Raising predatory babies: development from fertilization to sexual maturity of the hoplonemertean Emplectonema viride (Nemertea)

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Abstract

Marine ribbon worms from the class Hoplonemertea were presumed to have lecithotrophic development until the recent discovery of predatory larvae in several species, including *Emplectonema viride* Stimpson, 1857 —a common NE Pacific intertidal nemertean. Here we report the complete life cycle of *E. viride* from fertilization to sexual maturity, which takes about 9.5 months. Predatory larvae of this species were successfully raised to metamorphosis on a diet of planktonic crustaceans, including barnacle nauplii and cyprids, as well as an occasional calanoid copepod. The larvae swam and grew for 3–4 months in lab culture with abundant food, before settling as juveniles and starting to feed upon adult barnacles. Settlement was accompanied by a subtle but definite metamorphosis, which includes shortening of epidermal cilia, loss of the caudal ciliary cirrus, and behavioral changes. Larvae were positively phototactic, whereas juveniles were negatively phototactic. Pelagic larval duration of several months provides abundant opportunities for dispersal, and likely results in high genetic connectivity between populations. Population genetic studies on other hoplonemertean species reveal higher-than-expected gene flow, suggesting that planktotrophic macrophagy (predatory larvae), such as we describe here for *E. viride*, may be widespread within the class.

Introduction

Many marine invertebrates have a biphasic life cycle with benthic adults and planktonic larvae (Young et al. 2002). The adult phase has low dispersal potential, while the larval phase is capable of much larger dispersal distances, and thus potentially maintains gene flow between populations (Cowen and Sponaugle 2009). While in the plankton, larvae may be passively carried horizontally by ocean currents, but they can also exhibit regular (e.g. daily) vertical migrations, which can affect their horizontal dispersal (Cowen and Sponaugle 2009). The pelagic duration of larval stage is determined both by environmental and biological characteristics and can vary even between closely related species (Levin et al. 1987; Cowen and Sponaugle 2009). Planktotrophic larvae of marine invertebrates typically have a pelagic phase of weeks to months, depending on food quantity and quality, water temperature, and availability of settlement cues. Lecithotrophic larvae rely on yolk reserves with the egg, do not need to feed in order to reach metamorphosis, and tend to have shorter pelagic duration (hours to weeks). Many studies have linked the type of development (planktotrophic vs. lecithotrophic) to genetic connectivity between adult populations of marine species (i.e. Levin et al. 1987; Bowen et al. 2006; Hellberg 2007). The longer the pelagic duration of the larval stage, the higher the potential for long-distance dispersal.

Nemerteans are mostly free-living marine predatory worms, related to mollusks, annelids and other phyla within the clade of spirally-cleaving animals (Struck and Fisse 2008; Podsiadlowski et al. 2009; Laumer et al. 2019). The phylum is characterized by having a dorsal fluid-filled cavity that houses a muscular proboscis used for both predation and defense. Both lecithotrophic and planktotrophic development are found within the phylum, and within each of the three classes: Pilidiophora, Hoplonemertea, and Palaeonemertea (Thollesson and Norenburg, 2003; Maslakova and Hiebert 2014; Andrade et al. 2014; Strand et al. 2019). The easily recognizable pilidiophoran planktotrophic larvae (pilidia) are typically

shaped like a deerstalker cap, with a prominent apical tuft at the anterior end, and lobes (front and back “visors”) and lappets (“ear flaps”) at the posterior end. Pilidia feed on unicellular algae (von Dassow et al. 2013), and the juvenile develops inside the larval body from eight distinct rudiments (imaginal discs) over the course of weeks to months in the plankton. Once the juvenile is complete, the pilidium undergoes rapid and catastrophic metamorphosis, in which the juvenile erupts from and, typically, eats the larval body (Maslakova 2010). Hoplonemerteans and palaeonemerteans lack the pilidium. Instead, species with pelagic development have the so-called planuliform larva that superficially resembles the planula larva of some cnidarians — essentially, a planktonic juvenile (Maslakova et al., 2004; Maslakova and von Döhren 2009; Hiebert et al. 2010; Maslakova and Hiebert, 2014).

While pelagic larvae of most palaeonemerteans are planktonic macrophagous predators, hoplonemertean larvae were long thought to be lecithotrophic (Iwata 1960; Hyman 1951, Jägersten 1972; Stricker and Norenburg 2002; Maslakova 2010; but see Maslakova and Hiebert 2014). Indeed, some hoplonemertean species develop directly without feeding in the plankton (e.g. Maslakova and Malakhov 1999; Maslakova and von Döhren 2009). However, the presence in the plankton of conspecific larvae in a broad range of sizes (Maslakova and Hiebert 2014), or the strong gene flow among populations many kilometers apart (Andrade et al. 2011; Leasi et al. 2016; Mendes et al. 2018), are indications of species with a long-lived planktonic larva among hoplonemerteans. Indeed, recently von Dassow et al. (2022) published the first direct observation of planktotrophy through carnivorous feeding by the larvae of six hoplonemertean species including *Paranemertes californica*, *Paranemertes* sp., *Gurjanovella littoralis*, *Emplectonema viride*, *Carcinonemertes epialti*, and *Ototyphlonemertes* sp.

Emplectonema viride Stimpson, 1857 is one of the most common free-living intertidal hoplonemerteans along the Pacific Coast of North America (Griffin 1898; Roe et al. 2007 Mendes et al. 2021). Adults are found in the upper-middle rocky intertidal among barnacle and mussels in natural and anthropogenic environments, where they feed upon acorn barnacles. These are long and slender worms with a dark green dorsal surface and cream-colored or pale yellow ventrally. The planuliform larvae of this species have a characteristic green color, and are found in plankton samples in Oregon mostly during winter months (Hiebert, 2016). Early development of *Emplectonema gracile* (Johnston, 1837), the sister species of *E. viride*, was described by Iwata (1960) through formation of proboscis and stylet. However, later development remained unknown because the hatchlings died without food after about two weeks.

