Developmental modularity in the feeding structures of the predatory gastropod, *Amphissa columbiana* (Neogastropoda; Columbellidae)

by

Nova Hanson
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Supervisory Committee

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Abstract

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Developmental modularity may facilitate morphological evolution by allowing phenotypic change of a developing body component without negatively impacting other components. I examined foregut development in *Amphissa columbiana*, a predatory neogastropod with a highly derived foregut and in *Crepidula fornicata*, a phytoplankton-feeder with a less derived foregut, for evidence of developmental modules. Histological sections revealed that the post-metamorphic buccal cavity and radula of both species form as a ventral outpocketing (ventral module) from the larval esophagus (dorsal module). However, in *Amphissa columbiana* the ventral outpocketing is semi-isolated from the larval esophagus and also produces an "anterior esophagus" that is not developmentally homologous to the "anterior esophagus" of herbivorous caenogastropods. Semi-isolation of the ventral and dorsal modules of the developing neogastropod foregut allows precocious development of the post-metamorphic foregut during the larval stage without compromising larval feeding. Therefore, development of diverse variants of the post-metamorphic foregut are freed from larval constraints.

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1.0 Introduction

The Mollusca is one of the most diverse groups of animals and the second largest phylum, with an estimated 200,000 living species belonging to seven or eight classes (Ponder and Lindberg 2008). Within this phylum belongs the Gastropoda, a group that has seen extraordinary success, radiating to inhabit a variety of environments with much diversity in morphology, behaviour and physiology. In fact, the Gastropoda is the largest and most diverse molluscan class (Bouchet and Rocroi 2005) and the second most speciose animal class, with an estimated 150,000 living species (Aktipis et al. 2008). Much of this impressive diversity can be found within the Caenogastropoda, which accounts for 60% of all extant gastropod species (Ponder et al. 2008).

The earliest caenogastropods appeared during the mid-Paleozoic and they subsequently experienced significant radiations during the Jurassic, Cretaceous and Paleogene, leading to a diversification into a variety of habitats (Ponder et al. 2008). While the ancestral mode of feeding in gastropods is suggested to be herbivorous grazing (Ponder and Lindberg 1997), particular groups of caenogastropods have acquired different feeding strategies that have allowed them to utilize a variety of nutritional sources. Existing novel feeding strategies include suspension feeding, parasitism, grazing carnivory on sessile prey, and most notably predation on active prey (Taylor et al. 1980, Ponder et al. 2008). The emergence of predatory feeding may have been an evolutionary innovation within the Caenogastropoda, because it is correlated with a subsequent rapid rate of speciation and exploitation of novel feeding niches (Taylor et al. 1980, Kohn 1983, Ponder and Lindberg 2008). One caenogastropod group that has shown a remarkable capacity for generating novel, predatory feeding morphologies is the Neogastropoda, a clade that originated during the Cretaceous (Ponder et al. 2008). The most notable feature of the neogastropods is the presence of a well-developed proboscis and a particularly specialized foregut (Kantor 1996), which may have allowed these predators to utilize previously inaccessible food sources (Taylor et al. 1980).

1.1 Feeding systems within the Caenogastropoda

In order to understand the level of foregut specialization seen within predatory caenogastropod species, the basic morphology of ancestral caenogastropod feeding structures must be deduced. Currently, the gastropod clades that are suggested to be the most basal are the Patellogastropoda (true limpets) and the Vetigastropoda (*eg.* top snails, abalone, keyhole limpets)(Zapata et al. 2014), which show the plesiomorphic feeding condition of herbivorous grazing. At least some members of other gastropod clades, including the Neritimorpha, Caenogastropoda, and Heterobranchia, have also retained herbivorous grazing (Ponder and Lindberg 1997).The foregut of typical herbivorous gastropods begins with a mouth that opens into a short buccal tube that expands into the buccal cavity and continues posteriorly as the anterior esophagus (Figure 1A and B). The buccal cavity receives the ducts of the salivary glands. At the posterior of the buccal cavity, the radular sac exists as an outpocketing of the ventral wall, which secretes a tongue-like ribbon of recurved radular teeth, known as the radula. When fully formed, the radula extends from the radular sac to rest on the floor of the buccal cavity, near the mouth. In concert with complex musculature and supportive radular cartilages, the radula is protruded repeatedly from the mouth during grazing, to scrape algae or detritus off the substrate. A ciliated channel (known as the dorsal food channel, delineated by dorso-lateral folds)(Haszprunar 1988, Salvini-Plawen 1988), extends along the dorsal midline of the buccal cavity and down the length of the anterior and mid-esophagus, to where the mid-esophageal gland resides.

