REPRODUCTIVE AND LARVAL BIOLOGY OF THE NORTHEASTERN PACIFIC POLYCHAETE *OWENIA COLLARIS* (FAMILY OWENIIDAE) IN COOS BAY, OR

by

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A DISSERTATION

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An Abstract of the Dissertation of

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Title: REPRODUCTIVE AND LARVAL BIOLOGY OF THE NORTHEASTERN PACIFIC POLYCHAETE *OWENIA COLLARIS* (OWENIIDAE) IN COOS BAY, OR

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The polychaete worm *Owenia collaris* (Family Oweniidae) is found in soft sediment habitats along the northeastern Pacific coast, particularly within bays and estuaries. Seasonally, these small tubeworms spawn gametes freely into the water column where they develop into planktotrophic mitraria larvae. After three to four weeks at ambient temperatures, they undergo a dramatic metamorphosis and return to the bottom. The reproductive and larval biology of a population of *O. collaris* in Coos Bay, OR was investigated over several years. The development of this polychaete has several unusual features, including a stomodeum not derived from the blastopore and continued proliferation of trochoblast descendents, producing simple cilia on monociliated cells.

The description of larval and juvenile structures provided morphological characteristics useful for distinguishing this species from the congener *O. fusiformis*. The consequences of the unusual larval morphology of *O. collaris* (i.e. simple cilia, convoluted ciliated band) were investigated by comparing the feeding performance and growth of this species with those of invertebrate larvae representative of the more typical tornaria-type larval forms found in deuterostomes and trochophore-type larval forms found in the lophotrochozoa. Feeding and growth patterns were similar in the convergent mitraria and deuterostome larval forms. In an experiment designed to test the relationship between abiotic factors and the seasonal reproduction, the onset of breeding was cued by photoperiod, but seasonal trends in temperature, alkalinity, food availability for larvae, and salinity may drive reproductive patterns as well. Within the Coos Bay estuary, the intertidal distribution of *O. collaris* is related to adult salinity tolerances. Low salinity limits horizontal distribution and also reduces potential for reproduction. Most life-history stages are tolerant of a wide range of temperatures, both higher and lower than those typically seen in Coos Bay. There is little evidence to support the hypothesis that sediment characteristics limit distribution within the bay, although settling juveniles show some preference for small grain sizes and may not be able to recruit to mudflats that completely lack these size fractions.

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CHAPTER I

GENERAL INTRODUCTION

Polychaete worms are a morphologically and ecologically diverse group. Polychaetes display a variety of life history strategies, and the factors that control reproduction, larval biology, and adult distributions are as varied as these strategies. Reproduction ranges from direct development, to complex life cycles with planktonic larvae and benthic adults, to even more complex life cycles involving benthic adults that metamorphose into pelagic adults that disperse prior to spawning (epitoky) and produce either lecithotrophic or pelagic larvae. Adults can be free-living or epibiotic, parasitic or mutualistic, wandering, swimming, sedentary, or sessile. Within species that produce larvae, larvae can be short-lived, recruiting into the adult habitat after only a few hours or days, or long-lived, capable of broad dispersals. For larvae with relatively longer planktonic periods, they may be modified in morphology, behavior, or metabolism to assist in dispersal. In sedentary species, populations are connected primarily by larval dispersal and distributions and ranges often reflect larval ecology and reproductive strategies. Despite the overwhelming diversity within this group, some generalizations can be made about their development, reproduction and ecology.

Development of Polychaetes

Embryogenesis in polychaetes occurs via total, often unequal, spiral cleavage and organogenesis via determinate cleavage and protostomy (Okada, 1970). Gastrulation commonly occurs via epiboly (animal cells overgrow yolky vegetal cells), ingression

(vegetal cells divide inward, detaching and continuing to divide filling the blastocoel), or invagination (vegetal cells grow inward as a sac) (Shankland and Savage, 1997). Beyond embryogenesis, direct and indirect development are both common. Within those species that undergo indirect development, the resulting larvae are referred to as trochophores followed by a setiger larva (Nielson, 2001). The hypothetical ancestral trochophore larva is characterized by the presence of an apical tuft, a pre-oral prototroch derived from trochoblasts, an adoral ciliary zone, and post-oral meta-, gastro- and telotrochs, all of which are formed from multiciliate cells (Nielson, 2004). The setiger larva develops by addition of segments through teloblastic growth anterior to the anus (Schroeder and Hermans, 1975).

In a few cases, the ancestral trochophore has been lost or modified and the resulting larval forms lack some aspect of the trochophore's defining features. For example, larvae of *Marphysa* sp. have no distinct ciliary bands; instead the whole surface of the larva is ciliated (Aiyar, 1931). The endolarva of *Polygordius appendiculatus* and the mitraria larva of *Owenia fusiformis* develop the segmented body within the larval hyposphere (Wolterek, 1902; Okada, 1970; Wilson, 1932). Modifications in larval forms suggest modifications to cells fates, cleavage patterns, or organogenesis during embryogenesis.

Larval Performance

High feeding rates may result in shortened planktonic periods and increased size at metamorphosis, which may ultimately reduce mortality of larvae and juveniles of species with planktotrophic larvae (Havenhand, 1995; Hart and Strathmann, 1995;

Sogard, 1997). Larvae that feed using ciliated bands (e.g., the polychaete prototroch) can maximize the rate at which they clear particles from water by increasing the length or velocity of their cilia, the length of the ciliated band, or the number of cilia creating a current (Strathmann *et al*., 1972; Strathmann and Leise, 1979; Hart, 1996; Miner *et al*., 1999). These are effective ways of increasing the volume of water that passes within the capture zone of the feeding apparatus.

The changes to the cilia or ciliated band that can be made are constrained by the type of cilia present, which is generally constrained by phylogeny. These physical restrictions produce two strategies for increased feeding rates. Trochozoan larvae (found in the phyla Annelida, Mollusca, Nemertea, *etc.*) possess multiciliated cells and compound cilia (Nielson, 1985). Polychaete larvae with multiciliated cells can increase the length of cilia and the velocity of the effective stroke by fusing cilia together into compound cilia, which provide stiffness during the effective stroke that propels water and food (Sleigh, 1962). These larvae generally retain the circular band of the trochophore throughout development. Several polychaete larvae develop ciliary bands that increase in length over the course of larval development (i.e. mitraria larvae of the Family Oweniidae, rostraria larvae of the Family Amphinomidae). The growth patterns in these bands are similar to those of tornaria larvae of deuterostomes, despite being phylogenetically dissimilar. These growth patterns may confer a performance advantage for the modified polychaete larvae, allowing them to capture food at much higher rates than polychaetes that retain the ancestral trochophore and setiger larvae.

Seasonal Reproduction in Polychaetes

Seasonal reproduction (i.e., spawning or gametogenesis restricted to certain parts of the year) is common in temperate polychaetes. Nutrient availability, photoperiod, salinity and temperature are common environmental controllers of gametogenesis in polychaetes (Orton, 1920; Schroeder and Hermans 1975; Tenore, 1977; Olive, 1980; Garwood and Olive, 1982; Clark, 1988). Within habitats such as estuaries, a host of environmental variables vary seasonally and are likely to affect phenology and success of reproduction in invertebrates. Generally, seasonal species have developed reproductive systems in which either gametogenesis or spawning is strongly synchronized within a population or individuals are able to store and maintain gametes until the appropriate time. Synchrony provides the best chances for successful reproduction, ensuring availability of other gametes sufficient for fertilization, adequate environment for embryonic and larval development and survival, or excess energy for adults to apply to the production of gametes or brood care. Seasonal reproduction appears to have adaptive advantages in temperate waters, but the cues that entrain this seasonality vary substantially among taxa (Giese and Kanatani, 1987; Olive et al., 2000).

Control of Distribution Within Estuaries

Polychaetes are common members of estuarine ecosystems. For polychaete worms, community composition and reproductive ability can be highly dependent on salinity and temperature gradients (Pardal et al., 1993). Within temperate estuaries, salinity and temperature tend to covary along the estuarine gradient from river to mouth and interactions between these two physical factors may strongly influence species

distributions through their impacts on benthic and pelagic stages. Salinity alone, however, has been investigated in more detail than temperature in estuarine polychaetes. Low salinities cause mortality, lower fecundity, and prevent reproduction to varying degrees (Gasiunas, 1956; Bogucki, 1963; Kube and Powilleit, 1997; Daunys et al., 2000; Pechenik et al., 2000). Salinity tolerances in polychaete larvae often reflect adult distribution, with the most tolerant larvae having the broadest adult estuarine distribution (Lyster, 1965; Kube and Powilleit, 1997; Qui and Qian, 1997).

Polychaete species richness and diversity (Gambi and Giangrade, 1986), density (Scaps et al., 1998; Gutierrez et al., 2000), and community composition (Bilyard and Carey, 1979; Pardal et al., 1993; Scaps et al., 1998; Bromberg et al., 2000; Elias et al., 2001; Maggiore and Keppel, 2007) are related closely to sediment characteristics in intertidal and subtidal soft sediment habitats. These observed patterns may be related to preferences or tolerances of adults or the result of differential recruitment based on sediment type (Wilson, 1952; Hardredge et al, 1998; Duchene, 2004). However, polychaete larvae may not always differentially respond to sediment type, as exemplified by the lack of selectivity in some estuarine species (Grassle et al., 1992; Rohri, 1997).

Scope and Objectives

My primary objective in developing this dissertation project was to examine the connections between the biology of the polychaete *Owenia collaris* and its environment. *Owenia collaris* (Family Oweniidae) is found in soft sediment habitats along the northeastern Pacific coast, particularly within bays and estuaries (Blake, 2000; Smart, personal observation). This species is dieocious and ovoviviparous (Blake, 2000; Smart,

personal observation). Embryos develop in the water column into planktotrophic larvae (the "mitraria"), which later undergo a dramatic metamorphosis to the juvenile stage. The link between biology and the environment was examined through the use of manipulative laboratory and field experiments. This required developing techniques for culturing and maintaining embryos, larvae, juveniles, and adults in the laboratory and provided the opportunity to observe other aspects of the biology of this species in detail.

Chapters II and III of this dissertation focus on describing the development of *O. collaris* from zygote to juvenile worm and closely examining the consequences of unusual developmental aspects on larval performance. During the development of the congener *O. fusiformis*, Wilson (1932) noted that the ciliated band of the mitraria larva was unusual for a polychaete in that the band was much longer and more convoluted than that seen in typical polychaete larvae, although they retained the ancestral opposed band system (Nielson, 1995). Emlet and Strathmann (1994) further concluded that short, simple cilia on monociliated cells comprised the ciliated band of the mitraria larva, reminiscent of larvae of echinoderms and hemichordates (*very* distant relatives to the polychaetes). They proposed that the long, convoluted ciliated band of the mitraria (of unknown origin in the embryo) was an adaptation to enhance feeding rates and growth in the unusual mitraria. This hypothesis is tested in Chapter III by comparing feeding performance of mitraria of *O. collaris* to other species of larvae that represent the typical larval form of the lophotrochozoa (i.e., Annelids, Molluscs) and the typical larval form of the deuterostomia (i.e., Echinoderms, Hemichordates). In Chapter II, I identify the origin

of the unusual ciliated band in the mitraria larva and describe the particulars of development for *O. collaris*.

In Chapter IV, I focus on reproductive cycles in *O. collaris*. Embryos and larval cultures could only be obtained during spring and summer months, indicating that this species reproduced seasonally. I examined the effects of environmental factors on gonad development in *O. collaris* in two ways. Field samples of adult worms were collected on monthly intervals for two years in Coos Bay, OR. The stage of gonad development was determined for these samples and compared to environmental factors that vary on monthly and seasonal time-scales in temperate estuaries (e.g., photoperiod, salinity, temperature, primary production). A controlled laboratory experiment was used to determine this species' ability to respond to variations in photoperiod and food availability by regulating gametogenic cycles.

In Chapter V, I examine the relationship between the biology of the different life history stages of *O. collaris* and the distribution of this species within the Coos Bay estuary. I tested the effects of salinity and temperature on survival of embryos, larvae and juveniles in the laboratory and on survival and reproduction of adults in the laboratory and the field. The effects of sediment of different sizes on survival and reproduction of adults and recruitment of juveniles were examined in the field and the laboratory.

CHAPTER II

PROPERTIES OF PROTOSTOMY AND DEUTEROSTOMY IN THE EMBRYOGENESIS AND LARVAL DEVELOPMENT OF *OWENIA COLLARIS* (ANNELIDA: POLYCHAETA)

Introduction

Embryogenesis in annelids is defined by total, often unequal, spiral cleavage and protostomy (Okada 1970). Within these two constraints, however, embryogenesis and development are as diverse as the habitats and morphologies of the adults. Eggs can either be very yolky and produce stereoblastulae or have little to no yolk and produce coeloblastulae. The mechanism by which gastrulation occurs is generally determined by the amount of yolk with epiboly occurring in yolky embryos and invagination occurring in non-yolky embryos (Okada 1970). Direct and indirect development are both common. Within those species that undergo indirect development, yolky eggs frequently produce non-feeding larvae, whereas non-yolky eggs produce feeding larvae, with the ancestral larval form being that of the trochophore (Nielson 2001). The hypothetical ancestral trochophore larva is characterized by the presence of an apical tuft, a pre-oral prototroch derived from trochoblasts, an adoral ciliary zone, and post-oral meta-, gastro- and telotrochs, all of which are formed from multiciliate cells (Nielson 2004). Within polychaetes, this typical form also undergoes post-embryonic growth to form segments through teloblastic growth (Schroeder and Hermans 1975). Segments develop

sequentially from pockets of mesoderm which grow posterior to the progenitor segment and form new coelomic compartments through schizocoely, along with continued elongation of the epiderm.

In a few cases, the ancestral trochophore has been lost or modified and the resulting larval forms lack some aspect of the trochophore's defining features. For example, the endolarva of *Polygordius appendiculatus* develops the segmented body within the larval hyposphere (Wolterek 1902, Okada 1970). Larvae of *Marphysa* sp. have no distinct ciliary bands; instead the whole surface of the larva is ciliated (Aiyar 1931). One of the most modified forms is the mitraria larva of the family Oweniidae. The hyposphere of mitraria larvae is greatly reduced and they lack both gastro- and telotrochs. The segmental body in this family also forms within the larval body as a juvenile rudiment. Within the genus *Owenia*, the prototroch and metatroch are not formed from multiciliate cells, but rather monociliate cells (Emlet and Strathmann 1994), making this a highly unusual larval form.

D.P. Wilson (1932) first documented larval and juvenile development of *Owenia fusiformis* demonstrating both the unusual morphology of the mitraria and the unusual metamorphosis of this larva. However, there are no published studies on the early development of *O. fusiformis* or any species within the family Oweniidae, linking cell lineage and embryogenesis to larval morphology. The current study is an account of embryogenesis and development through the juvenile stage of *Owenia collaris* (Hartman 1969). My primary focus was to document the development of features that unite the Polychaeta: cleavage, gastrulation, ciliary bands. Secondarily, the development through

metamorphosis of this species was used to determine whether there are commonalities that can be used to define developmental patterns for the genus *Owenia* or to differentiate species within this genus.

Materials and Methods

Adult *Owenia collaris* were collected from mudflats in the Coos Bay estuary, Oregon, USA during spring and summer in the years 2004-2007 and were taken to the Oregon Institute of Marine Biology where individuals were held separately in 0.45 μm filtered seawater (FSW) at ambient seawater temperatures (10-13ºC). Worms were held until they spawned naturally. Both sexes released gametes from a pair of posterior pores. If worms failed to spawn, removing worms from tubes with fine forceps and piercing holes in the epidermis through which gametes could escape from the coelom could produce viable gametes.

Concentrated sperm were diluted in filtered seawater to a concentration of about 1 x 10^5/ mL and added to culture dishes containing a monolayer of eggs and 0.45 μm FSW. Cultures were covered and kept in an incubator set at 12°C. Occasionally polyspermy occurred, but at very low frequencies. Developing embryos and larvae were photographed *in vivo* using light microscopy (LM) or fixed for either confocal microscopy (CM) or scanning electron microscopy (SEM). To improve images, jelly coats were often removed after fertilization by placing eggs into a 1:1 mixture of 0.25M NaCitrate: 1M Sucrose and then passing fertilized embryos through a 73 μm sieve (Render 1982; Winesdorfer 1967). Time-lapse videos were made with a digital camera mounted on a Zeiss compound scope in a temperature-controlled box (Styrofoam with a

recirculating water bath). Images were captured and converted to video using bTV Pro for Mac OS 9 (Ben's Software, Inc.).

To observe organogenesis, specimens were fixed for CM in 4% paraformaldehyde for 45 minutes and then washed two to three times in 1X phosphate buffer solution (von Dassow, pers. comm.). Specimens were stored for no more than one week at 4ºC before they were stained with propidium iodide (PI) and either Alexafluor phalloidin or BODIPY-phalloidin (PH) (Molecular Probes, Inc.) and viewed on either a Zeiss 310 LSM or a BioRad Radiance 2100 CM.

Specimens for SEM were fixed two ways. 1) They were fixed with 2.5% glutaraldehyde for 30 minutes, followed by 2 washes in Millonig's phosphate buffer wash and then post-fixed with 4% osmium tetroxide in SW for 30 minutes, followed by 2 washes in Millonig's phosphate buffer wash. 2) Specimens were directly fixed in 4% osmium tetroxide for 30 minutes then washed twice with FSW and freshwater over a 24-hr period.

To examine the formation of cilia in embryos, some embryos at 20 and 22 hours post fertilization were fixed in 4% paraformaldehyde as described above for CM. However, these embryos were then stained with fluorescent-labeled tubulin antibodies (Molecular Probes, Inc.), which bind to microtubules found in cleavage spindles and cilia.

Larvae were reared through metamorphosis using standard techniques for polychaetes (Strathmann 1987). Larvae were kept in 4 L glass culture jars at a density of 1 per mL and gently stirred with plexiglass paddles. Cultures were cleaned every other

day with FSW and fed a mixture of *Rhodomonas lens* and *Isochrysis galbana* (5,000 and 10,000 cells/mL, respectively).

Results

General Embryology

Gametes are loose in the coelom in mature males and females and can flow between segments, although gametes are much more tightly packed in the mid-abdominal segments than in the posterior segments (Fig. 1.1A, B). Primary oocytes and spermatozoa either singly or in bundles (i.e., late stage rosettes with heads packed together and tails radiating outward) are spawned through paired pores at the posterior end of the animal (Fig. 1.1C, D). Upon contact with seawater, rosettes break apart into individual spermatozoa. Sperm have a small head (4 μm) and long flagellum (30 μm). Newly released eggs, whether spawned naturally or removed from ripe females, had an irregular shape, a pronounced germinal vesicle, a spawning membrane, and jelly coat upon release (Figs. 1.1C, 1.2B,C). After an hour or two, eggs became spherical and underwent germinal vesicle breakdown (GVBD). Eggs are clear to white and 70 μm in diameter. Removal of the jelly coat does not prevent fertilization or development, however, removal of the spawning membrane does. Eggs could be fertilized only after GVBD was completed.

Cleavage follows the typical spiral pattern through the 128-cell stage (Figs. 1.2-1.3). The first polar body is given off by the embryo approximately one-hour post fertilization at 12ºC, followed by a second after another hour (Fig. 1.2A). The first two divisions are meridional and occur at 3 and 4 hours post fertilization, respectively (Fig.

1.2B, C). Cleavage is dexiotropic and unequal at the 8-cell stage with the animal cells (micromeres) being slightly larger than the vegetal cells (macromeres) (Fig. 1.2D). The blastocoel first becomes apparent in the 16-cell stage (Fig. 1.2E, F) and is very pronounced beginning with the 32-cell stage and remains obvious throughout embryogenesis (Fig. 1.2G-I). At the 64-cell stage (Fig. 1.3A, B), the vegetal cells of the embryo thicken into the blastocoel, forming a flat plate (Fig. 1.3C).

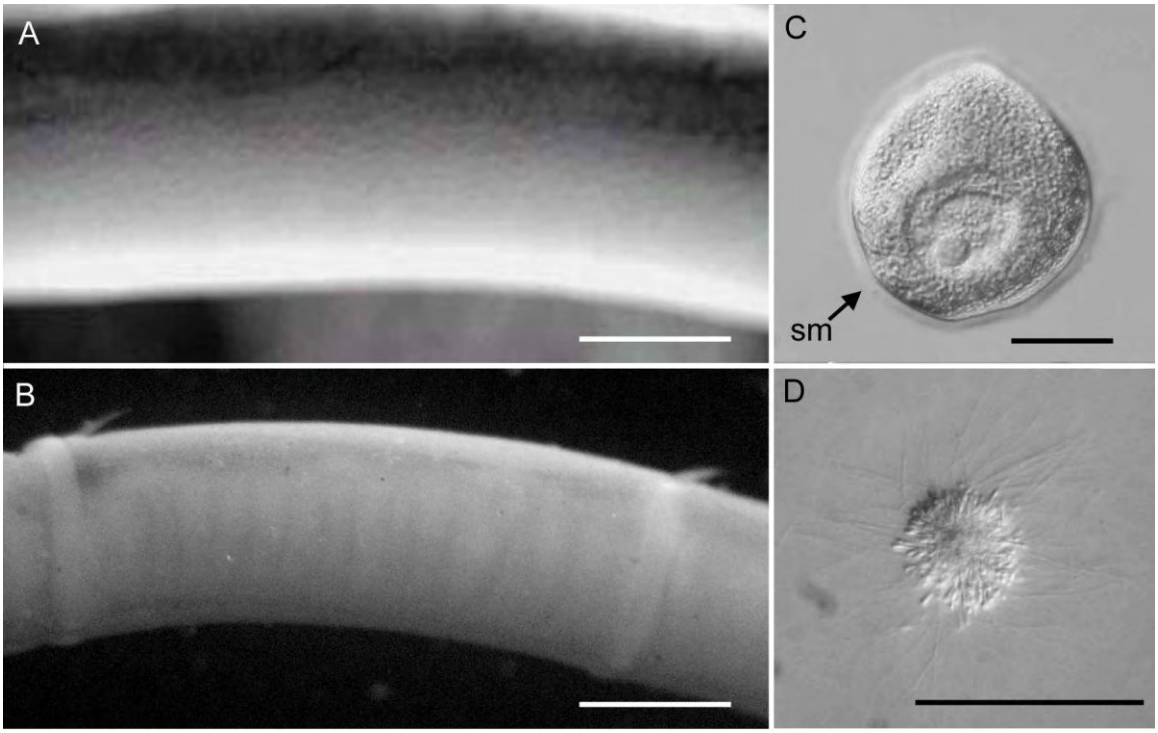

Figure 1.1. Light micrograph of adults and gametes of *O. collaris*: (A) adult female with free-floating oocytes concentrated along the ventral side of the coelom in an abdominal segment; (B) adult male with densely packed sperm bundles along the ventral side of the coelom in an abdominal segment. Scale bars: 1 mm. (C) Newly spawned primary oocyte with spawning membrane (sm) and jelly coat not visible in this photograph; (D) newly spawned sperm bundle (sperm in late stage rosette). Scale bars: 20 μm.

Gastrulation via invagination begins approximately 8-9 hours post fertilization, after the eighth cleavage, the 128-cell stage (Fig. 1.3D). Gastrulation is initiated when

the vegetal cells constrict at their apical ends and their basal regions extend into the blastocoel (Fig. 1.3F). After this initial rearrangement, the vegetal cells resume dividing and the archenteron takes up the majority of the blastocoel within four hours (Fig. 1.3F).

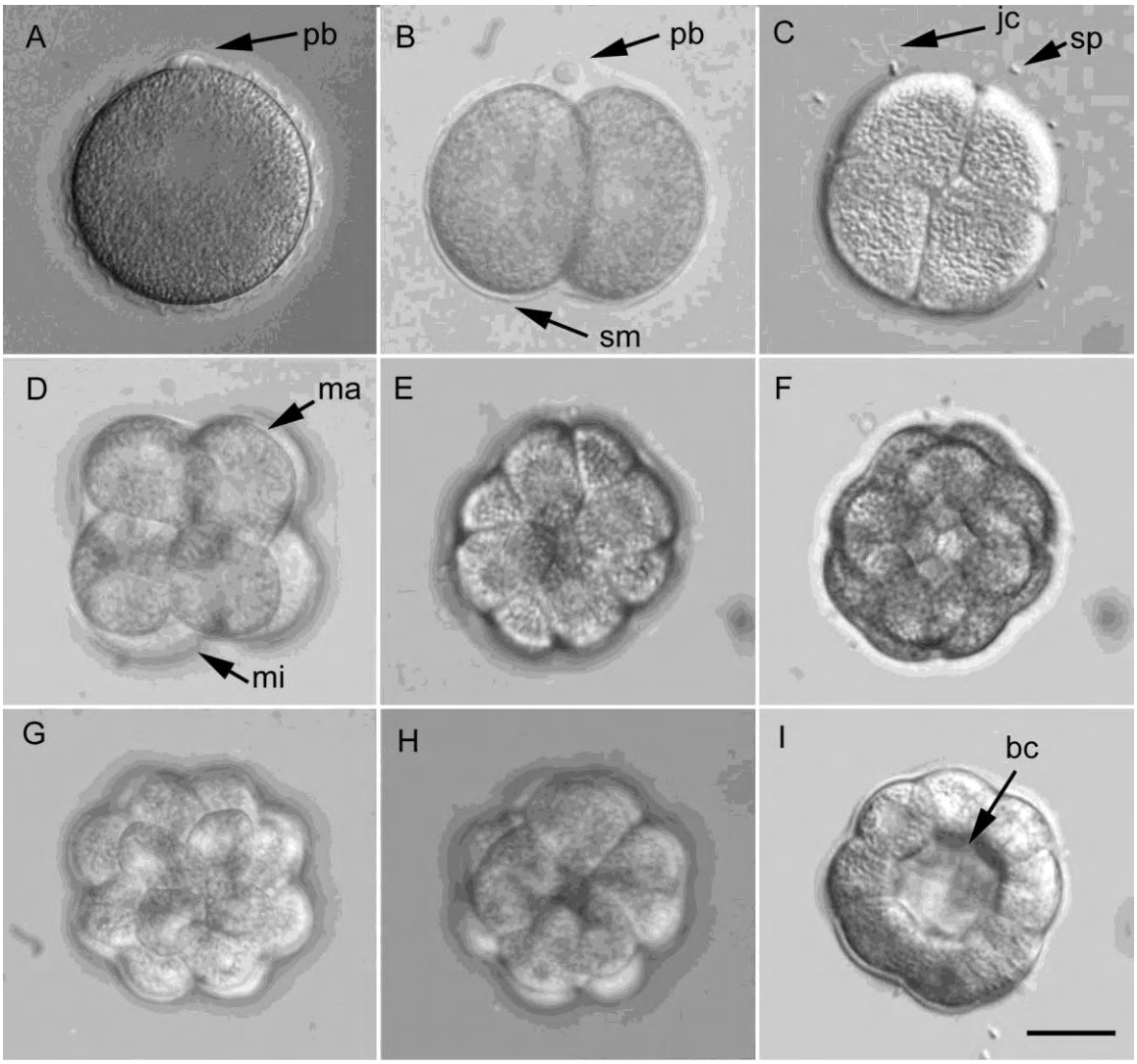

Figure 1.2. Light micrograph sequence of early embryonic development of *O. collaris*: (A) fertilized zygote with 2 polar bodies (pb); (B) 2-cell with spawning membrane (sm); (C) 4-cell with sperm (sp) in surrounding jelly coat (jc); (D) 8-cell viewed from vegetal pole showing small size of vegetal cross furrow and relative sizes of macromeres (ma) and micromeres (mi); (E) 16-cell viewed from animal pole (polar bodies out of focal plane); (F) 16-cell viewed from vegetal pole with cross furrow; (G) 32-cell viewed from animal pole; (H) 32-cell viewed from vegetal pole; (I) 32-cell blastula with blastocoel (bc). All photographs were taken at the same magnification. Scale bar: 20 μm.

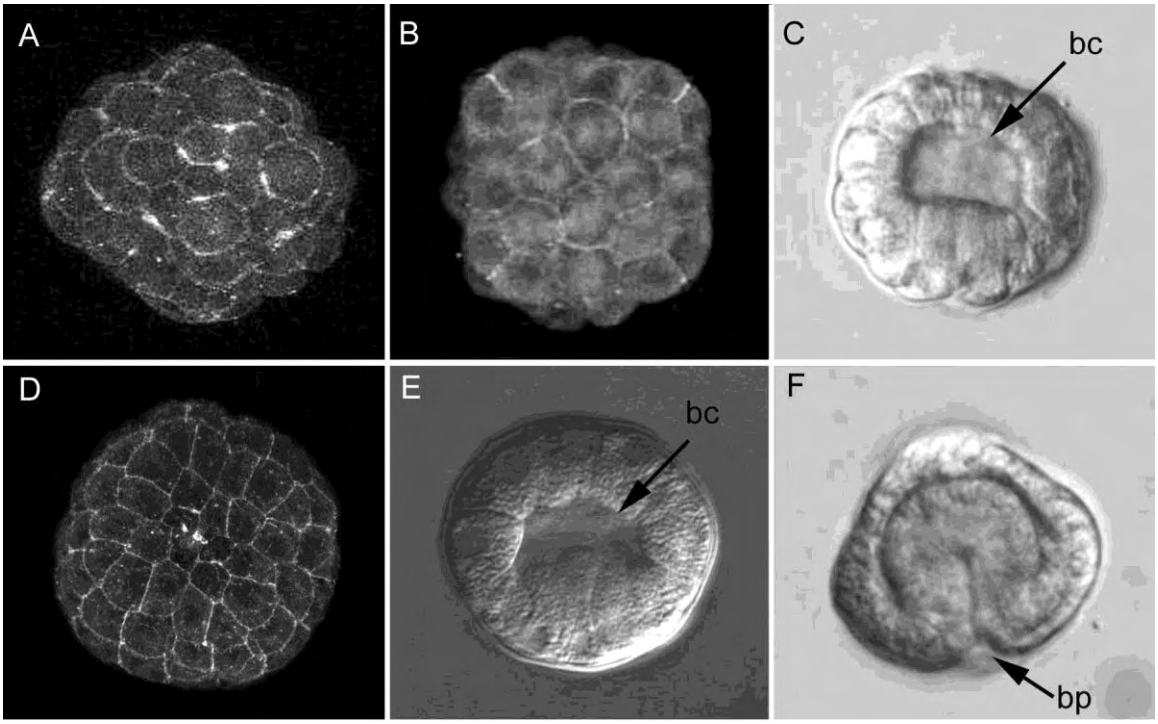

Figure 1.3. Light and confocal micrograph sequence of late embryonic development and gastrulation of *O. collaris*: (A) 64-cell viewed from animal pole, CM; (B) 64-cell viewed from vegetal pole, CM; (C) 64-cell viewed from side with flattened vegetal cells in blastocoel (bc), LM; (D) 128-cell viewed from animal pole, CM; (E) 9-h embryo with vegetal cells extending into the blastocoel, LM; (F) 13-h early gastrula with open blastopore (bp), LM. Scale bar: 20 μm.

Fate of the Blastopore

The blastopore initially opens 12-13 hours post-fertilization, after the invagination forming the archenteron takes place (Fig. 1.3F). The blastopore remains open throughout gastrulation and is situated in the center of the vegetal pole of the embryo (Fig. 1.4A-D). Once the archenteron reaches the apical end of the embryo, it folds over in the opposite direction from which mesodermal cells are proliferating in the blastocoel at the vegetal pole (Fig. 1.4A). Two hours after the archenteron makes contact with the apical pole, a group of cells grows out of the base of the archenteron and grows outward and upward

joining the top portion of the archenteron (Fig. 1.4B, C). On the opposite side of the archenteron, the epidermis of the embryo has extended outward just below the mesodermal pockets (Fig. 1.4C). This area forms the chaetal sacs and chaetae of the mitraria larva. At 21 hours post-fertilization, the gut has almost completely formed and one of two pairs of dorsal levator muscles connecting the chaetal sac to the apical epidermis have formed, confirming the location of the chaetal sac (Fig. 1.4D). At 22-hours the mouth and anus of the larva are both present and open (Fig. 1.4E). The location of the chaetal sac and the folding of the archenteron suggests that the blastopore is the opening immediately adjacent to the chaetal sac in the larva and thus is destined to become the anus. In contrast the mouth may form as a secondary opening of the archenteron with the anus between it and the chaetal sac or may form through the expansion of the blastopore to encompass both ends of the archenteron. At this point, the U-shaped digestive track is completely formed and 2 pockets of mesodermal cells have appeared around the presumptive intestine (Fig. 1.4F). Further growth of the epidermis and gut separate the mouth and the anus, but the digestive system remains U-shaped. Because of the strong curve of the digestive track, the ventral surface at this point consists of only the tissue between the mouth and anus at the vegetal end of the embryo. The ventral surface grows minimally by comparison to the dorsal surface of the larva, but cells in this region do proliferate over time.

