Development and Organogenesis of the Patellogastropod *Tectura scutum* (Gastropoda)

by

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We accept this thesis as conforming to the required standard

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ABSTRACT

The development and morphogenesis of the plate limpet, *Tectura scutum*, was studied from egg fertilization, through the larval phase, to 1 month post metamorphosis. *T. scutum* is a member of the order Patellogastropoda, the most basal offshoot of the Gastropoda. Live embryos, larvae, and juveniles were observed with light microscopy, and histological sections of various larval and juvenile stages were used to study the morphogenesis of internal structures. Scanning electron microscopy was also used to observe fine detail of external structures such as the velum and protoconch. Embryos developed into trochophore larvae within 16 hours post fertilization (hpf), at 12°C. Torsion, the ontogenetic process during which the visceropallium rotates 180° relative to the cephalopodium, occurred between 51 and 59hpf, with the entire rotation being completed in approximately 7h. Swimming, non-feeding larvae were metamorphically competent at 7dpf. During metamorphosis, the juveniles began to crawl on the substrate, the velum and operculum were lost, and teleoconch formation began. Several of the traits that were observed in larval and juvenile stages of *T. scutum* are similar to the derived condition of higher gastropods, despite the presence of the primitive form of the trait in adults. These data suggest that traits scored as primitive for the gastropods may be secondarily derived in this basal offshoot. This emphasizes the need for analysis of

developmental stages in addition to adult forms when attempting to determine evolutionary relationships or polarity of change.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

an	anus
arm	accessory retractor muscle
aso	apical sensory organ
at	apical tuft
bc	buccal cavity
bg	buccal ganglion
bm	buccal musculature
c	cilia
cc	cerebral commissure
cg	cerebral ganglion
ct	cephalic tentacle
dfc	dorsal food channel
dg	digestive gland
e	eyespot
es	esophagus
f	foot
fg	foregut
i	intestine
il	inner lip(s)
lg	labial ganglion
lk	left kidney
lpt	larval pallial tentacle
lrm	larval retractor muscle
m	mouth
ma	mantle
mc	mantle cavity
mtp	metapodium
ms	muscle
oc	odontophoral cartilages
ol	outer lip(s)
op	operculum
os	osphradium
osg	osphradial ganglion
pb	polar body
pc	protoconch
pdg	pedal ganglion
plg	pleural ganglion
ppc	propodial cleft
prc	prototroch cell(s)
prp	propodium
prt	prototroch
r	rectum
rad	radula
rk	right kidney
rs	radular sac
s	stomach
sd	stomodaeum
shg	shell gland
st	statocyst
tc	teleoconch
v	velum
vc	velar cilia
vg	visceral ganglion
vl	visceral loop
vm	visceral mass
xs	cross section

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INTRODUCTION

The Gastropoda, a class within the phylum Mollusca, is a very diverse group of animals that inhabit marine, freshwater, and terrestrial environments. The development of gastropod molluscs has been studied for many years, with many initial, classic studies performed between the late 1800s and early 1900s. This is partly due to the relative abundance of some species, but also because the regular, consistent pattern of early cell divisions (Verdonk and van den Biggelaar 1983) made them attractive subjects for studies on factors that direct and control development. Important pioneering studies on gastropod development include descriptions of embryonic and larval development (Pelseener 1911, Crofts 1937, 1955, Thompson 1958), cell lineage studies (Conklin 1897, Casteel 1904), and experimental work on fate specification during embryogenesis (Clement 1952, 1962). Continuing studies on gastropod development are important to our knowledge and understanding of gastropod relationships and evolution. Without a detailed understanding of larval morphology and development, all information about morphological evolution must be derived from adult structures, which can exhibit tremendous levels of convergence. Also, the larval stages may exhibit clade-specific apomorphic characters, which could be overlooked if only adult states are observed.

Recent phylogenies have placed the patellogastropods, or "true limpets" as the most basal clade within the Gastropoda, and as the sister group to the rest of the gastropods (Haszprunar 1988, Ponder and Lindberg 1997, Harasewych and McArthur 2000). However, studies of larval development and morphogenesis of patellogastropods have been relatively scarce (Boutan 1899, Smith 1935, Kessel 1964, Wanninger *et al.* 1999a and b, Wanninger *et al.* 2000, Kay and Emlet 2002), despite their importance as an

outgroup for the remainder of the Gastropoda. My study focuses on the development and organogenesis of the patellogastropod, *Tectura scutum* (Rathke, 1833) in order to provide more information on this important, basal group of gastropods.

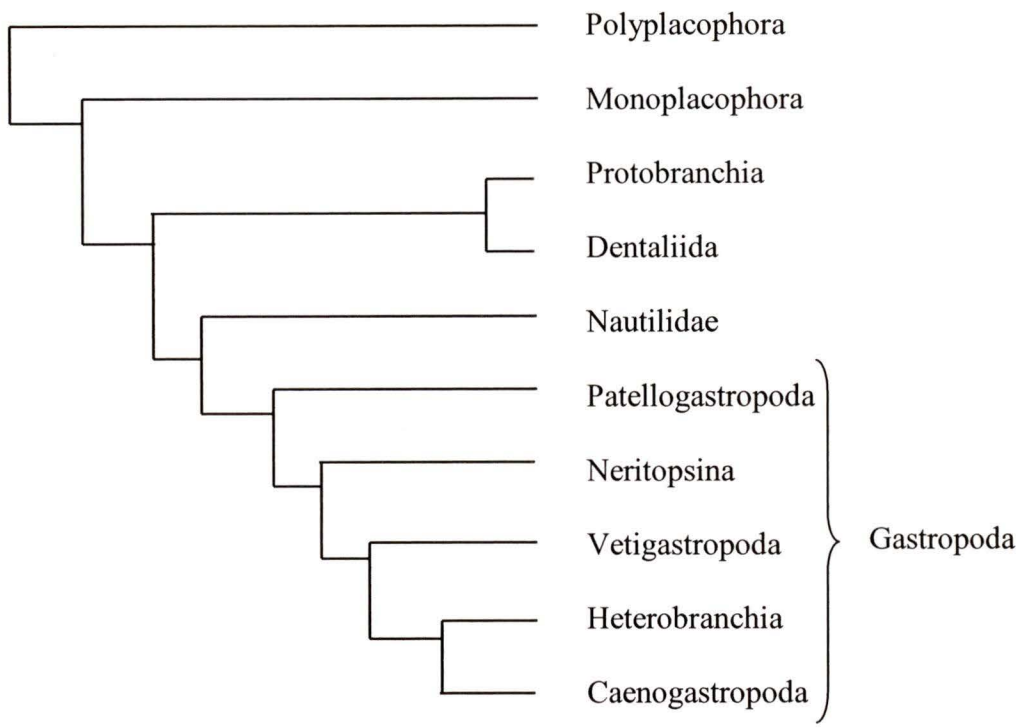
Classification and Taxonomy

The Patellogastropoda is generally regarded as the earliest offshoot of gastropods with members that are still extant (Haszprunar 1988, Ponder and Lindberg 1997, Harasewych and McArthur 2000). Recent molecular studies have supported the placement of patellogastropods within a monophyletic clade (Harasewych and McArthur 2000), as previously proposed by results of morphological studies (Haszprunar 1988, Ponder and Lindberg 1997). The other, more derived gastropod orders include: Neritomorpha (=Neritopsina), Vetigastropoda, Heterobranchia, and Caenogastropoda (Ponder and Lindberg 1997). It has been proposed that these four orders be grouped together in the super-order Orthogastropoda, separate from the Eogastropoda (=Patellogastropoda) (Ponder and Lindberg 1997). An abbreviated form of a phylogenetic hypothesis proposed by Ponder and Lindberg (1997) is shown in Figure 1.

Phylogenetic placement and taxonomy of the patellogastropods, or true limpets, have undergone several revisions over the past century. For many years, the true limpets were included as a suborder within the Archaeogastropoda, a group that included the vetigastropods (such as abalone, keyhole limpets, and turban snails) as well as the Neritomorpha, and later the hot-vent taxa (Lindberg 1988b, Bieler 1992). These groups share the characteristic of bipectinate ctenidia (Fretter and Graham 1962). However, bipectinate ctenidia are likely a primitive trait (plesiomorphy) of the gastropods, whereas

Figure 1. Gastropod phylogeny, adapted from Fig. 5 in Ponder and Lindberg (1997).

Outgroups are other molluscan classes; the cephalopods (Nautilidae) form the sister group to the gastropods. The patellogastropods are the basal offshoot within the Gastropoda and are a sister group to all other gastropods. Vetigastropoda includes the hot vent taxa, and other groups such as the Fissurellidae, Haliotidae, and Trochidae. Heterobranchia includes the opisthobranchs and pulmonates.


Polyplacophora
Monoplacophora
Protobranchia
Dentaliida
Nautilidae
Patellogastropoda
Neritopsina
Vetigastropoda
Heterobranchia
Caenogastropoda
Gastropoda

modern approaches to phylogenetic analysis accept only derived traits (apomorphies) as the basis for reconstructions of phylogenetic relationships. Golikov and Starobogatov (1975) first proposed that the patellogastropods be removed from the Archaeogastropoda and elevated to ordinal status, but few people accepted this change until the 1980s, when more data supporting this change became available (Haszprunar 1988, Lindberg 1988b). Another name that has been synonymous with Patellogastropoda has been Docoglossa, nomenclature that was based on radular structure (Fretter and Graham 1962, Haszprunar 1988). While this term was used frequently in past literature, its use is currently discouraged because members of two other molluscan classes, the Polyplacophora (chitons) and Monoplacophora, also have a docoglossate radula (Lindberg 1988b).

Characteristics of Patellogastropods

The patellogastropods possess many ancestral traits of the Gastropoda, but also have many unique, derived characters, as well as traits that have evolved convergently in several gastropod groups. Some of the plesiomorphic characters include pedal cords rather than consolidated pedal ganglia, widely separated statocysts that lie lateral to the pedal cords, lack of a tubular anterior pedal gland, a docoglossate radula, and a bipectinate ctenidium (although a ctenidium is absent altogether in some patellogastropods) (Ponder and Lindberg 1997). Autapomorphic characters include foliated shell structure, posterior aperture dilation, and three or more pairs of buccal cartilages (Ponder and Lindberg 1997). The non-crossing pedal muscle fibres that produce ditaxic pedal waves in patellogastropods are unique to this group of gastropods. Voltzow (1988) has suggested that monotaxic pedal waves are the ancestral condition for

gastropods, and possibly for all molluscs. Characters which have evolved convergently in the Patellogastropoda as well as other gastropod clades include the loss of the right auricle, the loss of the right (or both) ctenidia, simplification of the stomach, as well as the displacement of both renal organs to the right of the pericardium (Ponder and Lindberg 1997).

Some of the general trends in the anatomical evolution of patellogastropods, from more basal to more derived clades, include: a reduction in the number of intestinal loops, a reduction in the number of radular teeth, and a reduction in the number of gills (or a loss of gill structures) (Lindberg 1988a, b). Other possible trends include an earlier attainment of sexual maturity, and a shorter period of time during development from fertilization to the completion of torsion (Lindberg 1988a).

Development and Evolution

Developmental characters are important in evolutionary studies of the Gastropoda. In fact, larval characters are used to define the clade. For example, in establishing their morphology-based phylogeny of the Gastropoda, Ponder and Lindberg (1997) wrote that "the apomorphic characters defining Gastropoda (...), include torsion, the (larval) operculum, and the shape of the larval shell" (p.213). Although torsion is represented to some extent in the anatomy of all adult gastropods, torsion is essentially a developmental phenomenon (Raven 1958; Lever 1979). In trying to determine relationships and classification, adult characters can be confusing because of the prevalence of convergence in the different clades of gastropods (Wagner 2001). Knowledge of the developmental patterns for different species, and especially knowledge

of the developmental patterns of basal clades can aid in understanding the evolution of development and thus the evolution of adult morphology. For example, Lindberg (1988a and b) has suggested that heterochronic shifts, specifically progenesis, have been a pervasive theme during the evolution of patellogastropods, yet this hypothesis requires testing by detailed comparative studies of organogenesis and interpretation of these developmental data within a phylogenetic hypothesis.

While comparisons of developmental sequences can reveal much information about evolutionary relationships and changes, one must be careful not to try to derive information about the ancestor just from the developmental stages of extant species. Looking at ontogeny for answers to phylogenetic questions can lead to erroneous results if you are not careful to separate information about polarity of change from information about ancestral adult forms.

Gastropod Development - General

Gastropods are spiralians, and therefore also protostomes. Spiral cleavage produces an embryo which quickly develops into a trochophore larva, a larval form that is common to the Mollusca and Annelida (Fretter and Graham 1962, Collier 1997). Among the gastropods, only the patellogastropods and some vetigastropods have a free-swimming trochophore, whereas the trochophore stage occurs within an egg capsule in other gastropods. The trochophore becomes a veliger larva when it acquires rudiments of the shell (protoconch) and foot. The early veliger of gastropods undergoes the process of ontogenetic torsion before reaching metamorphic competence. During the processes of settlement and metamorphosis, the free-swimming veliger larva becomes a benthic

juvenile, which gradually develops into the adult form. A more detailed explanation of features of gastropod development follows.

Spiral cleavage of molluscan embryos is very regular and predictable, and many cell lineage studies have been carried out as a result of this (reviewed by Verdonk and van den Biggelaar 1983). Early cleavage can be unequal, in which a polar lobe may be formed during the initial cleavage stages, or equal, in which all blastomeres are the same size during the first few cleavage stages. Cell fate and dorso-ventral polarity of the embryo are determined early in species with unequal cleavage, while those with equal cleavage (such as patellogastropods) have a later determination of polarity and cell fate (van den Biggelaar and Guerrier 1983, Verdonk and Cather 1983, Verdonk and van den Biggelaar 1983, van den Biggelaar and Haszprunar 1996). In all cases, dorso-ventral polarity is definitively established when the 3D macromere divides to form the 4D macromere and the 4d micromere, also known as the mesentoblast (van den Biggelaar and Guerrier 1983). The D quadrant is dorsal, while the opposite B quadrant is ventral (van den Biggelaar and Guerrier 1983). The establishment of dorso-ventral polarity ensures the proper organization of the blastomeres and normal development.

Gastropod embryos differ according to the number of blastomeres present at the time the mesentoblast is formed. Van den Biggelaar and Haszprunar (1996) noted a correlation between time of mesentoblast formation, relative to overall number of blastomeres within embryos, and phylogeny. In patellogastropods and vetigastropods, embryos consist of 64 blastomeres at the time of mesentoblast formation, whereas caenogastropods have approximately 40 blastomeres, neritomorphs have 28-37, and heterobranchs have only 24 blastomeres at the time of mesentoblast specification.

Differences in blastomere number at the time of mesentoblast formation are due to differences in the cleavage rate of micromeres relative to macromeres. Thus, embryos of patellogastropods and vetigastropods (also those of chitons) have more micromeres when the mesentoblast arises than do embryos of caenogastropods and heterobranchs. The functional difference of this embryological variation among gastropods remains unexplained.

The trochophore larva is characterized by a distinctive shape, and by one or more equatorial bands of cilia that are used for swimming and capture and transport of food particles. The trochophore larva of gastropods becomes modified by further development to produce the veliger larval form (Raven 1958, Collier 1997). The anterior-posterior axis becomes bent such that the posterior end is now closer to the anterior end of the larva, and formerly dorsal regions of the trochophore become located posteriorly. Simultaneously, the embryo secretes a calcareous shell and begins to form a foot. The velum is the characteristic structure of the veliger larva, and is generally a bi-lobed structure on the apical surface of the larva. There is considerable variation in velum shape and size between different species: from a small, rounded disc-shape among non-feeding veligers, to a large, multi-lobed, elaborate structure in planktonic feeding veligers (Fretter and Graham 1962). The velum is lined with cilia along its circumference; this ciliary band is derived from the prototroch of the trochophore stage.

Ontogenetic torsion is one of the synapomorphies of the Gastropoda (Bieler 1992, Ponder and Lindberg 1997). It is a 180° clockwise rotation of the visceropallium (visceral mass, mantle, and shell) with respect to the cephalopodium (head and foot), as the animal is viewed from the anterior end (Raven 1958, Lever 1979, Signor 1985). Mechanisms

responsible for torsion have been debated extensively, with possibilities ranging from muscular contractions, to differential cell growth, to a combination of these factors (reviewed by Haszprunar 1988). The evolutionary advantage of torsion has also been debated, as the advantage could be for the veliger (ability to retract into its shell), or for the adult (retraction and water circulation) (theories reviewed by Ghiselin 1966, Lever 1979, Signor 1985).

Gastropod larvae exhibit a variety of life history patterns. The more basal clades, such as the Patellogastropoda and many vetigastropods, exhibit free-spawning and have swimming, non-feeding larvae that develop to metamorphic competence quickly. More derived clades have internal fertilization and brooded or encapsulated development of larvae that hatch to feed in the water column before subsequently becoming competent to metamorphose (reviewed by Fretter and Graham 1962).

Once metamorphic competence is achieved, the larvae of many species of gastropods require an exogenous cue to induce settlement and metamorphosis (Hadfield 1978, Morse 1990). During metamorphosis, many changes occur in morphology and behaviour (Fretter 1969, Bonar 1978, Page 2000). The velum is lost, the development of adult feeding systems is completed, the post-metamorphic shell (teleoconch) is initiated, and the juvenile begins to crawl on the substrate. In gastropods lacking a shell in the adult stage, larval metamorphosis involves loss of the larval shell (protoconch) and operculum. However, most gastropods retain the protoconch at the apex of the developing adult shell. Patellogastropods are unusual because the protoconch is lost from the apex of the teleoconch after several weeks or months into juvenile development (Smith 1935, Lindberg 1988a).