Although many of the less-derived morphological features of the foregut are retained within predatory caenogastropods, much variation exists. Whereas herbivorous grazers extend the radula in rhythmic movements from the mouth located in a ventral position on the head, caenogastropod predators, and most notably neogastropods, have acquired an extensible, muscular proboscis that protrudes from an orifice at the anterior terminus of the head, potentially allowing access to novel food sources (Taylor et al. 1980, Kantor 1996). When not in use, the proboscis can be retracted into a proboscis sac (Ponder 1973, Kantor 1996, Golding et al. 2009). Proboscis morphology is very diverse,

with many differences in retractor muscles and biomechanical operation, resulting in at least four proboscis types within the Caenogastropoda (Golding et al. 2009).

With the emergence of diverse proboscis types, the foregut of predators has also changed markedly from the ancestral condition. In order to accommodate the presence of an extensible proboscis, some portion of the foregut has become elongated from the ancestral prototype. In some lineages the anterior esophagus has been the source of elongation, with the buccal cavity and radula located at the apex of the proboscis (Kantor 1996, Page 2011)(Figure 1C); however, in conoideans, the buccal tube has been elongated, resulting in the buccal cavity residing at the base of the proboscis (Kantor 1996)(Figure 1D). At the posterior of the anterior esophagus, a valve of Leiblein has been identified in many predatory neogastropods, which is hypothesized to prevent the regurgitation of food (Graham 1941, Kantor 1996)(Figure 1C). In terms of the radula, much diversity exists across the Caenogastropoda with respect to the form, number and relative position of radular teeth (Simone 2011); however, examples of especially derived radular teeth have been identified within the Neogastropoda. The radular teeth are usually secreted as many rows of multiple teeth in each row that are attached basally to a ribbon of chitin. However, teeth produced by members of the superfamily Conoidea have the form of individual hollow harpoons, which are shot into prey using a ballistic mechanism (Schulz et al. 2004) to inject neurotoxins, thereby acting like a hypodermic needle (Kohn et al. 1972, Kantor and Taylor 1991, Castelin et al. 2012). In addition to these modifications (and in some cases, in collaboration), various glandular structures in neogastropods have derived from the original mid-esophageal gland, which often accommodate the functional needs of prey specialization (Andrews and Thorogood 2005). While in some, a gland of Leiblein provides secretions for digestion (Figure 1C), in other lineages, extremely derived glands have arisen, which potentially aid in prey immobilization (Kantor 1996). One such gland exists in the conoideans, the venom gland (Figure 1D), where neurotoxic peptides (conotoxins) are synthesized to be used in concert with the radular harpoons to paralyze prey (Olivera 2006). Accessory salivary glands with a potential paralytic function have also been reported in *Nucella* (Andrews 1991).

What is particularly interesting about the diverse feeding systems found within the Caenogastropoda is that all evolved within the context of a biphasic lifecycle, which begins with a larva that must undergo metamorphosis to produce the juvenile/adult. Moreover, this complex lifecycle often includes a herbivorous feeding larva. Due to the complex nature of such life histories, many questions have arisen as to how larval requirements may have imposed constraints on adult evolution, especially where the transition from herbivory to carnivorous predation occurs.