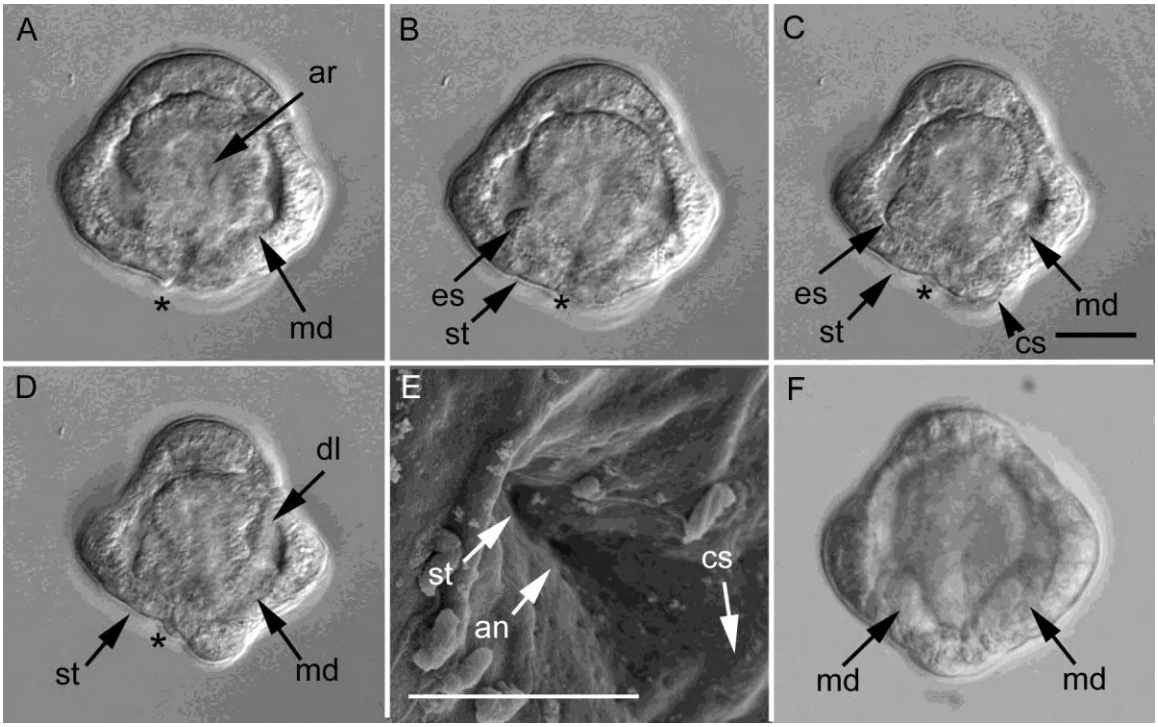

Figure 1.4. Light and scanning electron micrograph series of gastrulation in *O. collaris*: (A) 17-h, open blastopore at vegetal pole denoted by *, archenteron (ar) folding after making contact with the apical pole of the blastocoel and proliferating mesodermal pockets (md); (B) 18-h, outgrowth of archenteron forming presumptive esophagus (es) and indicating the location of the stomodeum (st); (C) 19-h, esophagus hollowed out, continued proliferation of mesodermal pockets and projection of epidermis forming chaetal sac (cs); (D) 20-h, archenteron complete, growth of dorsal levators (dl) connecting chaetal sac to apical organ; (E) 20-h, relative positions of stomodeum, anus (an), and chaetal sac, SEM; (F) 20-h late gastrula with 2 mesodermal pockets. Scale bars: 20 μm.

Prototroch Formation

Cilia form relatively late in embryos of *O. collaris*, and embryos lack obvious signs of trochoblasts ($1q^2$) becoming cleavage arrested. Time-lapse photography revealed that the daughter cells of the $1q^2$ continue to divide well beyond the typical point of cleavage arrest (Fig. 1.5A-G). The sizes of nuclei and cells continued to decrease throughout gastrulation in the region where trochoblast descendents occur in the embryo.

Nucleus diameter decreased from 5 μm to 3 μm to the point that they cannot easily be distinguished (Figs. 1.5A, E, H, respectively). The diameter along the animal-vegetal axis of these cells decreased from 11 to 7 to 5 μm at the same timepoints. Typical polychaetes become ciliated at the 64- to128-cell stage, which occurs in *O. collaris* at 8 hours post-fertilization, but cilia did not appear in embryos of *O. collaris* until 19 hours after fertilization at 12ºC (Fig. 1.5H, 6A). By this point, gastrulation has taken place and the archenteron is well developed. Tubulin antibody staining also demonstrated that the cells in the ciliated band are actively forming cleavage spindles, well beyond the point that there should have been a distinct band of very large, cleavage arrested cells in the band (Fig. 1.6B-D). When cilia first form, each is isolated from the others and although they appear to form in four regions around the embryo, at no point are compound cilia from a few large cells observed in embryos (Figs. 1.6B-D).

At 24 hours post-fertilization, larvae begin to swim off the bottom of culture dishes. Larvae do not hatch out of the spawning membrane, but rather the cilia of the apical tuft and prototroch grow through it (Fig. 1.6C). The epidermal outpocketing formed at the vegetal end of the embryo, just posterior to the anus, becomes the chaetal sac and soon the first pair of larval chaetae appears (Fig. 1.6E). The ciliary band is formed by a double row of simple cilia uniformly arranged around the embryo (Fig. 1.6E, F). Confocal imaging also revealed the vast number of cells in the ciliary band and the continued proliferation of these cells in larvae within the first few days post-fertilization (Fig. 1.7C-F).

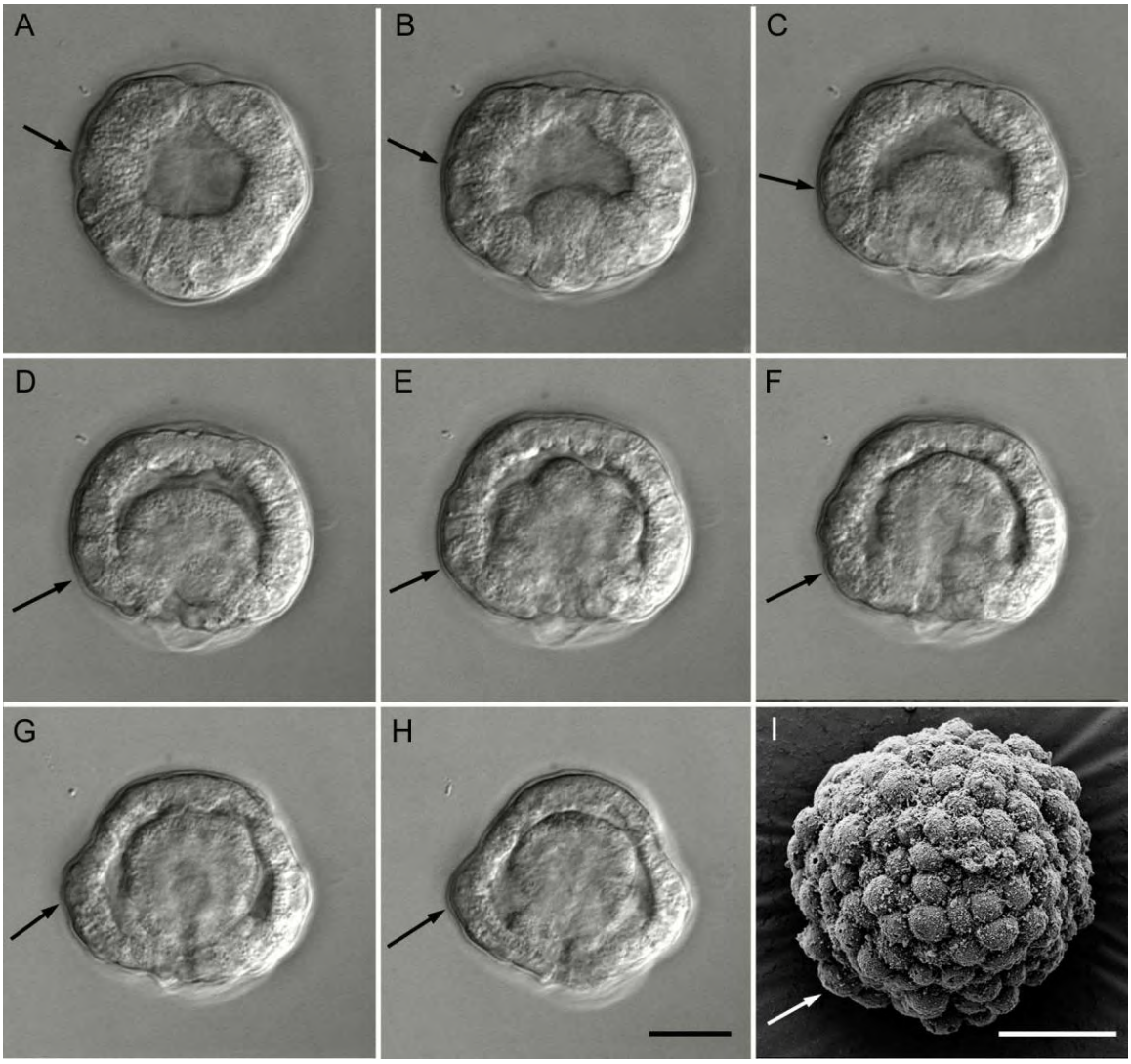

Figure 1.5. Light and scanning electron micrograph series of trochoblast division in *O. collaris*. Arrows indicate location of descendent of trochoblasts (1q2) and area of cilia development in image H. Embryos are 8, 10, 11, 12, 14, 15, 17, 19, and 18 h after fertilization, respectively. Reduction of nucleus and cell diameters to the point that individual cells are indistinguishable indicates continued division of trochoblast descendants. Scale bars: 20 μm.

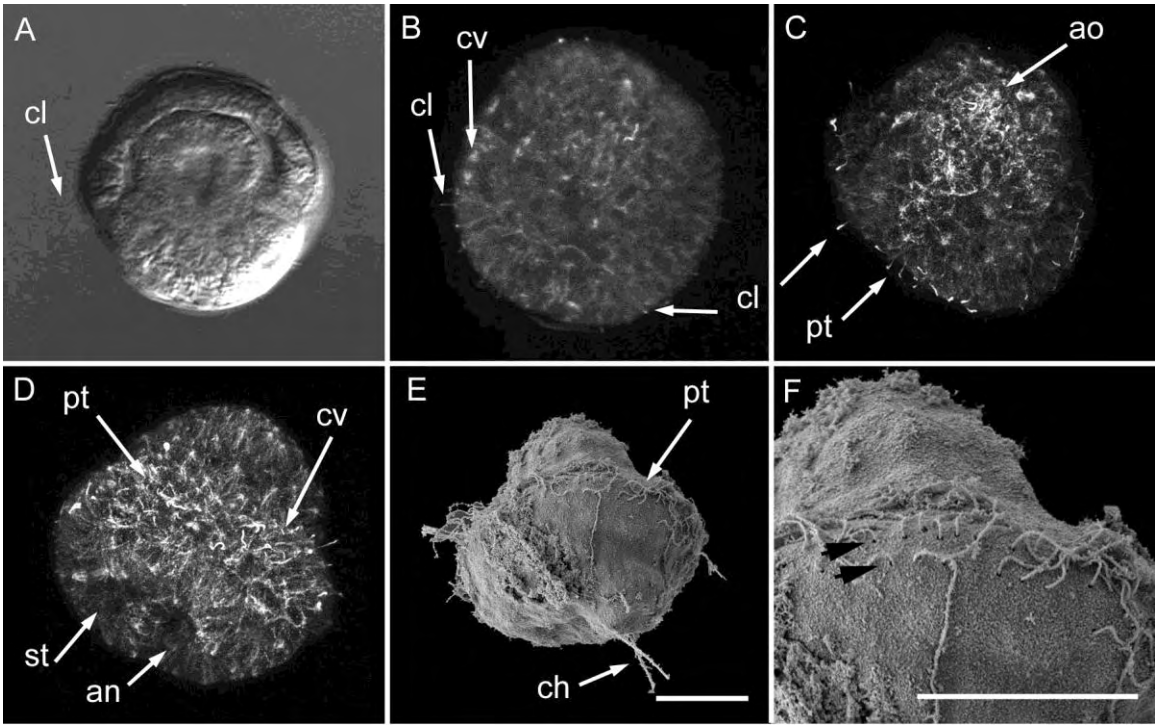

Figure 1.6. Formation of prototroch in *O. collaris*: (A) 22-h, weakly beating cilia (cl) viewed from side, LM; (B) tubulin-stained 20-h, oblique apical view, short cilia around equator and actively forming cleavage spindles (cv), CM; (C) tubulin-stained 22-h, apical view, equatorial cilia making up the prototroch (pt) and cilia at animal pole forming the apical tuft in the apical organ (ao), CM; (D) tubulin-stained 22-h, oblique vegetal view, with stomodeum (st), anus (an), equatorial cilia forming the prototroch, and cleavage spindles; (E) 22-h, oblique vegetal view, with first pair of larval chaetae (ch), SEM; (F) 22-h, with double row of prototroch cilia (arrows), SEM. Scale bars: 20 μm.

Larval Development

Larvae develop in the plankton for approximately 4 weeks at 12ºC and 3 weeks at 16ºC. The episphere of a new larva is relatively opaque with many cells closely packed together (Fig. 1.7A), but soon the episphere expands, the cells of the epidermis become less dense, and the episphere becomes nearly transparent (Fig. 1.7B). Within two to three days the mitraria has formed its first two to three pairs of chaetae and both the chaetal sac and esophagus have become highly muscular (Fig. 1.7C, D). The chaetal sac contains

two chaetal glands connected by a system of interwoven muscles that surround the glands in a figure-eight pattern. The dorsal levators increase in size in the next few days, becoming more distinct (Fig. 1.7C, E). These muscles connect the chaetal sac to the apical organ, which now contains a pair of red larval eyes. The chaetal sac is also connected to the esophagus by muscles running along the ventral surface of the larva (Fig. 1.7D). By this time, the ciliary band contains thousands of cells and is predominantly bright orange (Fig. 1.7D, F). The exact origin of the metatroch and food groove that complete the ciliated band system have not been identified, but are fully formed by this time (Fig. 1.7E, F). The ciliary band begins as a ring, but soon folds in the dorsal direction at the anterior and posterior ends of the larva (Fig. 1.8A). As the mitraria grows, folds also appear on either side of the larva forming lappets (Fig. 1.8B, C). Algal cells are often entrained into the broad food groove between the proto- and metatrochs from the posterior end of the larva and traverse along these folds to the anteriorly located mouth.

The juvenile rudiment in *O. collaris* first appears as an epidermal invagination on the larval ventral surface between the mouth and anus two weeks post fertilization at 12ºC (Fig. 1.8A). This invagination grows to occupy most of the hyposphere below the curve formed by the esophagus, stomach, and intestine. Within days, this fold grows up along the anterior portion of the intestine and forms a pocket which fills the space ventral to the gut (Fig. 1.8B, C). Approximately 18-21 days post fertilization four pairs of outpocketings form on the anterior and dorsal side of the rudiment (Fig. 1.8D-F). These pockets (thread glands in Wilson 1932) will become the first nephridia in the adult worm,

derived from the ventral epidermis of the larva. Several smaller pairs of nephridia can be found posterior to these between the fold on the rudiment invagination and the intestine. The basal portions of the juvenile rudiment are still connected to the ventral epidermis of the larva and the pocket remains open to the outside. Ventral to the nephridia, the trunk of the juvenile worm begins to surround the intestine (Fig. 1.8F).

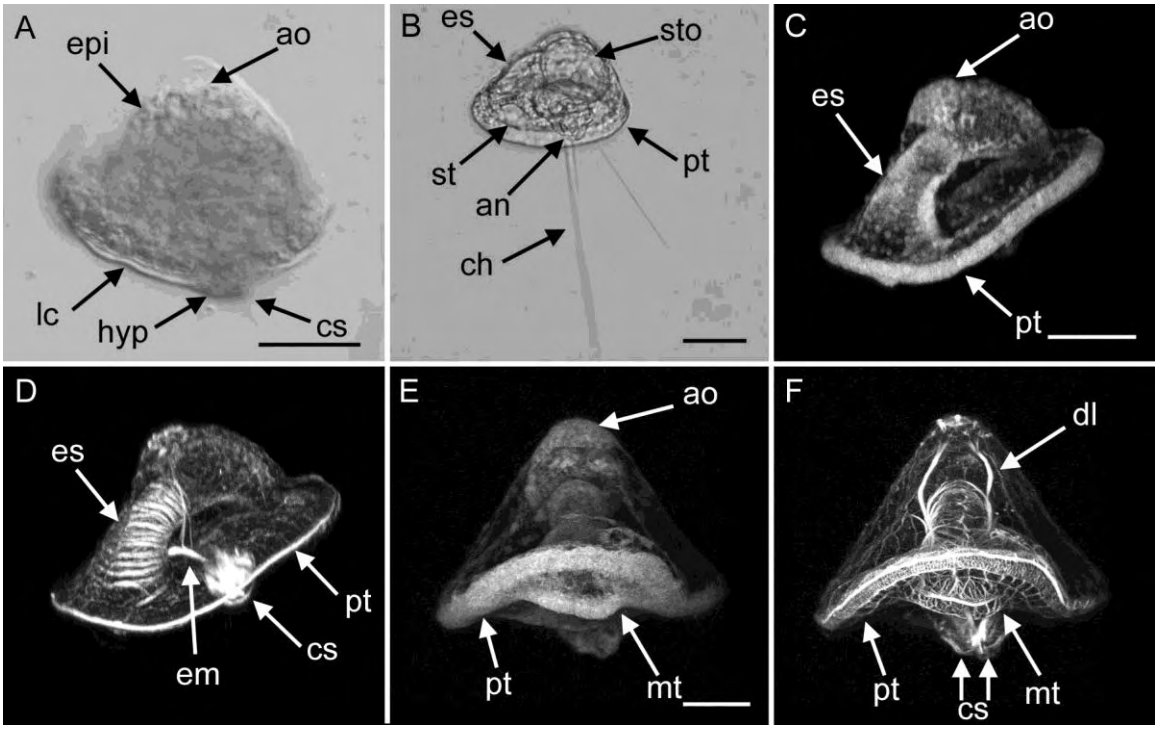

Figure 1.7. Early larval morphology of *O. collaris*: (A) 27-h, oblique apical view of episphere (epi) with spawning membrane/larval cuticle (lc), apical organ (ao), hyposphere (hyp) is out of focal plane but edge of chaetal sac (cs) can be made out; (B) 48-h, oblique apical view, with first two pairs of larval chaetae (ch) and fully formed digestive system with esophagus (es), stomach (sto), and intestine; (C) 3-day larva viewed equatorially, cell nuclei of all tissues, densest in prototroch (pt) and apical organ, PI-stained CM; (D) 3-day larva viewed equatorially, musculature of esophagus, chaetal sac, prototroch and connection between esophagus and chaetal sac (em), PH-stained CM; (E) 4-day larva, anterior view, cell nuclei of all tissues showing second ciliated band or metatroch (mt), PI-stained CM; (F) 4-day larva, anterior view, musculature connecting apical organ to digestive system, dorsal levators (dl), PH-stained CM. Scale bars: 50 μm.

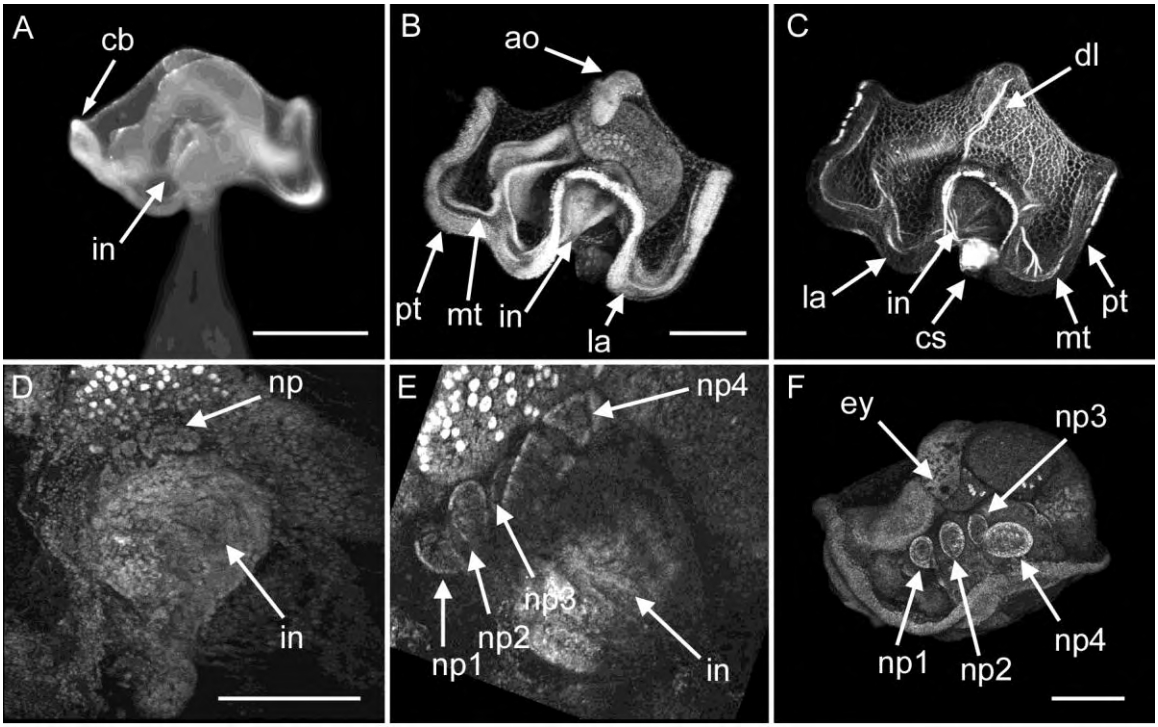

Figure 1.8. Juvenile rudiment development of *O. collaris*: (A) 14-day, side view showing folding of the ciliated band (cb) and invagination (in) of ventral epiderm, LM; (B) 17-day, expansion of rudiment invagination to fill the hyposphere below the digestive system, fully formed ciliated band system with prototroch (pt), metatroch (mt) and lappets (la), well developed apical organ (ao), PI-stained CM; (C) 17-day, fully developed larval musculature with dorsal levators (dl) and chaetal sac (cs), rudiment is now sac-like, PH-stained CM; (D) 19-day, outpocketing at apical end of rudiment forming first nephridium (np), PI-stained CM; (E) 24-day, first 4 pairs of nephridia, PI-stained CM; (F) 27-day, fully developed rudiment and apical organ with pair of eyespots (ey), PI-stained CM. Scale bars: 100 μm.

Metamorphosis

Prior to the onset of metamorphosis, larvae typically sink to the bottom of culture jars and the trunk segments begin to protrude from the larval hyposphere (Fig. 1.9A). The process by which tissues rearrange during metamorphosis mostly conforms to that described by Wilson (1932) for *O. fusiformis*, with a few exceptions.

Once the trunk segments protrude, the larval episphere begins to shrink and retract toward the apical surface of the gut (Fig. 1.9B). The cilia of the ciliary band still beat and propel the animal, but the band folds in on itself and slowly disintegrates (Fig. 1.9C). At the same time, the trunk begins to straighten out and this pulls the digestive system and the nephridia down into the trunk segments (Fig. 1.9D). In contrast to Wilson's description of the juvenile worm consuming the larval episphere, there is no compelling evidence of this occurring in *O. collaris*. The ciliary band remains distinctly bright orange, but this color is absent from the gut after the ciliary band has disappeared, and in most cases, the gut is empty after metamorphosis except for small clumps of consumed algae (Fig. 1.9D, H). It appears that the larval episphere simply collapses and is resorbed into the anterior end of the young worm. In Figure 1.9E-G, one can see the remainder of the larval episphere shrinking onto the dorsal side of the worm while the mouth of the juvenile remains unencumbered by this tissue on the ventral surface. The episphere continues to recede and the dorsal levators contract and pull the apical organ toward the esophagus until it becomes attached to the juvenile body (Fig. 1.9H). One of the final larval features lost is the chaetal sac (Fig 1.9D, E, H). The chaetae are shed once the episphere has collapsed and the sacs themselves are resorbed into the anterior collar of the worm.

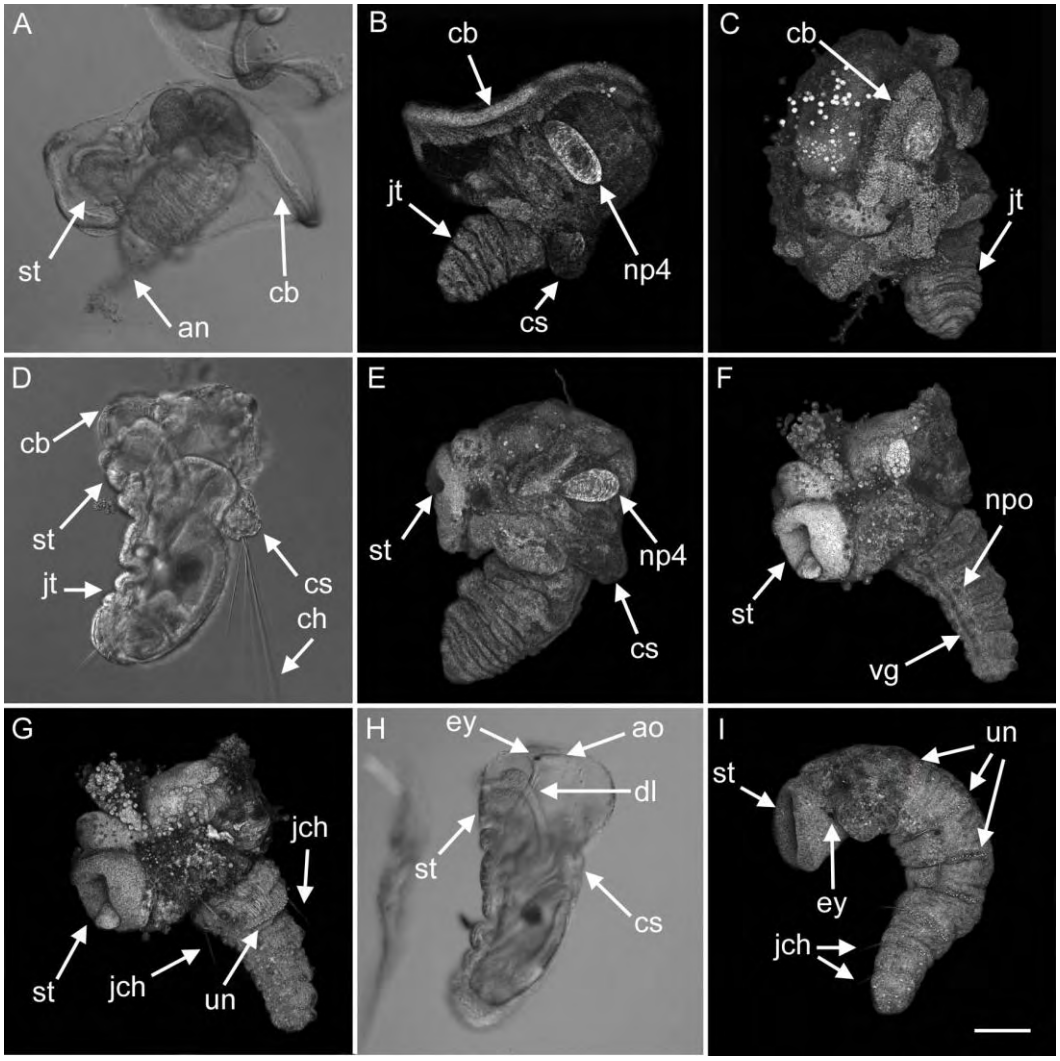

Figure 1.9. Metamorphosis of *O. collaris*: (A-I) 30-day: (A) initiation of metamorphosis by extension of rudiment just below stomodeum (st) and above anus (an) and breakdown of ciliated bands (cb), LM; (B) eversion of juvenile trunk (jt) including larval digestive system and nephridia (np) past the chaetal sac (cs), retraction of episphere, PI-stained CM; (C) folding and breakdown of ciliated bands, PI-stained CM; (D) retraction of larval episphere onto dorsal surface, trunk extended beyond chaetal sac and larval chaetae (ch), LM; (E) resorption of chaetal sac into dorsal surface, PI-stained CM; (F) elongation of trunk with ventral groove (vg) and nephridiopores (npo) visible, PI-stained CM; (G) musculature of elongated trunk with juvenile chaetae (jch) and uncini (un) demarcating segments, PH-stained CM; (H) retraction of dorsal levators (dl) pulling the apical organ (ao) with eyespots (ey) down to fuse with the trunk, stomodeum remains unencumbered by the degenerating larval episphere, LM; (I) juvenile worm with apical organ and eyespots fused to trunk, rows of uncini and juvenile chaetae can also be seen, PI-stained CM. Scale bars: 100 μm.

Juvenile Worm Morphology

The young worm is equipped with a head (prostomium and peristomium), one thoracic segment bearing two to three sets of chaetae, six or seven segments each bearing a single pair of chaetae and one pair of tori (rows of uncini located at the anterior of each segment), and a pygidium (Figs. 1.9I, 1.10A, B). The chaetae and uncini, which were present before metamorphosis, now can be seen clearly along the trunk segments (Figs. 1.9I, 1.10A, B). The juvenile worm retains the larval eyes as part of the fused pro- and peristomium, although they are generally lost in the adult (Fig. 1.10A). Internally, the most anterior two pairs of nephridia come to reside in the thoracic segment (np1 and 2), the first chaetigerous segment inherits the next nephridial pocket and the longest pair of nephridia (np3 and 4), which fuse as the juvenile develops. One pair of nephridia resides in each of the next three chaetigerous segments (np5-7) (Fig. 1.10C). Within the larva, the juvenile trunk is folded over itself, but at metamorphosis the juvenile epiderm unfolds and straightens to engulf the larval digestive system into the already segmented trunk. After metamorphosis, juveniles are approximately 815 μm in length and 125 μm wide.

Soon after completing metamorphosis, the juvenile begins to gather materials for its initial tube and often sticks to surfaces with its anterior end (Fig. 1.11A, B). The initial tube is generally made of clear mucus and small particles such as algal cells and bacterial film, but can also contain much larger items such as threads, larval chaetae, and the tubes of other worms (Fig. 1.11C). Within the first week, they also develop a pronounced sphincter at the opening to the stomach (Fig. 1.11D). When offered sediment, young worms commonly use the smallest grains to add to their tubes or will

shed the initial tube and replace it with one anchored into the sediment (Fig. 1.12A). At first, juveniles of *O. collaris* lack tentacles, but these soon form in pairs of buds (Fig. 1.12B). Within two weeks, juveniles grow three pairs of unbranched tentacles. Like the adults, juveniles are surface deposit feeders, consuming small grains of sediments as well as whatever is attached to them (Fig. 1.12B). When kept in the laboratory, juveniles grow to a length of about 1300 μm by 24 days after metamorphosis.