Development of Patellogastropods

While there have been very few developmental studies on *Tectura scutum* (see exceptions to this below), there have been relatively more studies on the development of other patellogastropods (mostly members of the family Patellidae). Smith (1935) provided an extensive study on the development of *Patella vulgata*, however his is the only study that describes larval development and morphogenesis (from gastrulation through to metamorphosis and early juvenile stages) of any patellogastropod. More recent works on *Patella* sp. include many genetic and cell lineage studies, as well as a limited number of morphological studies. There have been a number of recent studies on the developmental genetics of *Patella* (van Loon *et al.* 1993, van der Kooij *et al.* 1996, Damen *et al.* 1997, Lespinet *et al.* 2002, Nederbragt *et al.* 2002a and b), as well as cell lineage studies (van den Biggelaar 1977, van den Biggelaar and Guerrier 1979, de Laat *et al.* 1980, Arnolds *et al.* 1983, Dorresteijn *et al.* 1983, Kühtreiber *et al.* 1986, Serras and van den Biggelaar 1987, Serras *et al.* 1989, Serras *et al.* 1990, Damen and Dictus 1996a and b, van Loon and van den Biggelaar 1998). These studies have focused on very early developmental stages only (prior to ontogenetic torsion). Studies on later development have concentrated on development of muscles and their possible role in torsion (Wanninger *et al.* 1999a and b; Wanninger *et al.* 2000).

There have been few studies on development of species in the family Lottiidae. Kessel (1964) studied the development of *Acmaea (Tectura) testudinalis* (an Atlantic lottiid species), but her description lacked details of organogenesis and was restricted to superficial features, or the limited number of structures that were visible through the

larval shell (such as the retractor muscles). Recent work by Kay and Emlet (2002) on the development of two Pacific Coast members of the Lottiidae (*Lottia digitalis* and *L. asmi*) was more detailed than that of Kessel (1964), but neither study included histological work to reveal information about organogenesis and formation of internal structures.

Tectura scutum – Background

Tectura scutum (Rathke, 1833) is a marine gastropod: Order Patellogastropoda, Family Lottiidae (Lindberg 1986). It is a common species found in intertidal regions of the eastern shores of the Pacific Ocean. Commonly known as the plate limpet, it is found all along the Pacific coast, from Alaska to California (Test 1945). During the past century, the species has undergone many name changes at the genus level, from *Acmaea scutum* to *Notoacmea scutum*, and finally to *Tectura scutum* (Lindberg 1986).

The adult stage of *Tectura scutum* lives on rocks, and feeds by scraping algae off the surface of those rocks (Fritchman 1961). Growth rates were observed to be highest during the spring and summer, when the animals were lower in the intertidal zone; growth rates decreased in winter when the limpets migrated vertically to higher points in the intertidal (Phillips 1981). Adults release gametes into the water column (are free-spawners), and in California were observed to spawn in the fall and to a lesser extent in the early spring (Fritchman 1961). Larvae begin as swimming trochophores but rapidly become veligers; neither the trochophore nor veliger stage feeds.

Previous studies on *T. scutum* have included investigations of adult sensory structures (Phillips 1979), interactions with predators (Phillips 1975), ion and water balance (Webber and Dehnel 1968a and b), and adult natural history (Test 1945,

Fritchman 1961, Phillips 1981). Only a few studies have been conducted on developmental stages of *T. scutum.* Karp (1973) and Karp and Whiteley (1973) studied levels of RNA synthesis during early development of *T. scutum*, Collin and Voltzow (1998) described form and calcification of the larval shell, and Page (2002a) described the larval apical sensory organ. However, there has been no study of the overall development, morphogenesis, or organogenesis of *T. scutum*. My study of the development of *T. scutum* involves observations of live larvae as well as histological sections and electron microscopy to provide much needed information on morphogenesis and developmental characters in a scarcely studied family in the most basal clade of the Gastropoda.

MATERIALS AND METHODS

Adult Collection, Spawning, and Larval Culture

Adults of *Tectura scutum* were collected at low tide from the middle intertidal zone of several rocky shore locations along the southwest end of Vancouver Island between mid July and early September. Individuals were placed in separate glass finger bowls or Nalgene beakers containing 100ml of sea water from the recirculating sea water system at the University of Victoria. Spawning occurred naturally, 15 to 16 hours after the time when the low tide occurred. Eggs were removed from the adult finger bowl and were placed in beakers containing 500-1000ml of Millipore filtered seawater (MPFSW – 0.45μm filter), such that a sparse layer of eggs lay on the bottom of the beaker. Before being added to the beakers of MPFSW, aggregations of eggs were gently broken up by sucking them up into the wide, "wrong-end" of a pipet. A sperm suspension was prepared as follows: approximately 1ml of concentrated sperm was taken from bowls of spawning males and diluted in 500ml MPFSW. Of this diluted sperm suspension, 1-3ml were added to the beakers containing the freshly spawned eggs, which were periodically stirred for 0.5h Approximately 100 fertilized eggs were placed in glass culture beakers containing 500ml MPFSW at 12°C. Embryos and larvae were transferred to fresh culture beakers every 1 to 2 days by gentle sieving and pipeting. Once larvae had acquired the ability to crawl (7+ days after fertilization), metamorphosis was induced by transferring the larvae to small finger bowls containing 100ml seawater and small pebbles collected from the mid-intertidal zone of the adult habitat. Larvae were defined as metamorphically competent once they were able to crawl, and were morphologically similar to those larvae which responded to metamorphic induction (as described above).

Live larvae and juveniles were observed with a Zeiss Axioskop light microscope (with correction for left-right reversal), and photographs were taken with a 35mm camera, on Kodak T-Max100 black and white film. Negatives were scanned with a Polaroid SprintScan 35 slide/negative scanner, inverted to a positive image with SprintScan software, and were corrected for brightness, contrast, and sharpness in Adobe Photoshop 5.0.

Relaxation and Fixation

Larvae and juveniles to be fixed and processed for histological sectioning were first relaxed in artificial seawater with a high concentration of magnesium and a low concentration of calcium (Audesirk and Audesirk 1979). The larvae were initially placed in small vials (8ml) which were filled with 1 part seawater, 2 parts highMg-lowCa seawater. Vials were maintained at 12°C for 1-2h and the artificial seawater was replaced 3-4 times. Larvae younger than 4 days post-fertilization were further anaesthetized by drop-wise addition of a saturated solution of Chlorobutanol in seawater to the vials. Older larvae and juveniles were further anaesthetized by reducing the artificial seawater in the vial to approximately 2ml and floating a crystal of menthol on the water surface for 30-40min.

The relaxed larvae and juveniles were fixed in 2.5% glutaraldehyde in 0.2M phosphate buffer (pH 7.6) and 0.14M sodium chloride. Specimens were stored in this buffered fixative at 8-10°C for up to one month. The shells were then decalcified in a 1:1 mix of the glutaraldehyde fixative and 10% ethylenediaminotetracetic acid (disodium salt) for 1-2 h. Specimens were then post-fixed in 2% osmium tetroxide in 1.25% sodium

bicarbonate (pH 7.2) for 1h at room temperature before dehydration in a graded ethanol series (30%, 50%, 70%, 90%, 3X 100%; 10 min in each).

Histological Sections

Fixed and dehydrated specimens destined for histological sectioning were embedded in epoxy resin using propylene oxide as a transitional agent (three changes in propylene oxide; 10 min each). The embedded larvae were mounted on stubs and histological sections (0.75-1.0μm thick) of sequential developmental stages were cut in frontal, longitudinal, and cross-section with a Reichert UM-2 ultramicrotome using glass knives. Batches of five to seven sections were placed on a glass microscope slide, heated to adhere to the slide, stained with Richardson's stain (Richardson *et al.* 1960), and covered with a coverslip using Permount mounting medium (Fisher). Stained serial sections were observed with a Zeiss Akioskop that corrected for left-right reversal, and were photographed with a DVC digital camera. Digital images were captured with Northern Eclipse software and were corrected for brightness, contrast, and sharpness in Adobe Photoshop 5.0.

Scanning Electron Microscopy

Whole, fixed larvae and cleaned larval shells were examined by scanning electron microscopy. Whole larvae were relaxed, fixed, and dehydrated as described above for histological sections (with the decalcification stage unnecessary and omitted), and were then dried at critical point from liquid carbon dioxide. Shells of larvae and post-larvae were cleaned by feeding the specimens to scyphistomae of the scyphomedusae *Aurelia*

sp. After 12-48h, the shells were egested with soft tissues digested away. The egested shells were transferred to a small container of distilled water (dH_2O). The dH_2O was removed and replaced with a bleach solution (1 part double-strength Javex:9 parts dH_2O). This solution was changed once over 4-8 min before the shells were rinsed (twice) with dH_2O, then with 50% acetone, and finally with 100% acetone, in which they were stored at 4°C. The shells were pipetted onto lens paper to air dry.

Specimens (both whole larvae and cleaned shells) were transferred to double-sided adhesive tape on SEM stubs using an eyebrow hair mounted on an orange stick. They were then sputter-coated with gold and examined with either a JEOL SM35 scanning electron microscope or a Hitachi 7000N scanning electron microscope.

RESULTS

Spawning

One of every four to six *Tectura scutum* that were collected from the field during low tides occurring from mid July through early September spawned spontaneously in the laboratory. Individuals collected in May and June did not spawn. On each of 4 separate occasions when animals were collected during a morning low tide and subsequently held at 12-14°C in separate bowls containing 100ml seawater, spawning began between 15 and 16 hours after the time of lowest tide level. Initial spawners were invariably males and the first female spawners began releasing eggs at 0.5 to 1h after the onset of male spawning. Eggs released by different females were gold, caramel brown, or brick red in colour, although eggs released by a single female were all the same colour. Spawned eggs were 140μm in diameter and were invested with an egg envelope and transparent jelly coat. Eggs were easily dispersed by water jets from a pipet.

Overview of Development

The following account is a description of salient features of development for *Tectura scutum* as visualized from microscopic observations of whole mounted developmental stages, between egg fertilization and post-metamorphic juveniles. Body axes, orientations, and definitions of planes of section are as depicted in Figure 2. The sequence and timing of developmental stages and events for *T. scutum* is summarized in Table 1.

A. Early Development

Fertilization initiated the completion of meiosis by eggs of *Tectura scutum*. The first polar body was extruded at 10-20 minutes after fertilization (Fig. 3A), followed

Table 1. Summary of development in *Tectura scutum*, highlighting major events and developmental stages. Unless otherwise specified, times are given in hours post fertilization (hpf), and were recorded for development at 12°C.

Stage/ Event	Age
First polar body extruded	10-20 minutes post fertilization
2-cell stage	1.5hpf
4-cell stage	3.25hpf
Trochophore (ciliated bands, apical tuft)	16hpf
Larval pallial tentacle formed	28hpf
Veliger (shell, foot, velum formed)	34hpf
Larval retractor muscles visible	48hpf
Operculum visible	49hpf
Onset of torsion	51hpf
Completion of torsion	58hpf
Propodium visible	60hpf
Cephalic tentacles visible	85-90hpf
Pigmented eyespots visible	115hpf
Ability to crawl	7 days post fertilization

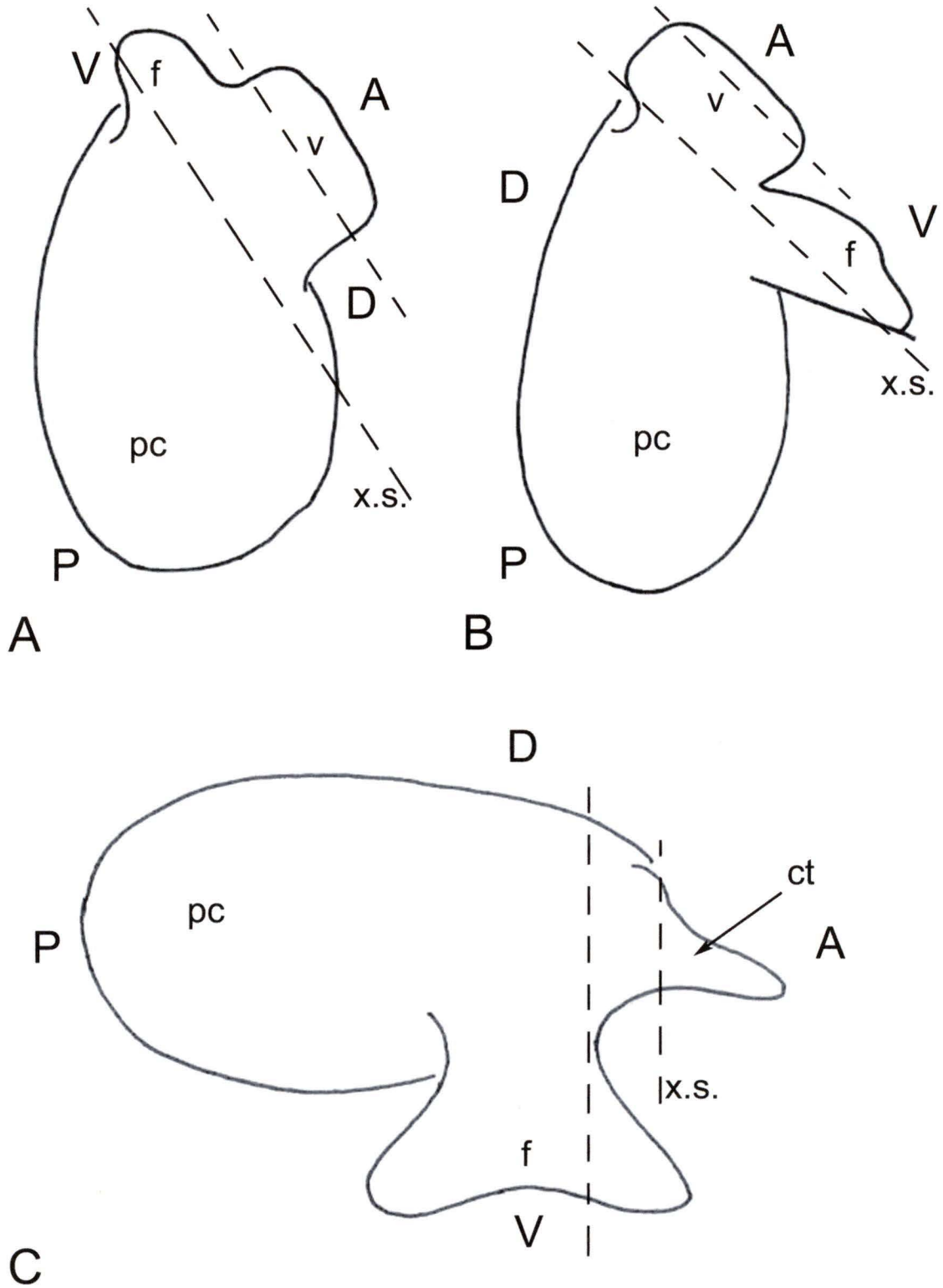
Figure 2. Orientation and definitions of body axes and planes of section. Not to scale.

A. Schematic sketch of a pre-torsional larva, viewed from the "left" side (pre-torsion).
B. Schematic sketch of a post-torsional larva, viewed from the right side (post-torsion).
C. Schematic sketch of a juvenile, post-metamorphosis, viewed from the right side.

Dorsal, ventral, left, and right are defined with respect to the cephalopodium prior to torsion and *both* the cephalopodium and visceropallium after torsion. Because left and right are always defined with respect to the cephalopodium, the pre-torsional right side of the visceropallium (includes the protoconch) is the left side of the post-torsional visceropallium.

All images in all figures will follow the orientations shown here, unless otherwise specified. A cross section (xs) is defined as a section cut in a plane that is parallel to the apical surface of the specimen (see Fig. 2A). A longitudinal section is defined as a section cut in a plane that is parallel to the page.

Axes: A, anterior; D, dorsal; P, posterior; V, ventral.
Abbreviations: ct, cephalic tentacle; f, foot; pc, protoconch; v, velum; x.s., cross section.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ct
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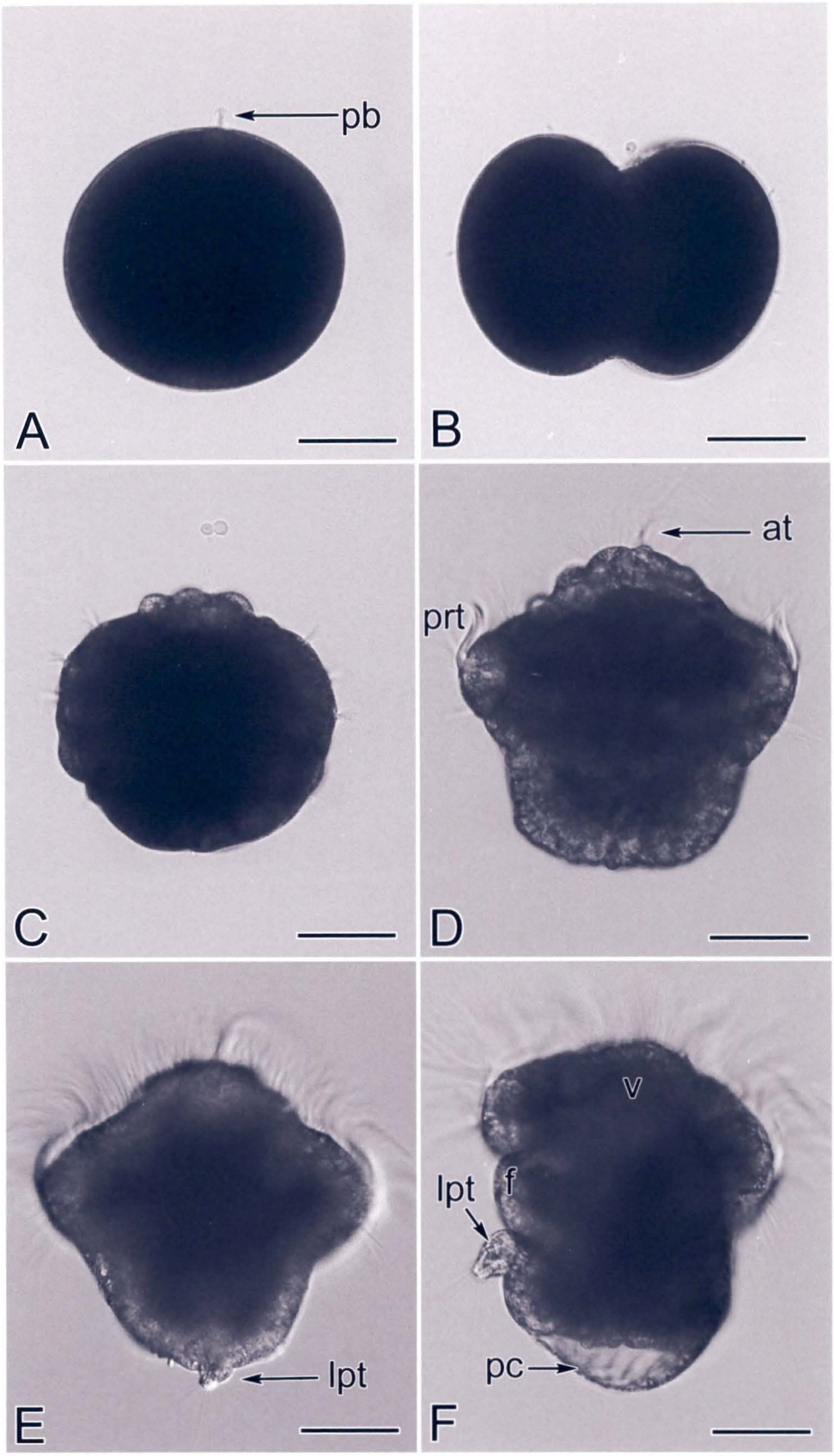
C

Figure 3. Early development of *Tectura scutum*. All figures (A-F) are light micrographs of live larvae in whole mount. Anterior is up for all figures.