We recently reported that planktonic larvae of *Emplectonema gracile* feed upon barnacle nauplii and cyprids (von Dassow et al. 2022). That study, however, only described feeding occurrences in wild-caught planktonic larvae mostly of advanced stages. Here we report the complete life cycle of these ubiquitous marine predators. We raised larvae of *E. viride* in the laboratory from egg to sexual maturity, documenting feeding behavior, developmental timeline, pelagic duration, and metamorphosis.

Material And Methods

Collecting adults

Clusters of acorn barnacles (mostly *Balanus glandula*) containing entangled adults of *Emplectonema viride* were collected from middle-low intertidal zone near the Oregon Institute of Marine Biology in Charleston, Oregon during between Fall 2019 and Spring 2020 under ODFW (Oregon Department of Fishing and Wildlife) collecting permits #22780 and 23609 (Table 1). The clusters were taken to the laboratory and placed into a sea table inside glass containers filled with filtered seawater until worms crawled out. Worms were kept in 150ml glass dishes in a sea table with running seawater at ambient sea temperature (12–15°C). The worms were observed under a stereomicroscope to assess the presence of gonads.

Table 1
Sampling locations and time of collection of reproductive *Emplectonema viride* along the Oregon Coast

Sampling location	GPS coordinates	Sampling dates
Charleston Marina	43° 20.63'N 124° 19.38' W	November 2019; February, May 2020
OIMB Boathouse dock	43° 20.96'N 124° 19.80'W	October 2019; May 2020
Bastendorff Beach	43° 21.10'N 124° 20.65'W	November 2019; February 2020

Obtaining embryonic cultures

About 20 mature animals were kept in the laboratory in 150ml glass bowls with frequent water changes (2–3 times a week). Since there is no reliable cue to induce spawning in hoplonemerteans, the bowls were checked for released eggs once a day. Once a spawning female was spotted, the eggs were collected with a glass pipette, washed twice in filtered seawater, and placed in a clean bowl with filtered seawater. Eggs were fertilized *in vitro* by dissecting ripe males, and adding a dilute suspension of sperm to eggs. The fertilized eggs in small glass dishes were placed on a thermoelectric cold plate and kept at 12ºC for the next 24 hours. During this period, at every hour, some embryos were mounted on a glass slide under a cover slip and photographed using a Spot 5.2 camera mounted on an Olympus BX51 microscope, equipped with DIC optics. After 24 hours the developing embryos were transferred to a 150ml glass bowl with filtered sea water, kept in a sea table at ambient sea temperature (12–15°C) and observed every 1–2 days to document development.

Culturing predatory larvae

Once the larvae had developed proboscis armature (stylets), putative prey items were added to the cultures and the behavior of about 400 larvae (94 in our first attempt, and about 300 in subsequent cultures) was observed under a stereomicroscope. First, candidate prey items — calanoid and cyclopoid copepods (adults and nauplii), barnacle nauplii, and decapod zoea larvae — were collected from the docks in the Charleston Marina (Charleston, OR) using a 150 um mesh plankton net — to test feeding preferences of *E. viride* larvae and observe feeding mechanism. Once acceptable prey was found, the cultures were fed with freshly hatched barnacle nauplii from *B. glandula* adults. The amount of prey added was adjusted to the rate of consumption by *E. viride* larvae to maintain food availability (about 10

nauplii per *E. viride* larva, per feeding event, once every two days). As soon as the larvae reached metamorphosis, newly settled balanid barnacles were also offered as food. The barnacles were obtained live in groups on small rock chips chiseled from nearby rocky shores. Feeding events were recorded using a Point Grey Grasshopper 3 camera operated by StreamPix 7, mounted on an Olympus BX51, equipped with DIC microscope for smaller younger larvae or on a Leica Z6 Apo macroscope for bigger older larvae and juveniles.

Two to three larvae were observed and photographed using a Spot 5.2 camera mounted on an Olympus BX51, equipped with DIC optics once a day for the first 10 days of development, and then twice a week until the first larva of the culture reached juvenile stage. After metamorphosis, the cultures were checked once a week.

Confocal microscopy

To document development 12 larvae per batch were relaxed in 1:1 mixture of 0.34 M $MgCl_2$ and filtered sea water for 10 minutes and preserved in 4% paraformaldehyde for 24 hours at the following stages: 2 days post fertilization (soon after hatching from the egg chorion), 4, 6, 8, 30 and 60 days, and upon metamorphosis). Larvae were permeabilized by rinsing 3 X 10 min in PBS with 0.1% Triton X-100 (PBT) and stained for 40–60 min in BodipyFL Phallacidin at 1U/100µl of PBT at room temperature. Stained larvae were mounted on glass slides coated with 1% poly-L-lysine either in 1X PBS or in 90% glycerol, covered with a glass cover slip, and sealed with nail polish. To better visualize internal structures three larvae of each stage were mounted on poly-L-lysine coated slides, quickly dehydrated in isopropanol series (1 min 70%, 1 min 85%, 1 min 95%, 1 min 100%, 1 min 100%), cleared in three 10-min changes of Murray Clear, mounted in Murray Clear on glass slides, and sealed with nail polish. Stained and mounted larvae were observed using an Olympus Fluoview 1000 confocal system mounted on an Olympus IX81 inverted microscope and imaged with 20X 0.85 NA, 40X 1.3 NA, or 60X 1.4 NA oil-immersion lenses. Each larva was scanned at 0.5 µm increments, and the confocal stacks were further processed using ImageJ (Wayne Rasband, National Institutes of Health, Bethesda, MD).