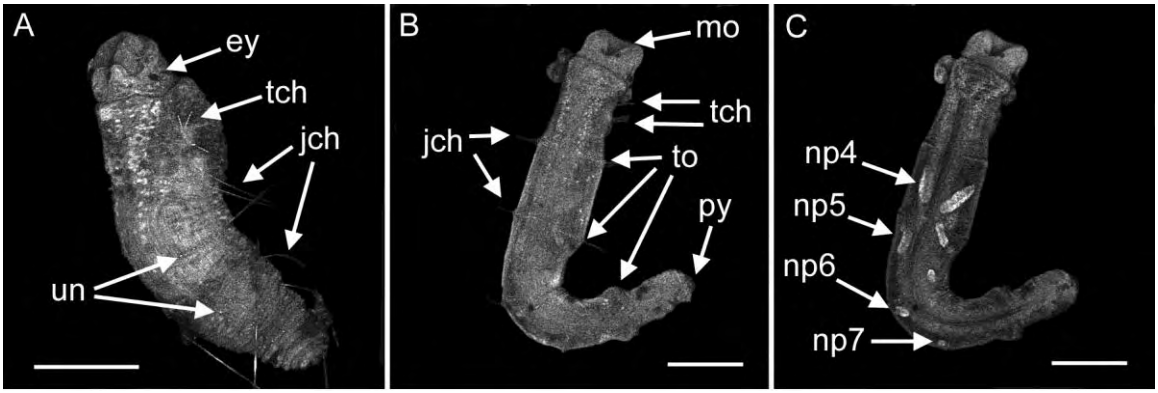

Figure 1.10. Confocal micrographs of early juvenile morphology of *O. collaris*: (A) juvenile 3 h after completion of metamorphosis, larval eye (ey) is retained and thoracic segment has two sets of chaetae (tch) whereas posterior segments have single pairs of chaetae (jch) and rows of uncini (un), PH-stained; (B) ventral view of fully extended 2-day old juvenile, open stomodeum (st), pygidium (py), tori (to), and juveniles chaetae, PH-stained; (C) ventral view of 2-day old juvenile,4 pairs of nephridia (np), PI-stained. Scale bars: 100 μm.

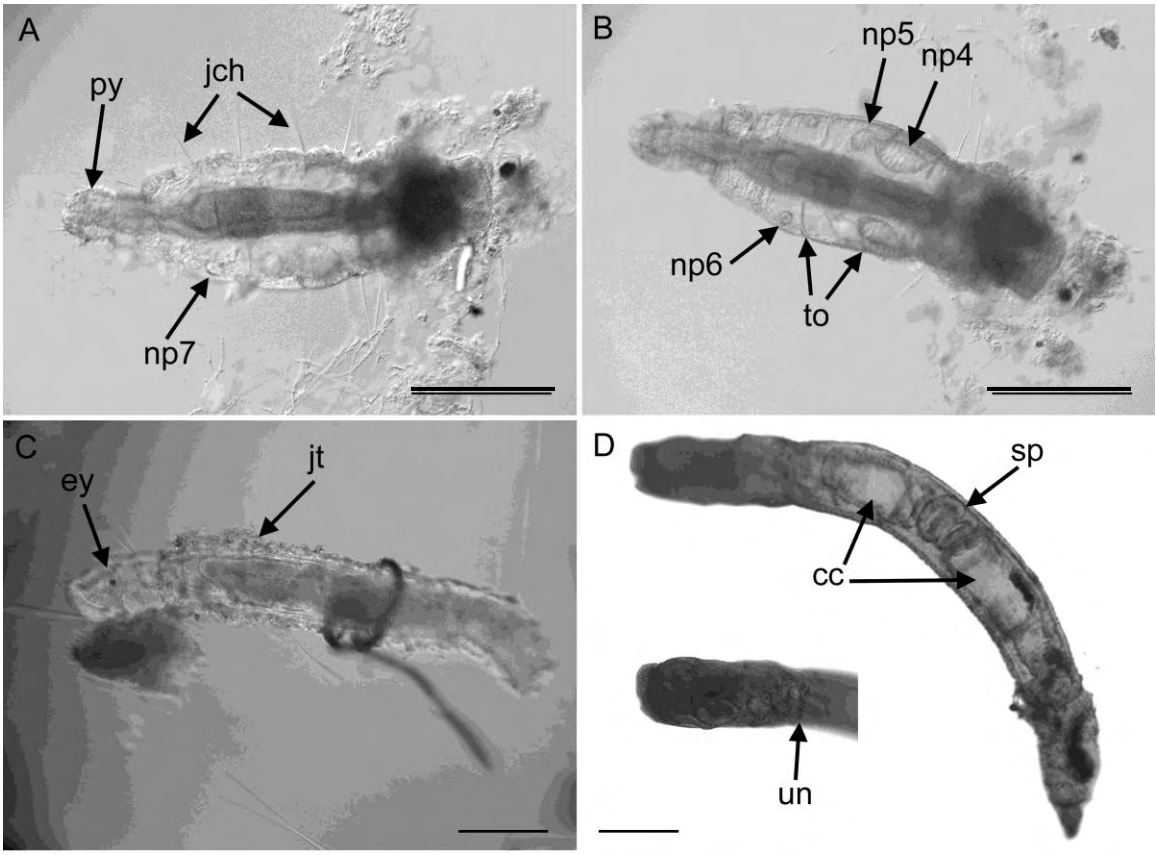

Figure 1.11. Light micrographs of juvenile morphology of *O. collaris*: (A) dorsal view of 3-day old juvenile beginning to gather particles together for initial tube with mucus at anterior end, gut has elongated through the worm, terminating in the pygidium (py), juvenile chaetae (jch) present on each segment and nephridia can still be seen through the translucent body wall (np); (B) ventral view of 3-day juvenile with tori (to); (C) 5-day old juvenile with complete juvenile mucus tube (jt), larval eye (ey) still present; (D) 1-wk old juvenile showing growth of trunk, coelomic compartments (cc), sphincter (sp) at the entrance to the stomach, and uncini (un) on head. Scale bars: 200 μm.

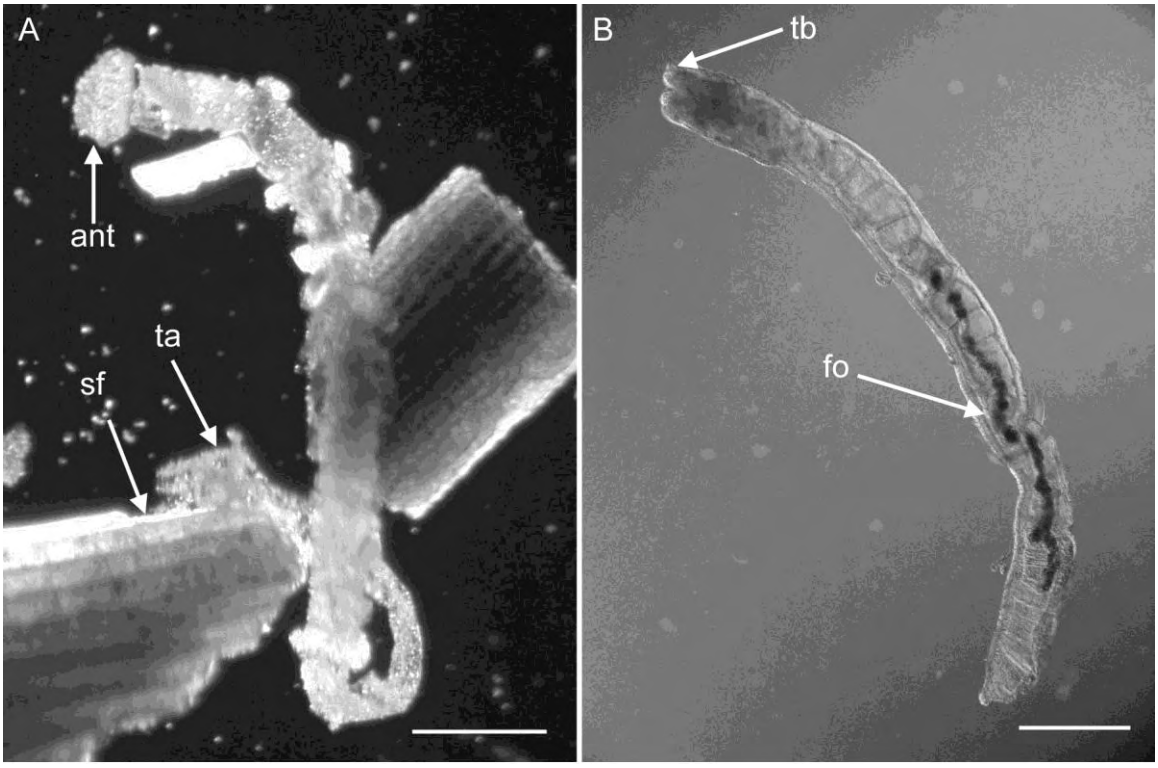

Figure 1.12. Light micrograph of advanced juvenile morphology: (A) 2-wk old juvenile with complete sediment tube anchored to a fragment of an urchin spine (sf) at the posterior end (ta) and unattached anterior end (ant); (B) 3-4 wk old juvenile removed from tube with feeding tentacle buds (tb) and partially digested food (fo) in the digestive system. Scale bars: 200 μm.

Discussion

Embryonic development in polychaetes does not follow one prescribed pattern. There is a range between those species that exemplify every aspect of accepted protostome development to those that possess modifications of cleavage patterns, gastrulation, and larval morphology. *Owenia collaris* exhibits shared features of development for annelids and protostomes, but also extreme modifications to the accepted patterns. Sperm are of the typical form for polychaetes with a small head and long flagellum (Okada 1970). At the 8-cell stage, animal cells (micromeres) that are

larger than the vegetal macromeres is a pattern similar to nemertean embryos but unlike most polychaete embryos (Shankland and Savage 1997). Embryos undergo holoblastic spiral cleavage to form a coeloblastula, which reflects the ancestral pattern for annelids. Past this point, several key features of typical protostome development cease to occur. The stomodaeum is not necessarily derived from the area in which the blastopore forms. It may form as either a secondary opening or by the lateral compression of the blastopore, followed by differentiation of the blastopore into a stomodeum and anus. However, the blastopore of *O. collaris* appears to remain a small, circular opening throughout gastrulation, suggesting that the anus alone develops from the blastopore (Nielson 2001). Although rare, this is not unheard of in polychaetes. In *Eunice kobiensis* the anus also forms in the region in which the archenteron opens and the stomodaeum forms as a separate, secondary invagination (Akesson 1967). However, in the case of *E. kobiensis*, the blastopore closes after gastrulation and reopens later in development as the anus.

Another modification found in *O. collaris* is the fate of the trochoblasts ($1q^2$). The derivatives of this lineage, those cells that generally form the prototroch in trochophores, do not become cleavage arrested and do not become multiciliated. The prototroch in *O. collaris* develops through continued proliferation of cells that may be derivatives of the original trochoblasts and form cilia much later in development than embryos of similar size (Rattenbury and Berg 1954, Okada 1970, Smith 1981, Strathmann 1987). The cells that make up the region of the prototroch are also much smaller than those of other protostomes, further supporting continued division. Estimates of prototroch cell diameters for *O. collaris* after ciliation are in the range of 4-5 μm,

whereas those for the nemertean *Carinoma tremaphoros*, the polychaetes *Nereis limbata* and *Polygordius* sp., and the limpet *Patella vulgata* are 25-50, 11-22, 15, and 30 μm, respectively (Maslakova et al. 2004, Costello 1945, Woltereck 1902, Damen and Dictus 1994). Although comprised of smaller cells, the prototroch of early trochophores of *O. collaris* covers approximately the same surface area relative to the total surface area of the larva when compared with early trochophores of other polychaetes (14% as compared to 11-38% for other polychaetes) (Costello 1945, Anderson 1959, Akeeson 1967). Adult *Owenia* sp. also possess deuterostome-like epidermis and nephridia, based on the fact that monociliated cells dominate both tissues (Gardiner 1978, Smith et al. 1987). Monociliated cells are also found in the larvae and adult *Magelona mirabilis* (Bartolomaeus 1995). The closest relatives of *Owenia* spp. and *M. mirabilis* within the polychaetes, however, possess multiciliated cells (Gardiner 1979, Pettibone 1982, Rouse and Fauchald 1997). Bartolomaeus (1995) suggested that in both *O. fusiformis* and *M. mirabilis*, the formation of monociliated cells occurred through a suppression of muliticiliarity in an early stage of ciliogenesis and thus monociliarity in these two lineages is secondarily derived. Gardiner's (1978) finding that some epidermal cells of adult *O. fusiformis* are biciliated further supports this idea.

The deuterostome-like characteristics found in *Owenia* may be the result of reversion to the plesiomorphic condition of the *Urbilateria* or they may be secondarily derived characters that resemble deuterostomes by conversion (De Robertis and Sasai 1996). Since the stomodaeum forms secondary to the blastopore in members of both the deuterostome and protostome lineages, this may indicate that the *Urbilateria* was a

deuterostome. If this is the case, protostomy in animals with complete digestive tracts developed in the ancestor to modern day protostomes, with loss of this character several times in individual genera (Aguinaldo and Lake 1997, Rouse and Fauchald 1997).

Comparisons between *O. collaris* and *O. fusiformis* reveal similarities in development, but also differences that can be used to distinguish the two species as larvae and juveniles (Wilson 1932). *Owenia collaris* and *O. fusiformis* vary in size throughout development, with the former species smaller at each stage (Table 1). Coloration patterns also vary between species, with the prototroch rim orange in the former and pale yellow in the latter and the photoreceptors red in the former and brown in the latter. Coloration in *O. collaris* is independent of the type of food consumed in culture. During metamorphosis the overall series of events is the same between species, although some differences occur. *Owenia collaris* resorbs its larval tissues rather than casting them off or consuming them. *Owenia collaris* has fewer nephridia and chaetigerous segments at metamorphosis than *O. fusiformis*. The difference in number of segments continues once tentacles form, probably due to the smaller size of *O. collaris*. This is also reflected in the relative size and number of segments in adults of these two species (Blake 2000, Koh and Bhaud 2003).

Table 1.1. Characteristics of juvenile worms of *O. collaris* and *O. fusiformis*. *Owenia fusiformis* values from Wilson (1932).

	O. collaris	***O. fusiformis***
# Nephridia	4	7
# Fused Thoracic Segs.	2	2
# Chaetigerous Segs.	6-7	11
Length (@ metamorphosis)	815 μm	870 μm
Length (3-4 weeks)	1300 μm	2560 μm

The study of embryogenesis and larval development in *Owenia collaris* supports both conservation and high levels of modification of characters, depending on the taxonomic scale examined. Within the genus *Owenia* there is variation in size and segmentation, but larval and juvenile development appears to be relatively conserved. Although typically thought of as determinant, spiralian cell fates have some plasticity, with different sets of cells becoming the stomodaeum in at least a few lineages and developmental arrest of trochoblasts is not universal. Ultimately, protostome development is much more plastic than was once thought, and occasionally the ancestral features may emerge or deuterostome characteristics can be secondarily derived (convergent evolution).

BRIDGE I

Embryogenesis in *Owenia collaris* produces a larva (mitraria) with a ciliated band composed of simple cilia on monociliated cells not derived from the typical polychaete trochoblasts cells. The cellular arrangement of this ciliated band prevents the formation of compound cilia, which can produce fast feeding and swimming currents by growing to longer lengths and moving faster through the water column. The ciliated band of mitraria larvae may be an adaptation to allow high feeding rates similar to the ciliated bands of other larval forms such as echinoderm plutei or bipinnaria, as suggested by Emlet and Strathmann (1994). This hypothesis will be tested in Chapter III by comparing growth and feeding rates of mitraria larvae with those of canonical larval types.

CHAPTER III

FUNCTIONAL MORPHOLOGY OF CILIATED BANDS: DOES PHYLOGENY OR FORM GOVERN THE PERFORMANCE OF THE LARVAL CILIATED BANDS OF THE POLYCHAETE *OWENIA COLLARIS* (FAMILY OWENIIDAE)?

Introduction

The role of a feeding larva can be considered from both evolutionary and ecological perspectives (Hart and Strathmann, 1995). The evolutionary view focuses on variation in larval form among taxa and over time, and explores the consequences of that variation on performance. The ecological view focuses within taxa on the population and community consequences of having planktotrophic larvae in the life cycle. Feeding larvae are sometimes viewed as machines for turning small eggs into larger (and therefore more fit) juveniles (Hart and Strathmann, 1995). Both approaches consider how feeding larvae have evolved to survive the planktonic period and recruit successfully into the adult population.

Feeding mechanisms of planktotrophic larvae have received much attention because they determine the rates at which different larval forms can remove food particles from suspension. High feeding rates may result in shortened planktonic periods and increased size at metamorphosis, which may ultimately reduce mortality of larvae and juveniles (Havenhand, 1995; Hart and Strathmann, 1995; Sogard, 1997). Larvae that feed using ciliated bands can maximize the rate at which they clear particles from water

(clearance rate) by increasing the length or velocity of their cilia, the length of the ciliated band, or the number of cilia creating a current (Strathmann *et al*., 1972; Strathmann and Leise, 1979; Hart, 1996; Miner *et al*., 1999). Larvae may also increase clearance rates by placing ciliated bands on ridges or other projections of the body (Emlet, 1991). Each of these is an effective way of increasing the volume of water that passes within the capture zone of the feeding apparatus. However, the potential changes to the cilia or ciliated band are constrained by the type of cilia present, which is generally constrained by phylogeny.

Physical restrictions imposed on cilia produce two major strategies for increased feeding rates. Trochozoan larvae (found in the phyla Annelida, Mollusca, Nemertea, *et al.*) possess multiciliated cells and compound cilia (Nielson, 1985). Larvae with multiciliated cells can increase the length of cilia and the velocity of the effective stroke by fusing cilia together into compound cilia, which provide stiffness during the effective stroke that propels water and food (Sleigh, 1962). These larvae can also increase the length of the ciliated band by incorporating accessory ciliated cells into the band (Damen and Dictus, 1994). Tornaria-type larvae (found in the phyla Hemichordata and Echinodermata), by contrast, possess monociliated cells and simple cilia and can only increase feeding rate by increasing the length of the ciliated band or the number of cells in the band (Nielson, 1985). Larvae with simple cilia on monociliated cells cannot increase the length or velocity of their cilia because the resulting increase in resistance would ultimately cause the cilia to cease functioning without the added support of a neighbor.

The morphology and function of ciliated band systems in both lineages is derived from programmed events that occur during embryonic development and from continued growth during larval development. The morphology of trochophore-type ciliated bands is initially set by the arrest of a limited number of trochoblast-derived cells in the embryo, which form the large, multiciliated cells found in the primary ciliated band or prototroch of hatched larvae (Nielson, 1985). The development of a second ciliated band, the metatroch, completes the morphology of the opposed-band system. Particles are captured by the interaction of currents between these two bands. Further growth of the ciliated bands in derived larvae (e.g. veliger larvae) by the addition of secondary trochoblast cells or accessory trochoblast cells and compound cilia increases the length of the ciliated band and the magnitude of the feeding current. The morphology of tornaria-type ciliated bands is initially set by the concentration of monociliated epidermal cells into a distinct band following a uniformly-ciliated blastula stage (Wray, 1997). With continued addition of epidermal cells, the ciliated band forms a convoluted loop of monociliated cells with simple cilia. This single band may split during larval development to form two sections of the loop, but particles are captured by a single current created by the band system and the reversal of ciliary beat to drive particles to the mouth. In both larval types, continued growth of ciliated bands by the increase in the number of cells increases the feeding and swimming performances of these bands.

The mitraria larva of the polychaete family Oweniidae is morphologically and developmentally very different from the trochophore larva typical of polychaetes. Mitraria larvae possess a sinuous ciliated band that is set out from the body on a

pronounced ridge, rather than the simple circular band characteristic of most trochophores. This band is comprised of simple cilia on monociliated cells that remain the same length throughout the larval period (Emlet and Strathmann, 1994). Although the cellular arrangement of this ciliated band is quite different from other trochophore larvae, mitraria still capture particles with an opposed band system consisting of the preoral prototroch and postoral metatroch. The unusual curvature of the ciliated band (and thus the increase in band length) in the mitraria has been proposed as an adaptation for increasing feeding rates since simple cilia cannot function if they increase in length or velocity (Emlet and Strathmann, 1994). However, this hypothesis has yet to be tested, and the feeding performance of this larval form has not been compared with those of other larval forms.

The purpose of this study is to compare the feeding performance (i.e. clearance rates) of mitraria larvae of *Owenia collaris* over the course of its development to larvae that represent the two typical larval forms, trochophore-type larvae and tornaria-type larvae, to assess if phylogeny (i.e. trochozoa) or form (e.g. opposed-band, simple cilia) determines function in ciliated band systems. Trochophore and setiger larvae of the polychaete *Serpula vermicularis* and trochophore and veliger larvae of the mussel *Mytilus californianus* are used to represent trochophore-type larvae. Larvae of *S. vermicularis* retain the typical circular ciliated band composed of multiciliated cells with compound cilia throughout development, while *M. californianus* moves through the trochophore stage to a veliger larva with a larger, but still circular, ciliated band. Echinoplutei of the urchin *Strongylocentrotus purperatus* and bipinnaria of the seastar

Dermasterias imbricata are used to represent typical tornaria-type larvae with their single bands of monociliated cells. The length of the ciliated band increases greatly during development in both of these species. A summary of larval characteristics can be found in Table 2.1.

Materials and Methods

The clearance rates and sizes of all species of larvae were measured in laboratory-reared cultures. Because of differences in breeding seasons, each species was cultured and tested at different times. *Dermasterias imbricata* were reared in July, 2007; *Mytilus californianus* in October, 2007; *Owenia collaris* in July, 2006 and August, 2007; *Serpula vermicularis* in September, 2006 and August, 2007; *Strongylocentrotus purpuratus* in March, 2007. Gametes were obtained from many parents of each species using standard techniques (Strathmann, 1987; see Ch. 2), and embryos were pooled after fertilization in order to minimize parental effects. Standard larval rearing procedures were used, with larvae of each species maintained in 4-L glass jars at a starting concentration of 1 embryo/mL of 0.45 μm filtered seawater (FSW) (Strathmann, 1987; Hart, 1996). A minimum of 15 culture jars were maintained for each species. Cultures were held in running seawater to maintain temperatures between 12 and 16ºC and they were stirred gently with plexiglas paddles. Once the gut formed, larvae were fed a mixture of cultured unicellular phytoplankton, *Rhodomonas lens* and *Dunaliella tertiolecta* for echinoderms and *R. lens* and *Isochrysis galbana* for polychaetes and mussels, at concentrations of 5,000 cells/mL. Cultures were cleaned every other day and given fresh food following each cleaning.

Table 2.1. Larval characteristics of species cultured to test for differences in function of ciliated band systems.

Species	*Larval Type*	*Band System*	*Cell Type*	*Cilia Type*	*Band Shape*
Dermasterias imbricata	Tornaria	Single	Monociliated	Simple	Convoluted
Strongylocentrotus purpuratus	Tornaria	Single	Monociliated	Simple	Convoluted
Mytilus californianus	Trochophore	Opposed	Multiciliated	Compound	Circular
Serpula vermicularis	Trochophore	Opposed	Multiciliated	Compound	Circular
Owenia collaris	Mitraria	Opposed	Monociliated	Simple	Convoluted

At various times throughout larval life, individuals of each species from each of five randomly chosen replicate jars were removed for measurements. Maximum clearance rates have been obtained for both bivalve veligers and echinoderm larvae at concentrations of 1,000 to 5,000 cells/ mL and can be dependent on preconditioning (Strathmann, 1971; Sprung, 1984; Hart, 1996), so clearance rates were measured using cultured *R. lens* at 5,000 cells/mL, a food that all larvae consumed in culture. Twenty larvae were removed from each of 5 culture jars and placed in 5 separate 20-mL scintillation vials with 20 mL of FSW and *R. lens*. *Rhodomonas lens* was added from a stock algal culture grown in f/2 media, which had been centrifuged, concentrated and the concentration determined by hemacytometer counts. Vials were held for 48 hours in a 16ºC incubator equipped with fluorescent lights set to mimic a 16:8 Day:Night cycle. Two control vials with *R. lens* but no larvae were also incubated alongside the five replicate larval vials.

At the end of the 48 hour experimental period, 10 mL samples were removed from each vial and transferred to a 15-mL centrifuge tube using a volumetric pipet. Immediately after this, all samples were killed with a drop of concentrated formalin and centrifuged at 3,000 rpm for 10 minutes in a Marathon 6K swinging bucket centrifuge (Fisher Scientific, Inc.). Algal cells in a pellet at the bottom of each tube were concentrated for counting by removing all but 1 mL of FSW and resuspending the pellet with a Pasteur pipet. A minimum of three subsamples for each vial was then counted

with a hemacytometer on an Olympus BH-2 compound microscope and the final cell concentration calculated.

Clearance rate (the volume of water cleared of particles) per animal over the 48-hour time interval was calculated from equations in Frost (1972). Analysis of control vials yielded an average algal growth constant, k, from:

$$C_1 = C_0 e^{\mathbf{k}(t_1 - t_0)} \qquad (1)$$

where C_0 and C_1 were cell concentrations in the control vials at times t_0 and t_1. A grazing coefficient *g* for each vial with larvae was calculated from:

$$C_1^* = C_0^* e^{(k-\mathbf{g})(t_1 - t_0)} \qquad (2)$$

where C_0^* and C_1^* were the cell concentrations in the larval vials at times t_0 and t_1. The clearance rate per animal over the 24-hour interval, F, was then calculated by:

$$F = Vg/N \qquad (3)$$

where V was the volume of the vial and N was the number of larvae in the vial.

The larval protein content was used as a proxy for the amount of metabolically active tissue in all species. Protein was determined by the Bradford method, which compares the absorption of samples stained with Coomassie blue reagent on a spectrophotometer with bovine serum albumin (BSA) standards of known concentrations (Bradford, 1976; Alexander and Griffiths, 1993). This method can be used with microgram quantities of protein, thus limiting the need for high numbers of larvae. One hundred larvae from each replicate culture jar were removed at the same time that clearance rates were measured, frozen in FSW with liquid nitrogen and held at –80ºC until analysis.

Ash free dry mass and ciliated band length also were measured as scaling factors for these experiments since there are no distinct stages within the larval period that are comparable for all species tested and the time from hatching to metamorphosis varies among species (Wilson, 1932; Young and Chia, 1982; Strathmann, 1987). For ash free dry mass, several hundred larvae were removed from each replicate culture jar at the same time that clearance rate and protein content were measured. Larvae were frozen at –20ºC in FSW and held until analysis. Samples were concentrated with a 53-µm mesh and rinsed with fresh water several times, transferred to pre-weighed foil pans and dried in a 60°C oven to a constant mass (Jespersen and Olsen, 1982). Samples were then ashed for 6 hours at 550ºC in a muffle furnace to remove all organic material and reweighed.

Ciliated band length was measured from fixed samples. Twenty larvae were held for a minimum of 5 minutes in a 1:1 mix of FSW: 7.5% MgCl in order to relax them and allow the ciliated bands to be viewed at their full size. Larvae were then fixed with 10% formalin buffered with sodium borate. Photographs of 10 larvae per replicate were taken from several different angles with an Optronics Microfire digital camera (Optronics, Inc.) mounted on an Olympus BX50 compound microscope. Segments of convoluted ciliated bands were traced and measured from series of images with Image J (National Institutes of Health). Circular ciliated bands were measured by calculating circumference from the measured diameter of the larva or velum.

Differences among performances of the various larval types were examined in several ways. Clearance rates, ciliated band lengths, and protein content were divided by the measured ash-free dry masses for each sample, thus standardizing these

measurements across all species by weight. Weight-specific measurements were then compared among the tornaria-type larvae (*D. imbricata* and *S. purpuratus*), the trochophore-type larvae (*M. californianus* and *S. vermicularis*), and the mitraria larva of *O. collaris* by analysis of variance with larval type as a fixed factor, species as a random factor, and with species nested within larval type. Analyses of variance were followed by Tukey's HSD post-hoc tests for larval type when differences were significant (Zar, 1999; SPSS 14.0). Band-length- specific and protein-content-specific clearance rates were also tested in this way.

Results

For the three larval types tested, clearance rates generally increased with growth in mass, band length, and protein content. The highest clearance rates were observed in the tornaria-type bipinnaria larvae of *Dermasterias imbricata*, followed by plutei of *Strongylocentrotus purpuratus* and mitrariae of *Owenia collaris* (Fig. 2.1). The trochophore-type larvae of *Mytilus californianus* and *Serpula vermicularis* cleared particles at much lower rates. However, tornaria-type and mitraria larvae grew to much larger sizes and differences in weight-specific clearance rates among larval types were not significant (Fig. 2.2; Table 2.2).

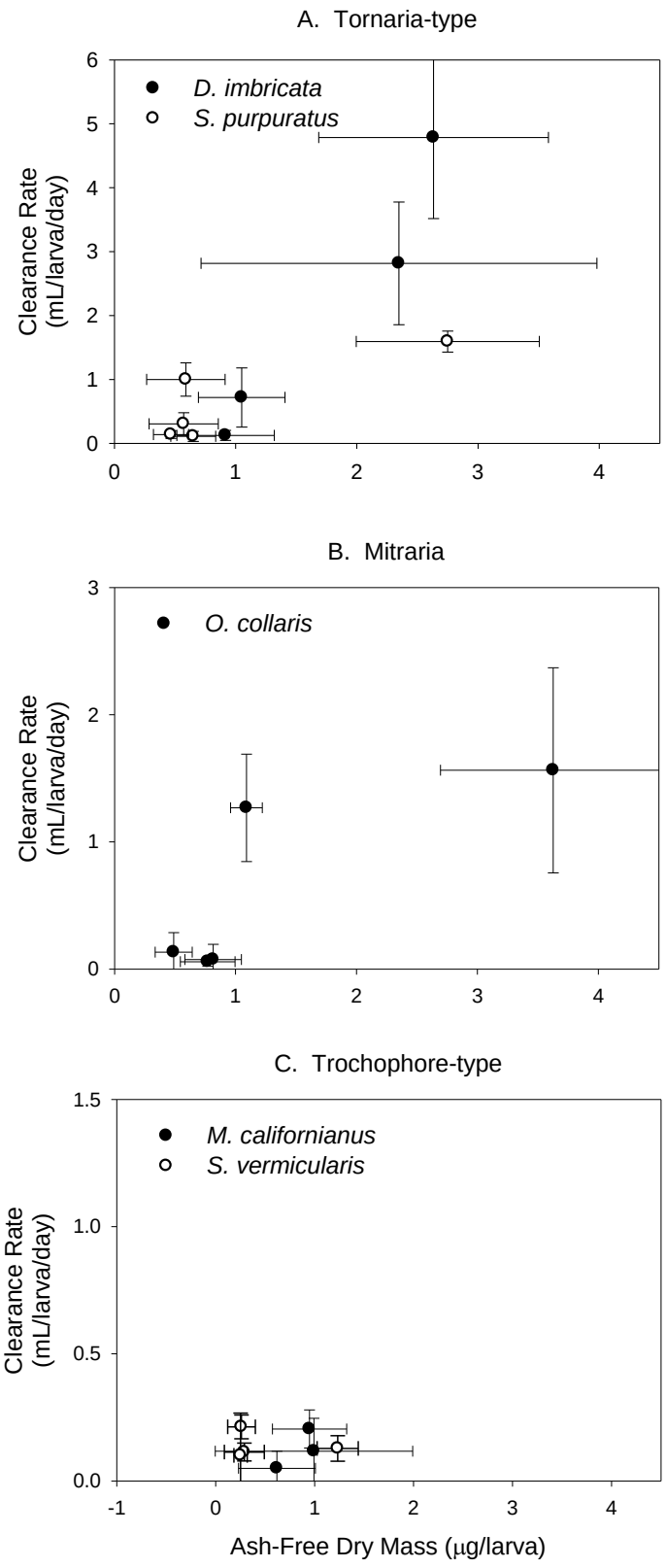

Figure 2.1. Clearance rates of (A) tornaria-type (*Dermasterias imbricata* and *Strongylocentrotus purpuratus*), (B) mitraria (*Owenia collaris*), and (C) trochophore-type (*Mytilus californianus* and *Serpula vermicularis*) larvae. Clearance rates and ash free dry masses were measured for each species over the course development for larvae randomly selected from 5 replicate culture jars. Data points represent means with standard deviations. Note the differences in y-axis scale between graphs.