A. Fertilized egg, with the first polar body extruded at the anterior end.
B. 2-cell stage. The two blastomeres are of equal size, and the polar body is still visible at the anterior end.
C. Larva, 9hpf. The apical surface has a pebbly appearance, and early tufts of cilia are present.
D. Larva, 16hpf. The typical trochophore morphology, including the prototroch (prc), is now apparent. A distinct tuft of cilia is present on the apical surface (at).
E. Larva, 28hpf. The larval pallial tentacle (lpt) is present on the posterior end of the trochophore.
F. Larva, 34hpf. The foot (f) and protoconch (pc) have now developed, and the trochophore has been transformed into a veliger larva. The larval pallial tentacle has shifted from the posterior end to a position just posterior to the developing foot.

Scale bars = 50μm.
Abbreviations: at, apical tuft; f, foot; lpt, larval pallial tentacle; pb, polar body; pc, protoconch; prt, prototroch; v, velum.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shortly after by release of the second polar body. At 12°C, first cleavage produced two equal sized blastomeres at approximately 1h 35min post-fertilization (Fig. 3B) and the 4-cell stage was produced at 3h 15min post-fertilization. Subsequent cleavages generated the four tiers of micromeres and a spherical embryo had formed by 9 hours post fertilization (hpf). A broad band of cells with relatively short, motile cilia (primary and secondary trochoblasts) ran circumferentially around these embryos, immediately anterior to the equator, and a tuft of much longer, non-motile cilia arose from the apical pole (Fig. 3C). The polar bodies were often loosely attached to this apical tuft of long cilia. By 16hpf, the embryos had acquired a typical trochophore shape (Fig. 3D), and the trochoblasts had become consolidated into a single band of large cells with actively beating cilia of increased length. This ciliary band is known as the prototroch (larval swimming organ) and at 16hpf the large cells that give rise to the prototroch formed a prominent ridge around the equatorial region of the embryos. Swimming larvae hatched from the egg membrane beginning at approximately 20hpf, before either the shell or foot was recognizable in whole mounted larvae.

By 28hpf, a small tentacle ('larval pallial tentacle') bearing a spray of very long, stiff cilia had developed from the vegetal (posterior) pole of free-swimming larvae (Fig. 3E). During the following 10 hours, the foot rudiment became recognizable as a low swelling on the ventral side of the trochophore larva and the protoconch (embryonic or pre-metamorphic shell) began to form as a lens-shaped structure covering the posterior pole of the larva (Fig. 3F). The onset of shell secretion and foot development transform a molluscan trochophore into a veliger larva, which is the characteristic larval type for many members of this phylum. As shell-secretion commenced, the larval pallial tentacle

shifted anteriorly to a position immediately beneath the protruding foot rudiment (Fig. 3F).

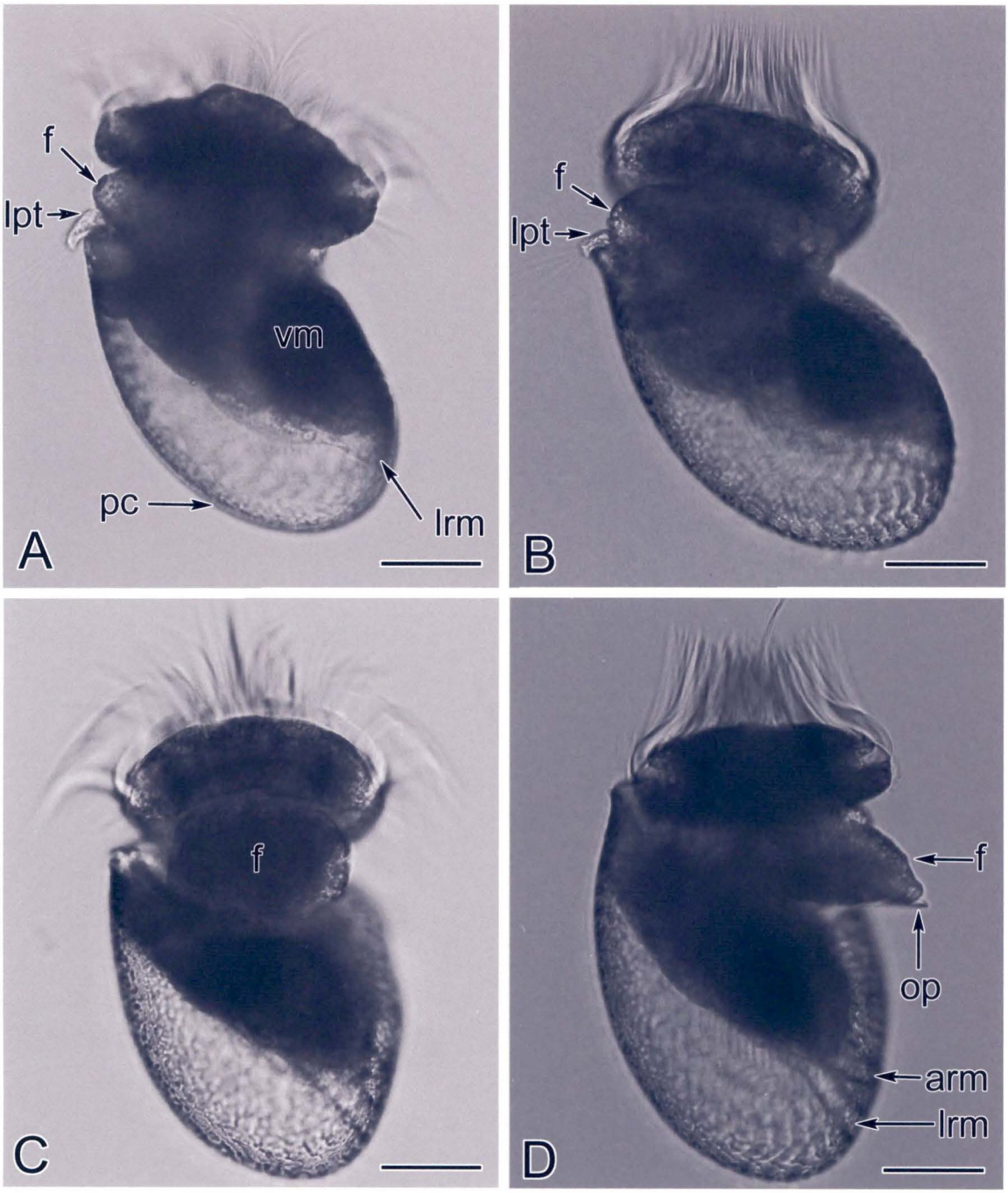
B. Torsion

The protoconch of *Tectura scutum* became obviously larger between 34 and 48hpf (compare Figs. 3F and 4A) and was ornamented with a pattern of wavy ridges. During the period preceding torsion, the larval pallial tentacle with its tuft of long, stiff cilia remained conspicuous in a location immediately beneath the foot. The yolky cells of the larval visceral mass eventually occupied only about half of the internal space contained within the protoconch (Fig. 4A). The torsional rotation began at 49 to 51hpf. During this process, the shell and visceral mass underwent a 180° clockwise rotation with respect to the velum and foot, as the veliger was viewed from the apical end. At the onset of this process, the larval pallial tentacle could be clearly distinguished (Fig. 4B), but it vanished from view by the time the foot and velum had completed half of the full 180° of rotation (Fig. 4C). All larvae sampled had completed torsion by 59hpf when cultured at 12°C (Fig. 3D). As shown in Fig. 3D, the trunks of two shell-attached muscles, the large larval retractor muscle and the smaller accessory retractor muscle, could be easily seen in live larvae at the completion of torsion. The protoconch measured approximately 200μm in length (Fig. 4D) at the end of the torsional rotation (59hpf) and no further shell growth occurred until after metamorphosis. Table 2 contains details of the timing of torsion as determined from sequential samples of 10 larvae. These data suggest that larvae of *T. scutum* require approximately 7 hours to complete ontogenetic torsion at a temperature of 12°C.

Figure 4. Torsion of *Tectura scutum* veliger larvae. All figures (A-D) are light micrographs of live larvae in whole mount. Anterior is up in all figures, and the future (post-torsional) dorsal side is kept to the left, such that the visceropallium remains in the same orientation in all figures.

A. Pre-torsion, 48hpf. Rotation has not yet begun, and the larval pallial tentacle (lrt) is still visible directly posterior to the foot (f). Note the presence of the larval retractor muscle (lrm).
B. Onset of torsion, 51hpf. The foot is no longer in the same orientation as in A, indicating that the rotation of the visceropallium relative to the cephalopodium has begun.
C. Mid-rotation, 55hpf. The foot is pointing out of the page, and torsion is half-completed (90^{o} rotation).
D. End of torsion, 58hpf. The 180^{o} rotation has been completed, as can be seen by the relative positions of the foot in A and D. The foot has grown noticeably larger in size during this time, and the operculum (op) is visible. The larval pallial tentacle is no longer visible.

Scale bars = 50μm.
Abbreviations: arm, accessory retractor muscle; f, foot; lpt, larval pallial tentacle; lrm, larval retractor muscle; op, operculum; pc, protoconch; vm, visceral mass.


f
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f
op
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lrm
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Table 2. Progression of ontogenetic torsion by larvae of *Tectura scutum* as assessed from microscopic examination of 10 larvae selected at random from two cultures at hourly intervals. Larvae were scored according to the following categories: pre-torsion (torsion not yet begun), early onset (torsion begun but not yet 1/4 complete), torsion 1/4 complete, torsion 1/2 complete, torsion 3/4 complete, and torsion complete (180°). Culture temperature was 12°C. Age is given as hours post fertilization (hpf).

Age	Proportion of larvae	Extent of Rotation
51hpf	10/10	Pre-torsion
52hpf	5/10	Pre-torsion
	5/10	Early onset
53hpf	8/10	1/4 complete
	2/10	1/2 complete
	0	3/4 complete
	0	Torsion complete
54hpf	2/10	1/4 complete
	6/10	1/2 complete
	2/10	3/4 complete
	0	Torsion complete
55hpf	0	1/4 complete
	7/10	1/2 complete
	3/10	3/4 complete
	0	Torsion complete
56hpf	0	1/4 complete
	1/10	1/2 complete
	8/10	3/4 complete
	1/10	Torsion complete
57hpf	0	1/4 complete
	0	1/2 complete
	7/10	3/4 complete
	3/10	Torsion complete
58hpf	0	1/4 complete
	0	1/2 complete
	2/10	3/4 complete
	8/10	Torsion complete
59hpf	0	1/4 complete
	0	1/2 complete
	0	3/4 complete
	10/10	Torsion complete

C. Later Larval Development, Metamorphosis, and Early Juvenile Development

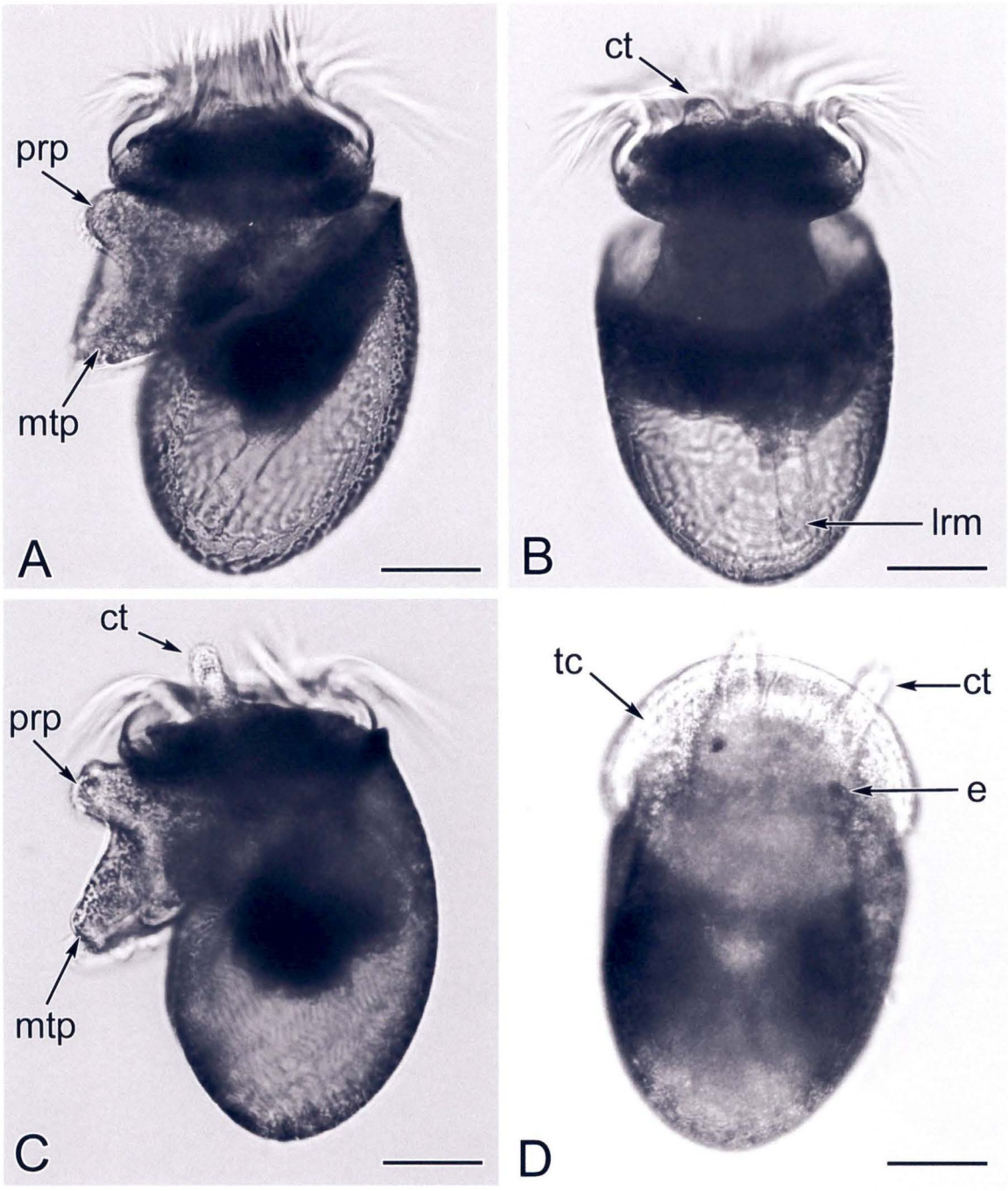
After torsion had been completed, the veliger larvae of *Tectura scutum* continued to develop for at least 5 more days before becoming competent to metamorphose into juveniles at 7dpf. The apical tuft of stiff cilia was lost between 6 and 12 hours after the end of torsion. Thus, whereas pre-torsional larvae had two conspicuous tufts of long, non-motile cilia, one arising from the middle of the apical surface and the other arising from the larval pallial tentacle, both of these distinctive ciliary structures were absent during most of post-torsional larval development. A sprinting/jumping behaviour, which was seen in young larvae up to the time of torsion, was not observed after the loss of these two ciliary tufts.

Enlargement of the foot was one of the most obvious post-torsional developmental events that could be seen in whole mounts of live veligers, although other notable events included the differentiation of cephalic tentacles (Figs. 5A-C) and pigmented eyespots. A larva at the stage of metamorphic competence is shown in Figure 5C. Small pebbles collected from the mid-intertidal zone promoted crawling behaviour by larvae that were 7dpf and older in age and more than 50% of these larvae subsequently underwent metamorphosis. Fewer than 1% of larvae cultured beyond 7dpf underwent metamorphosis when maintained in clean glass culture bowls without field-collected pebbles. The operculum and the ciliated cells of the velum were lost at metamorphosis and initial secretion of the post-metamorphic shell (teleoconch) was apparent within two days after velum loss. The newly secreted post-metamorphic shell was deposited as a visor-like rim along the outer apertural lip of the protoconch (Fig. 5D).

Figure 5. Later development (post-torsional veliger to juvenile) of *Tectura scutum*. All figures (A-D) are light micrographs of live larvae or juveniles in whole mount. Anterior is up in all figures.