Results

Larval development

Ripe females of *Emplectonema viride* appear pinkish to brownish ventrally due to color of the oocytes inside ovaries. Females often spawned immediately after a water change, but sometimes they spawned with no apparent cue. Spawned oocytes are round, opaque, and pinkish. Oocytes in one spawning event measured between 110 and 140 µm in diameter (n = 9), and were surrounded by a chorion (131–160 µm) and a jelly coat (204–351 µm). Fertilized eggs completed meiosis and then underwent equal spiral cleavage (Fig. 1A–G). At 12–14 ºC, the first polar body appeared 25 minutes post fertilization (PF); first cleavage was observed at 1 hour 30 minutes PF, second cleavage at 2 hours 20 minutes PF, third cleavage at 3 hours 20 minutes PF and fourth cleavage after 4 hours PF. Olive-shaped uniformly ciliated

larvae equipped with a thin apical tuft hatched after 36–38 hours PF (Fig. 1H). Two-day-old larvae swam actively, had a prominent apical tuft, and a thin caudal ciliary cirrus (Fig. 2A). The rudiments of the proboscis, cerebral ganglia, cerebral commissures, and lateral nerve cords could be identified by confocal microscopy in two-day-old larvae (Fig. 3A). By the fourth day, when larvae were first observed feeding, they had functional musculature, midgut and foregut (Fig. 3B), as well as the first two ocelli, and the central stylet (Figs. 2B; 4A). When relaxed, the larvae at this stage measured about 200 μm long and 110 μm wide.

After six days of development, the larvae assumed a more elongated shape (Fig. 2C). By the eighth day confocal microscopy revealed the accumulation of lipid droplets in the midgut. We did not detect the invaginations that correspond to the cerebral organs, but the cerebral organ openings were apparent in 12-day old larvae (Fig. 2D). The second pair of ocelli appeared around day 15 and the third — around day 18 (Fig. 2E–F). Around day 15 the larvae also acquired a greenish color in the epidermis (Fig. 2F–G). Between day 15 and day 30, no overt morphological changes were observed in the larvae, except for increase in size. By 30-days of age larvae had a larger gut, very dark epidermis, and an elongated shape (Fig. 5A) similar to the wild-caught larvae found in plankton tows (Mendes et al. 2021 – Fig. 5). The number of ocelli, however, did not change. At 45 days the larvae had four pairs of ocelli, but sometimes the ocelli were not paired, and the shape of the central stylet and basis resembled that found in juveniles and adults (Fig. 4). The fifth pair of ocelli appeared around the 76th day of development (Fig. 5B), when the midgut began to develop lateral diverticulae (Fig. 5F). At 76 days after fertilization, the larvae presented seven pairs of ocelli (Fig. 5C) and many midgut diverticulae (Fig. 5G).

We observed metamorphosis as early as 109 days of development, and 120 days on average. We considered a worm to be a juvenile when it no longer exhibited positive phototaxis and crawled, rather than swam most of the time. The juvenile had a very pale epidermis, but still seven pairs of ocelli (Fig. 5D–E). The animals at this stage possessed many diverticulae along the length of the midgut (Fig. 5H). Subtle morphological changes accompany the behavioral changes that we refer to as settlement: juveniles loose the caudal ciliary cirrus, which is present in all larval stages, and the body ciliation shortens dramatically (compare Figs. 5I and 5J).

Early development from hatchling to feeding larva was accompanied by dramatic changes in the epidermis, similar to that described in the lecithotrphic larva of *Paranemertes peregrina* and several other hoplonemerteans (Maslakova and von Döhren 2009, Hiebert et al. 2010 and references therein). The epidermis in the 2-day old *E. viride* larva was composed of a few dozens of large ciliated cells with groups of considerably smaller cells in the interstices between the large ones (Fig. 6A). The large cells were progressively replaced with small cells over the next few days of development (Fig. 6A–D). In the 4-day old larva the large cells remain but the epidermis is dominated by increasing numbers of small cells (Fig. 6B); the large cells almost disappeared in the 8-day old larva (Fig. 6D). In the 30-day old larvae, the epidermis is composed mainly of glandular and ciliated cells (Fig. 6E–F). The epidermis of the juvenile, however, is smoother, consisting of very small cells (Fig. 6G).

Feeding behavior and food preference

The larvae started to feed as soon as they developed a stylet, at 4-days old, and kept on feeding until metamorphosis. They showed a near-absolute preference for barnacle nauplii and cyprids; we observed only one feeding event upon a calanoid copepod. This copepod was not identified because there was no tissue left over after the feeding event. The nauplii and cyprids, however, were identified by DNA-barcoding as belonging to *Balanus glandula* and *Balanus crenatus* (Mendes et al. 2021), the two most common species of acorn barnacles in the region.

Emplectonema viride larvae reacted almost instantly to the addition of prey. They began to swim more actively and changed their body shape to something resembling a tadpole (Fig. 7A; supplemental video 1). However, they were not observed to attack barnacle larvae while this shape. Most observed encounters occurred when the nemertean larvae swam slowly near the bottom of the dish and attacked barnacle larvae already trapped by mucus previously laid by the *E. viride* larvae. In some cases, nemerteans seemed to sense prey caught by a line of trailing mucus, curving the head towards the tail, coiling around the prey, and then attacking using the stylet (Supplemental video 1).

The entire predation event took between 5 to 15 minutes, sometimes longer for smaller *E. viride* or when especially large cyprids were consumed. As stated above, the attacks on nauplii usually initiated after the nauplii were already caught by the mucus produced by *E. viride* larvae. Usually, in a successful predation event, *E. viride* would first attack the anterior region of a nauplius, near the point of attachment of antennulae to the carapace, using the proboscis, and repeatedly stabbing the prey (Fig. 7B; supplemental video 2). Following the attack, the prey gradually ceased moving, while the predator was still coiled around it or swam around the prey. Once prey movements reduced to mere spasms, *E. viride* larva inserted the everted foregut inside the carapace and sucked in semi-liquefied prey tissues, including the naupliar eye, typically leaving behind only the gut (Fig. 7C, supplemental video 3). If the *E. viride* larva was disturbed during this process, it would abandon the prey. Therefore, most recorded encounters happened when both *E. viride* larva and the nauplius were already between a slide and the cover slip. Perhaps, as a consequence, the recorded feeding events were less successful and took longer. In some instances, a single prey item was attacked by several, usually small, nemertean larvae.