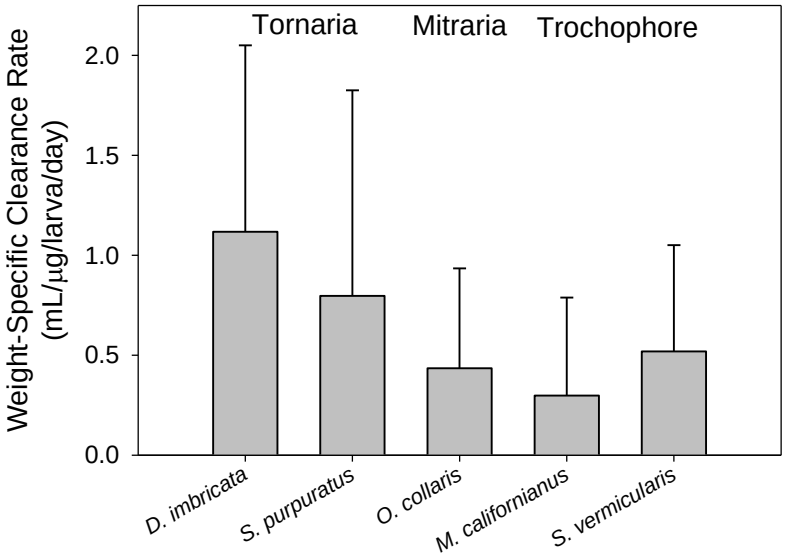

Figure 2.2. Weight-specific clearance rates of tornaria-type (*Dermasterias imbricata* and *Strongylocentrotus purpuratus*), mitraria (*Owenia collaris*), and trochophore-type (*Mytilus californianus* and *Serpula vermicularis*) larvae. Clearance rates and ash-free dry masses were measured for each species over the course of development for larvae randomly selected from 5 replicate culture jars. Bars represent means with standard deviations. Sample sizes range from 15 to 25.

Table 2.2. Results of 2-way nested ANOVA with larval type as a fixed factor and species as a random factor to test for differences in weight-specific clearance rates for tornaria-type (*Dermasterias imbricata* and *Strongylocentrotus purpuratus*), mitraria (*Owenia collaris*), and trochophore-type (*Mytilus californianus* and *Serpula vermicularis*) larvae.

Source	**df**	**SS**	**MS**	**F**	**p**
Larval Type	2	7.357	3.679	4.588	0.201
Species (Larval Type)	2	1.562	0.781	1.379	0.257
Error	100	56.639	0.564		

Tornaria-type and mitraria larvae developed much longer and more convoluted ciliated bands than the trochophore-type larvae during development. Weight-specific band lengths were not significantly different among larval types (Fig. 2.3, Table 2.3). Ciliated bands in all three larval types developed proportionally to the body size of the

larva. Clearance rates increased proportionally to the band length in the tornaria-type and mitraria larvae, but the relationship between these two factors was much less obvious in the trochophore-type larvae, probably due to their relatively small size (Fig. 2.4). Band-length-specific clearance rates did not differ significantly among larval types (Fig. 2.5, Table 2.3).

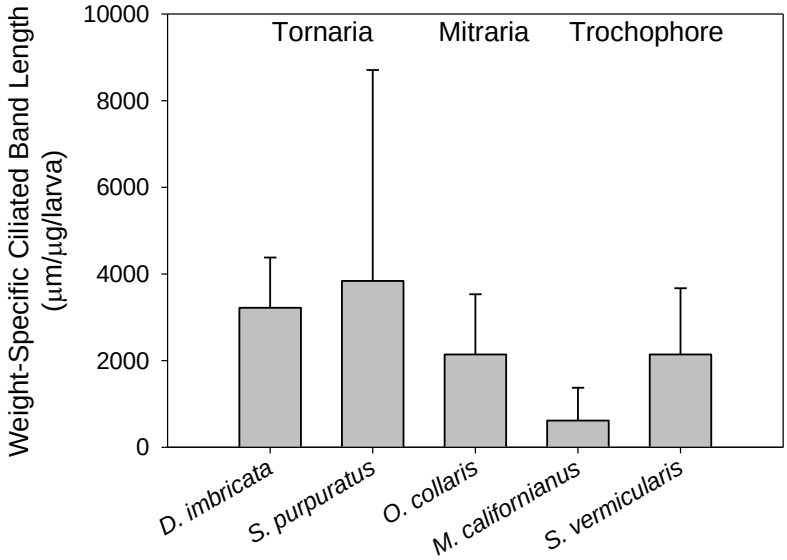

Figure 2.3. Weight-specific ciliated band lengths of tornaria-type (*Dermasterias imbricata* and *Strongylocentrotus purpuratus*), mitraria (*Owenia collaris*), and trochophore-type (*Mytilus californianus* and *Serpula vermicularis*) larvae. Ciliated band lengths and ash-free dry masses were measured for each species over the course of development for larvae randomly selected from 5 replicate culture jars. Bars represent means with standard deviations. Sample sizes range from 15 to 25.

Table 3. Results of 2-way nested ANOVAs with larval type as a fixed factor and species as a random factor and to test for differences in (A) weight-specific band length (B) ciliated-band-length-specific clearance rates for tornaria-type (*Dermasterias imbricata* and *Strongylocentrotus purpuratus*), mitraria (*Owenia collaris*), and trochophore-type (*Mytilus californianus* and *Serpula vermicularis*) larvae.

A. Weight-specific band length ANOVA

Source	**df**	**SS**	**MS**	**F**	**p**
Larval Type	2	9.33E07	4.67E07	3.693	0.230
Species (Larval Type)	2	2.42E07	1.21E07	1.751	0.179
Error	100	6.93E08	6.93E06		

B. Band-length-specific clearance rate ANOVA

Source	**df**	**SS**	**MS**	**F**	**p**
Larval Type	2	5.44E-07	2.72E-07	8.857	0.177
Species (Larval Type)	2	6.99E-08	3.50E-08	0.443	0.643
Error	100	7.89E-06	7.89E-04		

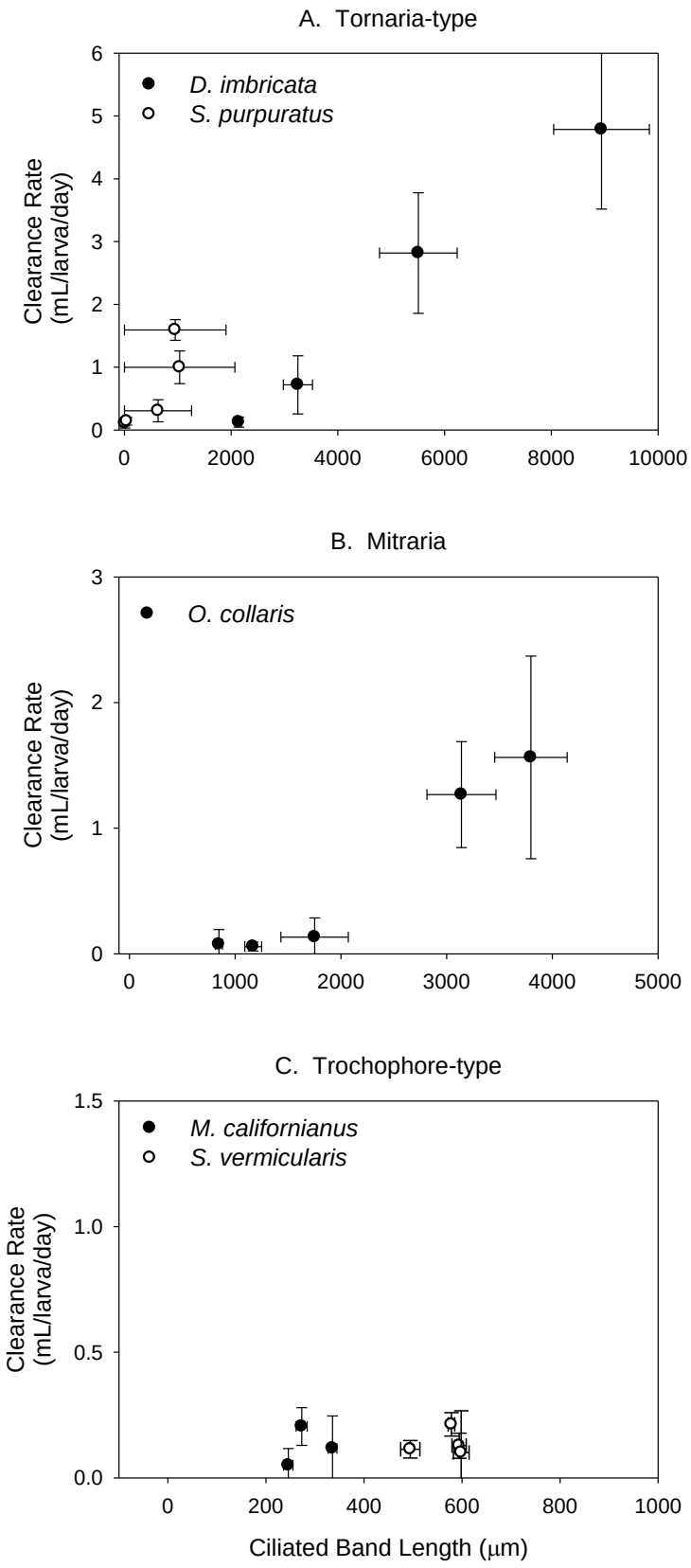

Figure 2.4. Ciliated band lengths of (A) tornaria-type (*Dermasterias imbricata* and *Strongylocentrotus purpuratus*), (B) mitraria (*Owenia collaris*), and (C) trochophore-type (*Mytilus californianus* and *Serpula vermicularis*) larvae. Ciliated band lengths and clearance rates were measured for each species over the course of development for larvae randomly selected from 5 replicate culture jars. Data points represent means with standard deviations. Note the differences in scale between graphs.

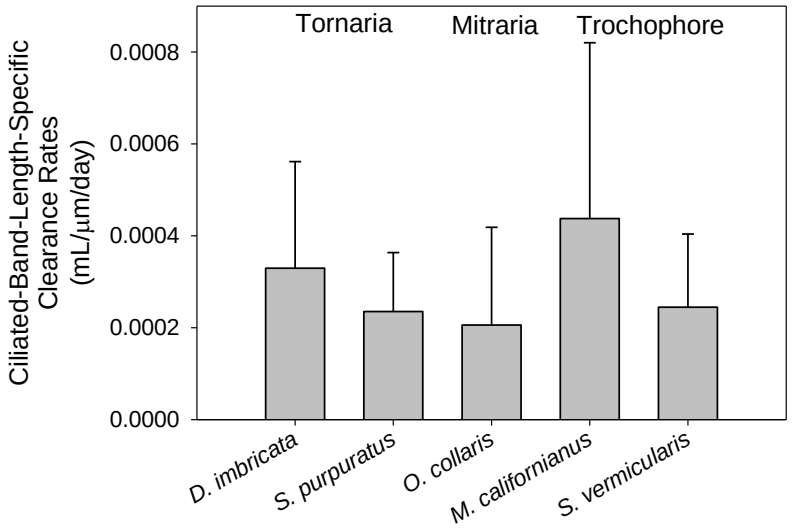

Figure 2.5. Ciliated-band-length-specific clearance rates of tornaria-type (*Dermasterias imbricata* and *Strongylocentrotus purpuratus*), mitraria (*Owenia collaris*), and trochophore-type (*Mytilus californianus* and *Serpula vermicularis*) larvae. Ciliated band lengths and clearance rates were measured for each species over the course of development for larvae randomly selected from 5 replicate culture jars. Bars represent means with standard deviations. Sample sizes range from 15 to 25.

Larval protein content increased as all larval types developed, but, again, tornaria-type and mitraria larvae possessed much higher amounts of protein than trochophore-type larvae later in development. Weight-specific protein content was not significantly different among larval types (Fig. 2.6, Table 2.4). For tornaria-type and mitraria larvae, the relationship between protein content and clearance rate was similar in shape to the relationship between protein content and ash-free dry mass (Fig. 2.7). Higher protein content did not coincide necessarily with high clearance rates in the trochophore-type larvae. Protein-specific clearance rates were similar among larval types, although rates of tornaria-type larvae tended to be higher than those of either mitraria or trochophore-type larvae (Fig. 2.8, Table 2.4). A summary of all results can be found in Table 2.5.

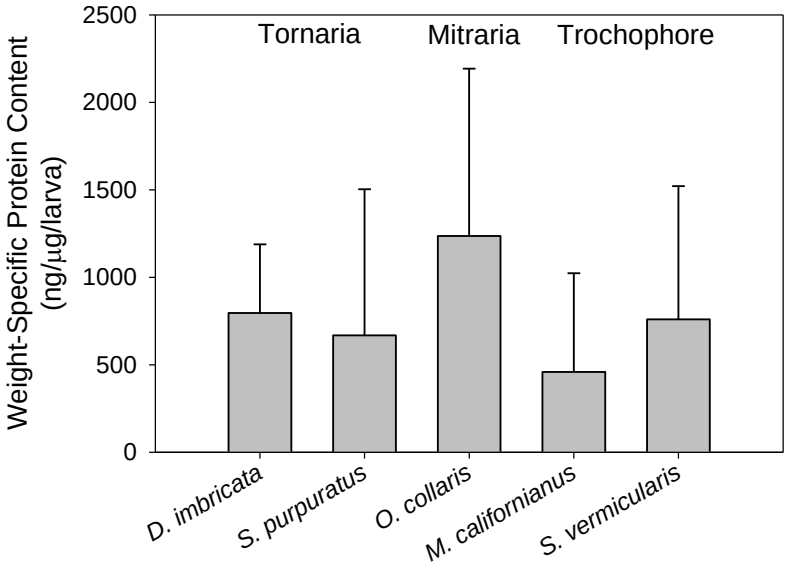

Figure 2.6. Weight-specific protein content of tornaria-type (*Dermasterias imbricata* and *Strongylocentrotus purpuratus*), mitraria (*Owenia collaris*), and trochophore-type (*Mytilus californianus* and *Serpula vermicularis*) larvae. Protein content and ash-free dry masses were measured for each species over the course of development for larvae randomly selected from 5 replicate culture jars. Bars represent means with standard deviations. Sample sizes range from 15 to 25.

Table 2.4. Results of 2-way nested ANOVAs with larval type as a fixed factor and species as a random factor to test for differences in (A) weight-specific protein content and (B) protein-content-specific clearance rates for tornaria-type (*Dermasterias imbricata* and *Strongylocentrotus purpuratus*), mitraria (*Owenia collaris*), and trochophore-type (*Mytilus californianus* and *Serpula vermicularis*) larvae.

A. Weight-specific protein content ANOVA

Source	**df**	**SS**	**MS**	**F**	**p**
Larval Type	2	6.18E06	3.09E06	6.579	0.169
Species (Larval Type)	2	9.58E05	4.79E05	0.839	0.435
Error	100	5.71E07	5.71E05		

B. Protein-content-specific clearance rate ANOVA

Source	**df**	**SS**	**MS**	**F**	**p**
Larval Type	2	2.66E-05	1.33E-05	31.173	0.094
Species (Larval Type)	2	1.01E-06	5.04E-07	0.385	0.681
Error	100	0.000	2.00E-07		

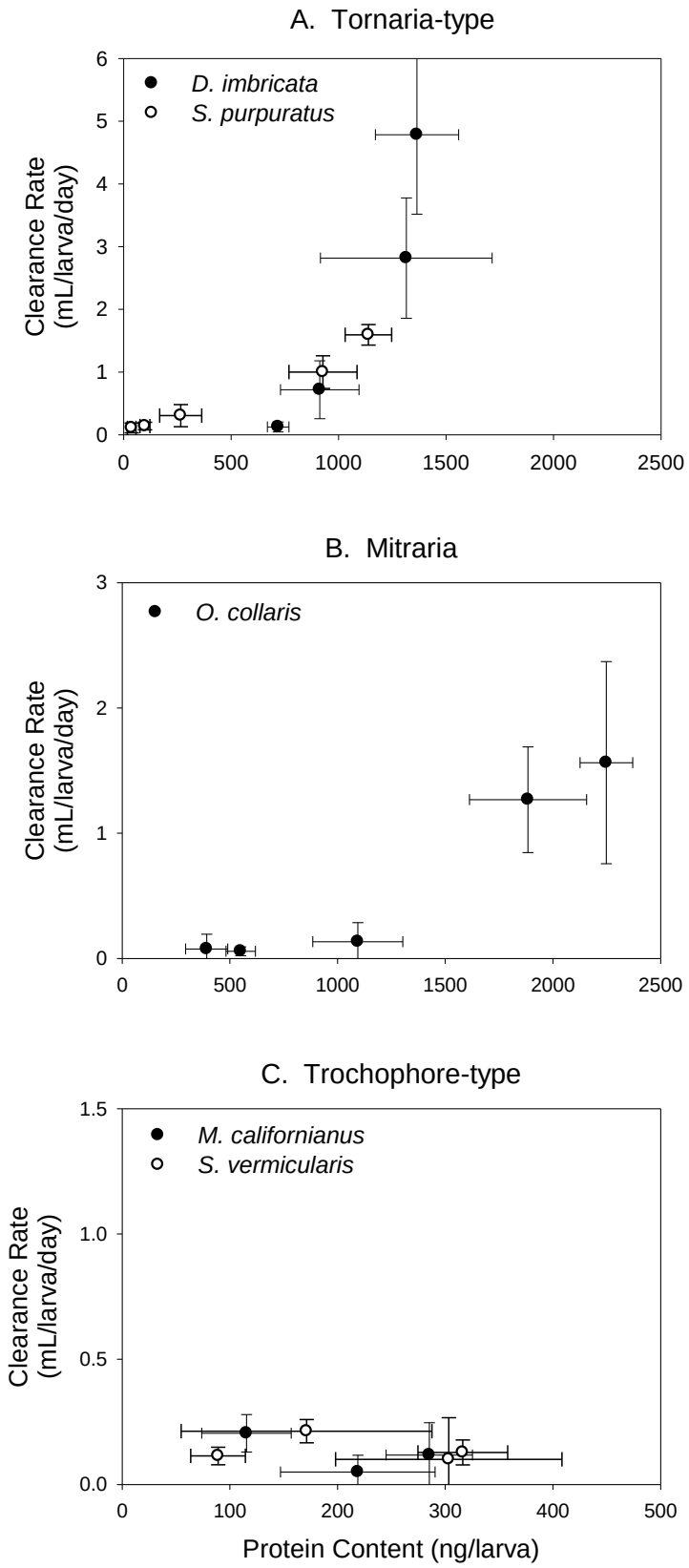

Figure 2.7. Protein content of (A) tornaria-type (*Dermasterias imbricata* and *Strongylocentrotus purpuratus*), (B) mitraria (*Owenia collaris*), and (C) trochophore-type (*Mytilus californianus* and *Serpula vermicularis*) larvae. Protein content and clearance rates were measured for each species over the course of development for larvae randomly selected from 5 replicate culture jars. Data points represent means with standard deviations. Note the differences in scale between graphs.

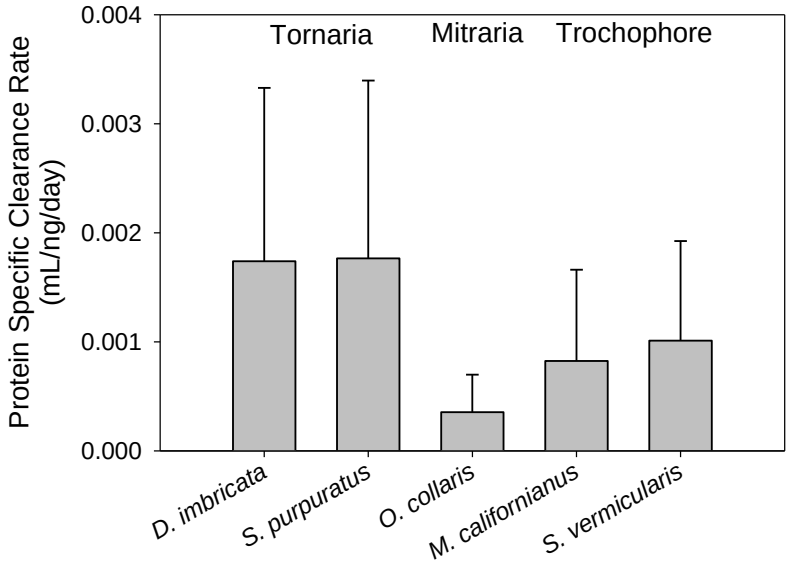

Figure 2.8. Protein-content-specific clearance rates of tornaria-type (*Dermasterias imbricata* and *Strongylocentrotus purpuratus*), mitraria (*Owenia collaris*), and trochophore-type (*Mytilus californianus* and *Serpula vermicularis*) larvae. Protein content and clearance rates were measured for each species over the course of development for larvae randomly selected from 5 replicate culture jars. Bars represent means with standard deviations. Sample sizes range from 15 to 25.

Discussion

The search for differences and similarities in the feeding abilities of different larval forms allows us to test general hypotheses about the connection between larval form, functional morphology and components of fitness in species with complex life cycles that include feeding larvae (Hart, 1996). Selection for efficient feeding in planktotrophic larvae is driven by the need to reduce mortality by either shortening planktonic periods or increasing size in the larval stage and at metamorphosis (Havenhand, 1995; Hart and Strathmann, 1995; Sogard, 1997). Among the species investigated, tornaria-type and mitraria larvae achieve higher clearance rates, masses, ciliated band lengths, and protein contents than trochophore-type larvae. Clearance rates

of fully developed tornaria-type and mitraria larvae are 6-18 times higher than those of fully developed trochophore-type larvae and ash free dry masses 2.5-3.5 times higher. Ciliated bands of fully developed tornaria-type and mitraria larvae were 3-20 times longer than those of trochophore-type larvae. Long, convoluted bands, despite being composed of short, simple cilia, provide greater particle capture ability than the circular, multiciliated bands tested here and are strongly associated with the larger body sizes found in tornaria-type and mitraria larvae.

The data collected for clearance rates and ash-free dry masses in this study (Table 2.5) are comparable to those obtained by other workers with the same larval types. Bipinnaria larvae of *Dermasterias imbricata* cleared particles at rates ranging from 0.1 to 4.8 mL/larva/day at sizes ranging from 0.9 to 2.6 μg/larva (Fig. 1), compared to bipinnaria larvae of *Acanthaster planci,* which cleared particles at a rate of 1.9 mL/larva/day at a size of 0.9 μg (Lucas, 1982). Echinopluteus larvae of *Strongylocentrotus purpuratus* cleared particles at rates ranging from 0.1 to 1.6 mL/larva/day at sizes of 0.5 to 2.8 μg/larva and (Fig. 2.1), compared to *Paracentrotus lividus*, which cleared particles at a rate of 0.9 mL/larva/day at 0.2 μg/larva (Strathmann, 1975; Fenaux *et al*., 1985). Veligers of *Mytilus californianus* cleared particles at 0.1 to 0.2 mL/larva/day at 0.6 to 1.0 μg/larva (Fig. 2.1). Other bivalve veligers investigated to date achieved clearance rates ranging from 0.05 to 4.1 mL/larva/day at sizes from 0.04 to 0.86 μg/larva (Walne, 1965; Gallager and Mann, 1980; Riisgard et al., 1981; Jespersen and Olsen, 1982; Gerdes, 1983; Sprung, 1984; Mann and Gallager, 1985). By comparison, gastropod veligers, which were generally larger, cleared particles at rates

ranging from 0.1 to 3.7 mL/larva/day and weighed between 0.6 and 5.0 µg/larva (Pechenik and Fisher, 1979; Pechenik, 1980; Hansen and Ockelmann, 1991). There are currently no other data available for clearance rates and masses of other polychaete larvae, but clearance rates and sizes of trochophores of *Serpula vermicularis* and mitraria of *Owenia collaris* fall within the ranges of all other larval forms.

Table 2.5. Summary of ash-free dry mass, clearance rate, ciliated band length, and protein content for tornaria-type (*Dermasterias imbricata* and *Strongylocentrotus purpuratus*), mitraria (*Owenia collaris*), and trochophore-type (*Mytilus californianus* and *Serpula vermicularis*) larvae measured in the current study. Values are means of 5 replicate samples.

Species	**AFDM (µg/larva)**	**Clearance Rate (mL/larva/day)**	**Band Length (µm/larva)**	**Protein Content (ng/larva)**
D. imbricata	0.92	0.12	2144.	718.8
	1.05	0.72	3251.	912.5
	2.34	2.82	5507.	1315.
	2.63	4.78	8940.	1364.
S. purpuratus	0.47	0.14	619.2	99.1
	0.57	0.31	2075.	265.6
	0.58	1.00	4195.	927.2
	0.65	0.11	344.5	37.4
	2.75	1.59	7413.	1139.
O. collaris	0.49	0.13	1751.	1884.
	0.77	0.06	1170.	550.2
	0.82	0.08	845.3	392.4
	1.09	1.27	3410.	1094.
	3.63	1.56	3797.	2248.
M. californianus	0.62	0.05	245.6	218.8
	0.95	0.20	272.9	115.4
	0.99	0.12	335.8	285.2
S. vermicularis	0.25	0.10	598.4	303.3
	0.26	0.21	578.4	171.2
	0.29	0.11	494.5	89.1
	1.23	0.13	594.1	316.3

The capture mechanism, i.e. single or opposed band, does not appear to affect either larval size or clearance rate. Mitraria larvae of *Owenia collaris* capture particles

with the opposed-band system using short, simple cilia, yet they clear particles at rates comparable to those of echinoplutei of *Strongylocentrotus purperatus* or bipinnaria of *Dermasterias imbricata,* both of which use a single-band system with short, simple cilia (Fig. 1). The trochophore-type larvae of both *Mytilus californianus* and *Serpula vermicularis*, on the other hand, appear to be limited in their clearance rates by their small size, not by their use of the opposed band system. Particle velocities measured in other opposed-bands of larvae and rotifers are generally higher than those of either mitraria or of single-band systems (Strathmann *et al*., 1972; Strathmann and Leise, 1979; Emlet 1983; McEdward and Strathmann, 1987; Gallager, 1988; Emlet, 1990; Emlet and Strathmann, 1994). Based on these velocities, opposed bands should produce higher clearance rates per unit length of ciliated band (Strathmann and Leise, 1979; Gallager, 1988). There is little evidence of this in the species tested in this study, although band-length-specific clearance rates tended to be higher in *M. californianus* than in the other species tested (Fig. 2.5). It is possible that the trochophore-type species tested here simply do not increase cilium length as much as other, larger species of veliger or setiger. These larvae also retain the circular ciliated band throughout development, rather than producing convoluted bands similar to those developed by larger veligers or rostraria larvae. Gastropod veligers with more convoluted bands clear particles at higher rates than *M. californianus* or *S. vermicularis* (Pechenik, and Fisher, 1979; Pechenik, 1980), further supporting the notion that opposed band systems are capable of creating feeding currents comparable to those of tornaria-type larvae. The cumulative effects of longer ciliated bands in tornaria-type and mitraria larvae (and larger veligers) overcome slower

particle velocities associated with short, simple cilia. High clearance rates are produced by both systems.

Other workers have found high variation in clearance rates within a given particle capture mechanism. Hart (1996) found that within the different echinoderm larval forms that possess the single band system, bipinnariae (asteroids) and auricullariae (holothuroids) clear particles at higher rates than either ophioplutei (ophiuroids) or echinoplutei (echinoids), although these rates were not corrected for larval size (Hart and Strathmann, 1995). Differences in the density of cilia within the ciliated bands in these groups accounted for differences in clearance rates. Hart (1996) proposed that ciliated bands of bipinnariae and auricullariae produced high clearance rates because they had more cilia per unit band length and could thus create faster currents. Of larvae with the opposed-band system, clearance rates of gastropod veligers vary with the length of prototroch cilia, not just with body size or band length (Strathmann and Leise, 1979; Gallager, 1988). It appears that across all larval forms investigated (bipinnaria, pluteus, veliger, trochophore, mitraria, etc.) the strongest relationship to clearance rate is larval body size and that variation in clearance rate within groups can be explained by the adjustments allowed within the different capture mechanisms (i.e. cilium length, cilium density, ciliated band length). There is currently no evidence to suggest inherent differences in the potential clearance rates or larval sizes that can be achieved by the two different capture mechanisms or lineages, since larvae with both capture mechanisms achieve large size and feed at comparable levels for their size.

Although Hart (1996) demonstrated that the clearance rates of echinoderm larvae, which possess simple cilia, increase proportionally to the lengths of the ciliated band, he also presented preliminary data suggesting that long ciliated bands are not the only way that different larval forms can compensate for low feeding rates. He hypothesized that different larval forms may take several different routes to increase their size at metamorphosis and to decrease the length of the larval period. Larvae may adjust their metabolic rates or the relative amount of metabolically active tissues as a way to compensate for low clearance rates. This may allow larvae with low clearance rates to develop and metamorphose faster than if they relied solely on food assimilation. Hart (1996) further suggested that larvae that employ morphological and physiological methods to maximize feeding efficiency could metamorphose sooner and at larger sizes than other larvae even if they start with the same egg size.

Protein content was used in this study as a proxy for the amount of metabolically active tissue in each species examined. Protein content, and therefore metabolic demand, increased dramatically along with clearance rate over the course of development for tornaria-type and mitraria larvae (Fig. 2.7). The trochophore-type larvae tested here do not reach such large sizes, and so metabolic demand and clearance rates remained low. The larger tornaria-type and mitraria larvae appear to maximize feeding rates for their high metabolic demand through the use of larger and more convoluted ciliated bands, meeting the demands of large larval bodies and juvenile tissues. However, clearance rates achieved by mitraria larvae do not increase as quickly as one would assume should be necessary to meet their increasing metabolic demands.

Mitraria larvae tended to contain more protein for their mass relative to the other two larval types investigated. Mitraria larvae develop two structures that probably account for their relatively higher protein content compared to other larval forms: the larval chaetal sac and the juvenile rudiment. The larval chaetal sac consists of muscle fibers arranged in a figure-eight pattern around two chaetal glands, which are used to erect chaetae when larvae are disturbed. The advanced juvenile rudiment possesses all of the segmental musculature of the juvenile worm within the larval hyposphere. Ciliated band growth and clearance rates of mitraria larvae may not be able to meet the metabolic demand from these highly muscular structures. Since the juvenile structures are not active in the larval stage, however, metabolic demand may not be as high as would be predicted by protein content alone.

Clearance rates do not scale isometrically with body volume, or presumably with metabolic demand, for other large polychaete larvae (Miner *et al*., 1999). Most polychaete larvae grow by posterior elongation, producing long-bodied larvae with relatively small heads and small, circular ciliated bands (Bhaud and Cazaux, 1987). This allometric relationship between volume and clearance rate may be unfavorable in these forms, either limiting further growth or causing larvae to develop alternative food-capture mechanisms. Mitraria larvae may be constrained by phylogeny in that the allometry between clearance rate and body size, or metabolic demand, becomes unfavorable to continued growth at large sizes. This, in turn, may have driven the evolution of a convoluted ciliated band to help offset this relationship. Perhaps this also drove the evolution of the internalized juvenile rudiment found in mitraria, which would reduce

drag as larvae move through the water column and minimize interference with flow around the ciliated band. The amphinomid rostraria larva, which extends its ciliated bands along anterior palps (Järgersten, 1972), and the endo-larva of *Polygordius appendiculata*, in which the juvenile trunk develops within the larval episphere (Cowles, 1903), may be other examples of larval forms that have evolved specialized morphology in order to cope with the unfavorable feeding allometry within the polychaetes.

The current research supports the hypothesis that the long, convoluted ciliated band of the mitraria larva is an adaptation to achieve high feeding rates, although the order in which this adaptation has come about is still unknown. The likeliest explanation is that mitraria larvae were constrained to simple cilia during embryonic development (see Ch. 2), which required that this larval form develop some mechanism other than a prototroch with long, compound cilia to allow for sufficient larval feeding rates and development to competent size (Emlet and Strathmann, 1994). Mitraria larvae of *Owenia collaris* develop a juvenile similar in size to that of *Serpula vermicularis* under similar laboratory conditions, but in almost half the number of days required by *S. vermicularis* (Young and Chia, 1982). It is plausible that the convoluted ciliated band either allowed for relatively fast growth rates or was produced by evolutionary pressure to decrease development time in the plankton. Together, evolutionary pressure (or pre-adaptation) along with the development of primarily simple cilia produced a larval form highly reminiscent of, and convergent upon, the tornaria-type larvae found among hemichordates and echinoderms in morphology. In many respects, though, mitraria larvae are a combination of morphology and performance of both ancestral larval types.

For all larval types, patterns in feeding performance are the result of continued larval and band growth not the result of phylogenetic constraints. Continued growth of both ciliated band types (opposed and single) increased feeding performance of larvae tested in this study and others. This increase overcame restrictions in cilia morphology imposed by phylogeny and development.