A. Post-torsional veliger, 85hpf, left side view, ventral is to the left of the page. The propodium (prp) and metapodium (mtp) have begun to form.
B. Post-torsional veliger, 85hpf, ventral view. Note the left displacement of the trunk of the larval retractor muscle (lrm). The developing cephalic tentacles (ct) are protruding from the apical surface of the velum.
C. Metamorphically competent larva, 7dpf. The propodium and metapodium are more developed (more lobed), and the cephalic tentacles have grown longer.
D. Young juvenile, post-metamorphosis, dorsal view. The velum has been lost, but the cephalic tentacles remain, and are longer. The protoconch is still present, and the teleoconch (tc) has also begun to form. Pigmented eyespots (e) are also visible at the base of the cephalic tentacles.

Scale bars = 50μm.
Abbreviations: ct, cephalic tentacle; e, eyespot; lrm, larval retractor muscle; mtp, metapodium; prp, propodium; tc, teloconch.


prp
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D

Morphogenesis of Specific Structures

A. Velum and Other Cephalic Structures

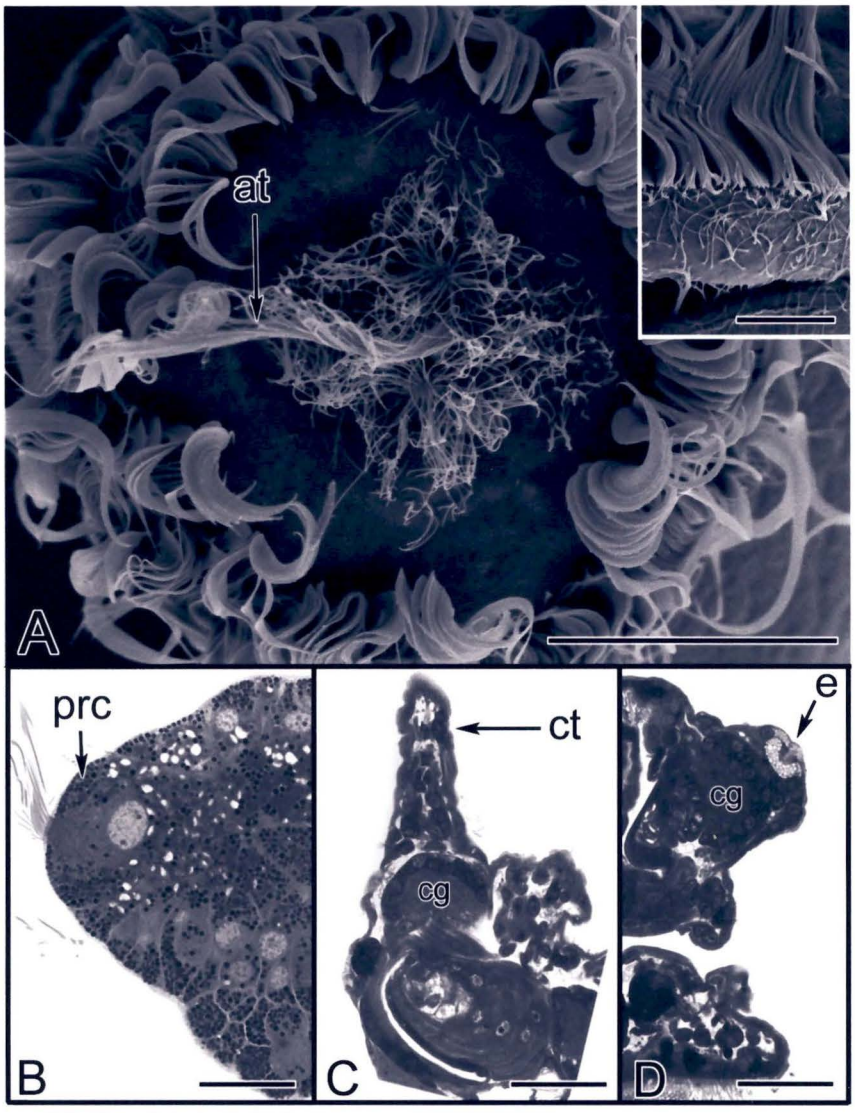
For most gastropod veligers, the large ciliated cells of the prototroch are mounted around the periphery of an expanded, bilobed outgrowth of cephalic epithelium and the entire structure is called the velum. However, the cephalic epithelium of *T. scutum* veligers did not become elaborated into bilobed expansions; the velum consisted almost entirely of the prototroch, which had a circular outline (Fig. 6A). Each large prototrochal cell gave rise to well over a hundred cilia that measured approximately 80μm in length at the end of torsion. The velum of *T. scutum* veligers also lacked a metatrochal ciliary band and food groove cilia, which are present in gastropod veligers that are capable of feeding on phytoplankton. Nevertheless, cells immediately above and below the prototrochal cells gave rise to relatively short, sparsely distributed cilia (Fig. 6A, inset).

The apical epithelium that was circumscribed by the prototroch bulged conspicuously at 16hpf and, in profile, could be seen to have a pebbly surface that gave rise to both the long apical ciliary tuft and to shorter cilia distributed over the apical surface (Figs. 3D, 6A). The apical cilia are superficial components of the apical sensory organ, a larval neuronal structure that has been described from ultrastructural data by Page (2002a). By the end of torsion, the apical surface no longer formed a conspicuous bulge when viewed in profile. All ciliary and neuronal cells of the apical sensory organ are destroyed at metamorphosis (Page, personal communication).

Figure 6. Velum and other cephalic structures of *Tectura scutum*, as seen with scanning electron microscopy (A and inset), and histological sections (B-D).

A. Scanning electron micrograph of the apical surface of a post-torsional veliger larva. The disc-shaped velum is lined by the cilia of the prototroch, and is marked by a long, apical tuft of cilia (at). Inset: side view of prototroch cells and their cilia. Anterior is up. Note the sparse cilia arising from the cells below the prototroch.
B. Prototrochal cells, 21hpf larva, longitudinal section. The prototrochal cell (prc) gives rise to the prototrochal cilia and is quite large compared with the other cells visible at this stage. Anterior is up.
C. Cephalic tentacle of a 1d juvenile, longitudinal section. The cerebral ganglion (cg) is located at the base of the cephalic tentacle (ct). Anterior is up, dorsal to the left.
D. Eyespot of a 1d juvenile, cross section. The left cerebral ganglion is located very close to the left eyespot (e). This section is just posterior to the base of the left cephalic tentacle. Anterior is up.

Scale bars: A and inset = 10μm; B-D = 25μm
Abbreviations: at, apical tuft; cg, cerebral ganglion; ct, cephalic tentacle; e, eyespot; prc, prototrochal cell.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The cephalic tentacles first appeared as small bumps on the apical surface of the velum at approximately 90hpf (Fig. 5B). The tentacles continued to elongate and were 50-60μm long by 7dpf (Fig. 6C). Pigmented eyespots could be resolved at 115hpf (=4.8dpf). These were located at the base of the cephalic tentacles and, like the cephalic tentacles, the eyespots were retained through metamorphosis (Fig. 6D). The prototrochal cells of the velum were lost during metamorphosis. At 24 hours after loss of the velum, the cephalic tentacles were much longer (approx. 80μm) and gave rise to tufts of non-motile cilia (Fig. 5D). As described in greater detail below, the cerebral ganglia differentiated immediately beneath the cephalic epithelium of the larva, on either side of the apical sensory organ. The developing mouth (stomodaeum) of *T. scutum* is described in the section on morphogenesis of the digestive tract.

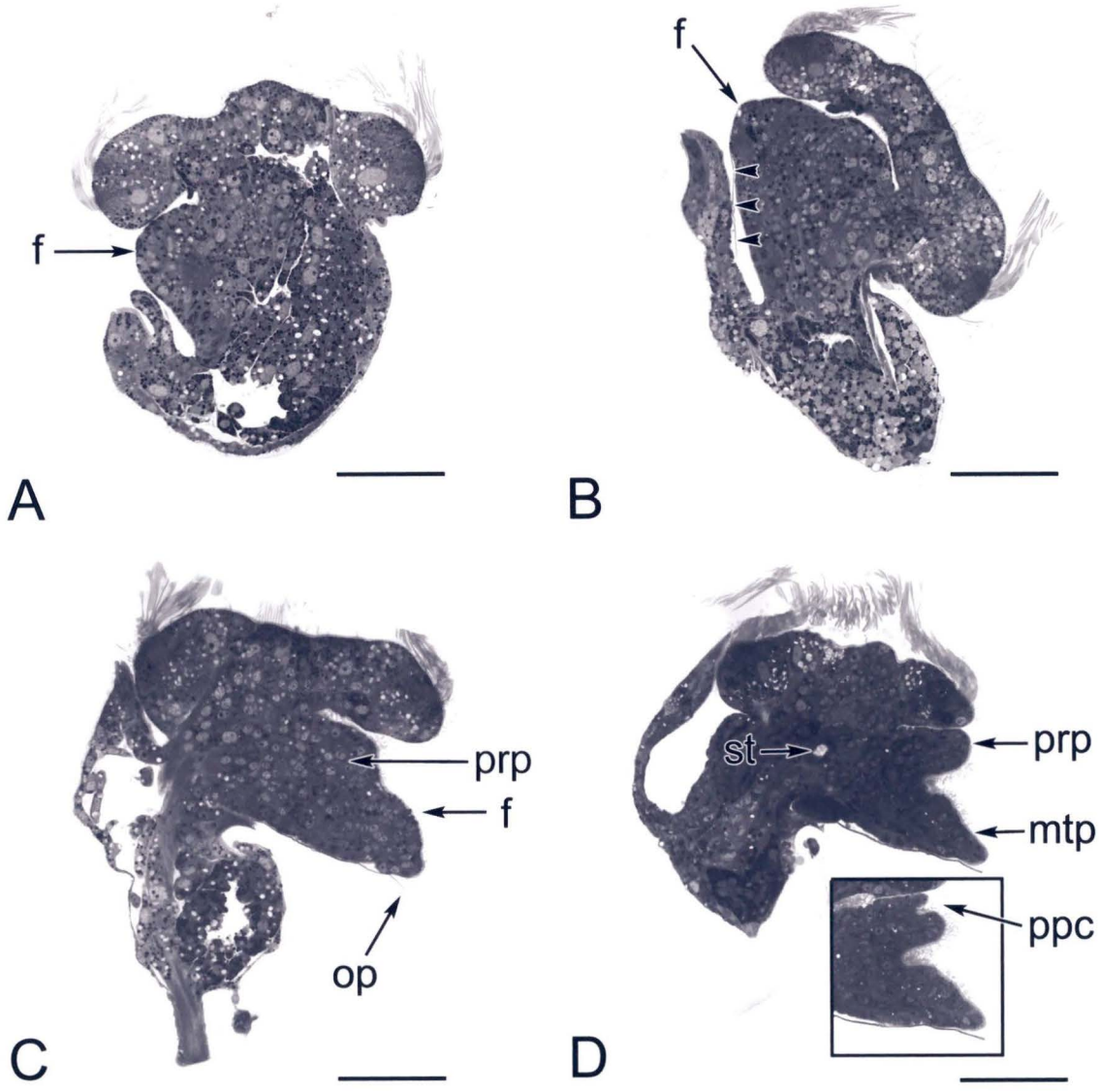
B. Foot

The foot was first recognizable in live larvae at 37.5hpf (Fig. 3F). Histological sections through larvae at this age and continuing to the end of torsion showed the developing foot as a protruding mass of densely packed, small cells located immediately beneath the ventrally located stomodaeum (Fig. 7A). A delicate operculum could be resolved in histological sections of larvae at the onset of torsion (Fig. 7B), but the initial enlargement of the propodium did not occur until after the end of torsion (Fig. 7C). The propodium was a swelling of the proximal area of the foot that, together with the more distal metapodium, provided the foot with a crawling surface (pedal sole). As the propodium enlarged following torsion, a transverse cleft appeared along its anterior edge (Fig. 7D, inset). In addition, a dense population of cilia differentiated over the entire sole

Figure 7. Histological sections showing the development of the foot of *Tectura scutum* from a pre-torsional larva to a recently metamorphosed juvenile.

A. Pre-torsional veliger larva, 37.5hpf. Longitudinal section, anterior is up, ventral (pre-torsion) is to the left. The foot (f) is a small mass of cells beginning to protrude from the body, past the edge of the velum.
B. Pre-torsional larva, 49hpf. Longitudinal section, anterior is up, ventral (pre-torsion) is to the left. The foot is now larger, and a delicate operculum has formed (arrowheads).
C. Post-torsional veliger larva, 60.5hpf. Longitudinal section, anterior is up, dorsal (post-torsion) is to the left. The foot is now much larger, and the propodium (prp) has begun to form. The operculum is also larger.
D. Post-torsional larva, 90hpf. Longitudinal section, anterior is up, dorsal (post-torsion) is to the left. The propodium and metapodium (mtp) are now well formed, as is the propodial cleft (ppc – see inset). A statocyst (st) is now visible.
E. Competent larva, 7dpf. Longitudinal section, anterior is up, dorsal (post-torsion) is to the left. The sole of the foot is covered with densely packed cilia (arrowheads).The pedal musculature (ms), pedal ganglia (pdg), and statocysts are found in the foot at this stage. The operculum is still present.
F. Juvenile, 1d post-metamorphosis. Longitudinal section, anterior is to the right, dorsal is up. The operculum has been lost. This section passes through the propodial cleft, and the pedal ganglion.

Scale bars = 50μm.
Abbreviations: f, foot; mc, mantle cavity; ms, muscle; mtp, metapodium; op, operculum; pdg, pedal ganglion; ppc, propodial cleft; prp, propodium; st, statocyst.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of the foot during later larval development. The pair of statocysts, which are gravity receptors located within the base of the foot of gastropods, was not recognizable in histological sections until 90hpf (Fig. 7D). Each statocyst was recognized by its open lumen (in contrast to the densely packed cells of the developing foot), and the statolith contained inside (Fig. 6E).

Although the foot at 90hpf was filled with a solid mass of cells, except for the lumen of the statocysts, by metamorphic competence (7dpf) the foot contained recognizable pedal muscles, pedal glands, and the paired pedal ganglia surrounded by hemal spaces (Fig. 7E). Although the operculum on the back of the foot was retained up to metamorphosis, it was shed within a day following metamorphic loss of the prototrochal cells (compare Figs. 7E and 7F).

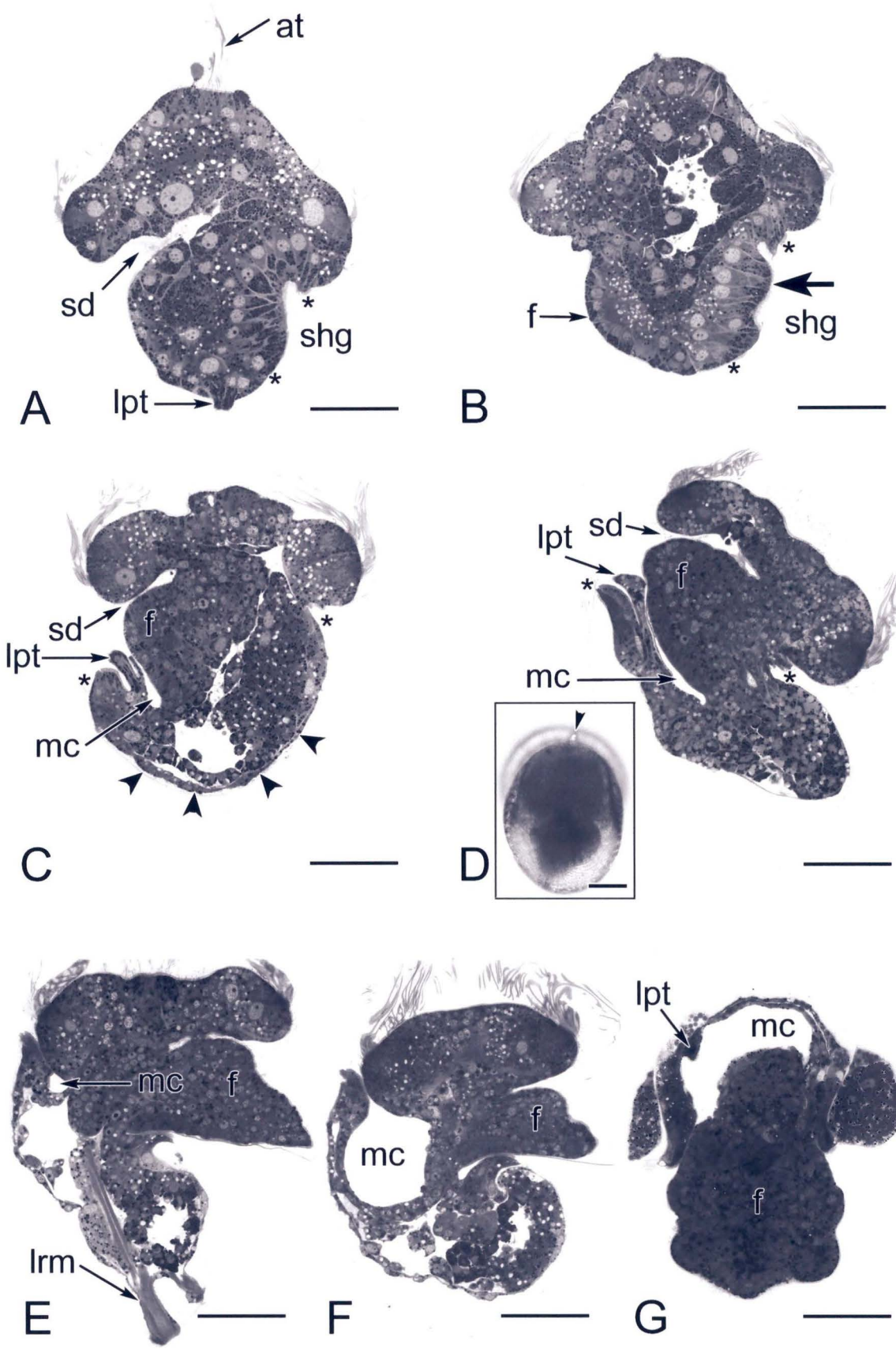
C. Mantle, Mantle Cavity, and Shell

The shell gland of *T. scutum* embryos, as in embryos of other conchiferan molluscs, is the progenitor of the mantle epithelium that secretes the shell and lines the mantle cavity. In *T. scutum*, the shell gland began as a field of tall columnar cells on the vegetal hemisphere of the embryo, opposite the stomodaeum. By 21hpf this epithelial field began to invaginate (Fig. 8A). The invagination continued to deepen up to 29hpf, at which time the central region of the inpocketing began to protrude through the external orifice of the gland (Fig. 8B, arrow). Subsequently, the epithelium of the gland spread around the entire internal mass of differentiating visceral cells, an action that was possibly effected by a change in shape of the gland cells. During the eversion and spreading process, the shell gland epithelial cells converted from a tall columnar shape to

Figure 8. Histological sections of *Tectura scutum*, showing features of the mantle (shell gland, mantle cavity, and larval pallial tentacle) of young larvae. The asterisks in A-D indicate the peripheral margin of the shell gland epithelium. Anterior is up in A-F.