All the observed feeding events upon cyprids (n = 12 events) had exactly the same sequence. First, the cyprid was caught in the mucus already present in the culture, which restrained some of its movements, and slowed it somewhat. After this, the *E. viride* larva coiled around the cyprid and stabbed it in the posterior region between the valves, inserting the proboscis and stylet many times (Fig. 7D). After being attacked, the cyprid moved antennules and sometimes thoracic appendages, but did not jump away or make any other obvious attempts to escape. The *E. viride* larva then prowled around the cyprid and, after about 1–2 minutes, inserted the foregut at the same place as before and began to suck in the tissues, including the eye and the oil droplets (Fig. 7E; supplemental video 1 [at 2x normal speed]). The ingestion took about 5–8 minutes.

Juvenile development and first reproductive adults

Two embryonic cultures were successfully started in October 2019, first containing 23 and second — 71 larvae. From these, 31 individuals reached metamorphosis, while 56 were fixed for confocal microscopy over the course of development. Juveniles from these cultures were offered recently-settled (hence small) barnacles as prey. At first no feeding was observed, instead the nemertean juveniles hid under rock chips bearing barnacles. Some empty barnacle shells were spotted after one or two days, but no feeding was witnessed until the cultures were observed under dim light. The observed feeding events took place after several minutes to 2 hours of monitoring. The worms crawled around the barnacles and attacked only barnacles that were alive but not moving vigorously. The worms initiated the attacks by inserting the proboscis between the operculum and the marginal plates or through the apertures, and apparently pierced the tissues with the stylet many times. Some seconds after the injection, the barnacle stopped moving and the worm inserted the foregut through the operculum and began evacuating barnacle tissues (Fig. 7F), leaving behind the empty capitulum after five to ten minutes. During feeding, it was possible to see liquefied barnacle tissues entering the worm's gut.

From the 31 worms that reached juvenile state, 26 kept on growing on a diet of juvenile barnacles, and five died attempting to escape the bowls. These 26 juveniles grew at different rates (varying around 2.5x in the same larval culture), the epidermis becoming darker as they grew. About eight months after hatching, these young adults started to show developing gonads, and after about 45 more days we observed spawning. Spawned eggs had the same characteristics as the ones spawned by their wild counterparts: round, opaque, and pinkish, and with similar size range.

Discussion

This is the first time a hoplonemertean with a predatory planktotrophic larva has been raised in laboratory conditions through its complete life cycle from fertilized egg to reproductive adult. The larval development of *Emplectonema viride* is similar to that described in other hoplonemerteans. One difference is that we did not observe the cerebral organ invaginations, such as those described in development of *Paranemertes peregrina* (Maslakova and von Doehren, 2009). It is possible that we missed the invagination stage since we did not preserve *E. viride* larvae younger than 2 days old (hatching) for confocal microscopy. Similar to other hoplonemerteans with described development, *E. viride* larvae progressively replace a transitory larval epidermis composed of large multiciliated cells by the much smaller cells of the definitive epidermis (Hiebert et al. 2010 and references therein). *Emplectonema viride* embryos develop proboscis and its armature within four days after fertilization, much faster than what is described for lecithotrophic species, which take up to three weeks to develop stylets (Stricker 1985; Chernyshev 2008; Maslakova and von Dohren 2009). However, the absence of a stylet is not always an evidence of lecithotrophic development, as seen in *Carcinonemertes errans* larvae (von Dassow et al. 2022).

It is noteworthy that the larvae of *E. viride* feed on the larvae of the same animals – barnacles – that *E. viride* preys upon as adult. It could be argued that this is a mere consequence of abundance and susceptibility, as a) barnacle nauplii are dominant members of the local plankton and don't swim as fast

as other abundant crustaceans such as copepods, and b) barnacle adults are among the most abundant, yet the least mobile, of benthic crustaceans. Alternatively, it could reflect a true selectivity. Although we offered a variety of prey items, the amount of each type of prey item in this microcosm (feeding bowl) reflected their relative abundance in the plankton. Some hoplonemerteans species are known to have specific feeding preferences, however when their preferred item is not available they can prey on alternative organisms (Roe 1993; Thiel and Kruse 2001).

The early development of *E. gracile* is described by Iwata (1960) and is very similar to what we observed for *E. viride* in this study. Although *E. gracile* embryos hatched earlier and no feeding was observed, the larvae had a developed proboscis and stylet within eight days, and died about 17 days after hatching. This is consistent with the need to feed, a possibility also raised by Iwata (1960), and by Chernyshev (2008) for another hoplonemertean, *Tetrastemma stimpsoni*.

Consequences of a long-lived feeding larva for hoplonemertean dispersal

Few hoplonemertean species have been observed through metamorphosis (Chernyshev 2008). Larvae of several species cultured in the lab by others died within a few days or weeks after hatching (e.g. Iwata 1960; Stricker and Reed 1981; Hiebert et al. 2010), suggesting that they require food to develop to metamorphosis. Several population genetics studies of hoplonemertean species (Andrade et al. 2011; Tulchinsky et al. 2012; Mendes et al. 2018) as well as observations of conspecific hoplonemertean larvae of varying sizes in the plankton (Maslakova and Hiebert, 2014) also suggest planktotrophy. The present study, as well as our recent work on several other hoplonemertean species (von Dassow et al. 2022) affirms that many hoplonemertean larvae are indeed planktonic predators.