BRIDGE II

During the investigations of embryogenesis and larval feeding, embryonic and larval material for *Owenia collaris* could only be obtained during the spring and summer months. Wilson (1932) also noted that *Owenia fusiformis* produced gametes only during the spring and summer months in Plymouth, U.K. The seasonal reproduction and the environmental factors that control this seasonality were investigated in Chapter IV using field and laboratory techniques.

CHAPTER IV

ENVIRONMENTAL CUES AND THE REPRODUCTIVE CYCLE OF A TEMPERATE POPULATION OF *OWENIA COLLARIS* (ANNELIDA: POLYCHAETA)

1. Introduction

Reproduction of most marine organisms is highly dependent on environmental factors. Beginning with Orton (1920), temperature has often been invoked as the primary influence determining onset of breeding, length of breeding period, and breeding success. Over time, more and more authors have recognized the importance of other environmental factors as well, either due to their interactions with temperature or their influence on reproduction independent of temperature (Giese and Kanatani, 1987). In reviewing reproductive cycles of polychaetes and other marine invertebrates, Olive (1995) delineated several exceptions to Orton's Rule of temperature dominance. Nutrient availability, photoperiod, endogenous long-term interval timers, and gamete growth rates can override or modulate reproductive responses to temperature (Tenore, 1977; Olive, 1980; Garwood and Olive, 1982; Clark, 1988; see Table 3.1 for details). Schroeder and Hermans (1975) also documented the effect of salinity on gametogenesis for several polychaete species. Within habitats such as estuaries, a host of environmental variables vary seasonally and are likely to affect phenology and success of reproduction in invertebrates. However, the effects of these variables are mostly untested.

Table 3.1. Summary of cues for onset of gametogenesis and or spawning in seasonally reproducing temperate polychaete worms. Documentation of patterns involved the use of correlation studies, isochronic experiments (experimentally examining the effects of environmental variables during the species reproductive season) or heterochronic experiments (experimentally examining the effects of environmental variables out of the species' reproductive season) experiments (Olive, 1984).

Species	**Family**	**Cues Tested**	**Evidence**	**Source**
Arenicola marina	Arenicolidae	air temperature	isochronic	Duncan, 1960
Bispira volutacornis	Sabellidae	water temperature	correlation	Nash and Keegan, 2003
Ditrupa arietina	Serpulidae	water temperature chlorophyll a	correlation	Charles et al., 2003
Capitella sp.	Capitellidae	water temperature	correlation	Tenore, 1977
Eulalia viridis	Phyllodocidae	water temperature	isochronic	Garwood and Olive, 1982
Harmothoe imbricata	Polynoidae	photoperiod	isochronic	Garwood and Olive, 1982; Clark, 1988
Hydroides dianthus	Spirorbidae	water temperature	heterochronic	Turner and Hanks, 1960
		water temperature	heterochronic	Olive, 1984
Kefersteinia cirrata	Hesionidae	photoperiod water temperature	isochronic	Olive and Pillai, 1983
Marenzelleria viridis	Spionidae	salinity	correlation	Bochert et al., 1996
		water temperature	correlation	Daunys et al., 2000
Nereis diversicolor	Nereidae	water temperature	isochronic; heterochronic	Olive, 1984
		Salinity	correlation	Gasiunas, 1956; Bogucki, 1963
Platynereis dumerillii	Nereidae	lunar cycle	isochronic	Hauenschild, 1955, 1956, 1960
Phragmatopoma lapidosa	Sabellariidae	wave disturbance photoperiod	isochronic correlation	McCarthy et al., 2003
Typosyllis prolifera	Syllidae	photoperiod water temperature lunar cycle	heterochronic	Franke, 1980

In polychaetes, phenology can be divided into two broad categories, non-seasonal and seasonal. Non-seasonal species are those that release gametes throughout the year or do not respond to seasonally varying cues. Within seasonally reproducing species, there is a range of reproductive strategies (Olive and Clark, 1978). Generally, seasonal species have developed reproductive systems in which either gametogenesis or spawning is strongly synchronized within a population or individuals are able to store and maintain gametes until the appropriate time. Olive et al. (2000) noted that two types of factors could maintain synchronicity, ultimate and proximate. Ultimate factors are evolutionary determinants that give a fitness advantage to animals with a particular spawning periodicity. Crisp's Rule is a commonly cited ultimate cause in which larval success (higher food availability, maximum opportunity for growth, favorable oceanography for recruitment) favors spring reproduction in the Northern Hemisphere (Crisp, 1954; Qasim, 1956; Crisp, 1959). Addition of adult food does not necessarily lead to out-of-season breeding in temperate invertebrates governed by Crisp's Rule because breeding is not controlled by immediate conditions but by those operating over a long period beforehand (Crisp and Klegg, 1960; Barnes, 1963; Crisp, 1966; Crisp and Patel, 1969). Selective pressures associated with fertilization and fecundity may favor seasonality (synchronicity provides better chances of fertilization and predator swamping; reviewed by Olive et al., 2000). Proximate factors are environmental inputs that are transduced by an organism allowing physiological responses to occur that organize a temporal sequence of cellular and physiological events that culminate in spawning. Within the polychaetes, proximate

factors vary considerably among and within taxonomic groupings, but the most common are temperature, photoperiod, and lunar cycle.

Seasonal reproduction appears to have adaptive advantages in temperate waters, but the cues that entrain this seasonality vary substantially among taxa (Giese and Kanatani, 1987; Olive et al., 2000). In the sabellid polychaete *Bispira volutacornis*, vitellogenesis occurs in response to the spring phytoplankton bloom whereas spawning is associated with the autumn bloom (Nash and Keegan, 2003). In this species, adults and larvae take advantage of seasonal peaks in food availability to complete the life cycle. Peak numbers of recruits of *Marenzelleria viridis* are linked to the autumn phytoplankton bloom in coastal waters of the southern Baltic (Bochert et al., 1996). In the North Atlantic, *Harmothoe imbricata* undergoes gametogenesis in the winter when low temperatures and lengthening days induce oocyte growth. Summer temperatures above 15ºC prevent vitellogenesis (Olive, 1984). Vitellogenesis is suppressed or prevented in *Eulalia viridis* below 5ºC but occurs independently of day length (Olive, 1984). Gametogenesis and spawning in the hesionid *Kefersteinia cirrata* and the nereid *Nereis virens* are controlled synergistically by temperature and day length (Olive and Pillai, 1983; Olive, 1995), whereas vitellogenesis in *Nereis diversicolor* is not affected by temperature or day length (Olive, 1984). In each of these species, synchronicity probably allows animals to take advantage of food availability, amenable water conditions, and availability of mates.

Relatively little is known about the phenology of breeding in the polychaete family Oweniidae. Seasonal reproduction has been documented for populations of

Owenia fusiformis in Plymouth, U.K. (Wilson, 1932) and the Bay of Seine, France (Menard et al., 1989) where reproductive adults and larvae are found in the spring and summer months. In contrast, a population of *O. fusiformis* from Biscayne Bay, Florida, was thought to have continuous or non-seasonal reproduction since adults with mature gametes were found in all months except June, September, and December (McNulty and Lopez, 1969). Seasonal cycles in environmental factors are not as dramatic in subtropical Florida as they are in Europe. It may be that these distinct populations vary in reproductive seasonality because the environmental cues that drive seasonality in Europe are not present at sufficient levels in Florida. However, the causes underlying these latitudinal differences remain a matter of speculation, since the relationship between breeding and environmental conditions has not been specifically addressed with experimental techniques. Moreover, reproductive traits have not been documented in any other species or populations within the family Oweniidae.

In this study, I chronicled the breeding season of a temperate population of *Owenia collaris* (Hartman, 1955) and identified the environmental factors that most influence this timing. Adults feed on surface sediments and the associated bacterial and algal communities. Gametes are free-spawned into the water column and feeding larvae develop for three to four weeks in the plankton, depending on food and temperature (Chapter 2). By reproducing seasonally in the spring and summer months, this species may be able to take advantage of the spring phytoplankton bloom either as adults or as larvae, as well as the presence of marine-dominated waters in the estuary and calmer conditions for establishment of new recruits. I report evidence for seasonal reproduction

associated with environmental fluctuations and give the results of a controlled laboratory experiment examining potential proximate factors organizing the seasonal breeding.

2. Materials and Methods

2.1. Natural Reproductive Cycle

Each month during one of the spring tide series, adult *Owenia collaris* were collected from Coos Bay (N 43º20, W 124º19) from March 2005 through February 2007 (Figure 3.1). Twenty to thirty animals were collected each month and preserved with 10% formalin buffered with seawater for 24 hours, then transferred to 70% EtOH. After preservation, worms were removed from their tubes and examined under a dissecting microscope for the presence of identifiable gametes (oocytes or spermatozoa) (Figure 3.2). Males can be distinguished from females by loops of gonadal tissue in the third and fourth segments, as well as sperm bundles free in the coelom. Female gonads are not so organized. Eggs are present all along the worm from the third segment to the pygidium. Gender and the presence or absence of gametes was scored for each individual collected each month. At the same time as the preserved collections were made, several live animals were examined for gametes. When gametes were present, eggs and sperm were dissected out of the worm and used to check for viability and fertilization success. In all samples in which gamete type could be identified, at least some gametes from each worm proved to be mature and viable. All animals with identifiable gametes are therefore referred to as "reproductive". A one-way ANOVA was used to examine differences in percent reproduction among months with month as a fixed factor. A Chi^2 Goodness of

Fit Test was used to test whether the observed sex ratio found in the field was different from the expected 50:50 ratio.

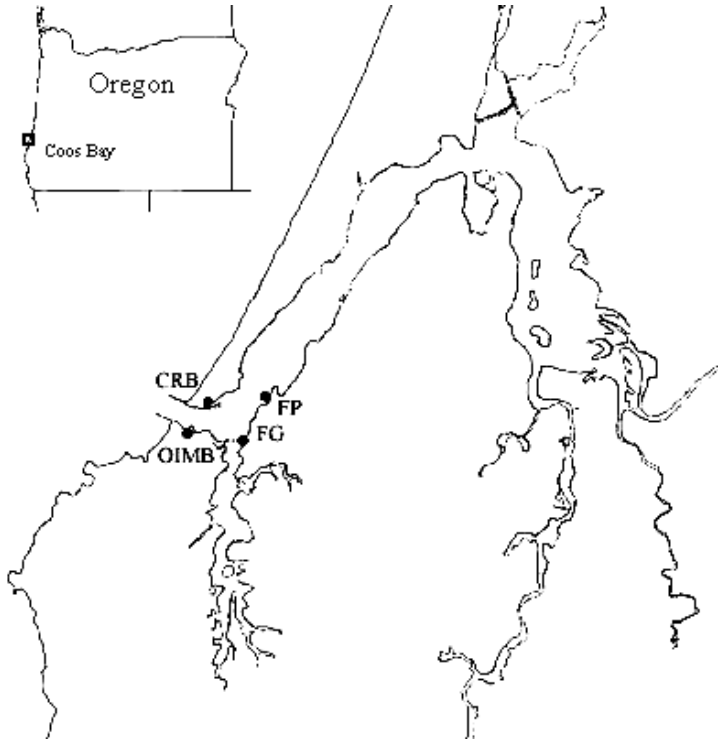

Figure 3.1. Map of Coos Bay Estuary, Coos Bay, Oregon. Worms and benthic chlorophyll samples used in natural reproductive cycle analysis were collected from the Fisherman's Grotto (FG) mudflat. Water quality and meteorological sampling stations maintained by the South Slough National Estuarine Research Reserve are located on the OIMB campus and adjacent to the Fisherman's Grotto mudflat, respectively. Worms used in gametogenesis experiment were collected from the Fossil Point (FP) and the Cribs (CRB) mudflats as well as Fisherman's Grotto.

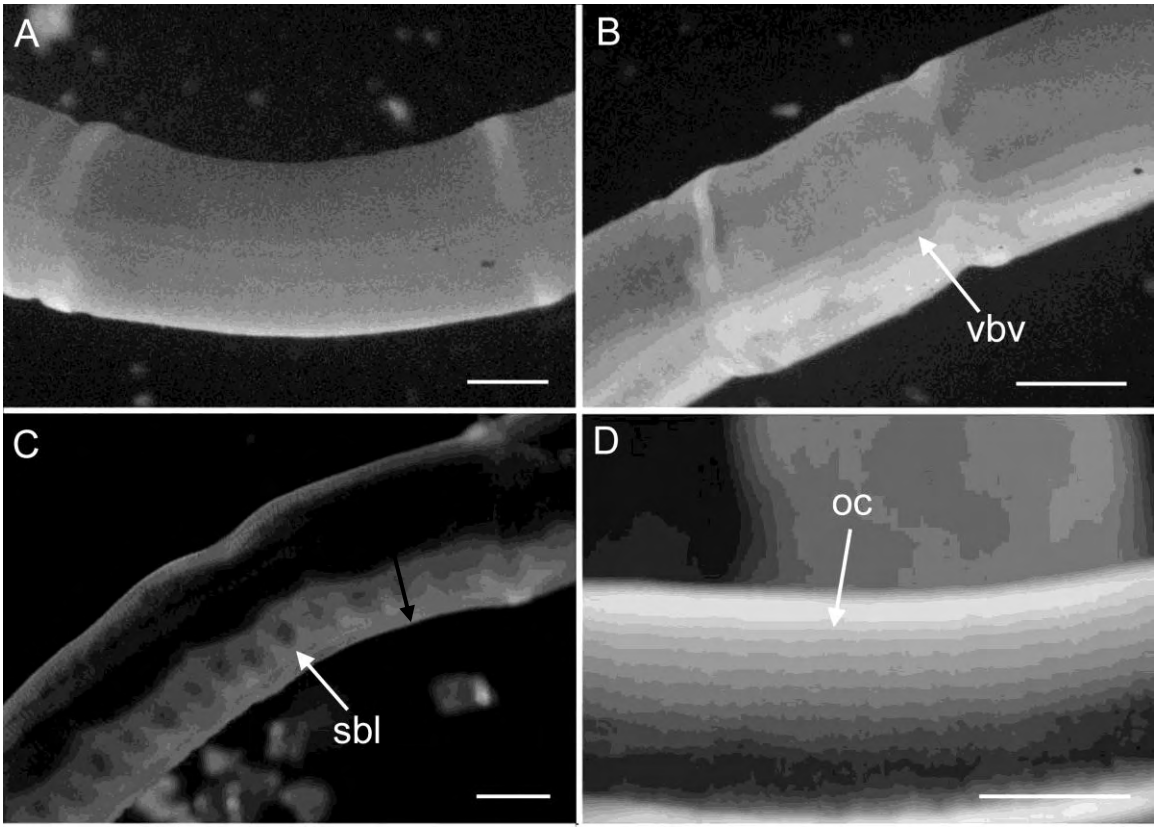

Figure 3.2. Stages of gonad development in *Owenia collaris*. A. Stage 0, where gonad is completely absent from the ventral portion of the abdominal segments. B. Stage 1, gonad appears as an opaque, cream-colored mass along the ventral blood vessel (vbv). C. Stage 2, male gonad fully formed as loops of tightly packed sperm bundles (sbl) in mid region of worm. D. Stage 2, female gonad fully formed with oocytes (oc) tightly packed in abdominal segments or free floating in posterior segments. Scale bars represent 1 mm.

Each month from March 2006 through February 2007, along with worm samples, three replicate sediment cores (0.5 cm depth) were collected using a 5 mL syringe with the end removed. Benthic chlorophyll was extracted with 90% acetone for 24 hrs in the dark and measured with a Turner TD700 Fluorometer (Turner Designs, CA). The percentage of the population with gametes (reproductive individuals) was correlated with benthic chlorophyll estimates over this 12-month period with a Pearson correlation (Quinn and Keough, 2002).

Environmental data were obtained from several online sources. Water quality and meteorological data for the South Slough National Estuarine Research Reserve Charleston sampling station were obtained from the Centralized Data Management Office, NOAA (http://cdmo.baruch.sc.edu/). Day length for the sampling period was calculated from sunrise and sunset data for Coos Bay obtained from the U.S. Naval Observatory (http://aa.usno.navy.mil/). Linear multiple regression was used to test which environmental variables had the greatest impact on reproduction in *O. collaris* (Quinn and Keough, 2002). Variables were chosen based on their lack of colinearity, the completeness of the data set, and Akaike's Information Criterion (AIC; Akaike, 1978; Quinn and Keough, 2002). Environmental variables considered were water temperature, salinity, pH, day length, and water-column chlorophyll. To adjust for colinearity between day length and both water temperature and chlorophyll, the unstandardized residuals of day length were used in this analysis. Data analyzed were mean monthly values for the two years for which worm samples were collected.

Spring transition dates for the Northwest Pacific were obtained from the Columbia River DART program at the University of Washington (http://www.cbr.washington.edu) but were not included in the statistical analysis because the data points are annual, not monthly. Benthic chlorophyll was not used in the linear regression analysis because these data were only collected during the second year of sampling.

2.2. Gonad Development Experiment

The influence of two environmental factors (day length and benthic chlorophyll) on reproductive condition was examined in depth using a controlled laboratory study. In June 2006, I collected 1000 adult *O. collaris* from mudflats within Coos Bay, OR (Figure 3.1). Worms with intact tubes were haphazardly assigned to one of six indoor aquaria at the Oregon Institute of Marine Biology (OIMB), Charleston, OR. These tanks were part of a temperature controlled, recirculating seawater system that was maintained at 12ºC throughout the course of the experiment. This system consisted of six tanks (150L each) pumped through a common titanium-coil seawater chiller set at 12ºC (Figure 3.3). Prior to the addition of worms, native sediment was collected from the same sites as the worms, sieved, rinsed with freshwater, and added to the bottom of the tanks to a depth of 1 cm. Surface sediment samples were also collected prior to the beginning of the experiment and placed in f/2 media (Guillard, 1975) in a 16ºC incubator with 16:8 Light:Dark cycle. The community of phytoplankton and bacteria grown in this manner was added to the sediment in all of the tanks to provide food for the collected worms, which can alternate between surface deposit and suspension feeding (Gambi, 1969).

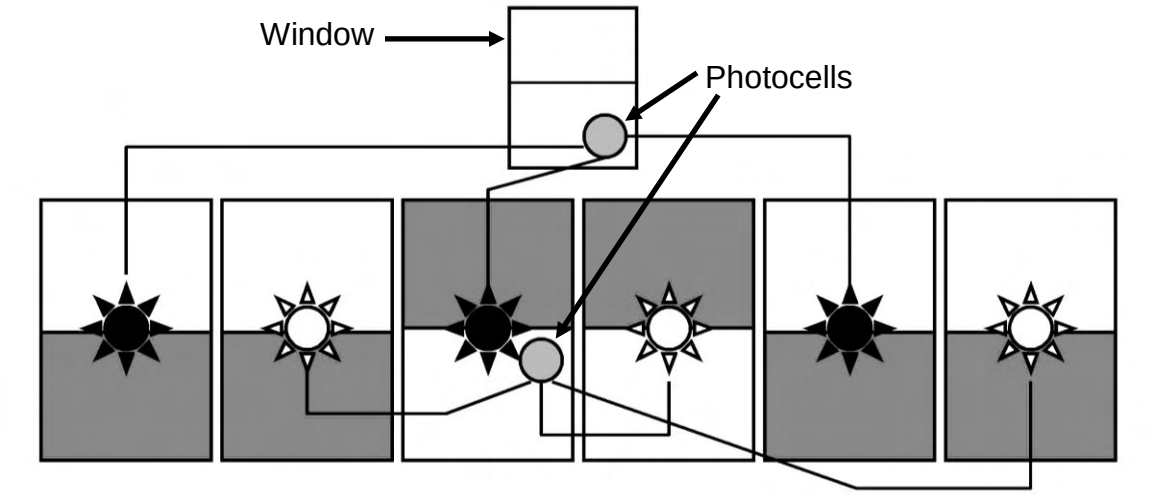

Figure 3.3. Diagram of setup for Gonad Development Experiment. Black suns indicate lights set to the reverse-photoperiod treatment six months out of phase with natural light cycles. Lights above these tanks were wired to a photocell placed in a window adjacent to the tank system. White suns indicate lights set to the in-phase photoperiod treatment that mimics natural light cycles. Lights above these tanks were wired to a photocell placed in view of one of the lights in the reverse treatment. Tanks are divided as described in the methods section into high (dark gray) and low (white) food treatments.

Each tank was darkened on all sides with black plastic and equipped with a light fixture and 15 watt fluorescent bulb. Three tanks were set in phase with the ambient light cycle (in-phase) and the other three tanks were set out of phase with the ambient light cycle (reverse) (Figure 3.3). Light treatment was alternated with every other tank receiving the same treatment. Each set of tanks was wired in series and controlled by a common system of photocells (Bingham, 1997). The first photocell was placed in a window and would cue the lights in the reverse series to turn off at dawn and on at dusk, producing light during nighttime hours. The second photocell was placed beneath one of the lights in the reverse series, and would thus cue the in-phase series to turn on at dawn when the reverse lights went off and to turn off at dusk. This produced an overall light system in which the in-phase treatment mirrored the natural seasonal light regime and the

reverse treatment was 6 months out of phase with the natural seasonal light regime and 12 hours out of phase with the normal daylight hour. For example, in the summer months if there were 15 hours between sunrise and sunset, the in-phase tanks received 15 hours of light and 9 hours of darkness and the reverse tanks received 15 hours of darkness and 9 hours of light.

Due to limitations of space, food or benthic chlorophyll treatments were created by dividing tanks into two identical halves, using a barrier to separate these two halves. Barriers consisted of plastic mesh sheets covered with cotton batting and 250-µm mesh to allow water flow between sides ensuring common water quality throughout all treatments while minimizing the exchange of particulates between sides of the tanks. Every two weeks over the course of the experiment, one of the two sides of each tank was spiked with the phytoplankton and bacterial mixture previously mentioned. The water was turned off in each tank, and the slurry allowed to settle for 24 hours. Food in the other half consisted only of that which continued to grow under the established light cycles after the initial addition. In this way, each tank within the two photoperiod treatments also received high and low food treatments. Food treatments were systematically assigned so that the high food side was not always on the same side of the tanks.

Once a week, the system was inspected for photocell and light function, temperature, and barrier integrity. When barriers became clogged, they were carefully cleaned using a Tygon aquarium vacuum.

Monthly, from June 2006 to February 2007 and again in May 2007, six individuals from each half of each tank were removed for gonad analysis. Animals were

held overnight in separate containers of seawater to allow for gut evacuation and then preserved in their tubes with 10% formalin buffered with seawater for 24 hrs followed by storage in 70% EtOH. Concurrently, a small sediment core was removed from each half of each tank using a 1-mL syringe with the end removed. I removed excess water from these samples, then extracted chlorophyll using 90% acetone as mentioned above. After extraction, sediment cores were dried and weighed, and these weights were used to normalize chlorophyll measurements for the volume of sediment sampled.

All worms were removed from their tubes after preservation, and each worm was scored for gonad stage. Individuals were examined under a dissecting microscope and scored for reproductive condition by the appearance of the gonad through the body wall and from coelomic fluid samples. The stages of gonad maturity were based on those used in Gentil et al. (1990) but simplified into three categories: 0 if no gonad was identifiable on the ventral surface of the worm, 1 if cream-colored gonad was present along the ventral surface of the worm, and 2 if the gonad extended dorsally beyond the midline of the worm (Figure 3.2). In this last stage, gametes could be identified as either sperm or eggs and gametes were also present in the coelomic fluid. The length of the fourth abdominal segment of each worm was measured, as Gentil and Dauvin (1989) found that this segment had the strongest correlation to the whole worm length in *O. fusiformis* and could be used in place of whole worm length when worms were damaged or had regenerated. This relationship was also valid in *O. collaris*.

Gonad stage was analyzed using an analysis of covariance (ANCOVA) with photoperiod and food treatments as fixed factors and worm length and chlorophyll as

covariates (Quinn and Keough 2002). Levene's Test of Homogeneity revealed significant heteroscedasticity ($F=2.5$, $p<0.001$) when month was included in the analysis. I was unable to transform the data in such a way as to equalize variances across treatments and the relationship between worm length and month also violated the assumption of equality of slopes for an ANCOVA. However, sample sizes were equal across all treatments, making this analysis relatively robust to heterogeneity of variances and the assumption of equality of slopes (Underwood 1995).

3. Results

3.1. Natural Reproductive Cycle

Gonadal tissue first appeared in *Owenia collaris* along the ventral blood vessel in the anterior abdominal segments at the end of February each year. This tissue thickened outwardly into the coelomic compartment, growing up along the sides of the worm. As more gametes reached their full size, the gonad extended into the posterior segments as loose aggregations of gametes, which was the typical state of worms from March through August. In samples collected in June and July of both years, the posterior segments usually were devoid of gametes, but the central segments remained full, indicating that spawning had occurred in these animals but that worms did not release all gametes in a single spawning event. Worms held in the laboratory in light and temperature conditions approximating natural conditions occasionally spawned without provocation. When this occurred, not all gametes were released and worms survived beyond this spawning event and into the next breeding season. In unprovoked spawning events, fecundity averaged

in the low thousands, whereas dissecting gametes could produce more than 40,000 mature oocytes per female.

There was a strong seasonal influence on the presence of gametes in *O. collaris*. Gametes were found in a majority of animals from March through August, followed by a precipitous decline in reproductive adults after September. By November, no reproductive adults were found in either year. There was a significant effect of month on the presence of reproductive products (Table 3.2). Post-hoc Tukey's HSD pairwise comparisons revealed specific differences between months. There were no significant differences between most of the spring and summer months (March through August), nor between the fall and winter months (October through January) (Figure 3.4). September was statistically similar to both spring/summer and fall/winter months, because of relatively higher variation between years in this month. It appears that these animals invest considerable energy in gametes starting in March and continuing through the summer. A drop in the presence of gametes characterized June and July, which probably indicates a high frequency of spawnings during the weeks preceding these samples rather than marking the end of the reproductive season, since there was a high frequency of gametes in August. In both years, September was a transitional month, with reproduction tailing off rather than coming to an abrupt halt. In all months when full-sized oocytes were present, dissected gametes were viable and capable of fertilization, although fertilization rates in March and September were depressed relative to April through August.

Table 3.2. Natural Reproductive Cycle of *Owenia collaris*. Results of 1-way ANOVA with month as a fixed factor and 2 replicated sampling years and post-hoc Tukey's HSD to test for differences between months.

Factor	**df_N, df_D**	**Mean Square**	**F**	**p**
Month	11, 12	2703.569	11.470	<0.001
Error	12	235.712		
Total	23			

More males than females were identified from the above samples from March through August during the first year of sampling (70:57 male:female ratio). This ratio, however, is not significantly different from the expected sex ratio of 50:50 (X^2 = 1.13, $F_{0.05}$ = 3.84, p=0.287). It is possible that many of the worms for which sex could not be determined were females whose gonads were not developed enough to be distinguished as eggs through the body wall. Many of these worms had a characteristic amorphous white mass in the anterior segments where gonads begin developing. When this material was examined under the microscope, it could not be identified positively as either developing eggs or sperm. The material examined, however, was that portion of the mass that was exuded when the animal was pierced with fine forceps. Any developing eggs or sperm that remained attached to the body wall might have been missed.

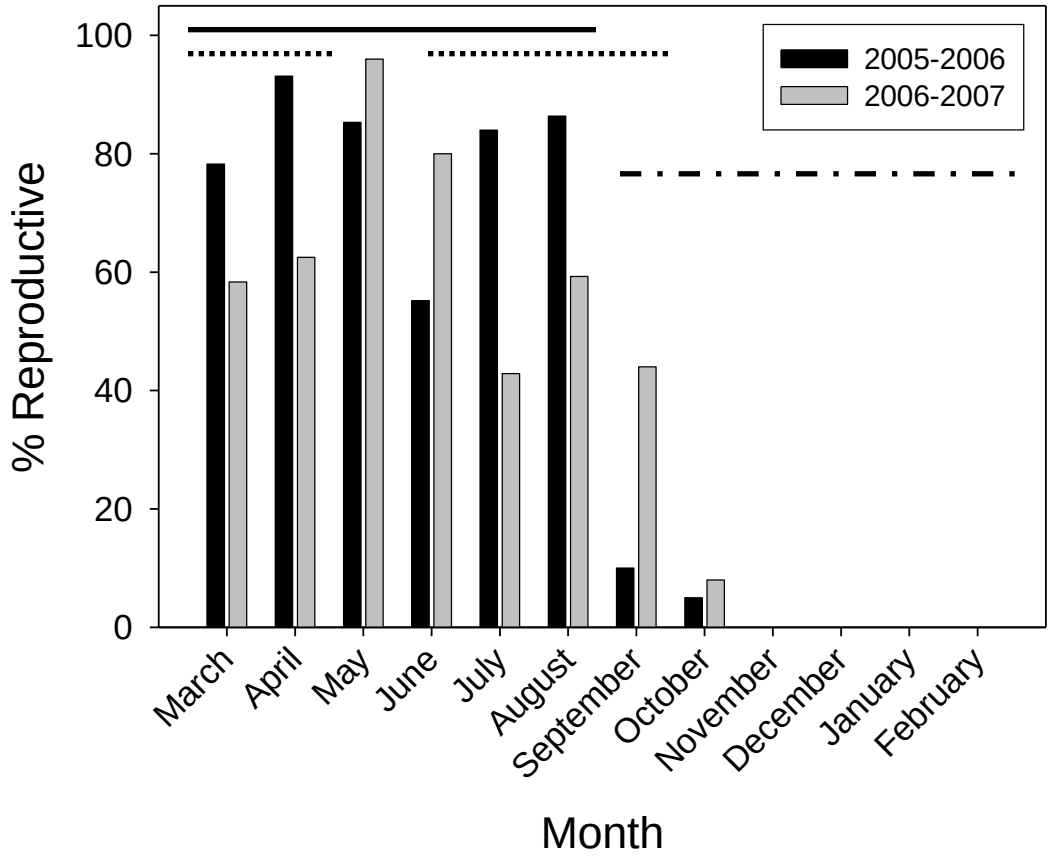

Figure 3.4. Natural Reproductive Cycle. Percentage of the population of *Owenia collaris* in Coos Bay, OR containing mature gonad during monthly collections for two replicate years. Horizontal lines above bars represent post-hoc Tukey's groupings from the 1-way ANOVA presented in Table 2, in which months covered by these lines are not significantly different from one another.

The presence of gametes in *O. collaris* was related to changes in several environmental variables (Figure 3.5). The best-fit model using AIC included alkalinity, water temperature, day length, and water-column chlorophyll (Table 3.3). As the water column became more basic in the spring, the percentage of the population carrying mature gametes increased dramatically and gradually decreased through the rest of the summer as the water column became less basic (Figure 3.5A). The frequency of worms

possessing gametes increased dramatically when water temperatures rose above 11ºC (Figure 3.5B). There was a similar jump between day lengths of 11 and 12 hours, from <10% to >40%, respectively (Figure 3.5C). Water-column chlorophyll did not account for a significant portion of the variation in reproduction between years, but the two peaks in reproduction (May and August) are associated with the highest concentration of chlorophyll in the water column (Figure 3.5D). Salinity was not included in the best-fit model due to its weak relationship with reproduction (Figure 3.5E). Overall, alkalinity, day length, and water temperature explained 74% of the variation in gamete presence between months and years. In the second year of sampling, there was no correlation between the benthic chlorophyll values and presence of gametes (r^2=0.031, Figure 3.5F).

Table 3.3. Natural Reproductive Cycle of *Owenia collaris*. Results of linear multiple regression using AIC between environmental variables and reproduction of *O. collaris*. Alkalinity, water temperature, day length, and water column chlorophyll were included in the model. Adjusted r^2=0.741.