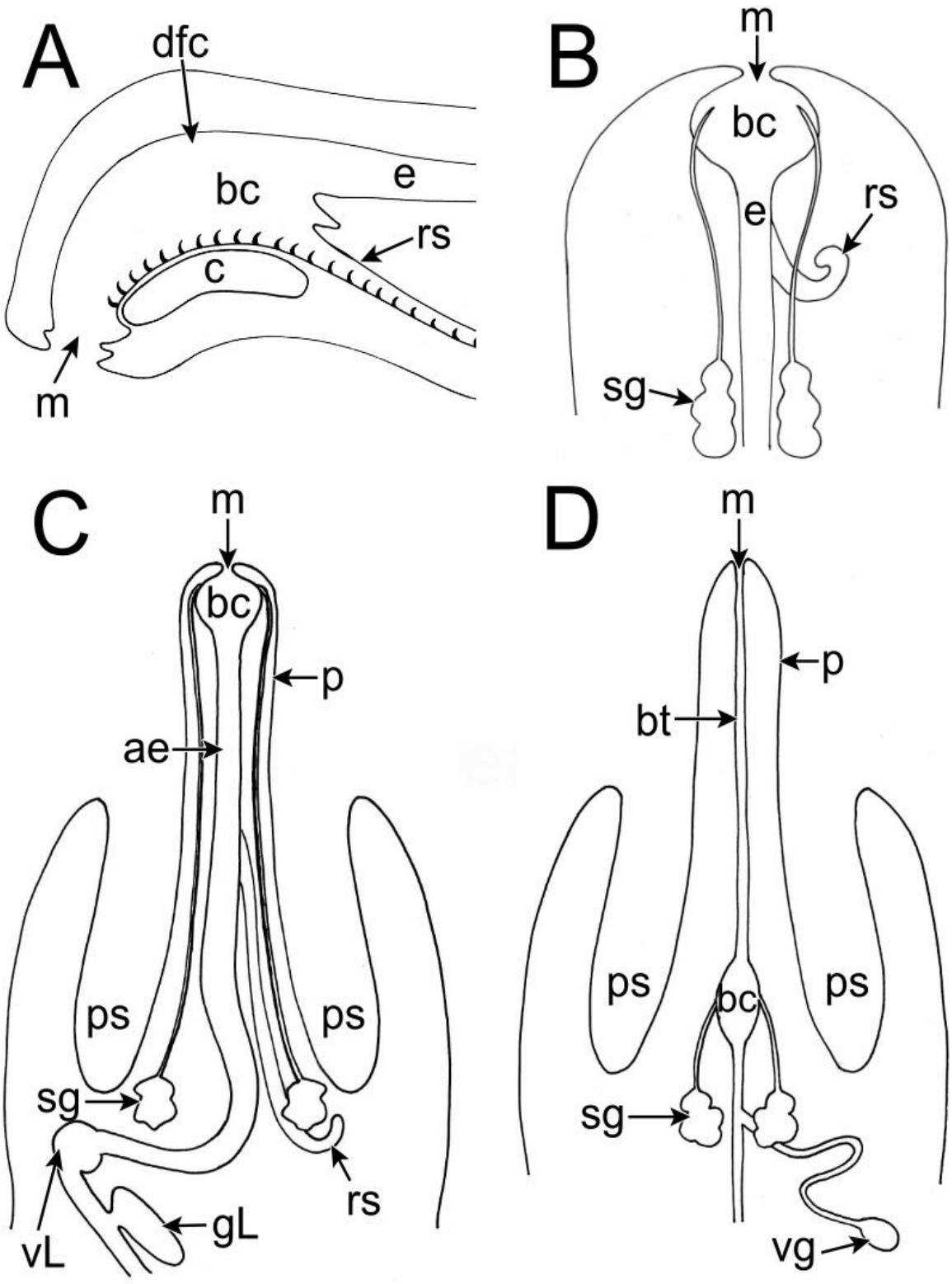

Figure 1. Foregut morphology of a herbivorous caenogastropod and two neogastropods. **A.** Lateral view of a herbivorous grazing caenogastropod, *Lacuna vincta*. **B.** Dorsal view of a herbivorous grazing caenogastropod, *Lacuna vincta*. **C.** Dorsal view of the predatory