Oregon populations of *E. viride* harbor low genetic diversity, with only two haplotypes present in this area (Mendes et al. 2021). The adult worms live among encrusting communities and have limited potential for long-distance dispersal. Our study demonstrates that this species has a months-long pelagic larval period (up to 120 days) which greatly increases potential for dispersal and gene flow within and between populations. The importance of larval dispersal for population connectivity in benthic species is recognized in many population genetic studies, and larval planktonic duration can be correlated to the amount of gene flow between populations (Palumbi 1994; Bohonak 1999; Hellberg et al. 2002; Hellberg 2007; Kelly and Palumbi 2010; Selkoe and Toonen 2011). It seems likely that the Pacific populations of *E. viride* are well connected, as can be inferred from the seemingly low haplotype diversity of the Oregon population. The sister species, *E. gracile*, also shows well connected haplotypes throughout the Northeast Atlantic Ocean until the North Sea (Mendes et al. 2021), which suggests a long-lived planktotrophic larva in this species as well.

A long planktotrophic larval phase seems to be more widespread among hoplonemertean species than previously thought, indicating that the intersection of life history evolution and biodiversity among nemerteans deserves a closer look.

Declarations

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Conflict of Interest

The authors declare no conflict of conflict of interest.

Ethics approval

All applicable international, national and/or institutional guidelines for sampling, care and experimental use of organisms for the study have been followed and all necessary approvals have been obtained. All material was collected under the permits #22780 and 23609 issued by the Oregon Department of Fishing and Wildlife.

Data availability

Video sequences of swimming and feeding behavior are available in the supplementary material.

Author's Contribution

Cecili Mendes – raised the larvae, documented their development and behavior, composed the figures and wrote the first draft

George von Dassow – Documented larval behavior, composed figures, wrote and reviewed the manuscript

Sónia Andrade – wrote and reviewed the manuscript

Svetlana Maslakova – supervised the lab work, wrote and reviewed the manuscript.

All authors conceived the project, contributed to the manuscript writing and approved the submitted version.

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Figures

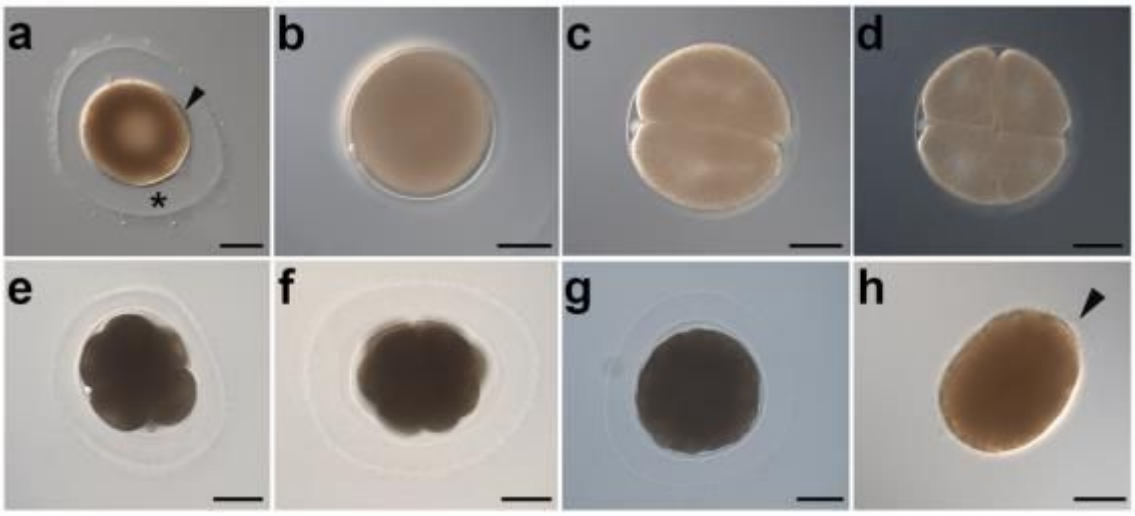

Figure 1

Embryos and a freshly hatched larva of *Emplectonema viride*. A: unfertilized egg surrounded by a tight chorion (arrow) and a jelly coat (asterisk) ; B: polar body formation; C: 2-cell stage; D: 4-cell stage; E: 8-cell stage; F: 16-cell stage; G: 32-cell stage; H: 36-hours post fertilization hatchling. Arrowhead indicates apical tuft. Scale bars: 50µm.