A) Model ANOVA

Factor	**df_N, df_D**	**Mean Square**	**F**	**p**
Regression	4, 19	6398.517	17.433	<0.001
Residual	19	367.039		
Total	23			

B) Individual Variables

Variable	**Standardized β**	**t**	**p**
Alkalinity	0.470	4.329	<0.001
Water Temperature	0.431	3.293	0.004
Day length	0.123	2.204	0.040
Water Chlorophyll	0.264	2.013	0.059

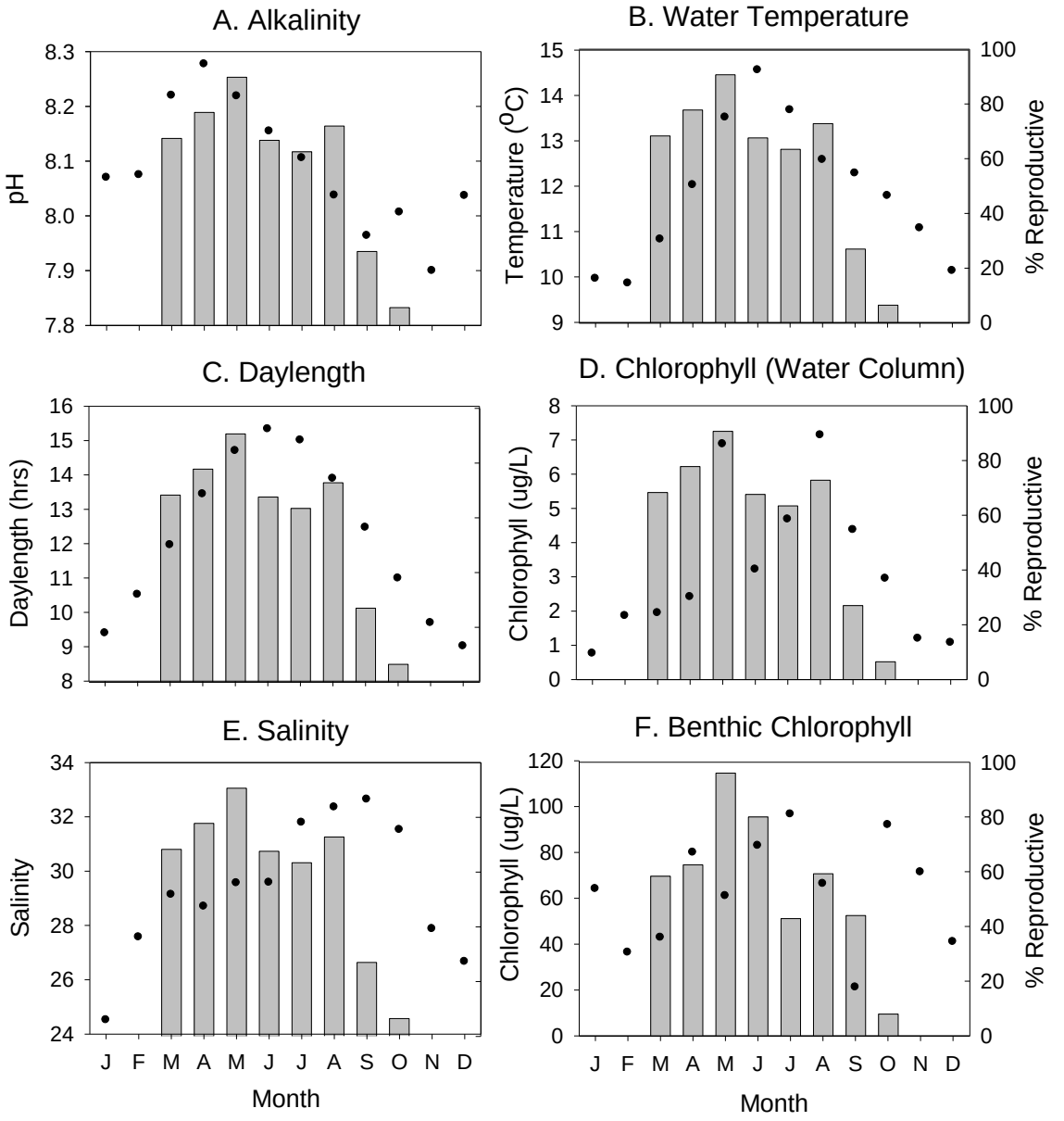

Figure 3.5. Relationship between environmental variables and percent reproduction in *Owenia collaris*. A-E) Data points represent mean monthly values for environmental variables averaged over two years and bars represent mean monthly values for percentage of population with mature gonad, averaged over two years. Variables are presented in order from highest to lowest p-value (Table 3.3). F) Data points represent mean monthly values of benthic chlorophyll from 3 replicate samples collected each month and bars represent monthly values for percentage of population with mature gonad in the second year of worm sampling.

3.2. Gonad Development Experiment

A 3-way ANCOVA with photoperiod, food, and month as fixed factors and chlorophyll and worm length as covariates was used to examine the results of the gametogenesis experiment (Table 3.4). Tank was used as the unit of replication (n=3). Results from the 3-way ANCOVA analysis indicate a strong interaction between photoperiod and food, which is best explained by the fact that those worms receiving high food with a reversed photoperiod possessed gonads at more advanced stage of development than those in any other treatment during the winter months (Figure 3.6). There was also a significant effect of both month and photoperiod on gonad stage. The effect of month is primarily due to the cessation of reproduction in the fall, which occurred in both photoperiod and food treatments. However, the samples collected in the winter months in the reverse photoperiod treatment possessed moderately developed gonad, indicating that increasing day lengths out of season slowed the cessation of gonad development or allowed it to continue through the winter months. Food availability had no effect on gonad development by itself. The lengths of worms decreased markedly over the course of the experiment, and length significantly covaried across all treatments.

Table 3.4. Gonad Development Experiment. Results of 3-way ANCOVA with photoperiod, food, and month as fixed factors and chlorophyll and worm length as covariates.

Factor	**df_N, df_D**	**Mean Square**	**F**	**p**
Worm Length	1, 78	0.406	6.192	0.015
Chlorophyll	1, 78	0.012	0.178	0.674
Photoperiod	1, 78	0.421	6.411	0.013
Food	1, 78	0.036	0.554	0.459
Month	9, 78	1.128	17.184	<0.001
Photoperiod*Month	9, 78	0.086	1.311	0.245
Photoperiod*Food	1, 78	0.399	6.076	0.016
Food*Month	9, 78	0.059	0.903	0.527
Photoperiod*Food*Month	9, 78	0.082	1.250	0.278
Error	78	0.066		
Corrected Total	119			

4. Discussion

Owenia collaris is a dioecious iteroparous species with a well-defined breeding season (March through September in Coos Bay, OR). Onset of gonad development occurs in early spring and worms advance rapidly to maturity in March. Two spawning peaks characterize the breeding season, the first occurring between April and May and a second shorter period in August. During the breeding season, a majority of the animals in the population contain mature gametes and individuals can spawn multiple times throughout the season.

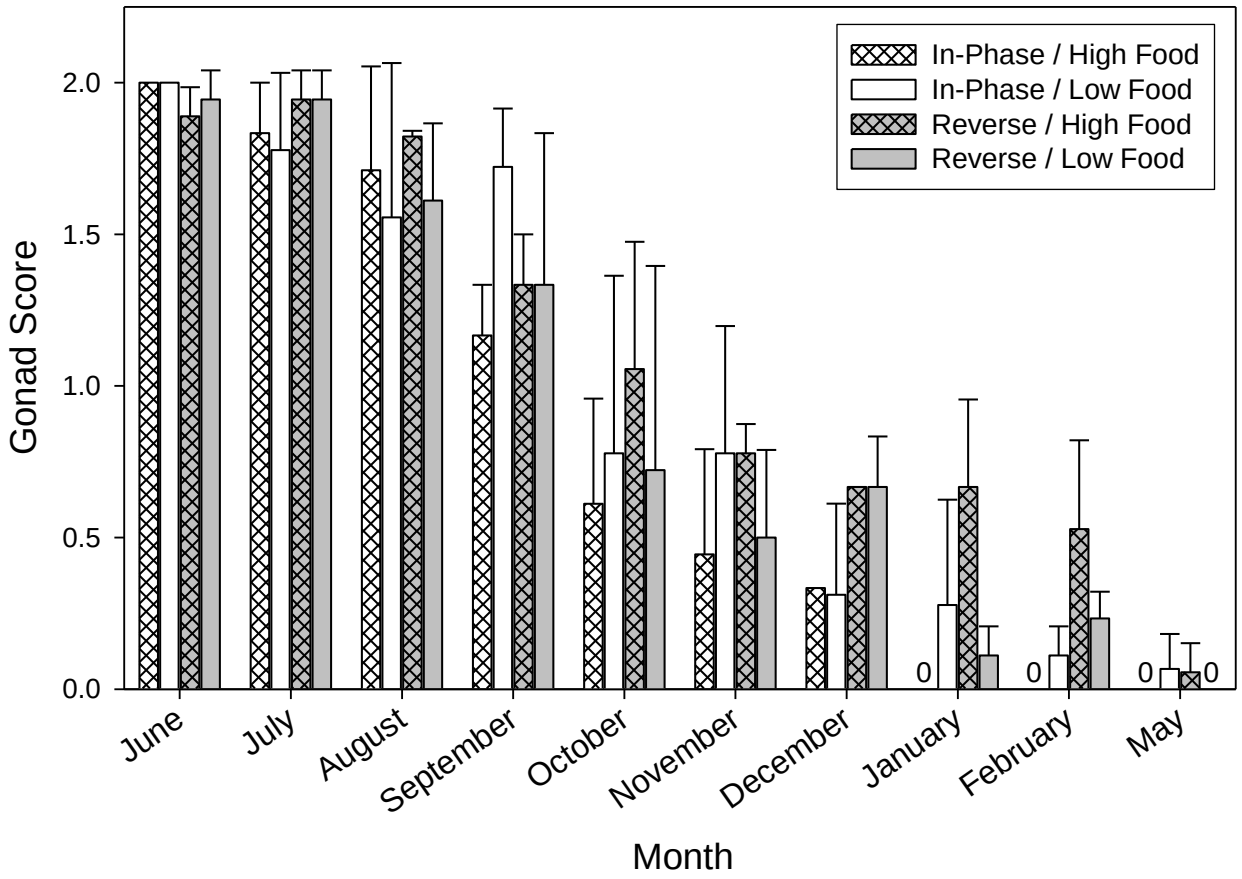

Figure 3.6. Gonad score for *O. collaris* exposed to either in-phase or reverse light cycles and either high or low food concentrations. Bars represent means of 3 replicate tanks with standard deviations. Zeroes represent treatments with gonad scores with mean values of 0.

The major influences on seasonality of reproduction in temperate invertebrates are food availability, temperature, and photoperiod (Giese and Kanatani, 1987). Often the increase in day length corresponds with an increase in water temperature and primary productivity, which in turn may allow invertebrates to begin devoting excess energy to reproduction. *Owenia collaris* adults are primarily direct deposit feeders, consuming surface sediments along with the associated algae, bacteria, and meiofauna (personal observation). They may therefore be able to take advantage of increasing day length and primary productivity in the surface sediments as winter progresses into spring. However, the lack of a strong correlation between benthic chlorophyll and the presence of gametes in *O. collaris* prior to the spring phytoplankton bloom indicates that food availability in the sediment is not necessarily a factor limiting the production of gametes for this species. By directly consuming the sediment, worms ingest not only benthic phytoplankton but also small infaunal organisms and bacteria, which may be readily available at all times of the year. Instead, the timing of reproduction in these animals most likely is determined by cues that are consistent between years, such as longer days and warmer water temperatures during spring and summer months.

The first mature individuals collected in 2005 were found in the second half of March. One female and one male of this group spawned a small number of gametes when removed from their tubes, but few of these eggs matured and developed. After this point, spawning events in the laboratory each month until September produced many more eggs and sperm and viable embryos. Presumably, spawning events in the field become increasingly common from March through June and continue throughout the

summer months. *Owenia collaris* produce planktotrophic larvae that remain in the plankton for three to four weeks, depending on temperature, before undergoing a dramatic metamorphosis into a juvenile (Chapter 2). With the onset of spawning in March or April, this should provide the larvae with high levels of phytoplankton and favorable water conditions and also provide juveniles with enhanced levels of surface primary productivity upon their return to the adult sediments. By spawning before September and October, the pelagic stage is completed typically before winter storms return, decreasing primary productivity and potentially washing new settlers away with increased wave action. Adults in Coos Bay often are found in looser, sandy sediment within mudflats and in protected embayments on the outer coast, which are prone to shifting and thus burying the smaller juveniles at the surface. Adults and juveniles of *O. collaris* are surface deposit feeders and suspension feeders and cannot tolerate extended burial below the surface. Moreover, they have limited ability to reconstruct damaged tubes.

Each year along the Oregon coast, winds from the north push surface waters offshore via Eckman Transport, thereby pulling cold, nutrient-rich water up from depth and promoting a spring phytoplankton bloom. This shift from winds from either the west or south to predominantly northerly winds is referred to as the spring transition (Huyer et al., 1979; Strub et al., 1987). Variations in the date of the spring transition strongly influence the production of commercially important species, including Dungeness crab and Coho salmon (Shanks and Roegner, 2007; Logerwell et al., 2003). Spring transition dates from 2005-2007 occurred in mid March or late April, after mature gametes had

developed in the Coos Bay population of *O. collaris*. This also indicates that the adults of this species do not convert increased primary production during the spring bloom to the production of gametes. However, the larvae that are produced at this time of year could take advantage of this bloom. This is further supported by the fact that there are two peaks in reproduction, one in May when chlorophyll concentrations within the water column are at their highest, and one in August when the chlorophyll concentrations again peak (Figure 3.5D). This observation supports Crisp's Rule (Crisp, 1954; Qasim, 1956; Crisp, 1959); if a high proportion of the population spawns in concert with these peaks in chlorophyll, larvae would have the advantage of increased phytoplankton productivity.

Changes in alkalinity can indicate changes in carbon dioxide concentration or, ultimately, phytoplankton concentration (Sverdrup et al. 2006). Reproduction in *O. collaris* is associated with the transition to more alkaline conditions in the spring, indicating an association with low levels of carbon dioxide or high phytoplankton. By itself, water alkalinity probably is not a factor driving reproduction in *O. collaris*, but rather an indication of the progress of the spring phytoplankton bloom followed by the increase in salinity as run-off diminishes in the summer.

Another advantage to reproducing in the spring and summer is that water temperature is higher. Laboratory cultures of larvae of *O. collaris* can be sensitive to temperature changes, and these affect both growth rates and the percentage of cultures that successfully undergo metamorphosis (personal observation). At 12ºC, larvae require up to 4 weeks to develop and metamorphosis occurs successfully in less than half of these larvae, whereas larvae grown at 15ºC require only 3 weeks to develop and nearly three-

quarters of these undergo metamorphosis successfully (Smart, unpublished data). In the field, spawning during times of warmer water temperatures would minimize loss through advection, predation, and incomplete development (see reviews by Thorson, 1950; Rumrill, 1990; Morgan, 1995).

Overall, reversing the photoperiod or adding food in the laboratory is not enough to induce out-of-season development of mature gametes in *O. collaris*, at least not over the span of a single breeding season. Polychaetes often have endogenous cycles that are maintained for months or years in the absence of external cues, so long as conditions are adequate for survival and reproduction (e.g. Franke, 1980; Olive, 1995). The temperature in the field during the months that most worms possess mature gametes is 11ºC or above and tanks were held at 12ºC. Sediment chlorophyll levels were comparable across the range of both high and low food treatments and under field conditions, suggesting that food may not have limited gonad development even in the low food treatments. In addition, water quality was maintained throughout the experiment. Water clarity was monitored and salinity was maintained by occasional additions of fresh seawater so salinity did not drop below a level (salinity of 28) at which worms do not normally produce gametes in the field (Figure 3.5E). Although worms collected at the end of the experiment were smaller than those collected at the beginning, they were still above the presumptive minimum size for reproduction since they were still similar in size to reproductive adults collected from the field. If gametogenesis is cued by or maintained by an endogenous rhythm in *O. collaris*, these animals may have continued the set cycle under these adequate laboratory conditions. It is possible, if not probable, that onset of

gametogenesis is cued by a change in one of the environmental variables not addressed in this experiment, and that adult *O. collaris* possess an internal clock that cannot be fully overridden under these laboratory conditions.

Reproductive cycles in *O. collaris* are strongly seasonal and associated with both proximate and ultimate environmental factors. Day length, water temperature, alkalinity and chlorophyll concentration in the water column all correlate with the time at which viable gametes can be produced and with the percentage of the population involved in reproduction. Gametes are produced rapidly in the springtime once day length increases above 11 hours and the mean water temperature reaches 11ºC. Gamete production trails off once days shorten back to 11 hours and water temperatures decrease in the fall. The effect of shorter days is somewhat reversible in the lab, and the reproductive season may be extended if light cycles are disrupted. Peaks in reproduction (and therefore in the production of larvae) mirror patterns of phytoplankton availability. Benthic food availability, however, has no effect on adult condition in the laboratory and adults in the field develop mature gonad before the onset of the spring phytoplankton bloom, suggesting that phenology in this species has evolved to allow larvae (rather than adults) to take advantage of enhanced phytoplankton production. One might postulate that the lack of a response to food may put this species at a greater risk of mismatching when larval food is available (Cushing, 1990). However, this species also appears to have an endogenous clock that reduces the production of gametes out of season and a relatively long breeding period encompassing the interannual variation in primary production cycles. These factors minimize the chances of larvae encountering unfavorable

conditions. Together, these environmental cues serve to synchronize reproduction in this species to ensure the best possible conditions for larval growth and recruitment.

BRIDGE III

Environmental factors play a major role in determining the reproductive biology of *Owenia collaris*. This species can be found in bays and estuaries along the northeastern Pacific coast, where the environment can change drastically on seasonal, monthly, and daily timescales. Within the Coos Bay estuary, *O. collaris* are abundant at several mudflats, but completely absent at others. The physical factors controlling the distribution of *O. collaris* in the Coos Bay estuary were investigated in Chapter V. In the following chapter, I concentrated on factors that vary along the estuarine gradient, salinity, temperature, and sediment, using laboratory and field technique

CHAPTER V

PHYSICAL FACTORS AND THE ESTUARINE DISTRIBUTION OF THE TEMPERATE POLYCHAETE *OWENIA COLLARIS* (FAMILY OWENIIDAE) IN COOS BAY, OR

INTRODUCTION

Estuaries represent marginal ecosystems for two communities, those with species adapted to brackish conditions and those with species adapted to marine conditions. The range limits of a principally marine species in an estuary may be set either by the extremes of its physiological tolerances or by its ability to tolerate the wide fluctuations in environmental variables that occur seasonal, daily, and or even hourly. The primary physical factors known to control distributions of benthic populations in estuaries include salinity, temperature, wave action, and sediment grain size (Lyster, 1965; Carriker, 1967; Tenore, 1972; Vernberg and Vernberg, 1974; Bulger et al., 1993; Dethier and Schoch, 2005). Variations in these factors among different portions of the estuary may lead to reduced growth, fecundity, or survival of benthic stages, thus setting limits to populations and producing observed patterns in species distributions.

Observed distributions of benthic organisms in marine soft-sediment habitats have been attributed largely to post-settlement mortality, whereas the larval stages may be distributed widely (Olafsson et al., 1994). Larvae dispersing within estuarine systems must either be tolerant of environmental fluctuations or exhibit behaviors that prevent

them from exposure to detrimental regions of the estuary. It is commonly assumed that the early life history stages are the most vulnerable to environmental variation (Orton, 1920; Hutchins, 1947; Passano, 1960; Anger, 2001; Sanford et al., 2006). However, a handful of published studies have demonstrated that larval tolerance of the range of a given physical variable does not always directly match patterns of occurrence of benthic adults (Lyster, 1965; Grassle et al., 1992; Bhaud, 1993; Bhaud, 2000). For example, Lyster (1965) found that the larval tolerances of estuarine polychaetes were much wider than would be expected based on benthic adult distribution. This pattern could be explained by narrower physiological tolerances or resource needs in earlier (e.g. embryo) or later (e.g. juveniles) life-history stages. Unfortunately, Lyster (1965) was not able to resolve the various alternative explanations.

Within temperate estuaries, salinity and temperature tend to covary along the estuarine gradient from river to mouth and interactions between these two physical factors may strongly influence species distributions through their impacts on benthic and pelagic stages. Often lower salinities increase lethal and sublethal impacts of other stressors such as pollution or high temperatures (see Carriker, 1967 for review). For polychaete worms, species' ranges can be highly dependent on salinity and temperature gradients (Pardal et al., 1993). Salinity alone can greatly influence survival and condition in multiple life-history stages. In the spionid *Marenzelleria* cf. *viridis*, low salinity restricted larvae from entering and colonizing lagoonal areas in the SE Baltic Sea and greatly reduced adult fecundity (Daunys et al., 2000). Oogenesis in *M.* cf. *viridis* failed at both low temperatures and low salinities. In the co-occurring *Hediste* (=*Nereis*)

diversicolor, oogenesis was also prevented at reduced salinities (Bogucki, 1963; Gasiunas, 1956). *Capitella* sp. I also fails to reach sexual maturity and exhibits reduced growth rates at low salinities (Pechenik et al., 2000). Kube and Powilleit (1997) demonstrated that another spionid (*Pygospio elegans*) was limited to brackish water by its lack of tolerance to marine salinities, primarily in the adult stage and secondarily in the earlier stages. Among larval stages, salinity tolerances in *Phyllodoce maculata*, *Scoloplos armiger*, *Notomastus latericeus*, *Pomatoceros triqueter*, and *H. diversicolor* reflect adult distributions, with the most tolerant larvae having the broadest adult estuarine distributions with respect to salinity. Temperature tolerances were important in *P. triqueter* but not the other species (Lyster, 1965). Larvae of the serpulid *Hydroides elegans* may be prevented from migrating into estuaries by their inability to survive low salinities (Qui and Qian, 1997).

The distributions of infaunal invertebrate species that inhabit soft sediments generally are correlated with the distributions of particular sediment types (Scheltema, 1974; Butman, 1987; James and Underwood, 1994; Snelgrove and Butman, 1994). Polychaete communities are no exception, with species richness and diversity (Gambi and Giangrade, 1986), density (Scaps et al., 1998; Gutierrez et al., 2000), and community composition (Bilyard and Carey, 1979; Pardal et al., 1993; Scaps et al., 1998; Bromberg et al., 2000; Elias et al., 2001; Maggiore and Keppel, 2007) being related to sediment characteristics on both intertidal and subtidal soft bottoms. These observed patterns may be established and maintained by behavioral preferences or physiological tolerances in any life-history stage. Habitat selection at settlement can produce differential recruitment

patterns. Thus, for example, larvae of *Ophelia bicornis* demonstrate strong selectivity for sediment particle size (Wilson, 1952). Juvenile *Arenicola marina* can migrate after settlement to find organically rich sediments without established adults (Hardredge et al, 1998). Settling larvae of the terebellid *Eupolymnia nebulosa* may respond positively to adult scented sediments as well as coarser sediments (Duchene, 2004). However, polychaete larvae do not always respond differentially to sediment type, as exemplified by the lack of selectivity between mud and sand in *Capitella* sp. I (Grassle et al., 1992) and *Marenzelleria* cf. *viridis* (Rohri, 1997).

The polychaete *Owenia collaris* (Hartman, 1969) (Oweniidae) has been documented in offshore and marine-influenced estuarine waters along the coasts of California (Blake, 2000), Oregon and Washington. Its overall distributional patterns are relatively unknown because often it has been confused with *O. fusiformis* in collection records and other works. *Owenia collaris* is a small (<4cm total length) tubiculous polychaete found in muddy and sandy sediments. *Owenia collaris* is potentially a very common and ecologically important member of infratidal soft sediment communities along the Pacific coast of North America. Dense patches of *O. fusiformis* (in some cases =*collaris*) stabilize sandy bottoms and reduce bedload transport (Fager, 1964; Nowell and Church, 1979; Eckman et al., 1981; Eckman, 1985), impacting recruitment of this species and that of other infaunal invertebrates as well (Levin, 1982; Desroy, 1984; Pinedo et al., 2000). *Owenia fusiformis* and *O. collaris* also serve as prey to both benthic and pelagic predators including turrid snails (Shimek, 1983), rays, and portunid crabs (Fager, 1964).

Adults of *O. collaris* are gonochoristic and free-spawning, producing planktonic embryos and larvae in the spring and summer (see Chapters 2 and 4 of this thesis). Larvae can remain in the plankton for several weeks (4 weeks at 12ºC and 3 weeks at 16ºC) before undergoing a dramatic metamorphosis into a juvenile worm (Chapter 2). After metamorphosis, juveniles begin forming the adult tube by gathering particles from surrounding sediments and joining these together via secreted mucus. Fager (1964) investigated particle selectivity in adult and juvenile *O. fusiformis* (probably *O. collaris*) and found that juveniles collected in the field prefer tabular heavy mineral grains of 62 to 80 μm. As the worm grows and the tube is extended, preference shifts to more equant particles such as shells, shell fragments, and quartz. No other data are currently available on the physical requirements of this species of the relationship between distribution and the physical environment.

As early as 1932, D.P. Wilson recognized that the presence of an appropriate substratum was necessary for successful metamorphosis and recruitment in laboratory experiments with *O. fusiformis*. Adults of this species are widely distributed in subtidal sandy sediments in the Mediterranean Sea (Picard, 1965; Zunarelli-Vandini and Cognetti-Varriale, 1978; Gambi and Giangrande, 1986, Ambrogi et al., 1990). Somaschini (1993) demonstrated that their distribution in the Tyrrhenian Sea is closely related to the proportion of silt and clay fractions (≤63 μm). In other populations, the highest densities of adult *O. fusiformis* are found in areas with greater than 4% by weight of the silt/clay grain-size class (Dauvin and Gillet, 1991 and Dauvin, 1992). Pinedo et al. (2000) demonstrated that recruitment densities of *O. fusiformis* in the Mediterranean Sea are

highly dependent on the proportion of small size fractions of sediment and that juveniles required very fine sand or silt (≤63 to 160 μm) to build tubes successfully. This study, however, found no effect of sediment characteristics on adult distribution. Within other genera in the family Oweniidae, physical variables do not always affect adult distribution directly. Ellingsen (2002) found that the abundance of adult *Myriochele oculata* off the coast of Norway was not related to depth range, sediment size, or sediment organic content. For this group of tubiculous worms, it appears that the physical environment may be important only in some areas or to some life stages.

The aim of my study was to document the distribution of *O. collaris* in a temperate estuary and to determine the impact of physical factors on the distribution of this species. Based on the sessile nature of the adults of this species, one would predict two alternate patterns: first, that the dispersive stages would be widely tolerant to variations in the estuarine gradient while the benthic stages would prove the limiting factor, or second, that dispersive stages are minimally tolerant to estuarine conditions and that the observed adult distribution is the result of limited dispersal or recruitment. Coos Bay, OR was chosen as a model population because of the abundance of worms in this estuary. Coos Bay is a large, temperate, well-mixed estuary with extensive tidal influence and a variety of soft sediment habitats. The effects of sediment on the biology of the congener *O. fusiformis* have been studied extensively in the mouths of estuaries and nearshore habitats, but there has been no work focused on estuarine populations of this genus. Here, I focus on the two factors that most vary most along the estuarine gradient, salinity and temperature, and also examine the effects of sediment grain size on

the biology of *O. collaris*. Havenhand (1995) recommended a "whole life cycle approach" when attempting to explain distributional patterns in benthic marine organisms because of the interdependence of all stages. This was done for *O. collaris* by (1) documenting and experimentally manipulating adult distribution in the field, (2) conducting laboratory experiments to determine physiological tolerances for all life-history stages and (3) testing the effects of sediment size on recruitment in the laboratory. Within the Coos Bay estuary, salinity, temperature, and sediment grain size characteristics change dramatically from the mouth of the estuary to head-of-tide, and these factors may covary as well, confounding individual impacts. This study attempts to separate the influences of salinity, temperature, and sediment grain size on the distribution of *O. collaris* through the use of controlled, manipulative experimentation.

METHODS

Field Survey

Distributional surveys were conducted in January 2007 over two consecutive spring tide series to determine the occurrence of *Owenia collaris* within and near Coos Bay, OR. In areas where *O. collaris* are present, they are found from the shallow subtidal to about 1 foot above mean lower low water (MLLW) (Smart, personal observation). I visited various soft sediment areas at low tide (Fig. 4.1) and walked back and forth parallel with the water line for twenty minutes, beginning just above the water line and working my way up, looking for tubes of *O. collaris*. Presence was confirmed by digging up potential tubes and visually inspecting them for live worms. In all cases where *O.*

collaris were found, tubes were located within the first five minutes of searching and these tubes always contained live worms. Site coordinates were recorded with a Garmin etrek Legend GPS unit.

Experiments with juvenile and adults worms of *Owenia fusiformis* indicate that fine sediments are important for juvenile recruitment, whereas adults require a broad range of sediment sizes, with no size being particularly important (Fager, 1964; Pinedo et al., 2000). These relationships have not been examined for *O. collaris*. The sediment characteristics of each site were examined by collecting three replicate 30-mL sediment cores during the survey. Cores were brought back to the Oregon Institute of Marine Biology, rinsed with freshwater, dried, and separated into size fractions by dry sieving. Each size fraction was then weighed. To determine if differences in the sediment grain size distribution differed significantly between sites where worms were present and those where worms were absent, I used a 2-factor ANOVA with occurence as a fixed factor, site as a random factor nested within occurrence and cores as replicates nested within sites. The percentage of fine-grained sediment or silt (63-125 μm) was the dependent variable.

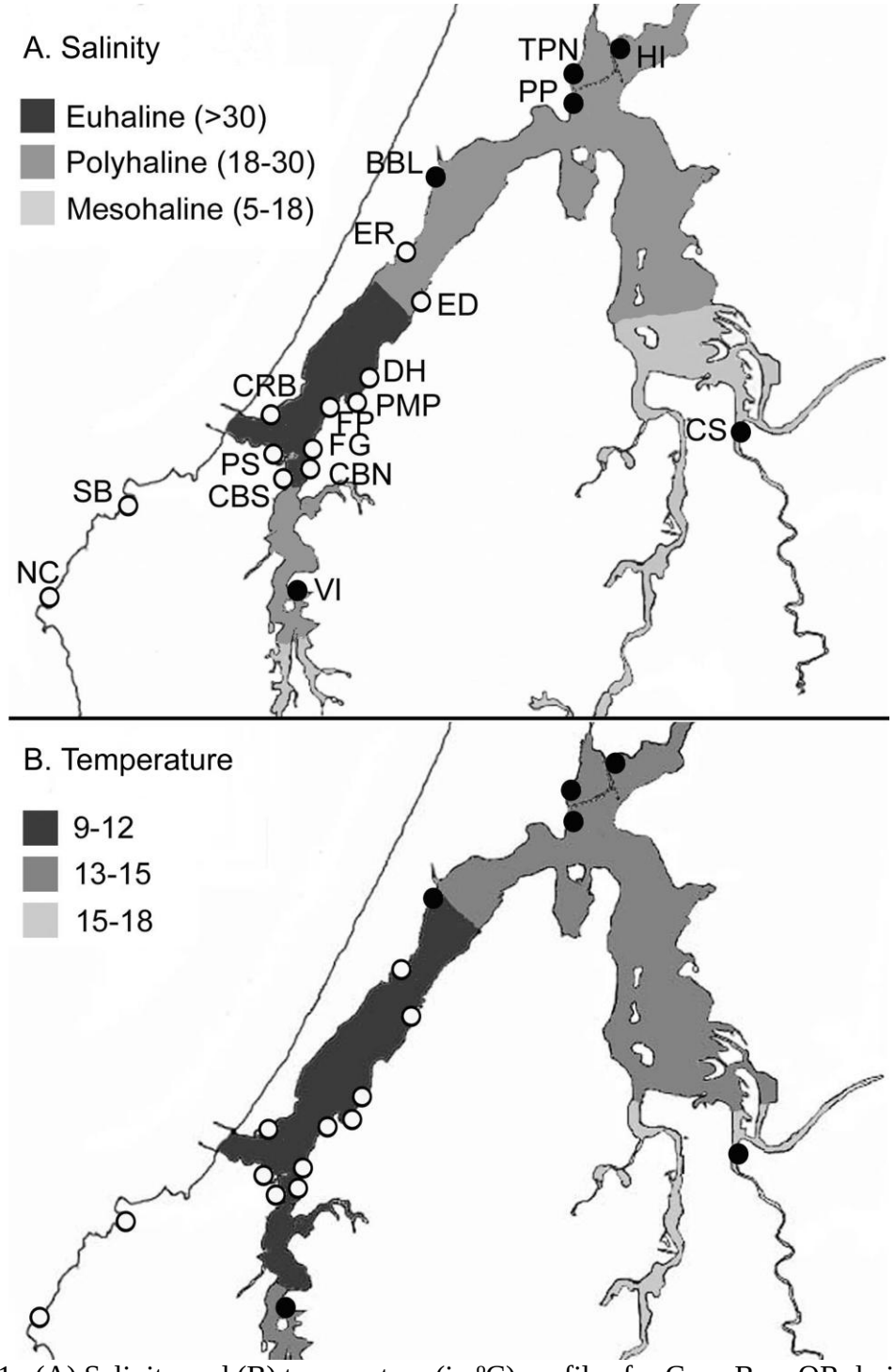

FIG. 4.1. (A) Salinity and (B) temperature (in ºC) profiles for Coos Bay, OR derived from Davidson (2008), McAlister and Blanton (1963), Arneson (1976), and SSNERR CDMO. Closed and open circles indicate absence and presence of adult *O. collaris*, respectively. Site names and coordinates for abbreviations shown here can be found in Table 4.1.