neogastropod *Nassarius mendicus*, with a partially extended proboscis. Note the elongate anterior esophagus, valve of Leiblein and gland of Leiblein. **D.** Dorsal view of the predatory neogastropod *Conus lividus*, with a partially extended proboscis. Note the elongate buccal tube and venom gland. Abbreviations: ae= anterior esophagus, bc= buccal cavity, bt= buccal tube, c= radular cartilage, dfc= dorsal food channel, e= esophagus, gL= gland of Leiblein, m= mouth, p= proboscis, ps= proboscis sac, rs= radular sac, sg= salivary gland, vL= valve of Leiblein, vg= venom gland. Adapted from Page (2000 and 2011).

1.2 Life history evolution within the Caenogastropoda

Gastropod life history patterns and life history transitions within clades must be considered in developmental studies. This is particularly true of gastropods with a complex life history because much of the larval body is typically carried forward into the juvenile stage. These life history patterns are exceptionally varied and can be categorized in many ways based on the presence or absence of swimming larvae, the nutritional source during the larval stage, and whether development involves a process of metamorphosis (Bonar 1978). However, gastropod life history patterns have undergone numerous transitions, losing and reacquiring traits, sometimes repeatedly.

In gastropods, two main life history patterns exist: direct and indirect (Figure 2). Embryos of direct-developing species develop directly into juveniles within an egg capsule, whereas embryos of indirect-developing species develop initially into a free-swimming larval stage, known as a veliger, which must undergo metamorphosis to become a juvenile. Indirect-developing larvae can be further subdivided into lecithotrophic (non-feeding) or planktotrophic (feeding). Lecithotrophic larvae are usually provisioned with maternal yolk, albumin, or a combination of the two, whereas planktotrophic larvae must feed on microalgae within the water column (Fretter and Graham 1994). Once the encapsulated or free-living larvae are sufficiently developed, they undergo metamorphosis to become juveniles. Metamorphosis most commonly involves the loss of morphological characters that are specific to the larval stage and the emergence of juvenile-specific characters (Haszprunar 1988, Hadfield et al. 2001), as well as an irreversible change in habitat and behaviour (McEdward and Janies 1993). Very often metamorphosis requires an environmental cue (Hadfield et al. 2001), which may be highly specific, and larvae of many species are capable of delaying metamorphosis until an appropriate environment is found based on the presence of the cue (Pechenik 1986, Haszprunar 1988, Hadfield et al. 2001). With regards to direct developing species, while some do pass through a veliger-like embryo stage (Fretter and Graham 1994), some do not and instead develop directly into the juvenile, effectively skipping metamorphosis (they are ametamorphic). In addition to this, many unique life

histories also exist, including adelphophagic species, which develop directly, consuming sibling embryos, or nurse eggs, within the egg capsule (Fretter and Graham 1994).