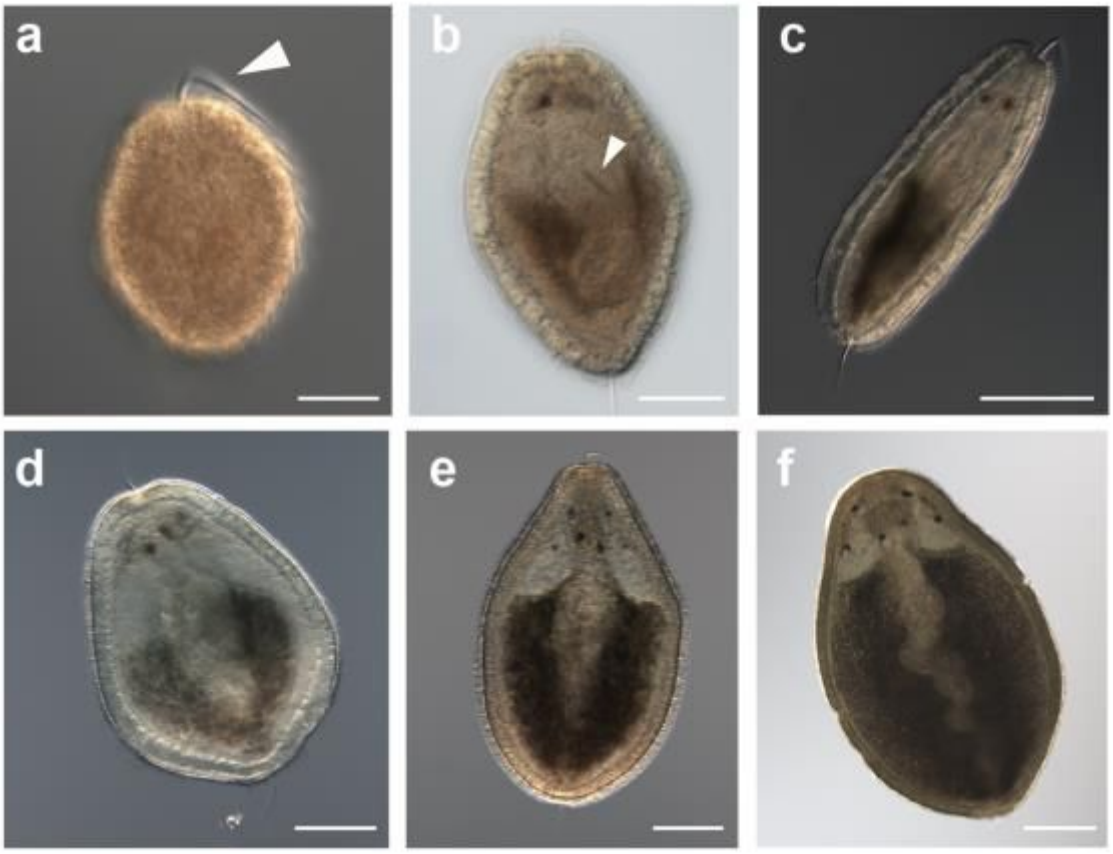

Figure 2

Initial development of *Emplectonema viride* larva. A: 2-day post fertilization; arrowhead indicates the apical tuft; B: 4-day post fertilization, arrow indicates the stylet; C: 6-day post fertilization; D: 15-day post fertilization; E: 18-day post fertilization, with six ocelli; F: 31-day post fertilization, note the change in color, from pale to dark green. Scales bar: 50 μm (A, B); 100 μm (C–F); 200 μm (G).

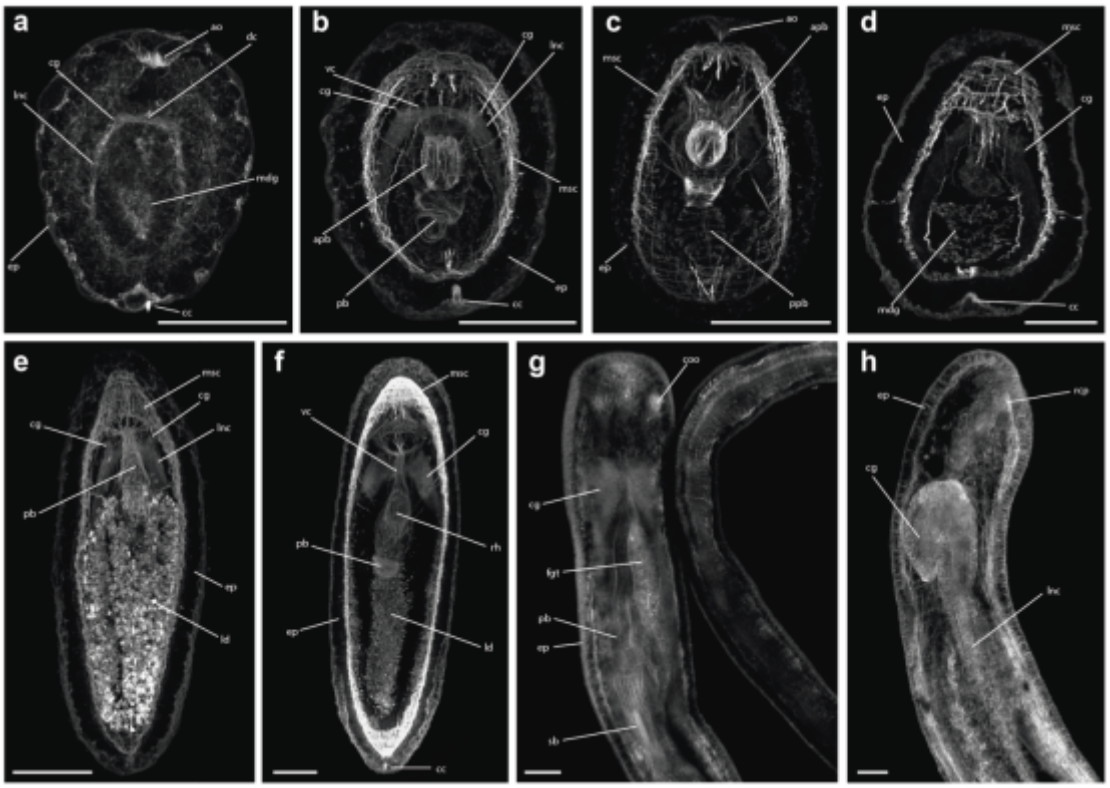

Figure 3

Confocal Z projections of substacks chosen to illustrate major morphological structures in phallacidin-labeled larvae and juveniles of *Emplectonema viride*. A-G frontal sections, H - sagittal sections. A: 2-day old; B-C: 4-day old; D: 6-day old; E: 8-day old; F: 30-day old; G-H: juvenile. apb: anterior proboscis; ao: apical organ; cc: caudal cirrus; co: cerebral organ; coo: cerebral organ opening; d: dorsal commissure of the brain; ep: epidermis; fgt: foregut; lnc: lateral nerve cord; mdg: midgut; msc: musculature; old: lipid droplets in the midgut; pb: proboscis; ppb: posterior proboscis; rcp: rhynchostomopore; rh: rhynchocoel; sb: stylet basis; vc: ventral commissure. Scale bars: 50 µm.