TABLE 4.1. Details of sites surveyed for adult *O. collaris* in Coos Bay, OR. Abbreviations are represented in Figure 1, GPS coordinates were collected during the surveys, salinity and temperature categories were derived from above, and dominant grain size was derived from Figure 4.

Site	**Abbreviation**	**GPS Coordinates**	**Distance Upriver (nautical miles)**	**Salinity**	**Temperature**	**Dominant Grain (µm)**	***O. collaris***
North Cove	NC	N 43º 18’ 463 W 124º 24’ 033	0	Euhaline	9-12ºC	125-250	Present
Sunset Bay	SB	N 43º 20’ 033 W 124º 22’ 424	0	Euhaline	9-12ºC	125-250	Present
The Cribs	CRB	N 43º 21’ 520 W 124º 19’ 825	0.5	Euhaline	9-12ºC	250-425	Present
Portside	PS	N 43º 20’ 576 W 124º 19’ 470	0.6	Euhaline	9-12ºC	250-425	Present
Fisherman’s Grotto	FG	N 43º 20’ 254 W 124º 19’ 105	1.5	Euhaline	9-12ºC	250-425	Present
Charleston Bridge South	CBS	N 43º 20’ 714 W 124º 19’ 769	1.5	Euhaline	9-12ºC	250-425	Present
Charleston Bridge North	CBN	N 43º 20’ 583 W 124º 19’ 463	1.6	Euhaline	9-12ºC	250-425	Present
Fossil Point	FP	N 43º 20’ 180 W 124º 18’ 906	1.9	Euhaline	9-12ºC	125-250	Present
Pump Station	PMP	N 43º 21’ 175 W 124º 18’ 908	2.0	Euhaline	9-12ºC	63-125	Present
Domehouse	DH	N 43º 18’ 960 W 124º 19’ 261	2.4	Euhaline	9-12ºC	250-425	Present
Valino Island	VI	N 43º 18’ 960 W 124º 19’ 260	3.7	Polyhaline	13-15ºC	250-425	Absent
End of the Road	ER	N 43º 23’ 503 W 124º 17’ 186	4.1	Polyhaline	9-12ºC	125-250	Present

TABLE 4.1 (continued).

Site	Abbreviation	GPS Coordinates	Distance Upriver (nautical miles)	Salinity	Temperature	Dominant Grain (µm)	*O. collaris*
Empire Docks	ED	N 43º 22’ 206 W 124º 17’ 877	4.2	Polyhaline	9-12ºC	125-250	Present
BLM Boat Launch	BBL	N 43º 24’ 986 W 124º 16’ 688	5.8	Polyhaline	9-12ºC	250-425	Absent
Pacific Power	PP	N 43º 26’ 202 W 124º 17’ 877	10.2	Polyhaline	13-15ºC	250-425	Absent
TransPacific HWY North	TPN	N 43º 26’ 266 W 124º 14’ 121	10.4	Polyhaline	13-15ºC	125-250	Absent
Haynes Inlet	HI	N 43º 22’ 206 W 124º 17’ 877	11.1	Polyhaline	13-15ºC	63-125	Absent
Catching Slough	CS	N 43º 26’ 266 W 124º 14’ 123	15.5	Mesohaline	15-18ºC	63-125	Absent

Temperature and salinity characteristics for each survey site were compiled from several sources. Davidson (2008) used data from Oregon Department of Fish and Wildlife and the South Slough National Estuarine Research Reserve collected over several years to divide Coos Bay into four salinity categories: euhaline (>30), polyhaline (18-30), mesohaline (5-18), and oligohaline (<5). These same sources were used to build temperature profiles throughout Coos Bay for this study (McAlister and Blanton, 1963; Arneson, 1976; NERR CDMO, http://cdmo.baruch.sc.edu). Only temperature data from April through August were included since most of my manipulative experiments were performed during this period and because temperature was uniformly cold (≤12ºC) throughout the fall and winter months. Coos Bay was divided into three temperature categories (9-12, 13-15, and 15-18ºC). These data were used to categorize survey sites and to assist in the selection of sites for further study.

Adult Transplant

Because *O. collaris* is primarily found in the lower estuary and on the outer coast, a transplant experiment was undertaken in the spring of 2007 to determine whether this pattern is the result of diminished survival or condition in the riverine regions of the estuary. Salinity and sediment grain size tend to decrease with distance from the mouth of the estuary and temperature tends to increase, worms may be limited to the lower estuary by an inability to survive in low salinity, warm water in silty bottoms. Transplants consisted of 3" diameter PVC pipe with the bottom covered with 100 μm mesh, allowing water to drain through the transplants but not allowing horizontal exchange of sediment between the transplant and surrounding mudflat. Sediment was

collected from three sites within Coos Bay according to their previously measured grain size profile by coring and immediately transferring undisturbed cores into prepared PVC pipes. Cores were visually inspected for worms, and if found, worms were removed. Cores were submerged in running seawater at the Oregon Institute of Marine Biology and held for two weeks until they were outplanted during the next spring tide series. Sediment treatments were: silt (dominant grain size 63-125 μm collected from Catching Slough), fine sand (dominant grain sizes 125-250/250-425 μm collected from Fisherman's Grotto), and medium sand (dominant grain size 250-425 μm collected from the BLM Boat Launch) (Fig. 4.1). Adult *O. collaris* with intact tubes were collected in April 2007, from two sites within Coos Bay, OR: Fossil Point and The Cribs (Fig. 4.1). Ten adults were haphazardly assigned to each transplant core. Worms were laid on the surface of each core in flowing seawater and given 48 hours to bury themselves (Fig. 4.2**A**, **B**). If any worms failed to bury, they were replaced with new worms which were given another 48 hours to bury themselves.

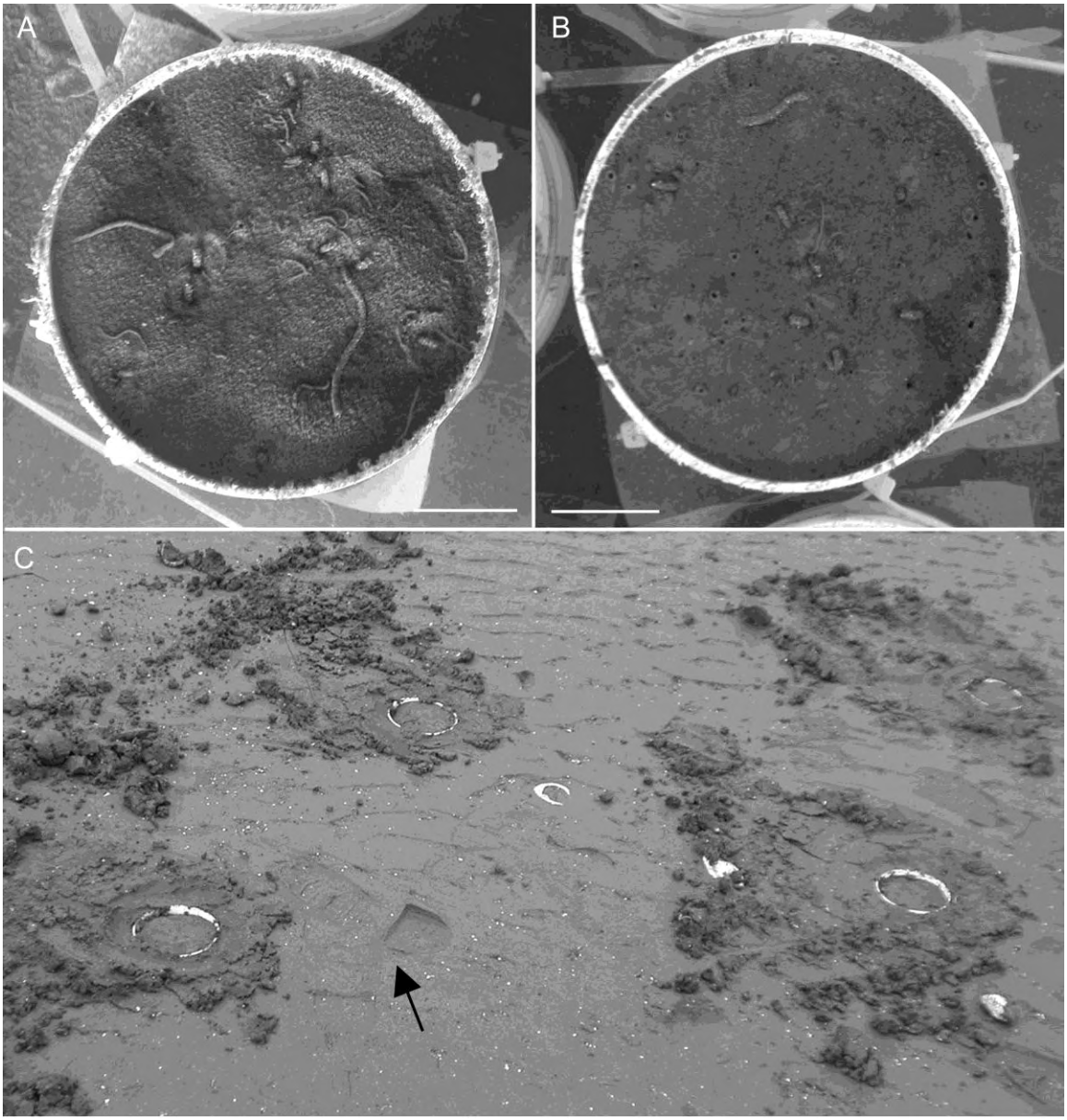

FIG. 4.2. Images of (A) 2 adult *O. collaris* burying themselves in a Sand core while the tubes of all others in the core stick vertically out of the sediment, (B) Silt core prior to outplant showing only established adults, and (C) four transplants deployed in the field at Fossil Point placed haphazardly in the low intertidal zone and buried flush with the sediment. Scale bars in A and B are 3-cm and a women's size 9 boot print (arrow) appears in C for scale.

During the subsequent spring tide series, cores were outplanted at six sites within Coos Bay, corresponding to Lower, Mid, and Upper estuarine locations. These distinctions generally corresponded to Davidson's (2008) categories of euhaline, polyhaline, and mesohaline, respectively. Four replicate cores for each sediment treatment were buried flush with the sediment (Fig. 4.2**C**) in the lower intertidal at each of two replicate sites per estuarine location, giving a total of 12 cores placed at each site. The Cribs and Fossil Point were assigned as the lower estuarine sites, BLM Boat Launch and Valino Island assigned as the mid estuarine sites, and Catching Slough and Haynes Inlet assigned as the upper estuarine sites (Fig. 4.1). Transplants remained in the field from April 2007 until July 2007, for a total of 3 months.

Upon retrieval from the field, cores were sieved through a 1-mm mesh on the day of collection and all worm tubes were examined for live *O. collaris*. Each live worm was also examined for signs of predation (i.e. segments missing or regenerating) and reproductive condition by carefully removing worms from their tubes and examining with a dissecting microscope. Reproductive condition is defined here as the stage of gonad development at the time of collection (see Chapter 4). Gonad development was divided into three categories and scored according to these categories (0 if no gonad was present along the ventral blood vessel; 1 if gonad was present along the ventral blood vessel but individual gametes were absent; 2 if gonad was present along the ventral blood vessel and individual gametes were also present in the coelom).

Due to loss of replicates, sites, and treatments, the Jonckheere-Terpstra (J-T) test was employed to examine differences in estuarine location and sediment separately,

combining replicates from sites within each treatment (S.A. Woodin, personal communication; Sokal and Rohlf, 1995). The J-T test is a nonparametric method that can be used to examine differences between treatments when there is a priori knowledge that can be used to order treatments in the null hypothesis such that one treatment is hypothesized to have a larger value than another. This test also has the advantages of ranking values to remove heterogeneity and is robust to unequal sample sizes. In this case, I hypothesized that survival and reproductive condition would be highest in the treatments that are most similar to those in natural habitats where worms were found (i.e. lower>mid>upper and fine sand>medium sand>silt). I also hypothesized that predation rates might be highest in loose sediment that would allow predators to move or dig freely (i.e. medium sand>fine sand>silt). In the case of predation, there was no *a priori* knowledge of potential predators in different areas of the estuary and so the Kruskall-Wallace test was employed to compare predation between estuarine locations.

Physiological Tolerances

The tolerance of varying temperatures and salinities by all life-history stages (adults, juveniles, larvae, and embryos) was tested to determine if either of these environmental parameters alone might explain why this species is limited to the lower estuary. The tolerances to short-term variations in temperature and salinity and the interactions between the two were determined between July 2005, and March 2008. Survival is used here as an indicator of tolerance. Adults of *O. collaris* were collected from mudflats near the mouth of the Coos Bay estuary. To provide embryos, larvae, and juveniles for experiments, adults were removed from their tubes and gametes obtained by

dissection. Males and females were kept separate to promote synchronous fertilization when dilute sperm were added to eggs from several females. Some of the resulting embryos were used to determine tolerances, while others were reared in culture in the laboratory using standard techniques for further study with larvae and juveniles (Strathmann, 1987; see Chapter 2).

To determine the tolerances of the early life-history stages of *O. collaris*, groups of embryos, larvae, and juveniles were exposed to a range of temperatures and salinities encompassing those commonly found in Coos Bay, and those outside the limits of what might be deemed normal in this area. Temperature treatments used were 5, 15, and 20ºC and salinity treatments were 5, 15, 25, and 35. Animals were held in 20-mL scintillation vials containing 0.45 μm filtered seawater (FSW) for 24 hours in each treatment. At the end of each experiment, the number remaining alive was determined with a dissecting microscope. Samples of 50 embryos, 40 larvae, and 10 juveniles were used in these experiments in each replicate vial. No acclimation period was used in these experiments due to the sheer number of vials and animals, and preliminary experiments had indicated that acclimation within these short-term experiments did not affect overall survival patterns. Mitraria larvae of two different ages (young and advanced) were tested in this manner. Young mitraria larvae were several days past the onset of swimming and advanced mitraria larvae had begun to develop the juvenile rudiment but were not yet competent to metamorphose. The juveniles were used within 5 days of metamorphosis. The number of juveniles available was limited, and so this stage was not exposed to either 5 or 20ºC at salinity of 5.

Adults were collected from the Fisherman's Grotto mudflat at low tide (Fig. 4.1). Five adults were placed in plastic 500-mL dishes with native sediment and seawater on the day of collection. Three replicate dishes were placed in either an incubator or water bath set to 5, 15, 20, 25, or 30°C, and placed in salinities of 5, 15, 25, or 35. Worms were checked for survival by gently squeezing their tubes with a pair of forceps and looking for movement of the animals under a dissecting scope. Replicates were checked one day and 5 days day after exposure began, and once a week until 47 days after exposure began. The experiment was terminated after 47 days because many of the containers were overgrown with an algal film, which may have interfered with the animals' ability to feed.

Survivorship was analyzed for the early life-history stages using a nonparametric nominal logistic model with life-history stage, temperature and salinity as fixed factors (JMP 7). Survival analysis was used to calculate the time-to-death of 50% of adult worms (td50) in each treatment (Nuzhdin et al. 2005; Ajuzie 2007). Td50 was then analyzed by a full-factorial two-way analysis of variance with salinity and temperature as fixed factos, followed by post-hoc Tukey's tests when main effects were significant (Quinn and Keough 2002).

Settlement and Sediment Choice

It is possible that differences in the recruitment or growth of juveniles between the upper and lower estuary is explained by the availability of appropriate sediment for tube building. These differences, in turn, could also explain the adult distribution. To examine potential effects of sediment size or type on recruitment, I exposed competent

mitraria larvae to a variety of sediments in the laboratory and examined subsequent recruitment in two separate experiments.

Adult Selection: Cultured mitraria were reared until the juvenile rudiment was well advanced and the larvae began to sink to the bottom of culture jars. In the first experiment, four hundred competent larvae were introduced to a common garden (Latin Square design) in which three replicate petri dishes were filled with one of four sediment treatments: fine sand (dominant size classes 125-250 μm) conditioned with adults, medium sand (dominant size class 250-425 μm) conditioned with adults, sterilized fine sand, and sterilized medium sand. The medium sand was collected from The Cribs near the mouth of the estuary and the fine sand was collected from the Domehouse mudflat 3.8 nautical km from the mouth of the estuary (Fig. 4.1). Sediment was sterilized by placing dishes into an autoclave for 8 minutes. Sediment was conditioned with adults by laying two adults in dishes of sterile sediment in FSW for 48 hours. All petri dishes were submerged in a 500-mL dish containing FSW and held in a 15ºC incubator (Fig. 4.3**A**). Competent larvae were added haphazardly to the dish. After two days, each petri dish was examined for juveniles and larvae undergoing metamorphosis. Data were analyzed using a 2-factor ANOVA with Sediment Type and Condition as fixed factors.

Grain Size Selection: In a second experiment, 60 competent larvae were introduced to each of 3 divided petri dishes filled with FSW, in which they were given a choice of sediment of different size classes: 63, 125, 250 μm, or the negative control with no sediment (Fig. 4.3**B**). After 24 hrs, dishes were examined for the presence of either larvae or juveniles adhering to the bottom. Data were analyzed using a Chi-squared test.

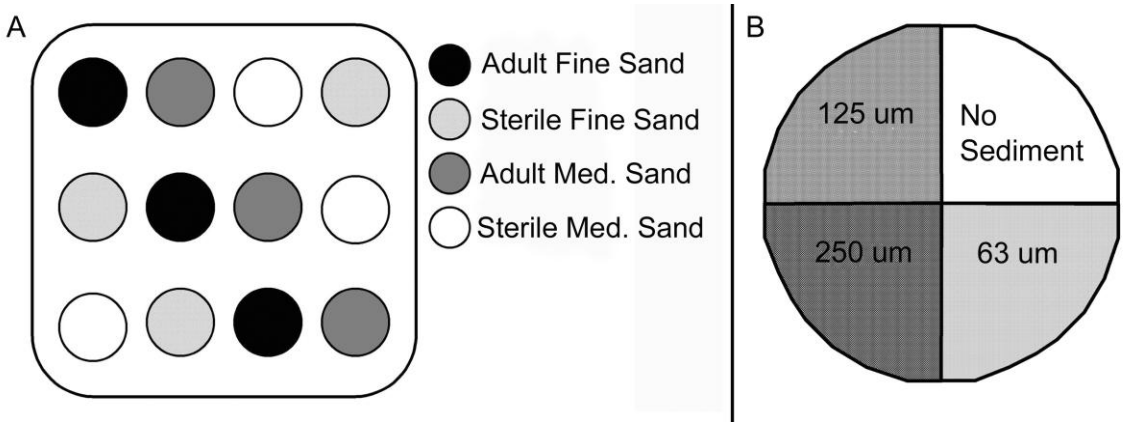

FIG. 4.3. Schematic representation of settlement/ sediment choice experiments using competent mitraria larvae of *O. collaris*. (A) Adult Selection Experiment: competent mitraria were added to a single dish containing replicate petri dishes holding two types of sediment (fine sand or medium sand) and adult conditions (adult or sterile) in a fully crossed model. (B) Grain Size Selection Experiment: competent mitraria were added to replicate divided petri dishes holding clean sediment of different sizes (63, 125, 250 μm or no sediment).

RESULTS

Field Survey

Owenia collaris were present only in the lower estuary, in cold, euhaline waters with a mix of small grain sizes (63-250 μm), with the exception of 2 sites in polyhaline waters (End of the Road and Empire Docks; Figs. 4.1 and 4.4; Table 1). Adults were absent at sites in the mid and upper estuary that contained high proportions of small sediment (i.e. Haynes Inlet and Catching Slough). Adults were absent from two mudflats surveyed in the mid estuary that share temperature and salinity characteristics with other sites that contain worms (BLM Boat Launch and Valino Island). These are sites composed of relatively higher proportions of medium-grained, loose sediment and, particularly at the BLM Boat Launch, negligible amounts of fine sediment. There was no

statistical difference between the percentage of fine-grained sediments between sites with and without *O. collaris*. The source of variation in grain size came primarily from variation among sites (Table 4.2).

Adult Transplants

Over the the three months that the adult transplants were in the field, many replicate cores were lost, leaving an unbalanced designed with no replication in some cases for site and sediment. Modifications in analysis were undertaken accordingly. In reviewing Davidson's (2008) data and GPS coordinates, the transplants placed at Haynes Inlet actually fell within his "polyhaline" category at the time that the transplants were carried out. All transplants at the BLM Boat Launch, originally a mid estuary site, also were lost over the course of three months so treatments were reassigned and data were analyzed using nonparametric tests in which sites were pooled within estuarine location. The Cribs and Fossil Point represent lower or euhaline sites, Valino Island and Haynes Inlet represent mid or polyhaline sites, and Catching Slough served as the only upper or mesohaline site.

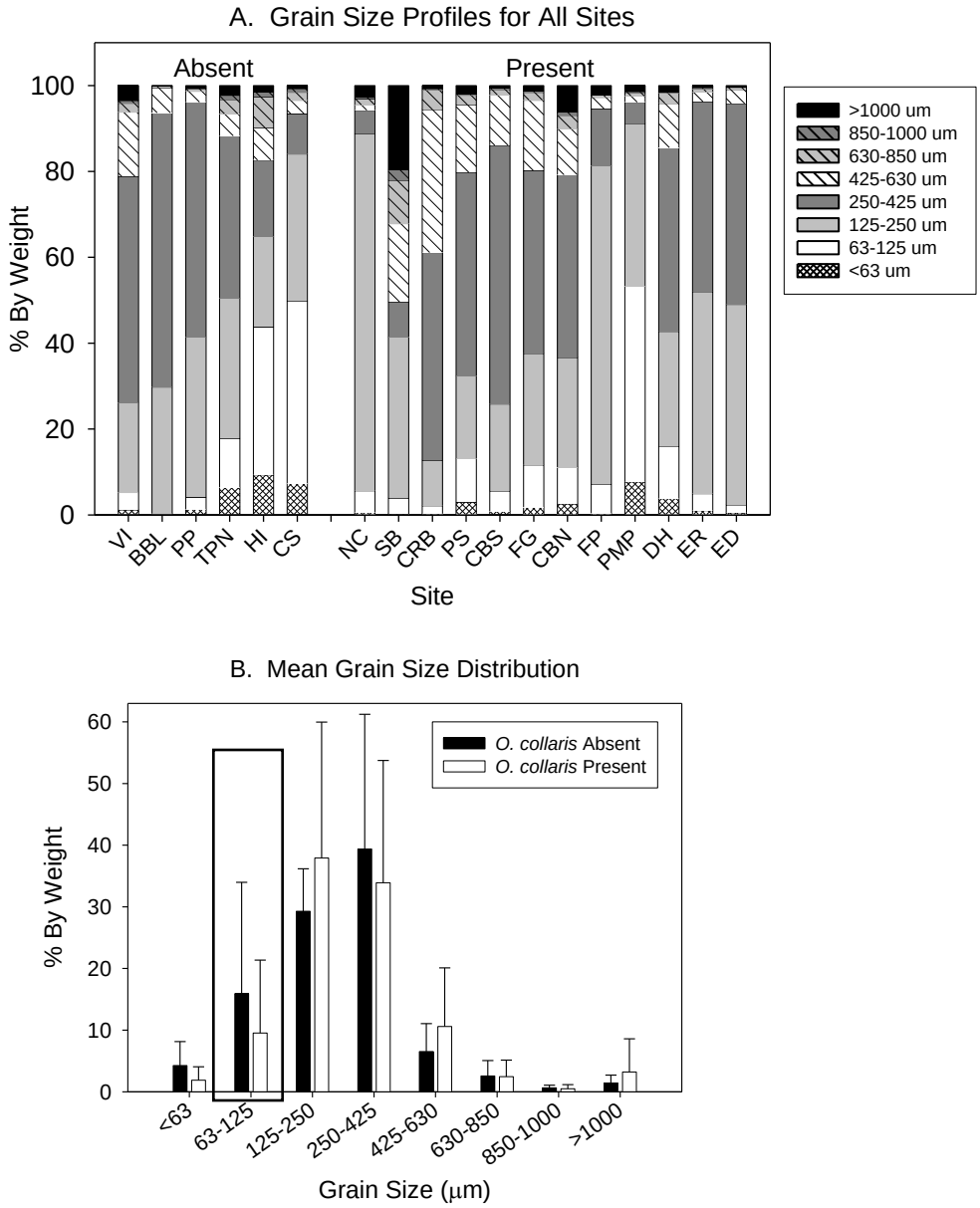

FIG. 4.4. Sediment grain size profiles for mudflats with and without adults of *O. collaris* in Coos Bay, OR. (A) Percent by weight of each size class for each site grouped by presence or absence of *O. collaris*. Bars represent means of 3 replicate cores per site. (B) Mean percent by weight of each size class with standard deviations. The boxed data points represent those used to examine differences in relative abundance of fine-grained sediment or silt (63-125 μm) between sites with and without *O. collaris*.

TABLE 4.2. Two-way ANOVA for differences in relative abundance of fine-grained sediment in sites with and without adult *O. collaris*.

Source	**df_N, df_D**	**MS**	**F**	**P**
Occurence	1, 16	490.544	0.825	0.377
Site(Occurrence)	16, 36	594.675	20.357	<0.001
Core(Site(Occurence))	36	29.212		
Total	53			

Survival and reproductive condition were higher in the lower estuary than in the mid estuary, and no worms were able to survive in the upper estuary for the full three months of the experiment (Fig. 4.5**A** and **B**). A majority of worms survived in the lower estuary, while only one-third survived in the mid estuary. Worms held in the lower estuary possessed nearly fully developed gonads, whereas most worms held in the mid estuary possessed depleted or undeveloped gonad. Differences in survival and reproductive condition were significant among the three estuarine locations tested (Table 4.3). Survival and reproduction were similar across all sediment treatments tested (Fig. 4.5**A** and **B**). Incidence of predation was similar in both the lower and mid estuary and among sediment treatments, with a few worms regenerating body parts in both the lower and mid estuary (Fig. 4.5**C**; Table 4.3).

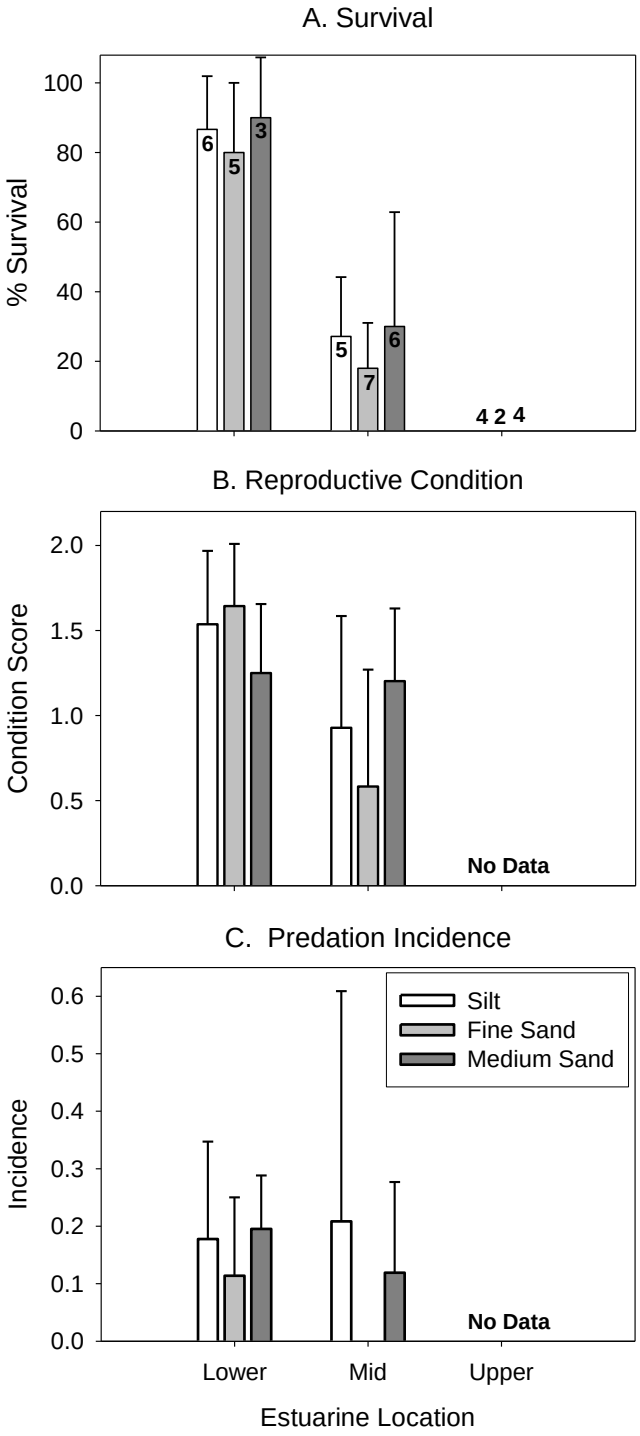

FIG. 4.5. Results of adult transplant experiment showing (A) survival, (B) reproductive condition, and (C) predation three months after deployment. Bars represent means with standard deviations. Numbers within bars represent the number of cores recovered for each category.

TABLE 4.3. Statistical analysis of transplantation of adult *O. collaris* in Coos Bay, OR. (A) Jonckeere-Terpstra test for differences in (1) survival and reproductive condition due to estuarine location and sediment and (2) predation between sediment. H_a for estuarine location is upper<mid<lower and for sediment is sand<silt<mud. (B) Kruskal-Wallis test for differences in predation between estuarine locations.

A. Jonckheere-Terpstra results

		Estuarine Location	**Sediment**
1. **Survival**	$J_{observed}$	564.00	321.00
	$J_{critical}$	383.00	360.00
	P	**<0.001**	0.493
Reproductive Condition	$J_{observed}$	166.00	132.00
	$J_{critical}$	115.00	204.00
	P	**0.001**	0.390
2. **Predation**	$J_{observed}$		104.00
	$J_{critical}$		204.00
	P		0.457

B. Kruskal-Wallis results

		Chi-Square	**df**	**p**
Predation	Estuarine Location	2.792	1	0.102

Physiological Tolerances

In general, physiological tolerances of short-term exposures to various temperatures and salinities increased with age in the early life-history stages of *O. collaris* (Fig. 4.6). A full range of data was not available for salinity of 5, and so this treatment was removed from statistical analysis. The effects of salinity, stage, and temperature on survival were all significant, as were all of the interactions among factors except the interaction between temperature and salinity (Table 4.4). Very few individuals of any stage could survive salinities of 5 and upper estuarine conditions (warm and low salinity) decreased survival in both embryos and larvae. Embryos were the least tolerant to high temperatures and low salinities, while juveniles were the most tolerant of both.

Survival of young and advanced mitraria larvae and juveniles at all salinity and temperature treatments combined tended to be similar, while embryo survival was generally reduced when compared to these stages. With all stages and salinities combined, survival was generally similar at all temperatures. Survival was generally highest at 35 (full strength seawater) across all stages and temperatures.

Adults of *O. collaris* were held for approximately seven weeks at a range of temperatures and salinities. A Weibull distribution was used for the parametric survival analysis. The effects of salinity, temperature, and the interaction between salinity and temperature were significant (Table 4.5). Although survival at all treatments except salinity 5 was 100% after one day, differences in survival became apparent as the experiment continued. Over the 47-day time period, adults exposed to the highest temperatures and lowest salinities experienced the highest mortality and the only treatments in which a majority of the animals survived were those most similar to the lower or mid estuary (cold and high salinity) (Fig. 4.7). Adults were more tolerant than the early stages to upper estuarine conditions, surviving a temperature of 25ºC for several days and at a salinity of 15 for several weeks. However, their intolerance of low salinities over extended periods of time was similar to the intolerance of low salinities by larvae and juveniles over shorter periods. Across all salinities, td50 was significantly higher at 5ºC than 15, 20, 25, or 30ºC. Td50 was not significantly different across all salinities within the latter temperature treatments. Across all temperatures, td50 was significantly higher at salinity 25 than at salinities of 5, 15, or 35.