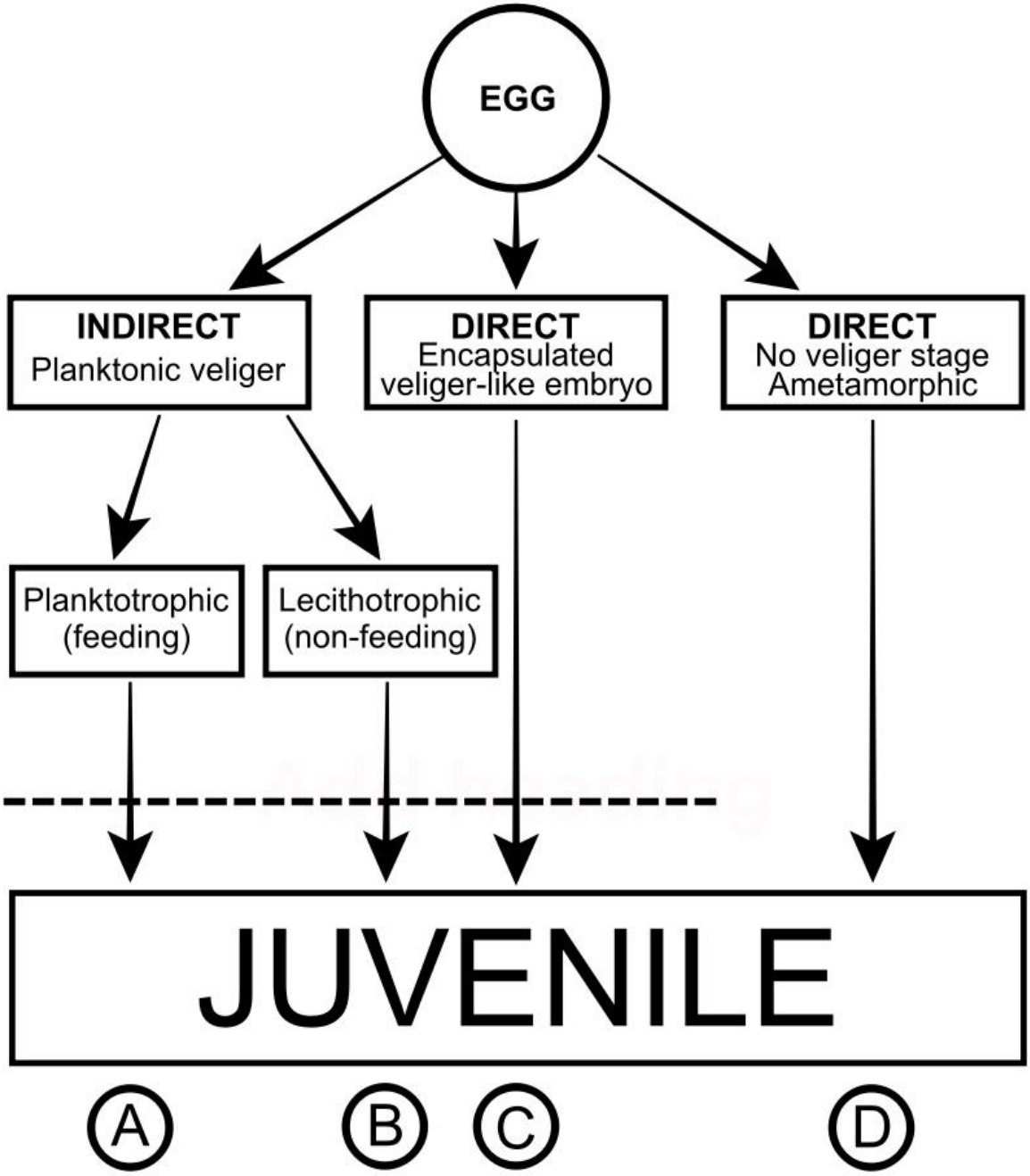

Figure 2. Summary of major life history patterns found in gastropods. **A.** Indirect planktotrophic veliger. **B.** Indirect lecithotrophic veliger. **C.** Veliger-like embryo that develops directly within the capsule, either as non-feeding or adelphophagic. **D.** Complete capsular embryogenesis that does not undergo metamorphosis to achieve the juvenile stage. Adapted from Bonar (1978). Dashed line indicates the metamorphic transition.