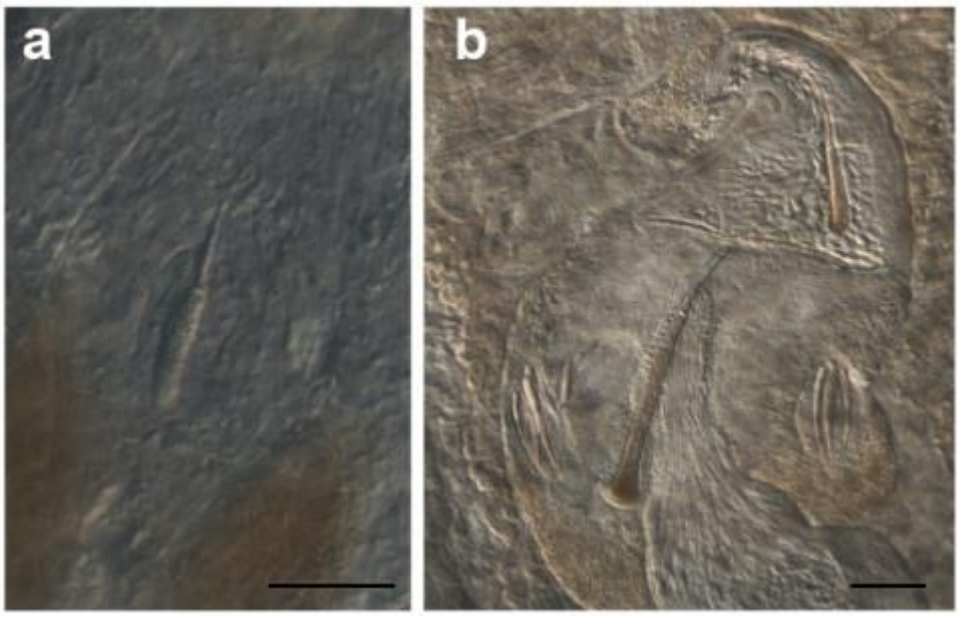

Figure 4

Development of proboscis armature in *Emplectonema viride*. A: 4-day old larva; B: juvenile. Scale bars: 20 µm (A–B); 50 µm (C–D).

Figure 5

Advanced larvae and juveniles of *Emplectonema viride*. A: 43-day old larva; B: anterior region of 76-day old larva, showing proboscis and stylets; C: anterior region of 100-day larva; D: anterior region of 109-day old juvenile; E: general view of 109-day post fertilization juvenile; F: posterior region of 76-day old larva; G: posterior region of a 126-day old larva; H: juvenile posterior region. Note the lack of caudal cirrus and shorter cilia in the juvenile. Scale bars: 50 µm (I); 100 µm (B, D, F, J, C, G,H); 200 µm (A); 500 µm (E).

