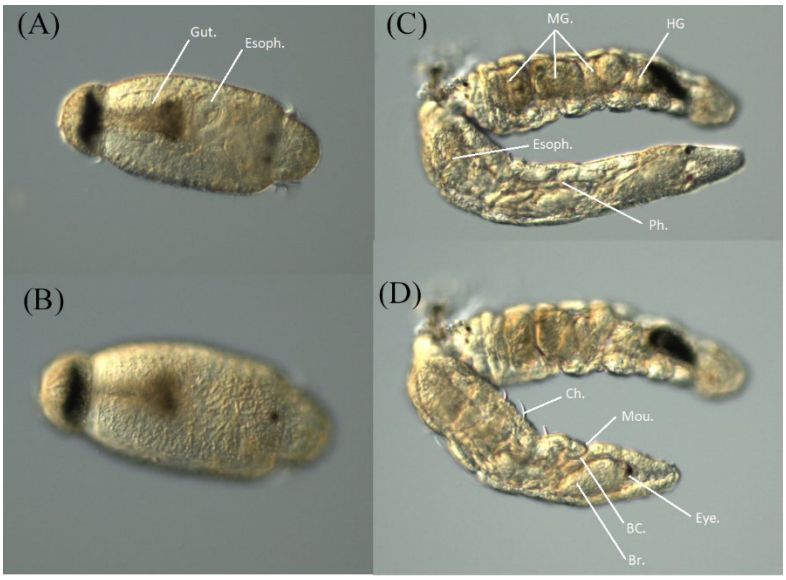

Figure 12: Pre and post-settlement capitella B

This image shows two sets of before and after images of capitella B settlement. (A) has a focal plane focusing on the internal anatomy (gut (Gut.), esophagus (Esoph.)), matched with (C) which shows a similar focal plane shot of the post-settlement juvenile. Midgut (MG.), hindgut (HG), esophagus (Esoph.), pharynx (Ph.). (B) is a focal plane on the surface of the pre-settlement larva, showing mild segmentation and organization of surface bumps. (D) is matched up with (B) and shows a more surface-focused image displaying chaetae. Chaetae (Ch.), mouth (Mou.), brain (Br.), buccal cavity (BC.), eyespot (Eye.). Specimen may have been damaged.

Identification

DNA Analysis

Tran et. al (2021) performed DNA barcoding but was unsuccessful in identifying larval genome and identity of those found in plankton samples.

Adult Breeding

Neither captured adult produced larvae like those collected from the plankton.

The adult worms found in the mudflats of the Charleston boat basin that were tentatively identified as *Capitella capitata* were barcoded as *Capitella teleta* (Tran et. al. 2021). Their larvae hatched from tube-brooded sacks and settled within hours of release. The other worm, *Mediomastus californiensis*, had traditional planktotrophic larvae. Once hatched from the eggs and developed into trochophores, the larvae consumed *Rhodomonas*, an easy algal food source.

Discussion

Capitellid larvae utilize carnivory as a larval development strategy

The unknown capitellid larvae are predators of planktonic larval prey. They demonstrate a characteristic feeding behavior, which includes wrapping, swimming with, and engulfing their prey, that barely deviates between individuals. This suggests that the behavior was not an anomaly, but a strategy utilized by these organisms. They deploy mucous to restrain their large, armored, and flailing prey, which could be up to twice the size of the predator capitellid. They were also seen targeting smaller prey like ciliates, although in lab culture this was not a frequent occurrence. In addition, prey selectivity could be demonstrated, as capitellids would forgo eating polychaete larvae when offered and might be able to detect and select their prey from a distance. Orientation towards a selected prey item before wrapping initiation was observed multiple times during the course of the experiment, though further experimentation and more data is needed to conclusively suggest targeted intent.

These observations are not enough to confirm a carnivorous larval development strategy, however. Proving that the larvae develop on a carnivorous diet is important, and I discovered this during my experiment. Growth 1 and 2 experiments, although not focused directly on morphological development, show a clear growth, segmentation, organ formation and behavioral shift. This suggests digestion and nutritional extraction from engulfed prey.

The Development experiment, however, is the most illuminating. While the "Fed" culture developed as expected, mirroring the changes seen in Growth 1 and 2, the "Starved" culture withered and vanished from the bowls without ever changing

morphologically from their initial collection. No tiny juveniles were seen in the bowl, suggesting that without a carnivorous diet, the larva do not develop. This means they are likely obligate (required) carnivores. They were not fed suitable algae like *Rhodomonas* during the experiment because they had no larval feeding structures and were assumed to not consume it, though algae was present in the bowl while feeding. An interesting future experiment could provide larvae with only a source of algae like *Rhodomonas* to see if they could or would consume algae if they could not access their traditional carnivorous diet.

From these experimental findings, I can reasonably infer that capitellid larvae utilize carnivory as a way to extract resources from the plankton for development and growth. Joining the other groups of invertebrates where this has already been observed (nemerteans, paleonemerteans, and polyclad flatworms) (D,D), my thesis adds to evidence of the widespread nature of macrophagous carnivory in planktonic organisms.

Partial documentation of full larval lifecycle

This thesis partially achieves another goal, which is to successfully document the larval development of this capitellid. All stages of development, from the basic larvae collected from the plankton to the non-feeding metamorphically competent larvae, were recorded for numerous specimens. Their growth was measured, demonstrating a rough doubling of size and a quadrupling of volume over the course of development. Large quantities of video footage and documentation of their behavior during these stages was also recorded, which includes feeding preference and characteristic feeding behavior.

Unfortunately, this goal could only be partially accomplished, considering what is still available to document. Primarily, no eggs were recovered, which leaves a missing stage between hatching and presence in the plankton. Additionally, only one specimen was successfully found after settlement, due to a lack of viable specimens during the summer when the settlement experiment was undertaken. Considering this specimen may have also been damaged, there is very little information on the healthy juvenile stage of these larvae. Future experiments, done during winter and spring when the larvae are plentiful in the plankton, could easily mass-settle fed cultures with sediment to observe the juvenile stage of this organism. Perhaps it would even be possible grow them to adulthood and breed them to completely document the lifecycle.

Missing larval identity

Determining larval identity is a very difficult task with these organisms. The overall group of Capitellidae, and the *Capitella* species complex can be cryptic and distinguishing between individuals via morphology is difficult (Grassle & Grassle 1976; Blake 2009). Thus, more intensive methods are required to pin down the species. Unfortunately, neither route, which included collecting possible adults and DNA barcoding of samples by Tran et. al. (2021), resulted in any firm identification.

The cryptic nature of adults may also prevent morphological distinction between larvae. During my experiment, I noted some differences among larvae, which included their final sizes, their diverse prey preferences, and the shape of their pygidium and pigment blotch. These may have been due to competition, differences in prey size, or other variables, but a possibility of encountering multiple species must be considered and may explain what was seen in the experiment.

To recognize the full potential of the findings in this thesis, DNA barcoding of the larvae must be prioritized for any future continuation of this project, due to morphological similarity between species. This is likely the only way a species-level identification might be achieved in these organisms.

Conclusion

From this project, I present enough data to conclude that the unknown capitellid larvae found in Coos Bay require a carnivorous diet to successfully develop and undergo metamorphosis into a juvenile. My conclusion allows the findings of this thesis to contribute to a growing body of research that highlights the widespread nature of macrophagous carnivory as a larval life-history strategy. However, a lack of species-level identification prevents the findings of this project from being applied to their full potential, and DNA barcoding of the cryptic larvae in future experiments is an absolute requirement for further study.

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