A. Pre-torsional larva, 21hpf. Longitudinal section, ventral (pre-torsion) is to the left. The larval pallial tentacle (lpt) is at the posterior end of the larva, and the shell gland (shg) has begun to invaginate, opposite the stomodaeum (sd).
B. Pre-torsional larva, 29hpf. Longitudinal section, ventral (pre-torsion) is to the left. The cells of the shell gland have begun to evaginate (arrow), and the foot rudiment (f) has begun to enlarge.
C. Pre-torsional larval, 37.5hpf. Longitudinal section, ventral (pre-torsion) is to the left. The mantle cavity (mc) has formed, and the larval pallial tentacle has changed position (compare with A.) The shell gland epithelium has spread out over a greater area, and it has secreted the periostracum of the protoconch (arrowheads).
D. Pre-torsional larva, 49hpf. The mantle cavity has deepened, and the larval pallial tentacle, located posterior to the foot (f), has emerged from the mantle cavity and is visible in images of live larvae (as shown by the arrowhead on the whole mount of a live larva: inset). The protoconch is larger.
E. Post-torsional larva, 60.5hpf. Longitudinal section passing through the left side of the larva. Dorsal is to the left.
F. Post-torsional larva, 60.5hpf. Longitudinal section through the right side of the same larva shown in E. The mantle cavity is much larger on the right side than it is on the left. Dorsal is to the left.
G. Post-torsional larva, 64hpf. Cross-section through the posterior regions of the mantle cavity and foot. Dorsal is up. The remnant of the larval pallial tentacle is visible on the right side of the mantle cavity.

Scale bars = 50μm.
Abbreviations: at, apical tuft; f, foot; mc, mantle cavity; lpt, larval pallial tentacle; lrm, larval retractor muscle; shg, shell gland; sd, stomodaeum.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an extremely thin, squamous shape (compare Figs. 8B and 8C). The asterisks on the images shown in Figures 8A-D indicate the positions of the peripheral margin of the shell gland epithelium in histological sections of sequential stages of development. As the peripheral margin of the gland spread around the vegetal hemisphere of the larval body, it secreted the proteinaceous covering (periostracum) of the embryonic shell (protoconch). The space between the periostracum and the evaginated and spread epithelium of the shell gland is the space within which biomineralization occurred. Identifying the exact time when shell mineralization began was difficult, but the fact that the protoconch of squashed larvae at the onset of torsion showed obvious cracking, rather than simple compressive distortion, suggested that mineralization of the protoconch had started to occur by this stage (the stage shown in Fig. 4A).

Histological sections showed that the mantle cavity began to form between 29 and 37hpf as a shallow inpocketing of proliferating epithelial cells lying between the enlarging foot rudiment and the peripheral margin of the spreading shell gland (compare Figs. 8B and 8C). The larval pallial tentacle, which initially differentiated at the extreme posterior end of embryos prior to the appearance of the foot rudiment and evaginated shell gland (Fig. 8A), eventually became positioned within the infolded epithelium that represents the initial mantle cavity (Fig. 8C). The larval pallial tentacle elongated as the mantle cavity further deepened prior to torsion so that the distal, ciliated tip of the tentacle emerged from the apertural margin of the mantle cavity and protoconch. (Fig. 8D). Sections through developmental stages fixed during torsion revealed that the pallial tentacle is pulled into the deepening mantle cavity during the first half of torsion, which explains why it cannot be seen in whole-mounted larvae after the mid-torsion stage (Figs.

3C, 3D). The larval pallial tentacle degenerates rapidly following torsion and is almost completely atrophied by 64hpf (Fig. 8G).

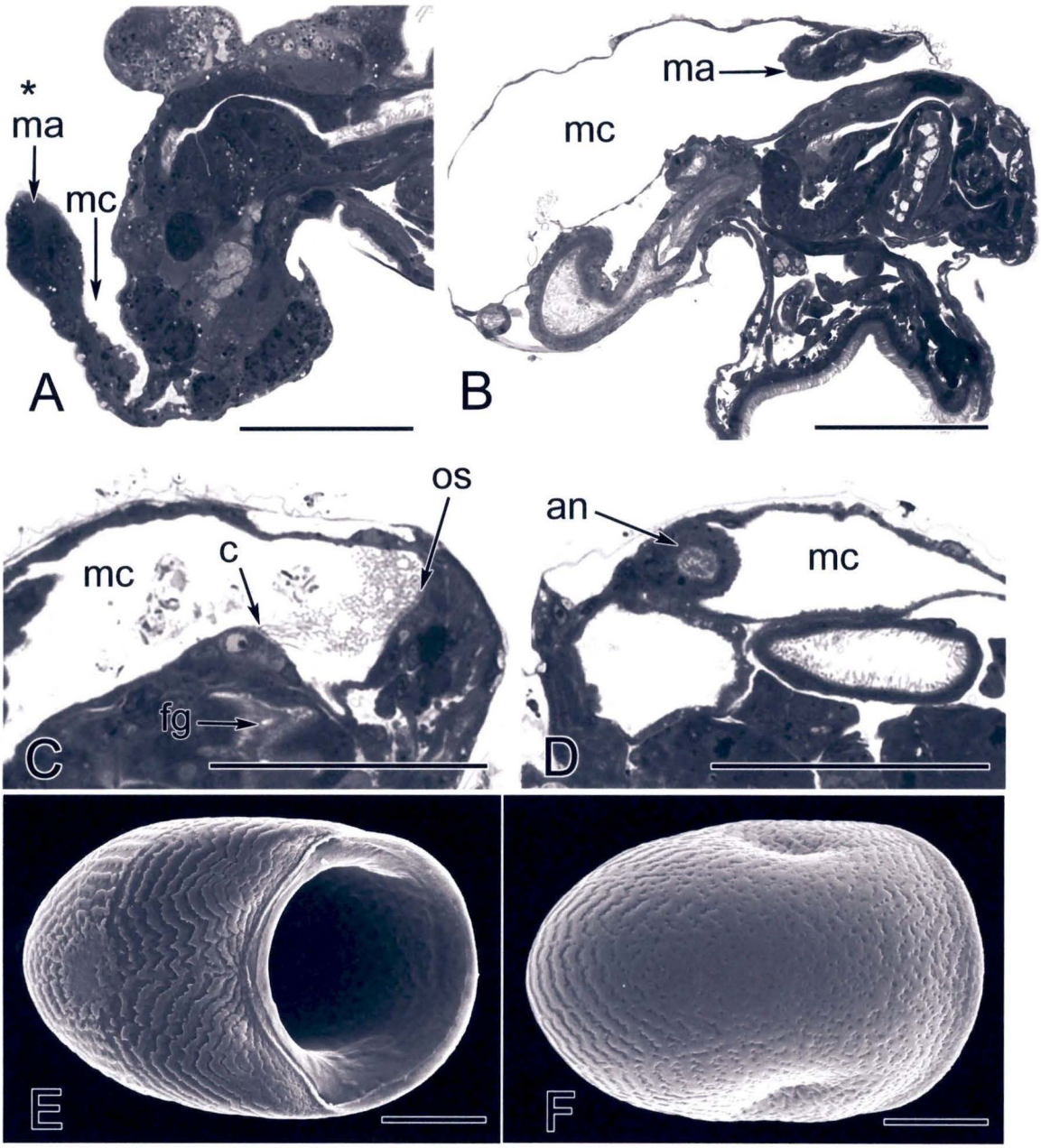
The position of the mantle cavity with respect to the foot, mouth, and head of the larva changed drastically during ontogenetic torsion. Before torsion, the mantle cavity was immediately beneath the foot on the ventral (stomodeal) side of the larva (Fig. 8D) and the larval pallial tentacle was located at the mid-sagittal plane (Fig. 8D, inset). After torsion, the mantle cavity was located on the side *opposite* the foot and stomodaeum (Figs. 8E, 8F). Nevertheless, the mantle cavity did not have a completely symmetrical shape immediately following torsion, in that the cavity was much deeper on the right side than on the left. Figure 8E is a longitudinal section passing slightly to the left of the mid-sagittal plane of a larva that was fixed shortly after completing torsion (note the shell-attached trunk of the larval retractor muscle, which lies on the left side). The mantle cavity in this section is much shallower than the mantle cavity on the right side, as shown by a second longitudinal section through the right side of this larva (Fig. 8F). Furthermore, the larval pallial tentacle shifts slightly to the right side within the mantle cavity during the torsional rotation of the visceropallium (Fig. 8G).

During the remainder of the larval phase, the mantle cavity continues to deepen until it extends to the posterior extremity of the larval body (Fig. 9A). In addition, the peripheral rim of the mantle fold detaches from the apertural rim of the protoconch and recedes posteriorly within the shell cavity (Fig. 9A). Indeed, no additional shell material is added to the apertural rim of the protoconch after the end of ontogenetic torsion. Scanning electron micrographs of the protoconch of *T. scutum* are shown in Figs. 9E and 9F.

Figure 9. Later development of the mantle, osphradium, anus, and protoconch form of *Tectura scutum*. A-D histological sections, E-F scanning electron micrographs.

A. Competent larva (7dpf). Longitudinal section, anterior is up, dorsal is to the left. The mantle (ma) has retracted from the aperture of the protoconch (position marked by asterisk), and the mantle cavity (mc) has deepened.
B. Juvenile, 1d post-metamorphosis. Longitudinal section, anterior is to the right, dorsal is up. The mantle is once again at the aperture of the shell, and the mantle cavity extends to the extreme posterior end of the juvenile.
C. Cross-section through the mantle cavity of a juvenile, 1d post-metamorphosis. Dorsal is up, the osphradium (os) is located on the left side of the mantle cavity. A second field of cilia (c) arises from a central bulge on the floor of the mantle cavity.
D. Cross-section through the mantle cavity of a 1d juvenile, more posterior than the section of the same specimen shown in C. Dorsal is up, and the anus (an) opens to the right side of the mantle cavity.
E. and **F.** Scanning electron micrographs of the protoconch: E is a ventral view, F is a dorsal view. Anterior is to the right. A pattern of wavy ridges as well as two lateral indentations are visible.

Scale bars = 50μm.
Abbreviations: an, anus; c, cilia; fg, foregut; ma, mantle; mc, mantle cavity; os, osphradium.


*
ma
mc
A
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E
F

At metamorphosis, the periphery of the mantle fold reestablishes connection with the apertural rim of the shell. Much of the remainder of the mantle epithelium, including that which forms the floor of the mantle cavity and the mantle fold underlying the shell takes the form of a thin and delicate sheet (Fig. 9B). Two ciliary structures were distinguished within the mantle cavity of juveniles between 1 day to 1 month after metamorphic loss of the velum. One of these was an elongate tract of cilia running down the floor of the mantle cavity above the level of the buccal mass (Fig. 9C). The second ciliary structure was a smaller patch of cilia on the extreme left side of the mantle cavity (Fig. 9C). The latter was tentatively identified as cilia associated with the osphradium, a sensory structure within the mantle cavity of gastropods. Sections through juveniles fixed at one month after metamorphosis showed no evidence of a gill rudiment or of a developing osphradium on the right side of the mantle cavity, although both of these structures are present in the adult stage of *T. scutum*.

The anus opened into the mantle cavity, on the right side (Fig. 9D). Details of digestive tract development will be discussed below.

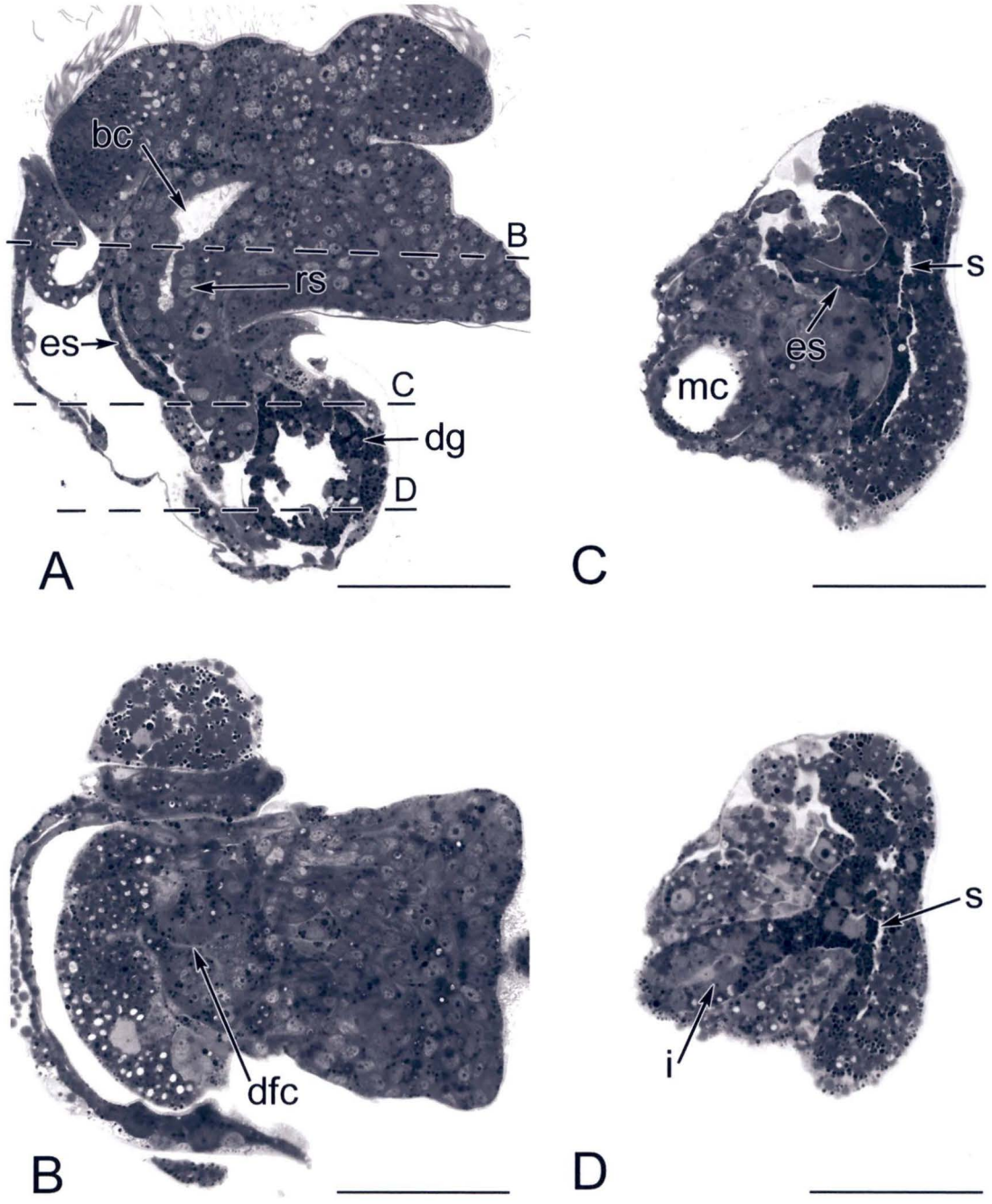
D. Digestive Tract

In its early stages of development, the digestive tract of *T. scutum* was little more than an opening (the stomodaeum), which led into an epithelium-lined tube that ended blindly within the interior of the larva body (Fig. 8A). During most of larval development, the future stomach, digestive gland, and intestine were merely a mass of large, yolk-filled endodermal cells. In post-torsional veligers, the buccal cavity and radular sac had begun to form (Fig. 10A), and the tubes of the future esophagus, stomach,

Figure 10. Histological sections of the digestive tract of *Tectura scutum*, 60-64hpf larvae.

A. Longitudinal section of a larva, 60.5hpf. Anterior is up, dorsal is to the left. The stomodaeum is not present in this plane of section, but the buccal cavity (bc) is visible. The radular sac (rs) has begun to form, and is a relatively simple blind-end sac. The esophagus (es) can be seen on the dorsal side of the visceral mass as a narrow, ciliated tube. The digestive gland (dg), which opens into the stomach, can also be seen. The lettered, dashed lines represent the approximate plane of section of the cross sections shown in B–D.
B–D. Cross-sections through a 64hpf larva. Left side of larva is up, dorsal is to the left of the page. The dorsal food channel can be seen in B, and is a very narrow channel, with a tripartite form. The narrow openings of the esophagus and stomach (s) can be seen in C; both structures are lined by darkly stained cells. This section also passes through the posterior region of the mantle cavity, which is displaced to the right side. The beginnings of the intestine (i), extending from the stomach, can be seen in D. As with the esophagus and stomach, the cells lining the intestine are more darkly stained than the surrounding tissue.