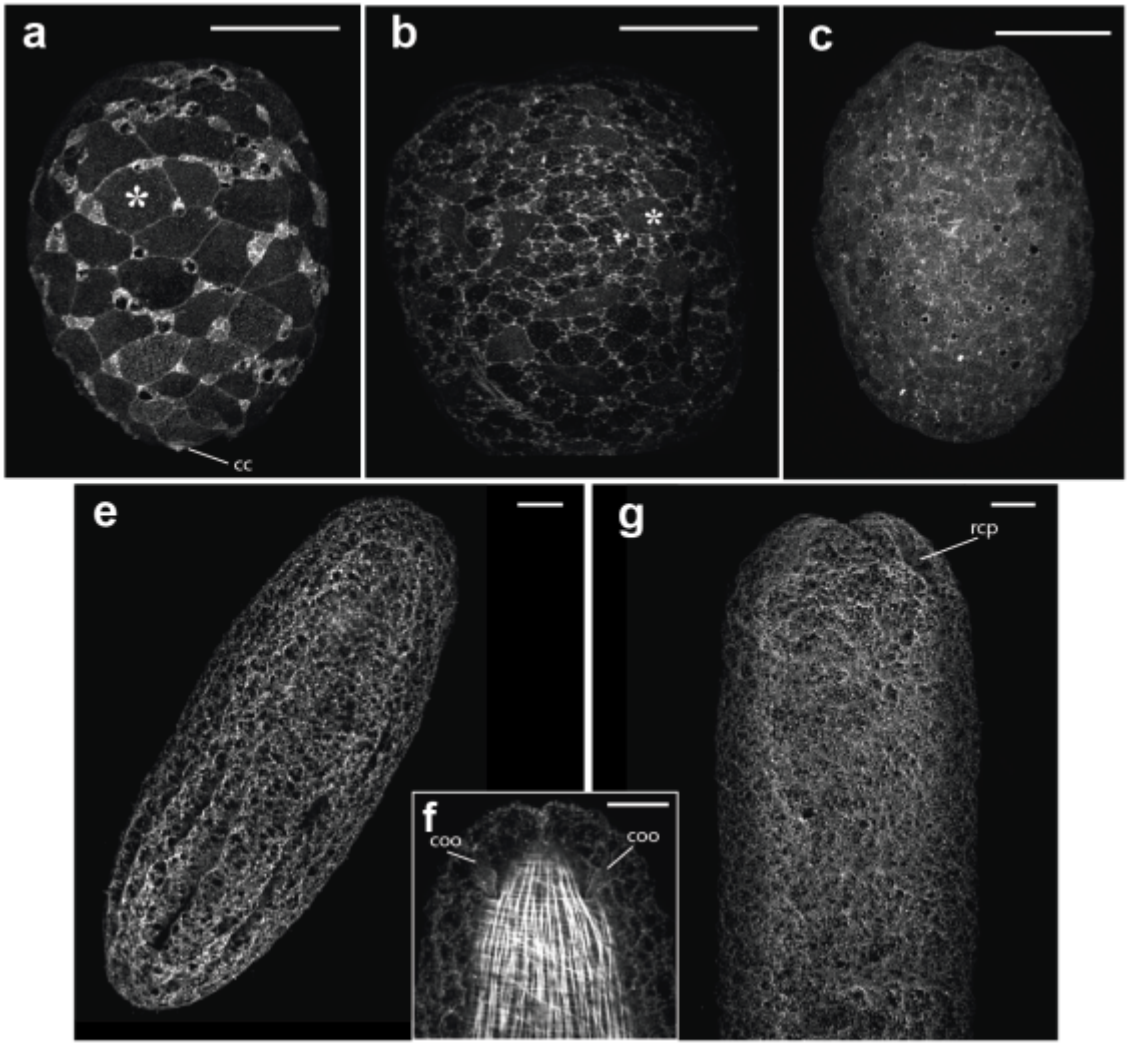

Figure 6

Confocal Z-projections of *Emplectonema viride* larvae illustrating gradual replacement of the transitory larval epidermis (large cells) by the smaller cells of the definitive epidermis in the course of planktonic development. A: 2-day old larva; B: 4-day old larva; C: 6-day old larva; D: 8-day old larva; E: 30-day old larva; F: 30-day old larva, showing cerebral organ openings; G: 60-day old larva; H: juvenile. cc: caudal cirrus; coo: cerebral organs opening; rcp: rhynchostomopore. Scale bars: 50 µm.

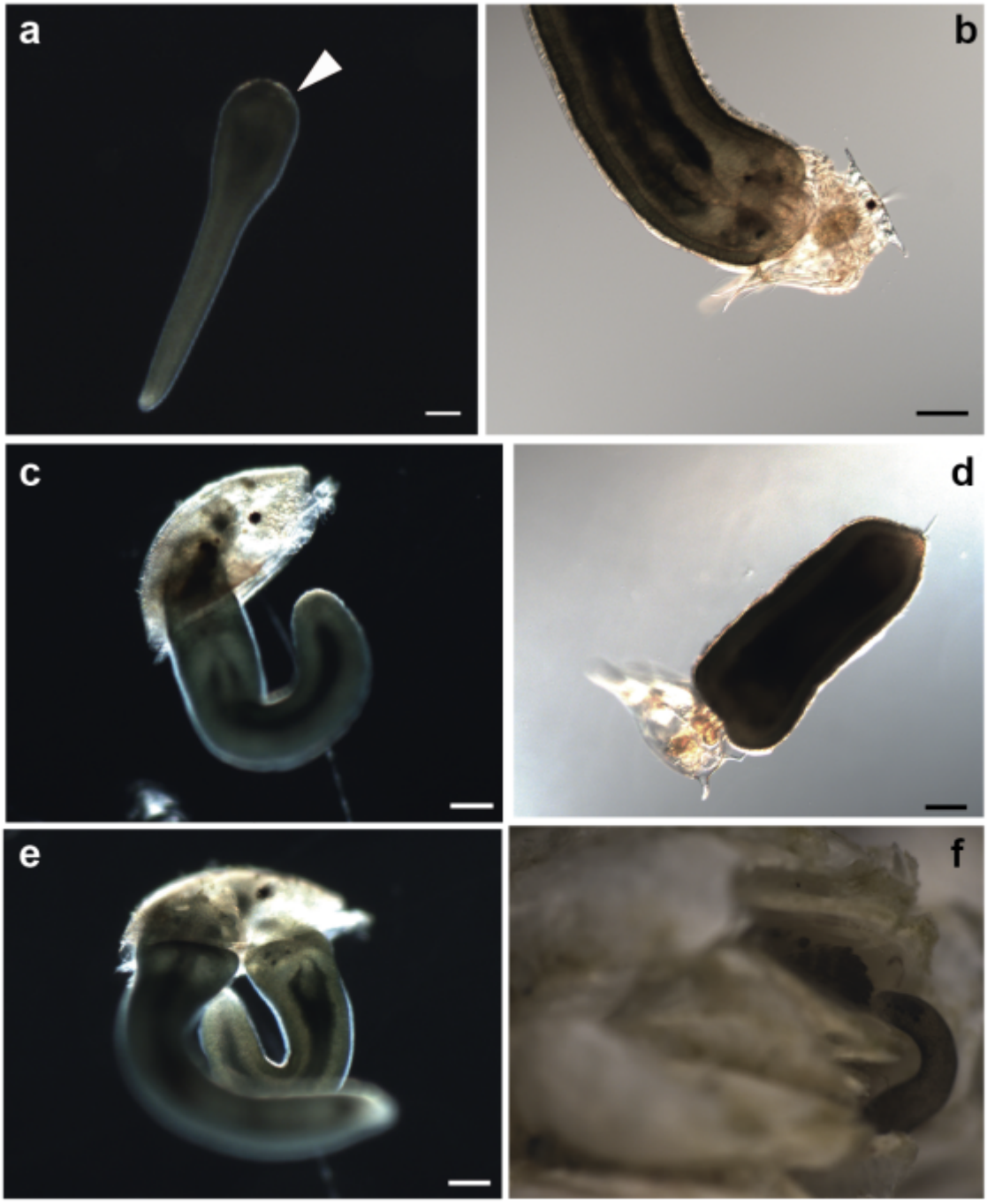

Figure 7

Feeding behavior of *Emplectonema viride* larvae (A-E) and juvenile (F). A: typical shape of a swimming 45-day old larva; B: 45-day old larva attacking a barnacle nauplius; C: larva feeding upon a barnacle nauplius; D: larva attacking a barnacle cyprid; E: larva feeding upon a barnacle cyprid; F: juvenile feeding upon a newly settled *Balanus glandula* in 3.5 zoom. Scale bars: 50 µm (A); 100 µm (B–E).

Supplementary Files

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- EmplectonemaEatsCypridLoMagsm.mp4
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