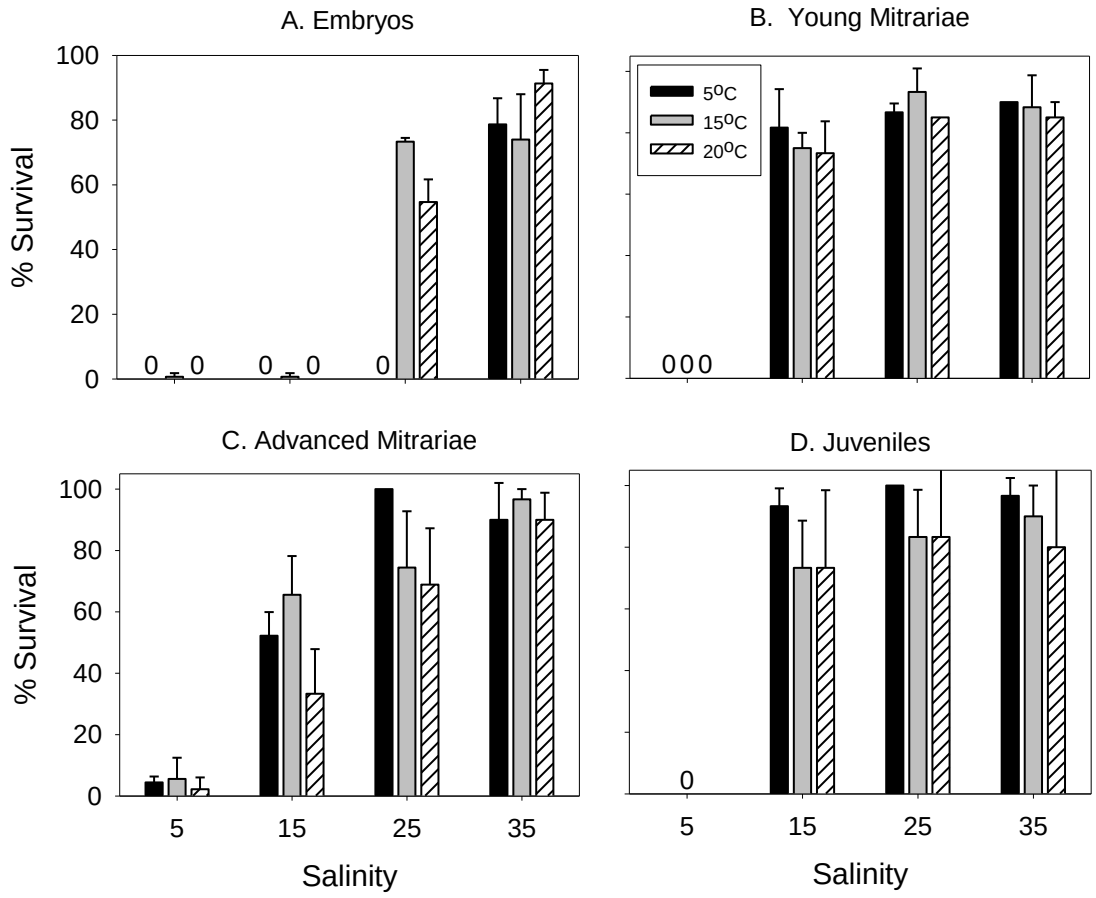

FIG. 4.6. Results of short-term exposure to variations in salinity and temperature for the early life-history stages of *O. collaris*. Survival of (A) embryos, (B) young mitraria larvae, (C) old mitraria larvae, and (D) juveniles after 24 hours. Bars represent means with standard deviations (n=3). Zeros are placed where means are 0, but do not indicate lack of data.

TABLE 4.4. Statistical analysis of survival after short-term exposure to variations in salinity and temperature for the early life history stages of *O. collaris*.

Source	**df**	X^2	**p**
Stage	3	479.07	<0.001
Salinity	2	111.93	<0.001
Temperature	2	6.14	0.047
Stage*Salinity	6	177.33	<0.001
Stage*Temp	6	33.18	0.010
Salinity*Temp	4	1.70	0.792
Stage*Salinity*Temp	12	133.94	0.001
Repl (St*Sal*T)	24	125.35	<0.001

TABLE 4.5. Statistical analysis of td50 after long-term exposure to variations in salinity and temperature for adult *O. collaris* held in the laboratory.

Source	**df_N, df_D**	**MS**	**F**	**p**
Salinity	3, 40	8.09 E 70	6.068	0.002
Temperature	4, 40	8.04 E 70	6.026	0.001
Temp*Sal	12, 40	7.94 E 70	5.952	<0.001
Error	40	1.33 E 70		

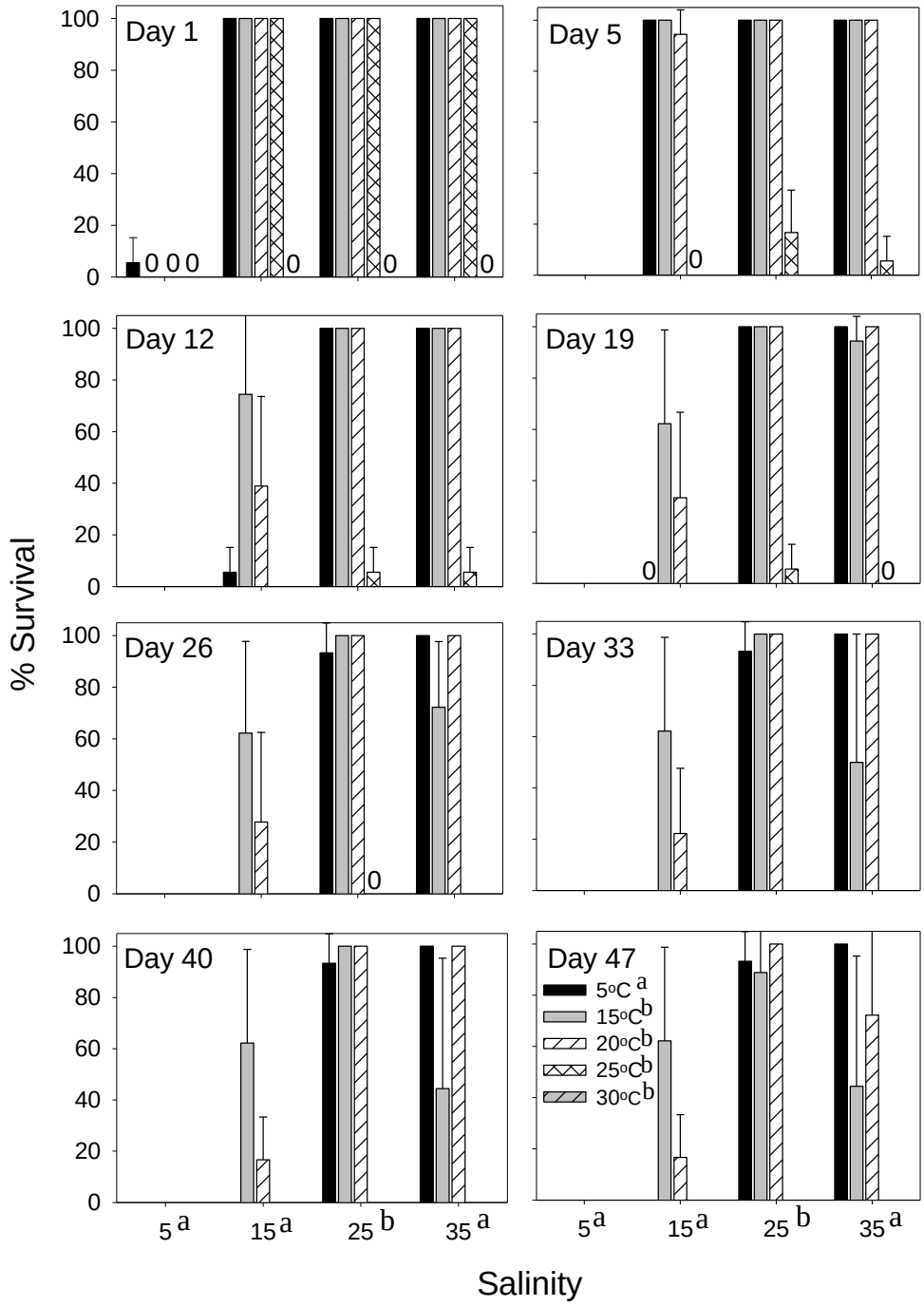

FIG. 4.7. Results of long-term exposure to variations in salinity and temperature for adult *O. collaris* held in the laboratory for 47 days. Bars represent means with standard deviations (n=3). Zeros are shown only for the sample day on which a treatment first experienced complete mortality. Letters represent Tukey's HSD post hoc groups.

Settlement and Sediment

There were no significant differences in the number of recruits among treatments in the adult selection experiment (Fig. 4.8**A**; Table 4.6), indicating that worms showed no preference for sediment with adults or sediment of the grain size profiles tested. There were more juveniles in sterile fine sand, which consisted of a higher proportion of smaller grains than medium sand. The number of settlers recovered in the grain size selection experiment was low, but a Chi-squared analysis indicates that the differences among treatments were significant ($G=95.4$, $X^2_{crit}=7.81$, $p=0.05$; Fig. 4.8**B**). The highest number of settlers was found in the 63-µm treatment, with the other three treatments having similar numbers of recruits.

Juvenile tubes collected from sites in the lower estuary typically have a variety of small grain sizes, whereas adults utilize a variety of larger grains (Fig. 4.9).

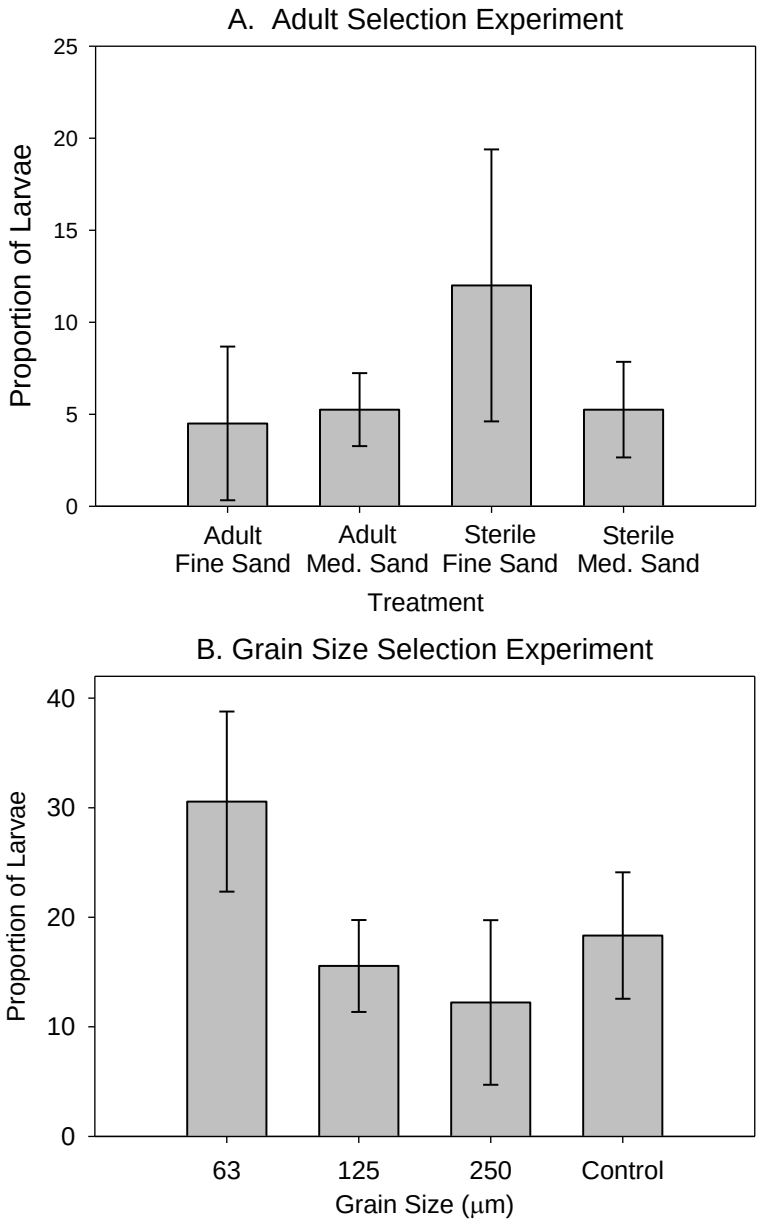

FIG. 4.8. Percent of competent larvae recruiting into (A) sediment of different size profiles and adult condition in a latin-square experiment after 48 hours and (B) sediment of different sizes in a choice experiment after 24 hours. Bars represent means with standard deviations (n=3).

Table 4.6. Statistical analysis of recruitment into sediment of different size profiles and adult condition in a common garden experiment using a 2-way ANOVA.

Source	**df**	**SS**	**MS**	**F**	**p**
Condition	1	0.020	0.020	1.792	0.218
Sediment	1	0.003	0.003	0.230	0.644
Condition* Sediment	1	0.021	0.021	1.840	0.212
Repl(C*S)	8	0.091	0.011		
Total	11	0.135			

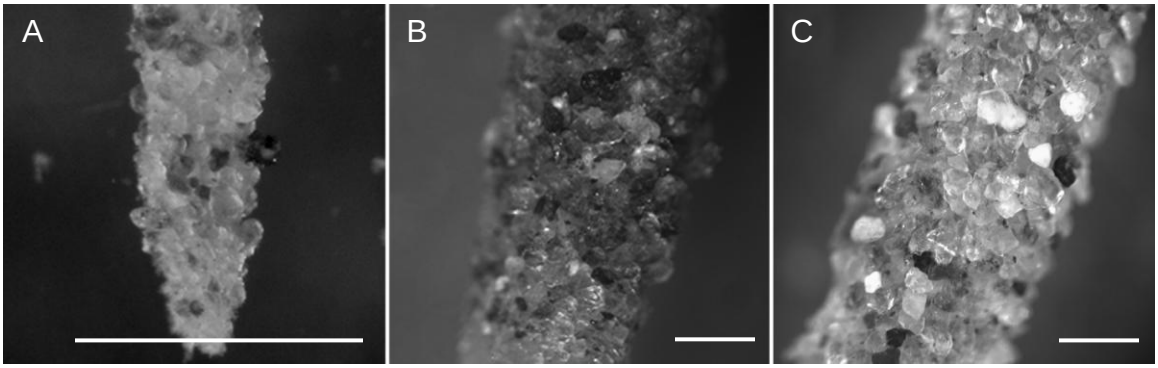

FIG. 4.9. Tubes of *O. collaris* showing relative sizes of sediment used in construction of the tube. (A) The juvenile tube, (B) the bottom portion of the adult tube, and (C) the top of the adult tube (the newest portion). Scale bars represent 1 cm.

DISCUSSION

Adult *Owenia collaris* exhibit a very specific distributional pattern in the Coos Bay estuary. Worms are found most commonly in cold, euhaline areas with relatively little fluctuation in temperature and salinity relative to other parts of the estuary. They are not restricted to euhaline waters alone but also can be found in polyhaline areas (Fig. 4.1). They are found in several types of sediment, from areas where silt dominates to areas in which medium sand dominates (Fig. 4.4, Table 4.1). Laboratory and field experiments further indicate that the estuarine distribution of *O. collaris* is regulated in all

life-history stages by the salinity gradient in the Coos Bay estuary and perhaps by sediment grain size in the juvenile stage. Water temperature may set range limits at large geographic scales for this species, but does not regulate distribution in this temperate estuary.

Physical Limitations by Environmental Variation

A wide range of sediment sizes is available throughout the estuary. Juveniles utilize a variety of smaller grains to build their tubes, and show no requirement for sediment or a chemical from conspecific adults to undergo metamorphosis (Figs. 4.8, 4.9). Juveniles, however, do show a preference for small grains, a preference that may become a necessity with continued growth (Fager, 1964). Sediments of different types or grain sizes could provide inadequate organic content, interfere with feeding activity, or allow predators to move more freely. However, there is no indication that sediment grain size affects survivorship, reproductive condition, or predation in adult *O. collaris* (Fig. 4.4). The congener *O. fusiformis* adjusts feeding behavior based on conditions and can readily switch between surface deposit and suspension feeding (McCall, 1977; Taghorn et al., 1980; Gambi, 1989). It is likely that *O. collaris* also can augment low returns by one mechanism with the other if a given type of sediment is unsuitable for consumption or prevents one type of feeding. Adult *O. collaris* potentially can live in the silty sediments found in the upper estuary or the medium sand found in many areas in the mid estuary where they are absent.

The patterns in survival, reproductive condition, and predation are probably influenced by one of the other two physical factors (salinity and temperature) associated

with the estuarine location in which transplants were placed. There was no differential predation across the estuary, although there were no worms or tubes remaining to examine in the upper estuary (Fig. 4.5**C**). High predation in this area seems unlikely, as no cores were disturbed by the activities of burrowing predators that could completely remove tubes and no tracks from snails were found on the surface of these cores. The most common sign of predation in *O. collaris* is the loss of the head or feeding tentacles, as would occur from nipping or cutting. Juvenile Dungeness crabs (*Cancer magister*) were commonly found in or near cores placed in the lower estuary and are a likely predator. Dungeness crabs are found throughout the main body of the bay (P. Dunn and S. Yamada-Behrens, personal communication), and so differences in survivorship among estuarine locations cannot be attributed to predation.

The upper estuary, or mesohaline or warm waters, cannot support adult *O. collaris*. Even worms transplanted into polyhaline waters in the field exhibited decreased survival compared to worms transplanted into native euhaline waters. Decreased survival and reproduction could either be attributed to decreases in salinity by 10 or 20, or it could be attributed to the slight increase in temperature of 4 or 6ºC (Fig. 4.1). Although adults can survive mid-estuarine conditions for at least three months, limited production of viable gametes in the polyhaline reaches of the estuary indicates that this is an inappropriate habitat for *O. collaris* over longer time scales because the worms are unable to complete the life cycle under these conditions. For *O. collaris*, low salinity is probably the factor driving the reduction of gamete production. The temperatures encountered in the upper estuary at the time that this experiment was conducted were within in the range

at which reproduction occurs in *O. collaris* (see Ch. 4), although the day-to-day fluctuations may have had some impact. Salinity toward the riverine end of the polyhaline region is probably well below that which is typical at the time of year that *O. collaris* produce gametes in the lower estuary and outer coast (see Ch. 4).

Reproduction in *Marenzelleria* cf. *viridis* is also limited by hyposaline conditions, even though adults can survive at much lower salinities (Bochert et al., 1996; Fritzsche, 1997). Low salinities also produce sterile sub-populations in *Capitella* sp. I (Pechenik et al., 2000). Larvae and juveniles of *Capitella* sp. I can survive short-term exposure to low salinities, and adults can survive long-term exposure, but often brooding and fecundity are greatly reduced or prevented at low salinities. Sterility in this case was probably the direct result of reduced feeding and growth rates caused by salinity stress. Juvenile *O. collaris* that may recruit and grow to adult size in polyhaline areas represent a sink for this population, produced by the lower estuary or outer coast portion of the population.

Long-term exposure to conditions that mimick the three primary areas of the estuary indicate that physical factors probably do not control distribution of adult *O. collaris* (Fig. 4.7). Adults can survive for up to 47 days in polyhaline conditions (25) and at reduced levels at mesohaline conditions (15). Ultimately, mortality increases with the length of the exposure to strong hyposaline or mesohaline conditions. If this experiment had continued, survivorship patterns may have matched those seen in the field experiment. The temperatures that resulted in mortality were well outside the range of water temperatures typically encountered in Coos Bay (<5ºC and >20ºC) and the probability of prolonged exposure such as those experienced in the lab are very unlikely

in the field, where extremes probably occur as pulses. Even if worms are exposed to high air temperatures during low tide, they are only exposed for several hours at a time and the surrounding sediment and tube may act as thermal buffers to mitigate stressful conditions. Salinity variation throughout the bay, on the other hand, is a much more predictable stressor for sessile, benthic invertebrates, and is the most likely physical limitation to distribution of *O. collaris*. Salinity stress in the polychaete *Capitella* sp. I reduced feeding rates and growth of adults (Pechenik et al., 2000). *Terebellides parva* were unable to regulate water and ion balance under salinity stress and this rapidly resulted in mortality (Ferraris et al., 1994). While some polychaetes, e.g. *Neanthes succinea, Mercierella enigmatica,* and *Arenicola marina,* are able to regulate water and ion balance under salinity stress or withstand this stress (Freel et al., 1973; Skaer, 1974; Shumway and Davenport, 1977), others, such as *T. parva,* are more limited in these abilities and their distributions generally reflect this. Salinity may limit the distribution of *O. collaris* in Coos Bay by decreasing the ability of worms to feed or grow but the observed adult distribution is still narrower than would result from adult tolerance alone.

Physical factors may not be as limiting to the early life history stages as would be predicted based on adult distribution and tolerances. For a temperate species, the early life-history stages of *O. collaris* also are tolerant to wide variations in salinity and temperature, which may reflect their broad range along the west coast of North America (southern CA to Puget Sound, WA) (Blake, 2000; Smart, personal observations). Like many other invertebrates, the earliest stages (embryos) are the most vulnerable to environmental stress, with tolerance increasing in subsequent stages (Bayne et al., 1976;

Claudi and Evans, 1993; Sanford et al., 2006). When gametes are spawned into the water column, thin mucus accompanies the negatively buoyant eggs (Smart, personal observation). The result of this mixture is that eggs are often retained around the female's tube for several hours in gently flowing water, where they may develop without risk of exposure to water conditions other than those experienced by the adult. At higher flows, the negatively buoyant eggs are swept up into the water column thus exporting them from the adult habitat and potentially into harmful conditions. If embryos somehow remain close to the adult population at least until hatching (24 hrs) before potentially being washed up into the estuary on incoming tides, the pelagic larvae and young juveniles can withstand exposure during early development (Fig. 4.6). Again, the water temperatures that cause mortality in these stages are well above recorded observations in Coos Bay and the most vulnerable stages (embryos and larvae) should not be exposed to air temperatures. Salinity, on the other hand, plays a major role in limiting the pelagic and early benthic stages from the upper estuary. The absence of adults from most areas within the polyhaline section of the bay suggests that long-term exposure of either juveniles or larvae to salinities at the low end of this range (5-15) prevents recruitment densities high enough to establish adults in these areas, either through mortality or reduced growth (Lambert et al., 1994; Qui and Qian, 1997; Pechenik et al., 2000), or that there are other post-settlement factors that prevent juveniles from growing to the adult stage (Lyster, 1965; Olaffson et al., 1994).

It is possible that larvae in polyhaline regions do not encounter the appropriate substrata in which to settle. Juvenile *Owenia fusiformis* and *O. collaris* prefer silt (≤63

μm) with which to build their tubes (Fager, 1964; Pinedo et al., 2000). Larvae in the Coos Bay population showed slight preference for silt, but were able to successfully undergo metamorphosis in a variety of sediments (Fig. 4.8). They do not appear to require a certain type of sediment or even any sediment at all to undergo metamorphosis, as long as they have access to relatively small particles with which to begin forming tubes (Fig. 4.9). All sites surveyed in this study contained at least some fine-grained sediment (≤250 μm) that would be suitable for early tube formation. Although Dauvin (1992) found that *O. fusiformis* required at least 4% silt, *O. collaris* were abundant in sites around Coos Bay with much less silt (The Cribs and Empire Docks). The BLM Boat Launch was the only site with negligible levels of silt and this site had no adults, despite the fact that it was adjacent to two sites where *O. collaris* occurred abundantly (Empire Docks and End of the Road). Juvenile *O. collaris* may require a mixture of small grain sizes that includes silt at a later time than those tested in these experiments and that this site alone cannot provide this minimum necessity. These data together suggest that *O. collaris* are limited in their extent to the Coos Bay estuary primarily by the salinity regime at all life-history stages and secondarily, perhaps, by sediment characteristics in the juvenile stage after metamorphosis. However, there are other post-settlement factors (i.e. juvenile predation, competition) that have yet to be addressed for this species.

Consequences for Dispersal Models

Havenhand's (1995) "whole life cycle approach" was used to test the effects of key estuarine physical factors on the distribution of the polychaete *O. collaris* in Coos

Bay, OR. Tolerances of all life history stages (embryos, larvae, juveniles and adults) were compared and related to the documented benthic distribution. Although the earliest life-history stages exhibited reduced survival at low salinities and the highest temperatures tested, this species may be very capable of undergoing broad dispersal into the nearshore environment, as would be predicted from their broad geographic range (Blake, 2000; Smart, personal observation). Within the estuary, however, dispersive stages cannot survive in the upper estuary because of their intolerance of low salinities, much like the adults. Substratum preference does not appear to play a very strong role in determining where juveniles can establish, so distribution is most likely a function of salinity acting on survival in the pelagic stages.

It is, of course, possible that the larvae of *O. collaris* do not actually disperse farther upriver from where they are spawned and that there is a perfect match between the pelagic and benthic distributions. Invertebrate larvae are strong enough swimmers to control their vertical position in the water column, thereby reducing transport by remaining in the benthic boundary layer or enhancing transport by swimming to the top of the water column where flows are much higher (Mileikovsky, 1973; Chia et al., 1984). Postlarvae of the polychaete *Pectinaria koreni* display selective tidal stream transport by swimming up into the water column only during flood tide, thus moving shoreward and eliminating seaward transport during ebb tide (Thiebaut et al., 1996). Precompetent larvae of *O. collaris* do not exhibit a distinct swimming direction in either still or gently mixed water but are phototactic to some extent (Smart, personal observation). When exposed to haloclines, these same larvae can swim across steep discontinuity layers and

will also swim up into detrimental salinities at the surface (Smart, unpublished data), similar to what would be produced at times of high river discharge. Larvae of this species do not exhibit behavior that would retain them near the bottom, out of low salinity water masses, limit their movement up river or out of the estuary, or retain them near the adult population. However, field tests of estuarine transport for this or any polychaete other than *P. koreni* do not exist (see Forward and Tankersley, 2001, for review).

Bhaud (2000) outlined distributional patterns for species with complex life histories that are subject to variations in multiple physical conditions across their ranges. Tolerance of physical variations set the boundaries of pelagic larval and benthic adult distributions, with the area in which the entire life cycle can be completed defined by the overlap in these distributions. For sessile animals, the early life history stage distribution (and therefore tolerances) must be equal to or wider than that of the adults, since migration is impossible. For those found in estuaries, the early stages must therefore be tolerant of fluctuations in physical parameters such as salinity and temperature that are wider than or equal to those found within the adult distribution. Competent larvae may need low specificity for sediment types at metamorphosis because these too can vary throughout an estuary. There are relatively few examples of species for which physical requirements are known for all life history stages.

Owenia collaris in the Coos Bay estuary follows Bhaud's (2000) description for the categories of sub-populations in sessile marine invertebrates in that the dispersive stages are probably wide ranging but there are areas within that range in which the whole

life cycle cannot be completed. In the upper reaches of the estuary, dispersive stages may live for a very short time, but cannot grow to metamorphosis and the benthic stages cannot survive for long periods of time. In the mid-region of the estuary, dispersive and benthic stages can survive, although early stages may not survive through their entire development. Juveniles have suitable sediment at most sites in the mid estuary with which to construct their tubes, but adult fecundity is reduced within this subpopulation due to salinity stress. It is only within the lower estuary that the entire life cycle of *O. collaris* can be completed: embryos can develop to hatching, larvae can develop to metamorphosis, juveniles can recruit, and adults can grow to their full reproductive capacity.

CHAPTER VI

GENERAL CONCLUSION

Polychaete diversity can apply to a wide variety of topics: phylogenetics, morphology, ecology, and developmental biology. Within this broad group, there are few universal rules that can be employed to describe the group as a whole. The discovery of segmentation within the polychaete families Siboglinidae and Echiuridae lends credence to the universal truth of segmentation within the polychaetes. Protostome development through a trochophore stage is generally thought of as another universal truth within the polychaetes. The family Oweniidae is morphologically, ecologically, and genetically allied to other families of tube-building worms within the Polychaeta (including Sabellariidae, Serpulidae, and Siboglinidae). However, morphological similarities are only found within the adult stage of oweniids. The larval morphology is highly unusual when compared to typical polychaete larvae. Observations of the development of the larval form within the family Oweniidae called into question whether developmental characterics really are universal within the polychaetes or not (Wilson, 1932; Emlet and Strathmann, 1994). Observations of the adult habitat and juvenile recruitment patterns of *Owenia fusiformis* called into question whether or not species within this family followed the general ideas applied to the biology of soft-sediment, seasonally reproducing, and estuarine species. The body of work presented in this dissertation was undertaken in an

attempt to answer these questions using a representative species within the Oweniidae, *Owenia collaris*.

Similar to typical polychaete embryos, embryos of *Owenia collaris* undergo holoblastic, spiral cleavage and form the archenteron via invagination of the vegetal cells. Unlike typical polychaete embryos, embryos of *O. collaris* do not form the stomodeum from the blastopore and do not develop trochoblasts that cleavage arrest and grow multiple cilia, which in turn form compound cilia for swimming and feeding. Instead, embryos of *O. collaris* develop the anus from the original blastopore and the stomodeum from a secondary opening to the u-shaped gut. The trochoblasts continue dividing throughout development and the prototroch forms from hundreds (eventually thousands) of monociliated cells. Development to the swimming larva requires 24 hours at ambient seawater temperatures and larval development to competency requires approximately four weeks, potentially allowing for high dispersal. The juvenile rudiment develops internally, similar to that described for *O. fusiformis* (Wilson, 1932), and metamorphosis occurs gradually over a day. There is no evidence that the newly formed juvenile consumes cast off larval tissues as described for *O. fusiformis* by Wilson (1932), and juvenile *O. collaris* are smaller and have few segments than *O. fusiformis*. Cultures of *O. collaris* are simple to maintain in the laboratory and can provide numerous larvae and juveniles with which to conduct experiments.

I demonstrated that mitraria larvae of *O. collaris* feed and grow at rates similar to tornaria-type larvae of two species of echinoderms, but not unlike trochophore-type larvae. These two larval types obtain much greater sizes and ciliated band lengths than

do trochophore-type larvae of the polychaete *Serpula vermicularis* and the bivalve *Mytilus californianus*. These longer band lengths allow the mitraria and tornaria-type larvae to clear particles from the water column at much higher rates. However, these feeding rates appear to merely keep pace with the larger size of these larvae and there is no difference in either the weight specific or protein specific clearance rates among larval types.

Breeding in *O. collaris* is strongly seasonal in the Coos Bay estuary, occurring in spring and summer. Seasonality is related to photoperiod, temperature, primary production, and water chemistry, but probably is cued by photoperiod and controlled by an endogenous timer. The breeding season is adaptive in that it encompasses the spring phytoplankton bloom and subsequent high levels of primary production during the coastal upwelling season. Adults do not respond to increased food levels by producing gametes out of season. This indicates that breeding seasonality in *O. collaris* takes advantage of better conditions for larval growth and subsequent juvenile recruitment, rather than excess energy for adults.

Along the estuarine gradient, adult *O. collaris* are found most commonly in cold, euhaline waters. Their distribution does not appear to be controlled by sediment characteristics, as adults can survive in a variety of sediments and juveniles can successfully metamorphose in a variety of small-grain sediments. Survival and reproduction, however, are affected by both salinity and to a lesser extent temperature. Temperature tolerances are much broader than the ranges typically seen in the Coos Bay estuary, but may affect the large-scale geographical range of *O. collaris*. Intolerance of

low salinities indicate that this environmental factor controls the distribution of *O. collaris* within the Coos Bay estuary, affecting potential larval dispersal, juvenile recruitment, and long-term adult health.

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Chapter VI

Wilson, D. P. 1932. On the mitraria larvae of *Owenia fusiformis* Delle Chiaje. Philos Trans. R. Soc. Ser. B 221: 231-334.