Scale bars = 50μm.
Abbreviations: bc, buccal cavity; dfc, dorsal food channel; dg, digestive gland; es, esophagus; i, intestine; mc, mantle cavity; rs, radular sac; s, stomach.


bc
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and intestine were also visible (Fig. 10A-D). By contrast, at the stage of metamorphic competence, the complex components of the buccal mass had achieved a much greater degree of differentiation than the endodermal components of the digestive tract. A well developed radular sac and ribbon of radular teeth could be resolved in larvae at 7dpf (Fig. 11). Details of gut differentiation were difficult to resolve using sections for light microscopy because the various components were packed within a very small volume that occupied only a portion of the space within the protoconch (*e.g.*, see Fig. 5C).

In the post-metamorphic individuals that were sectioned (1d, 12d, and 1 month juveniles), the gut was complete. Figures 12A-H show features of the digestive tract of a 1d juvenile. The mouth was bordered by two sets of lips: the outer and inner lips (Fig. 12A). The radula was well developed, and the odontophoral cartilages and accompanying buccal musculature were quite prominent (Figs. 12B-D). The anterior portion of the esophagus had a central, dorsal food channel, and two lateral "pouches", all with long cilia (Fig. 12C). In contrast, the more posterior portions of the esophagus were round in cross-section, and were lined with long, dense cilia (Figs. 12F, 12G). Where the esophagus opened into the stomach, there was a change in the cilia lining these structures, clearly distinguishing the esophagus from the stomach (Fig. 12H, inset). The lumen of the stomach was continuous with the lumen of the digestive gland, in the posterior regions of the juvenile body (Fig. 12H). The cilia of the stomach were shorter and much less dense than those of the esophagus. The intestine looped in a path that traveled anterior and to the right (dorsal to the esophagus), then posterior again, along the left side of the viscero-pallium, then posterior to the stomach, before the rectum opened to the right side of the mantle cavity (Figs. 9D and 12H).

Figure 11. Histological sections of the digestive tract of competent larvae of *Tectura scutum*, 7dpf.

A. B. and **C.** are all longitudinal sections through the same larva, anterior is up, dorsal is to the left. The section in A passes through the mouth (m), foregut (fg), and radular sac (rs). The foregut is a ciliated channel which becomes the esophagus (es), as seen in B and C. The radular sac is darkly staining, and is the structure that gives rise to the radula (rad) (shown in B, inset). The profile of the radular sac is best seen in B, while its beginning and end are also seen in A and C. respectively. The inset of B is a cross section through the buccal cavity (bc), the common channel anterior to the branch between the foregut and radular sac. The buccal cavity is immediately dorsal to the radula apparatus.

Scale bars = 50μm (including inset)
Abbreviations: bc, buccal cavity; es, esophagus; fg, foregut; m, mouth; mc, mantle cavity; rad, radula; rs, radular sac; v, velum.

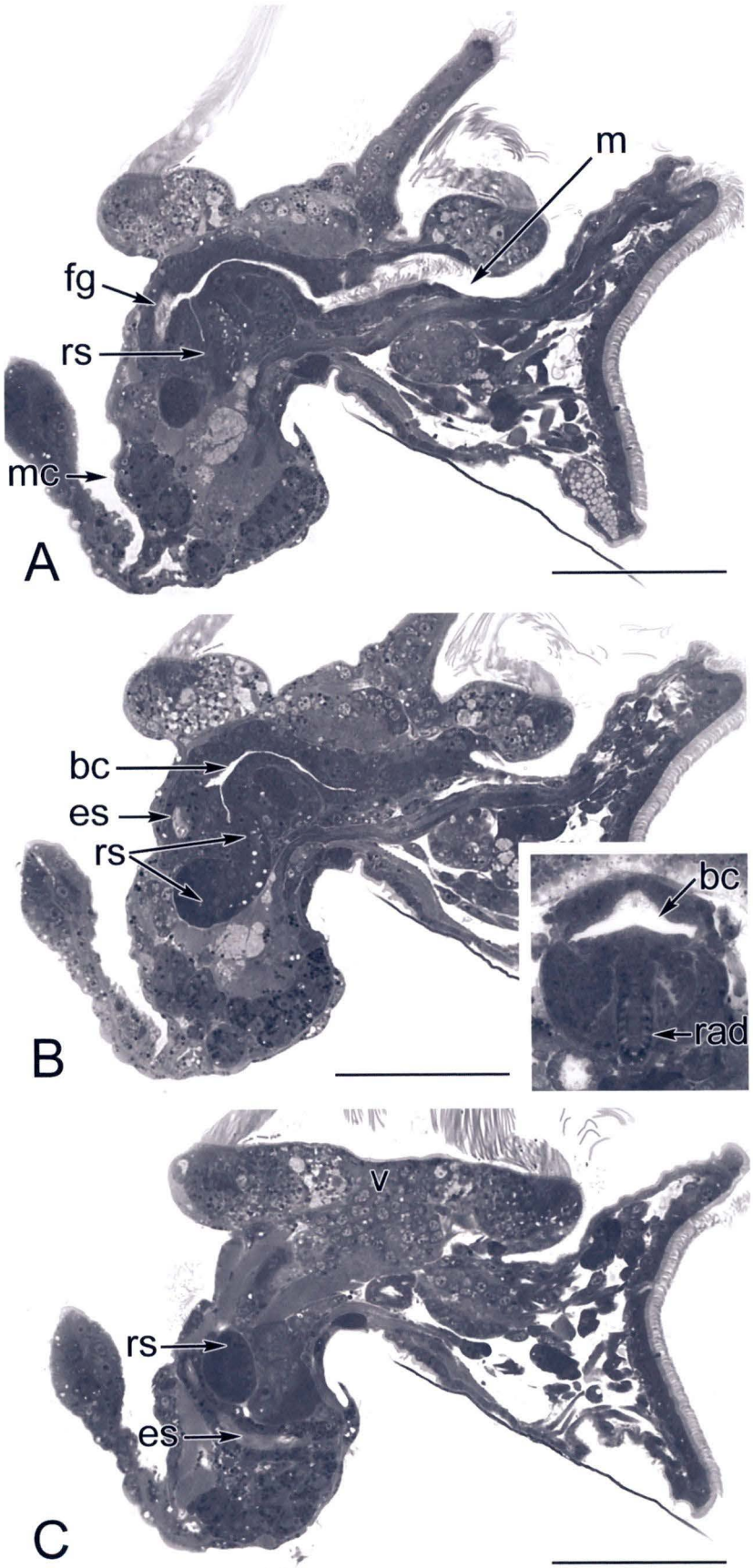
m
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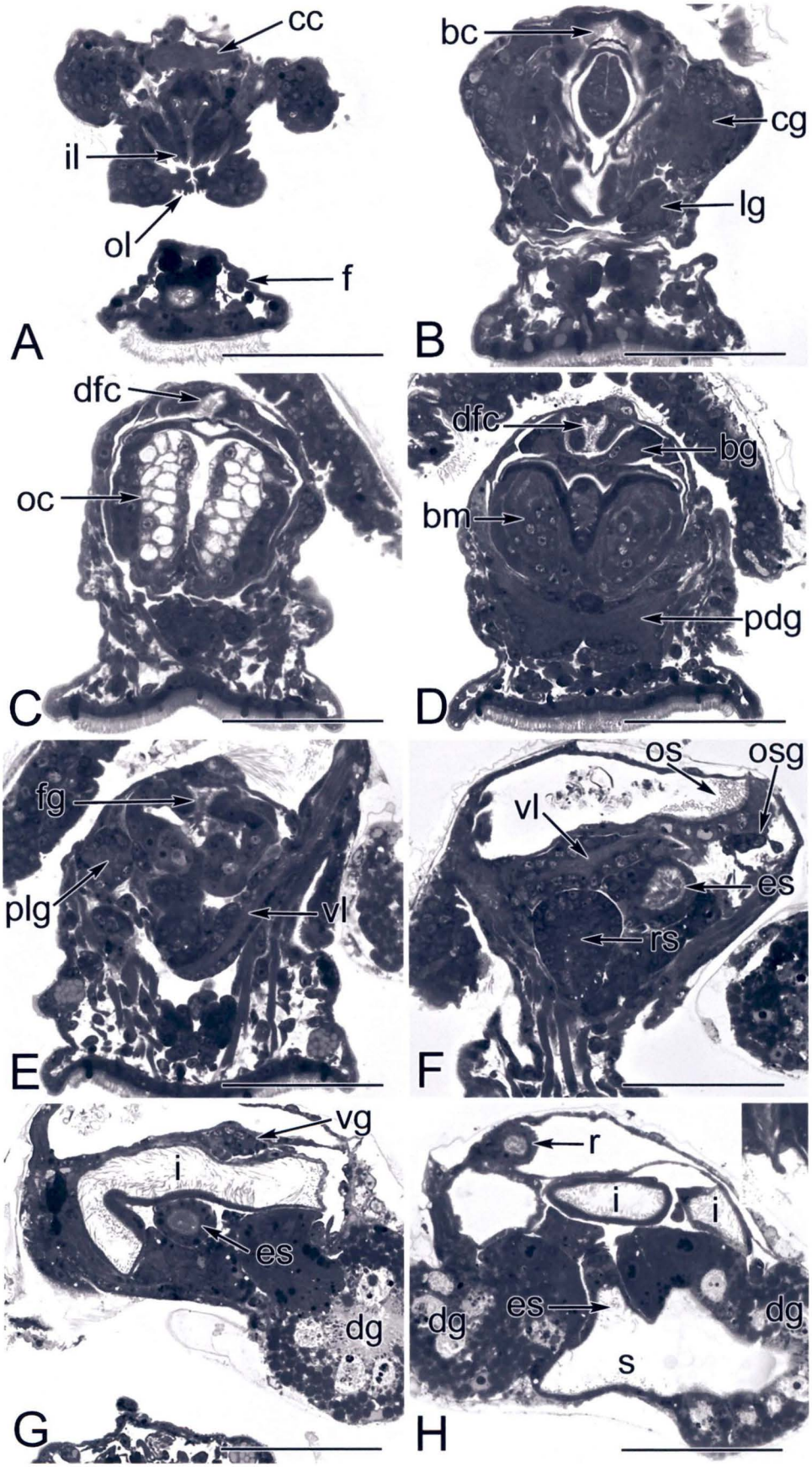
Figure 12. Histological sections of *Tectura scutum*, 1d juvenile, highlighting structures of the digestive tract and nervous system. A-H are all cross sections through the same specimen: A is the most anterior section, H is the most posterior. Dorsal is up, right side of the juvenile is to the left side of the page.

A. Digestive tract: the inner and outer lips (il and ol) of the mouth are visible. Nervous system: the cerebral commissure (cc) is a wide band which connects the two cerebral ganglia (cg, in B).
B. Digestive tract: the buccal cavity (bc) of the digestive tract sits dorsal to the radula. Nervous system: the paired cerebral and labial ganglia (cg and lg) are visible. The labial ganglia sit just posterior to the lips (il and ol, see A).
C. Digestive tract: the ciliated dorsal food channel (dfc) sits dorsal to the large odontophoral cartilages (oc), part of the radular apparatus.
D. Digestive tract: the dorsal food channel is now flanked on either side by lateral pouches, and the buccal musculature (bm) of the radular apparatus is visible. Nervous system: the paired buccal ganglia (bg) are present, lateral and ventral to the foregut. The large pedal ganglia (pdg) are also present in the foot.
E. Digestive tract: the foregut (fg) is larger than in previous sections, and is becoming more rounded. Nervous system: the right pleural ganglion (plg) is present, and the left pleural ganglion has already given rise to the sub-esophageal visceral loop (vl).
F. Digestive tract: the rounded, ciliated esophagus (es) as well as the darkly staining radular sac (rs) are present in this section. Nervous system: the supra-esophageal visceral loop (vl) has extended from the right pleural ganglion, and the osphradial ganglion (osg) is visible at the base of the osphradium (os).
G. Digestive tract: the esophagus is visible, along with a large portion of the looping intestine (i), and the large digestive gland (dg). Nervous system: the visceral ganglion (vg), the most posterior structure of the nervous system, is seen dorsal to the intestine.
H. Digestive tract: the esophagus opens to the lumen of the stomach (s), which is continuous with the lumen of the digestive gland. This section passes through the looping intestine twice, and the rectum is seen on the right side. The inset shows the distinctive change in cilia between the esophagus and the stomach.

Scale bars = 50μm
Abbreviations: bc, buccal cavity; bg, buccal ganglion; bm, buccal musculature; cc, cerebral commissure; cg, cerebral ganglion; es, esophagus; dfc, dorsal food channel; dg, digestive gland; f, foot; fg, foregut; i, intestine; il, inner lips; lg, labial ganglion; oc, odontophoral cartilages; ol, outer lips; os, osphradium; osg, osphradial ganglion; pdg, pedal ganglion; plg, pleural ganglion; r, rectum; rs, radular sac; s, stomach; vg, visceral ganglion; vl, visceral loop.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In older juveniles, the gut was further developed, and became more twisted and looped. In 12d juveniles, the radula and associated structures (odontophoral cartilages, buccal musculature) were still quite prominent, and the "pouches" of the anterior esophagus were a little larger, with longer cilia in the dorsal food channel and shorter cilia in the lateral pouches.

E. Nervous System

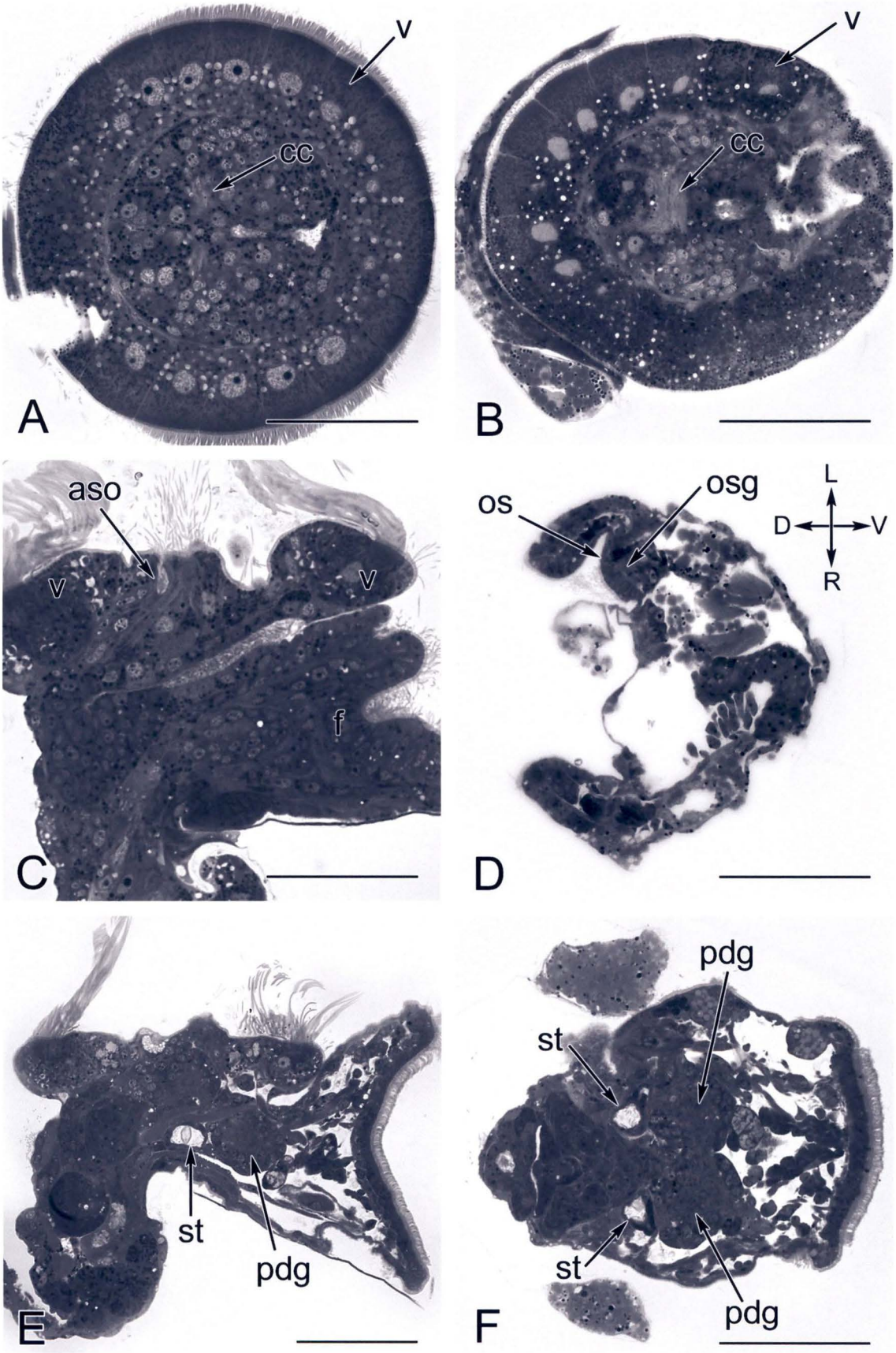
Young larvae of *T. scutum* showed very few structures of the nervous system. The cerebral commissure was first seen in larvae that were 56hpf (Fig. 13A). Although this is the commissure that ultimately connects the paired cerebral ganglia, the outline of the cerebral ganglia could not be resolved in 1μm sections until much later in development (90hpf). The apical sensory organ (ASO), a larval neural structure that has been previously described for *T. scutum* (Page 2002a), was seen in 90hpf larvae. It is an apical cluster of neurons, marked externally by tufts of cilia, and an invaginated pit on the surface of the velum (Fig. 13C). Larvae that had reached metamorphic competence possessed many different ganglia, and the nervous system closely resembled the juvenile nervous system morphology.

There were five main pairs of ganglia observed for the nervous system of *T. scutum*: the cerebral, labial, buccal, pedal, and pleural ganglia, as shown in a 1d juvenile (Fig. 12). The cerebral ganglia were located at the base of the cephalic tentacles, and were connected by a wide commissure that is located anterior to the ganglia (Figs. 12A, 12B). The labial ganglia were much smaller than the cerebrals, and were located ventral to the cerebrals, just posterior to the lips (Fig. 12B). The buccal ganglia were located on either side of the foregut, dorsal to the buccal mass (Fig. 12D). They were connected to

Figure 13. Histological sections of *Tectura scutum* showing features of the larval nervous system.

A. Cross section through the velum of a 56hpf larva (mid-torsion), showing the fibrous cerebral commissure (cc).
B. Cross section through the velum of a 64hpf larva (post-torsion). The cerebral commissure is more developed than in the younger, 56hpf larva (in A).
C. Longitudinal section through the anterior portions of a post-torsional veliger larva, 90hpf. Anterior is up, dorsal (post-torsion) is to the left. The invaginated pit of the apical sensory organ (aso) can be seen in the velum, below a field of cilia.
D. Cross section through a competent larva, 7dpf. Axes are as shown on the figure. The cilia of the osphradium (os) can be seen on the left side of the larva, and the osphradial ganglion (osg) can be seen as a cluster of cells at the base of the osphradium.
E. Longitudinal section through the left side of a competent larva, 7dpf, anterior is up, dorsal is to the left. The left pedal ganglion (pdg) can be seen in the foot, and the left statocyst (st) can clearly be seen immediately dorsal to the pedal ganglion.
F. Cross section through a competent larva, 7dpf. Axes are as shown for D. Both pedal ganglia can be seen, with the statocysts located immediately dorsal to them.

Scale bars = 50μm
Axes: D, dorsal; L, left; R, right; V, ventral.
Abbreviations: aso, apical sensory organ; cc, cerebral commissure; f, foot; os, osphradium; osg, osphradial ganglion; pdg, pedal ganglion; st, statocyst; v, velum.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each other via a connective that passed below the foregut. While they were quite small in 7dpf larvae, they were much larger in 1d juveniles. The pedal ganglia were large structures located in the foot, and were connected by a short, wide commissure (Fig. 12D). The pleural ganglia were much less obvious than the other pairs of ganglia described and appeared not to be in close association with either the cerebral or the pedal ganglia (Fig. 12E); they were more dorsal and posterior than the pedal ganglia. The visceral loop extended from the pleural ganglia: the left pleural led to the sub-esophageal visceral loop whereas the right pleural (larger and more prominent than the left) led to the supra-esophageal visceral loop and the visceral ganglion – the posterior-most portion of the nervous system (Figs. 12E-G). A small cluster of neurons were also observed at the base of the osphradium (Fig. 13D).

A pair of statocysts were first observed in histological sections of 90hpf larvae (Fig. 7D). They were located in the foot, dorsal to the pedal ganglia (Figs. 13E, 13F), and remained in this position in juveniles as old as 1 month post-metamorphosis.

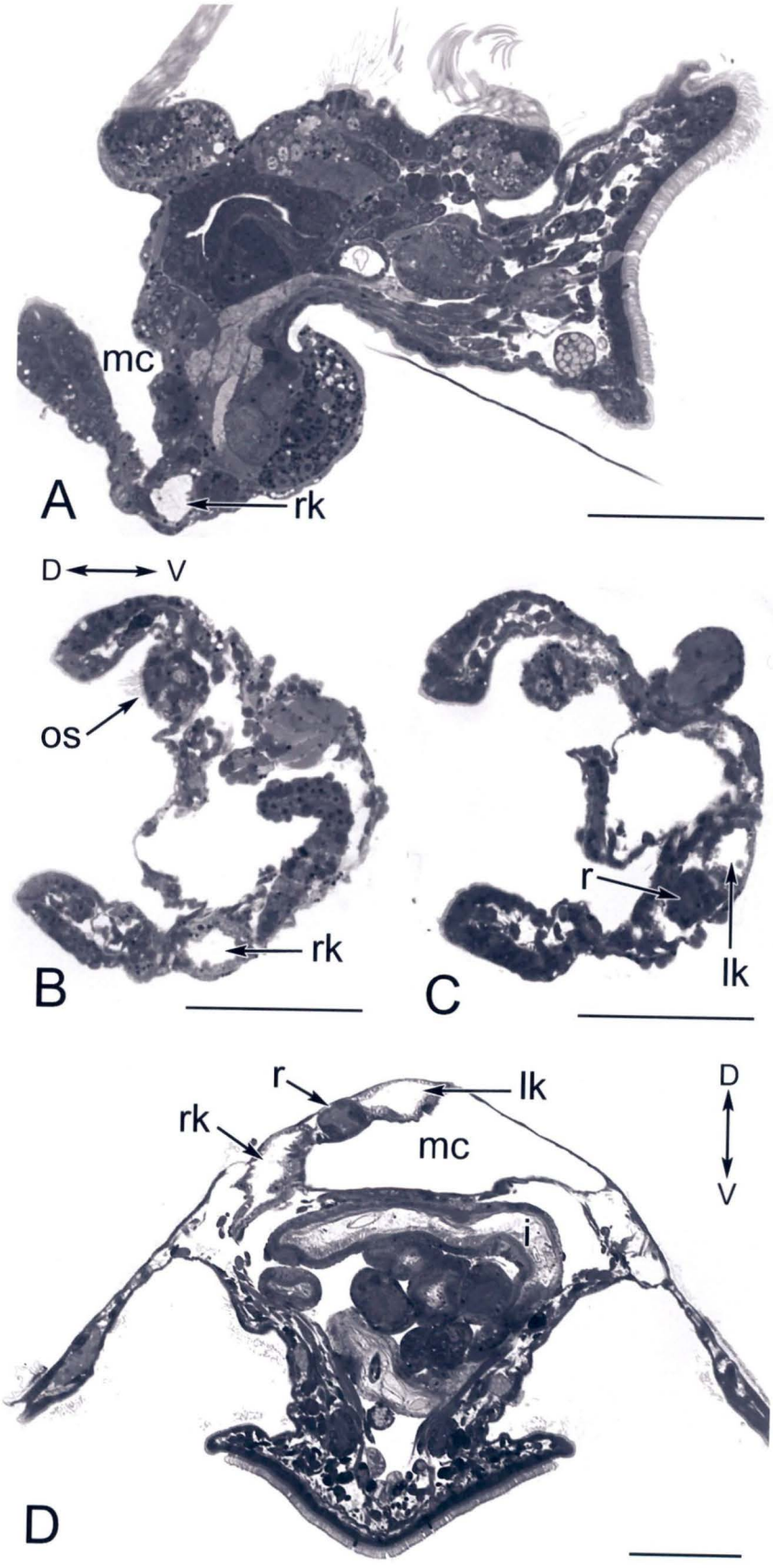
F. Renopericardial System

The kidneys were not observed in young larvae; they were first seen in competent larvae (Figs. 14A-C) and they became more obvious in juvenile stages. There were two, one left and one right, but both were displaced to the right side of the body. They were located dorsally in the visceral mass, on either side of the intestine/rectum (Fig. 14D). Both the left and right kidneys appeared to be approximately the same diameter (compare Figs. 14B and 14C). I saw no evidence of a larval or adult heart.

Figure 14. Histological sections of *Tectura scutum*, showing the left and right kidney.

A. Longitudinal section through a competent larva, 7dpf. Anterior is up, dorsal is to the left. This section is through the right side of the larva, and shows the right kidney (rk), near the posterior-most regions of the mantle cavity (mc).
B. and **C.** Cross sections through a competent larva, 7dpf. Left side of larva is up, dorsal is to the left. The right kidney is to the right side of the rectum (r), and the left kidney is to the left side of the rectum. The osphradium (os), is on the left side of the larva, while both kidneys and the rectum are displaced to the right side.
D. Cross section through a juvenile, 1 month post-metamorphosis. Both the left and right kidneys are still present on either side of the rectum, and all of these structures are still displaced to the right side of the juvenile.

Scale bars = 50μm
Axes: D, dorsal; V, ventral.
Abbreviations: i, intestine; lk, left kidney; mc, mantle cavity; os, osprhadium; r, rectum; rk, right kidney.


mc
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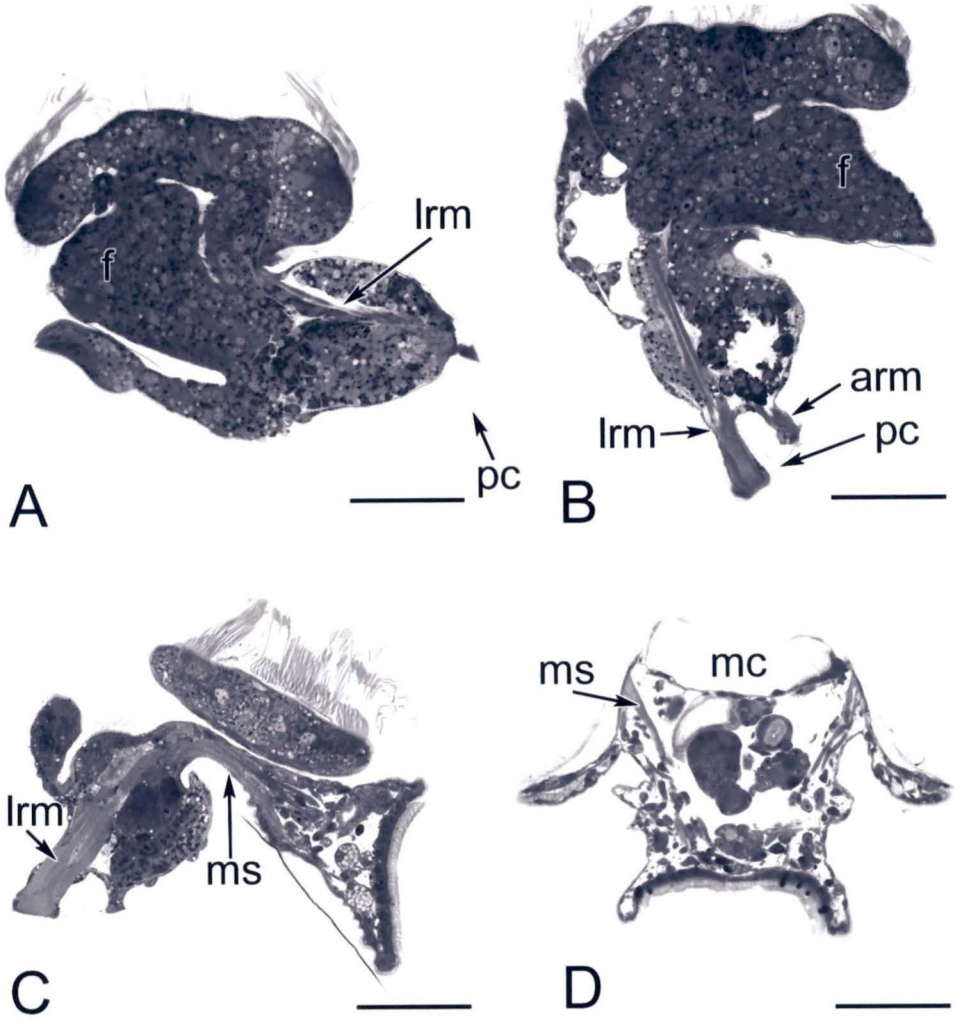
G. Muscles

The larval retractor muscles were the first muscles to develop in *T. scutum* larvae. The main larval retractor muscle was first seen in pre-torsional veligers, 49hpf (Figs. 4A, 15A). The muscle extended from the visceral mass, and attached to the inside of the shell at its posterior end, right of center (after the visceropallium rotated during torsion, the shell attachment of the main larval retractor muscle was positioned to the left of the mid-sagittal plane of the cephalopodium). A second larval retractor muscle (accessory retractor muscle) was also present, somewhat paralleling the main larval retractor; its attachment to the shell was seen in 60.5hpf larvae and was more central than the attachment site of the main larval retractor muscle (Fig.15B). Evidence of muscle-shell attachments was obtained only from light microscopy; transmission electron microscopy would be required to make definitive statements on the attachment of the muscle to the shell. There were also muscle fibers that ran from the posterior region of the visceral mass to the dorsal base of the velum, and to the proximal regions of the foot. By 7dpf, many muscles were seen in the larval foot (running in a dorsal-ventral orientation, from the central portions of the body to the base of the foot), and also near the base of the velum (Fig. 15C). The muscle cells of the larval retractor muscles were very wide, and appeared to be striated. After metamorphosis, the larval retractor muscles were absent, and there were many muscles connecting the foot to the shell. These muscles ran in a dorsal-ventral orientation, compared with the anterior-posterior orientation of the larval retractors (Fig. 15D).

Figure 15. Histological sections of *Tectura scutum*, showing muscles before and after torsion, and after metamorphosis.

A. Longitudinal section of a pre-torsional larva, 49hpf. Anterior is up, ventral (pre-torsion) is to the left. The larval retractor muscle (lrm) is visible, extending from the visceral mass to the most distal portions of the protoconch (pc).
B. Longitudinal section through the left side a post-torsional larva, 60.5hpf. Anterior is up, dorsal (post-torsion) is to the left. The larval retractor muscle and accessory retractor muscle (arm) both extend to the posterior regions of the protoconch, and appear to connect to the shell.
C. Longitudinal section through the left side of a competent larva, 7dpf. Anterior is up, dorsal is to the left. The larval retractor muscle is quite wide, and a tract of muscle fibers extending into the foot is also visible.
D. Cross section through a juvenile, 12d post-metamorphosis. Dorsal is up, right side of larva is to the left side of the page. Muscle tracts are visible extending from the dorsal surface, ventral to the foot.

Scale bars = 50μm.
Abbreviations: arm, accessory retractor muscle; f, foot; lrm, larval retractor muscle; mc, mantle cavity; ms, muscle; pc, protoconch.


f
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DISCUSSION

A. Development of *Tectura scutum*: comparisons with other Patellogastropods

The development of *Tectura scutum* was quite similar to that reported for *Patella vulgata* (Smith 1935), *Acmaea (Tectura) testudinalis* (Kessel 1964), and for *Lottia digitalis* and *L. asmi* (Kay and Emlet 2002). The overall form of the larva, including the velum, foot, larval pallial tentacle, shell, and main larval retractor muscle are similar among these five species. However, there are also several differences. Furthermore, my use of histological sections has allowed me to extend observations made on live larvae of two species of lottid patellogastropods by Kay and Emlet (2002), and my incorporation of an effective technique for anaesthetizing older larvae and juveniles prior to fixation has permitted more accurate observations on the developing form of the mantle and mantle cavity than was given by Smith (1935) for *Patella vulgata*. My observations on the events and timing of ontogenetic torsion in *T. scutum* add to accumulating data that appear to discredit Boutan's (1899) early report that torsion is accomplished within 2-3 minutes in patellogastropods; a report that has been often quoted in subsequent literature and has strongly influenced speculation about the evolution of torsion. A more detailed discussion of several characters (larval muscles, torsion, features of the mantle cavity, the digestive tract, and nervous system) follows below.

1. Early Development

Two polar bodies were formed on the fertilized egg of developing *T. scutum* (see Figs. 3A, 3B), similar to those reported by Kay and Emlet (2002) for *L. digitalis* and *L. asmi*, and Kessel (1964) also described the formation of two polar bodies in fertilized

eggs of *Acmaea (Tectura) testudinalis*, although they were not included in her drawings of embryonic development.

The first two cleavages of *T. scutum* were equal, with no evidence of polar lobe formation (see Fig. 3B). Equal cleavage has also been reported for other patellogastropods, including *Patella vulgata* (van den Biggelaar 1977), and is generally considered to be the pattern for all patellogastropods (van den Biggelaar and Haszprunar 1996). Similarly, the fertilized eggs of heterobranchs also divide equally during first cleavage and do not form polar lobes. This differs greatly from the early developmental patterns of caenogastropods, in which the first cleavage plane subdivides the egg cytoplasm unequally. In some caenogastropods, a polar lobe is formed that is equivalent in size to the first two blastomeres (van den Biggelaar and Haszprunar 1996). Polar lobe formation may allow for earlier determination of the mesentoblast in these species, which may have important ramifications for their development. The significance of mesentoblast determination and cleavage rates will be discussed in greater detail below.

Karp (1973) and Karp and Whiteley (1973) reported on the rates and timing of RNA synthesis in embryos, larvae, and veligers of *T. scutum*. However, there is very little detail given in either of these papers on developmental events, stages, and timing. Also, rearing temperatures were not given in either of these studies. Because temperature is known to have a significant impact on developmental rate, the timing data provided by these authors cannot be directly compared to my observations on *T. scutum*. Most of the general developmental information that was mentioned does basically parallel the results of the current study: trochophore larva stage at approximately 28hpf (Karp 1973), mid-veliger stage at approximately 74hpf (Karp and Whiteley 1973). However, their report

that sixth cleavage occurs at 16.5hpf differs greatly from this study in which a trochophore larva was fully formed by 16hpf (see Fig. 3D). In any case, the marked increase in RNA synthesis of *T. scutum* embryos at the stage of sixth cleavage (regardless of how long after fertilization this may have occurred), is still significant in that this is the stage when gastrulation begins, when the mesentoblast is determined, and dorso-ventral polarity is set down (Karp 1973, van den Biggelaar and Guerrier 1983).

2. Larval Muscles and Torsion

The general morphology of the main and accessory larval retractor muscles of *T. scutum* was the same as was previously reported for *Patella* (Smith 1935, Wanninger *et al* 1999a and b, Wanninger *et al* 2000). Observations of whole larvae (light microscopy) of *Acmaea (Tectura) testudinalis* (Kessel 1964) and two *Lottia* species (Kay and Emlet 2002) also included mention of the larval retractor muscles. In all but Smith (1935), the main and accessory larval retractors were present before the onset of torsion, while Smith did not report the appearance of the accessory larval retractor muscle ("ventral retractor") until after the completion of torsion. This may simply have been an artifact of the tools of microscopy available at that time. In all cases, the main larval retractor muscle attached to the shell at the posterior region, offset to the definitive left side, while the accessory retractor muscle had a slightly more central attachment point (see Figs. 5B and 15B). These observations were made with light microscopy only, so can only be an indication of where the attachment points could be located; electron microscopy would be required to confirm the physical attachment of the muscle to the shell. It has been hypothesized that this asymmetric attachment of the main larval retractor muscle could be responsible

for the 180° rotation of the visceropallium (with respect to the cephalopodium) that occurs during ontogenetic torsion (Crofts 1937 and 1955). If there were a second retractor muscle that provided symmetry, or if the attachment point were central (*i.e.*, not offset to the left), there would not have been an unequal torque exerted on the larva, therefore a twist due to muscular contraction could not have occurred. However, in a recent study by Page (2002c), torsion was observed in abnormal larvae of two vetigastropods that failed to form an attachment of the larval retractors to the shell. This suggests that the larval retractor muscle is not involved in, or not required for, the rotation of ontogenetic torsion, at least in some gastropod species. To further test the possible role of muscular contraction in the process of torsion, with or without attachment of the retractor muscle(s) to the shell, pre-torsional larvae could be experimentally manipulated to have inhibited muscle activity, and then observed to determine whether or not they were subsequently able to undergo torsion.

Whether or not the larval retractor muscles are involved in ontogenetic torsion, they do perform an important functional role for *T. scutum* larvae. In contrast to adult morphology, larvae of this limpet have a more typical gastropod (snail) shape, with a protoconch, and an operculum (see Figs. 4D and 7C). The larval retractor muscles can pull the cephalopodium (velum and foot) of a post-torsional veliger back into the shell, such that the whole veliger larva is protected by its shell and operculum. Adults of most gastropod taxa are able to withdraw into their shells, using their columella muscle (Ruppert and Barnes 1991). This is very different from the adult limpet morphology and behaviour, since the adult lacks the narrow aperture, snail-shaped shell, and operculum, but instead possesses a very strong foot that can anchor the limpet to its substrate and pull

the limpet shell down tightly to cover and protect the entire adult body. The larval retractor muscles, larval shell (protoconch), and operculum are lost either during or shortly after metamorphosis, along with the accompanying withdrawal behaviour. Since veligers are unable to retreat into the protoconch until after the completion of torsion, one may wonder why the larval retractor muscle develops before the onset of torsion. Possibly it is a preparation for the requirements of a post-torsional veliger: a laying-down of the structures required for a behaviour that the veliger may need to use immediately after torsion.

Torsion in *Tectura scutum* was monophasic: it occurred in one, uninterrupted morphogenetic movement. Wanninger *et al.* (2000) reported that torsion in *Patella caerula* was also monophasic, in contrast to the many, earlier reports that torsion occurred in two phases for many gastropods (Crofts 1937 and 1955, Smith 1935, others reviewed by Wanninger *et al.* 2000). The reports of torsion occurring as a biphasic process led to theories that the process is controlled by two separate mechanisms: muscular contraction and differential growth. One phase would be controlled by muscular contraction and would occur quickly, while the other phase would be a result of differential growth and would occur more slowly. If torsion is not biphasic, however, and occurs as one, monophasic movement, this erodes the argument that two separate mechanisms act at two separate times. The process could involve more than one mechanism, however, they need not be mutually exclusive.

3. Mantle, Shell Gland, and Mantle Cavity

The larval pallial tentacle with its terminal tuft of long, stiff cilia (see Fig. 3E, 3F and Fig. 10A-C), has been described for several other patellogastropods: *P. vulgata*

(Smith 1935), *Acmaea (Tectura) testudinalis* (Kessel 1964), and *L. digitalis* and *L. asmi* (Kay and Emlet 2002). The ciliary tuft on this tentacle has often been called the 'telotroch'; however, its homology with the telotroch of other trochophore larvae has not been studied so this term should be used with care. The larval pallial tentacle appears to be unique to the patellogastropods, and may be involved in the jumping/ sprinting behaviour observed in these larvae. Kay and Emlet (2002) described a sprinting movement for larvae of *L. digitalis* and *L. asmi*, and proposed that this avoidance/ escape behaviour was mediated by sensory receptors of the larval pallial tentacle. This behaviour was not observed in post-torsional veliger larvae, when the larval pallial tentacle was no longer present. Post-torsional veligers, unlike the pre-torsional stage, were able to retreat into their protoconch for protection, and may no longer require the sprinting behaviour to avoid danger.

The mantle epithelium (derived from the shell gland) retracted from the aperture of the protoconch prior to metamorphosis (Fig. 9A), but reestablished an association with the aperture of the developing teleoconch after metamorphosis (Fig. 9B). This withdrawal of the mantle epithelium from the edge of the protoconch, near the end of the larval phase, has been observed in many different opisthobranchs (Thompson 1958, Switzer-Dunlap and Hadfield 1977, Bickell and Chia 1979, Bickell and Kempf 1983), and also for the vetigastropod *Haliotis kamtschatkana* (Page 1997). Although this withdrawal was originally thought to be related to the loss of shell exhibited by nudibranch larvae during metamorphosis, the observation of this phenomenon in species that do not lose their shell at metamorphosis (*Aplysia* spp., *H. kamtschatkana* and *T. scutum*) suggests that it is not

related to shell loss. Possibly it is related to the change from protoconch to teleoconch formation.

A shallow, anterior mantle cavity, as present in adult patellogastropods and other symmetrical limpet groups, is generally considered to be a primitive trait among gastropods (Haszprunar 1988). While adult patellogastropods, including *Tectura scutum*, do have a shallow mantle cavity, the post-metamorphic juvenile has a very deep mantle cavity, extending from the apertural rim of the shell, along the entire dorsal surface to the posterior-most regions of the shell cavity (see Fig. 9B). Since this patellogastropod passes through a developmental stage that is similar to the more "derived" condition seen in adults of other gastropods (such as the more derived caenogastropods), it seems likely that the deep mantle cavity is not a condition unique to more derived groups. Instead, the shallow mantle cavity seen in adults of patellogastropods is likely a derived condition, possibly related to the limpet-shaped shell. Implications of this for gastropod phylogeny will be discussed below.

Within the mantle cavity, very few structures were seen. The anus, which opens to the right side of the mantle cavity, a central ciliary structure likely involved in the movement of water throughout the cavity, and a single osphradium were observed (see Figs. 9C, 9D). Although Smith (1935) reported that two osphradia (left and right) were present in older juveniles of *Patella vulgata*, he did not show this in a figure, or specify the age of the juveniles, or state if there was a stage in which only the left osphradium was present. In this study, I looked at juveniles as old as one month post-metamorphosis, yet observed evidence of a left osphradium only. This identification requires confirmation

from electron microscopy, as well as examination of older juveniles, to determine when the second osphradium eventually develops on the right.

4. Foregut

The anterior portion of the digestive tract of *T. scutum* develops before metamorphosis; the radula, radula sac, odontophoral cartilages, and the anterior portions of the esophagus developed in the non-feeding veliger larva (see Fig. 8D). The more posterior regions of the digestive tract do not develop until after metamorphosis. This is in a direct contrast to the pattern seen in the protected, encapsulated development of many caenogastropods and heterobranchs, in which a feeding larva eventually hatches from the egg capsule (van den Biggelaar and Haszprunar 1996). The larvae of these more derived gastropod groups have a larval gut that is partially distinct from the adult, or definitive digestive tract and the stomach, digestive gland, and intestine are fully differentiated before foregut elaborations related to post-metamorphic feeding have begun to form (Page 2002b). The early development of a complete, functional gut is important for these larvae because they hatch out of their protective capsules and feed in the plankton for a time before metamorphosis. Because the veliger larvae of *T. scutum* are non-feeding, a complete gut is not needed in larval stages and gut differentiation moves directly toward formation of the definitive, post-metamorphic digestive tract. These differences between formation of a non-feeding larva with no larval gut and a feeding larva with a functional larval gut may be correlated with differences in the timing of mesentoblast determination (van den Biggelaar and Haszprunar 1996), an association that is described more fully below.

The patellogastropods have an early cleavage pattern that involves rapid divisions, but a late specification of the mesentoblast mother cell 3D. The other macromeres (3A, 3B, 3C) divide before 3D, and in fact induce 3D to delay its division, which will subsequently form 4D and the mesentoblast 4d (van den Biggelaar and Haszprunar 1996). In caenogastropods and heterobranchs, the mesentoblast mother cell is determined at an earlier stage of development (40 or 24-cell stage, as compared with 63-cell stage for patellogastropods), which means that the blastomeres can have their fate determined at an earlier stage of development (van den Biggelaar and Haszprunar 1996). This early cell-fate determination may allow for the early, quick development of a larval gut in the caenogastropods and heterobranchs, to allow for a feeding larval stage. Patellogastropods, with a free-swimming (non-encapsulated), non-feeding veliger larva, must develop quickly, but need not develop features such as a digestive tract quickly. The later-stage determination of cell fate in patellogastropod larvae does not allow for the early development of a complete gut, but the rapid cleavages can still allow for rapid development of the larva as a whole. Strathmann *et al.* (2002) related rates of cell division to the level of protection of developing embryos. Those embryos that were at greatest risk (*i.e.*, not protected, such as patellogastropods) exhibited faster cell divisions than those species with embryos that were protected by egg capsules and/or brooding.

The lack of a complete, functional larval gut in the patellogastropod *T. scutum* may be explained by the hypotheses described above, but why would the anterior portions of the gut develop in advance of any differentiation in the posterior regions? Once the macromere 3D has formed and dorso-ventral polarity is determined, the micromeres of the ventral quadrant divide faster than those of the dorsal quadrant

(Verdonk and Cather 1983). This may facilitate the early determination and development of structures associated with the anterior/ ventral portions of the gut, including the buccal cavity, radula, odontophoral cartilages, and anterior esophagus.

5. Nervous System

The overall pattern of the larval and juvenile nervous system of *T. scutum* is somewhat similar to that reported for patellogastropods in general (Bullock and Horridge 1965), and for *P. vulgata* by Smith (1935): the position and timing of development of the pleural, pedal, visceral and cerebral ganglia are similar to those observed in the current study, while the buccal and labial ganglia exhibited some differences. The pleural ganglia are dorsal and slightly posterior (and slightly more lateral) to the pedal ganglia, while the cerebral ganglia are more anterior and dorsal than either of these pairs of ganglia (Figs.12B-E and 13E; and compare with Fig. 22a and b in Smith 1935). The cerebral ganglia are observed early in development (before the onset of torsion), as they were in *P. vulgata* by Smith (1935), however, no other ganglia were observed in *T. scutum* until close to the time of metamorphosis. This is unlike the pattern seen in *P. vulgata* (Smith 1935), where the pedal ganglia and statocysts were observed before the onset of torsion, and the pleural ganglia began to develop during torsion. The visceral loop and its ganglia were late to develop (after torsion), indicating that torsion is not directly responsible for the figure-eight twist in the visceral loop. While the loop itself had not formed before torsion, it is possible that the stem cells of the future visceral loop were already defined before torsion began, causing its twisted morphology (Smith 1935).

Similar to the pattern seen in the development of the digestive tract, the anterior portions of the nervous system differentiated earlier than the more posterior ganglia. This also may be related to the rapid division of the ventral micromeres with respect to the slower division of the dorsal micromeres (Verdonk and Cather 1983).

It has been suggested that the position of the pleural ganglia, in relation to the pedal and cerebral ganglia, may be useful in classification and phylogenetic relationships of the Gastropoda (Haszprunar 1988). The hypoathroid condition is reported for the "Archaeogastropoda" (as used by Haszprunar 1988: includes the Patellogastropoda, Nertopsina, Vetigastropoda and some hot vent taxa), and describes a situation where the pleural ganglia are "adjacent" to the pedal ganglia (Haszprunar 1988). In contrast, the epiathroid condition, which is characteristic of most caenogastropods and heterobranchs, places the pleural ganglia "adjacent" to the cerebral ganglia. Fretter and Graham (1962) describe a third, intermediate type, dystenoid, in which the pleurals are "well separated from the pedals..., but have not yet migrated to the proximity of the cerebrals: they still lie ventral to the gut (308)." Haszprunar (1988) restricts use of the term dystenoid to the Viviparidae (a more derived "Archaeogastropod"), and has changed its definition to describe a neural organization which is hypoathroid on one side and epiathroid on the other. The Patellogastropoda are reported as being hypoathroid, with the epiathroid condition evolving only once, in the Caenogastropoda (Haszprunar 1988). The pattern observed here for the patellogastropod *T. scutum* appears to fit Fretter and Graham's (1962) definition of dystenoid: the pleural ganglia are not connected to the pedals, but they are still ventral to the gut and are not in close proximity to the cerebrals (Figs. 12D,

12E). This calls into question the strength of classifications based on the hypoathroid/epiathroid condition.

B. Ancestral Characters and Polarity of Evolutionary Transitions for Gastropods

Heterochrony is the change in the timing or sequence of developmental events (Smith 2001). It has been proposed that heterochrony has been a pervasive theme during gastropod evolution. Lindberg (1988a) reviewed several developmental characters of gastropods, with particular reference to the patellogastropods. In this review, as in most cases where character state polarity was determined, outgroup comparisons were made with the Monoplacophora and/or Polyplacophora (*e.g.*, Lindberg 1988a, Ponder and Lindberg 1997, Harasewych and McArthur 2000). Many of the gastropod traits which have been scored as "primitive" were classified as such simply because they have been observed in the outgroups and the basal Patellogastropoda. However, many of these so-called primitive traits may have evolved convergently in monoplacophorans, polyplacophorans, and patellogastropods. One such trait is the morphology of the pedal cords/ pedal ganglia.

In monoplacophorans and polyplacophorans, the tracts of the nervous system in the foot are in the form of pedal cords, whereas more derived gastropods generally have compact pedal ganglia (Fretter and Graham 1962, Bullock and Horridge 1965, Ponder and Lindberg 1997). Adults of patellogastropods (including *Tectura scutum*) and vetigastropods (such as the abalone, *Haliotis* and the key-hole limpet, *Diodora*), have pedal cords, similar to those seen in mono- and polyplacophorans (Fretter and Graham 1962). However, they pass through a developmental stage in which compact pedal

ganglia are observed (see Fig. 12D) before eventually stretching out into the pedal cords. Because individuals in these basal gastropod clades pass through a stage which exhibits the derived gastropod condition (pedal ganglia), the later appearance of the primitive state (pedal cords) is likely an example of convergent evolution between the outgroups (monoplacophorans and polyplacophorans) and the basal gastropod groups, patellogastropods and vetigastropods. There are other examples of convergence of this trait, including the presence of pedal cords (and not concentrated ganglia) in a few species of more derived gastropods (Haszprunar 1988).

Another example of patellogastropods exhibiting the derived state before finally demonstrating the primitive condition is in the position of the statocysts. The primitive condition for position of the statocysts is for the statocysts to be located lateral to the pedal cords (or ganglia), whereas the derived condition places the statocysts dorsal (or postero-dorsal) or anterior to the pedal ganglia (Haszprunar 1988, Ponder and Lindberg 1997). The primitive condition was defined as such because it was observed in the outgroups (monoplacophorans and the cephalopod *Nautilus*) and also patellogastropods (Ponder and Lindberg 1997). However, this is not always the case for patellogastropods. As seen in the present study on *T. scutum*, the statocysts are located dorsal to the pedal ganglia, not lateral to them, for up to one month following metamorphosis (see Figs. 13E, 13F).

The form of the pedal ganglia (vs. cords), location of the statocysts, and depth of the mantle cavity (as discussed above) are three examples of traits for which the patellogastropod *T. scutum* expresses the derived condition during development before ultimately expressing the primitive condition as an adult. The so-called derived

conditions (*i.e.*, pedal ganglia, statocysts dorsal to the pedal ganglia, and a deep mantle cavity) that are ordinarily observed in the adults of caenogastropods and heterobranchs could be a part of the developmental program of all gastropods, but are only transient in patellogastropods because later developmental elaborations occur that alter these characters. Thus, the adult traits of *T. scutum* that are convergent with outgroups could have arisen by peramorphosis – the addition of new descendent characters to the ancestral ontogeny (Lindberg 1988a). Alternatively, the more derived gastropods may have simply retained the juvenile character states (paedomorphosis), and do not go on to complete the ancestral or "normal" developmental program as seen in patellogastropods (Ponder and Lindberg 1997).

My observations that the patellogastropod *T. scutum* exhibits, during development, the so-called derived adult condition of a number of traits could also have an impact on the hypothesis (Haszprunar 1988) that the non-coiled, limpet-shaped shell is ancestral for the Gastropoda. *T. scutum* exhibits several traits related to a limpet-shaped shell (pedal cords, lateral statocysts, and shallow mantle cavity) only after passing through a developmental stage where forms of those traits associated with a coiled gastropod morphology are observed. This suggests that patellogastropods may have secondarily evolved the so-called primitive forms of these traits to support a limpet shape as an adult. This would suggest that the gastropod ancestor was coiled, and that the limpet shape is the derived condition for the Patellogastropoda. Because this argument against the hypothesis that the limpet morphology is ancestral for the Gastropoda was a result of studies of developmental stages, it further highlights the importance of the

examination of traits throughout development, rather than just in the adult, for determining information about relationships and polarity of change.

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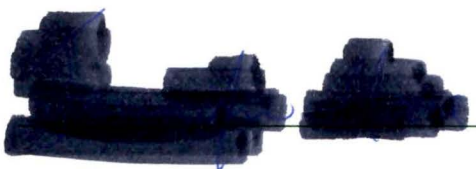
Author 

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September 23, 2003