Depending on the developmental mode and the lineage, veliger larvae can differ in morphology; however many features can be generalized across this life stage. All veliger larvae have velar lobes, a larval shell and a foot (Fretter and Graham 1994). The velar lobes, although quite variable, usually consist of two lobe-like extensions of the head bearing two opposed bands of cilia: the prototroch and the metatroch (Thompson 1959, Fretter and Montgomery 1968, Strathmann and Leise 1979). While the prototroch (preoral band) consists of a band of long compound cilia, used for swimming, the metatroch (postoral band) consists of shorter cilia that beat toward the prototroch (Strathmann and Leise 1979). By virtue of the bands beating toward each other, microalgae are captured and transported to the mouth along the ciliated food groove, which is located between the two bands (Thompson 1959, Fretter and Montgomery 1968, Strathmann and Leise 1979). Despite the fact that encapsulated, veliger-like embryos do not capture microalgae, ciliated velar lobes are often still present in these species and have been hypothesized to have roles in albumin uptake (Rivest 1992) and gas exchange (Hunter and Vogel 1986), because they allow the larvae to rotate within the egg capsule.

Current consensus suggests that the life history of the ancestral gastropod was that of an indirect lecithotrophic veliger, a plesiomorphy which still persists in extant representatives of the most basal clades, the Patellogastropoda and the Vetigastropoda (Haszprunar et al. 1995, Ponder and Lindberg 1997, Lindberg and Guralnick 2003, Aktipis et al. 2008). However, the emergence of planktotrophy is theorized to have occurred in the ancestor to the remaining major gastropod clades: the Neritimorpha, Caenogastropoda and Heterobranchia (Lindberg and Guralnick 2003, Aktipis et al. 2008, Ponder and Lindberg 2008)(Figure 3). While the majority of extant species in these clades have retained planktotrophic larvae, secondary loss of feeding larvae has occurred independently many times within the Caenogastropoda (Page and Hookham 2017). Remarkably, the larvae of calyptraeids, a family of sedentary filter feeding marine limpets have been found to have numerous modes of development, including planktotrophy, lecithotrophy, direct development, as well as adelphophagy (Collin 2004). Moreover, when life history patterns were mapped onto a highly resolved phylogeny for calyptraeid species, evidence was found for reaquisition of larval planktotrophy within lineages where planktotrophy had been previously lost (Collin et al. 2007).

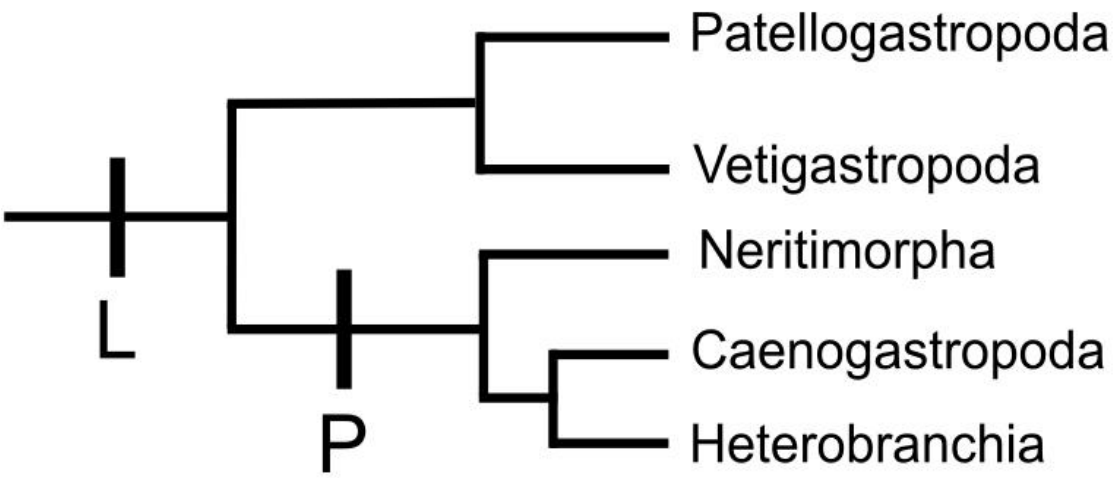

Figure 3. Recent phylogenetic hypothesis of major gastropod clades.
L represents the presence of a life history that includes indrect lecithotrophic veligers. P represents indirect planktotrophic veligers, which are very common throughout the Neritimorpha, Caenogastropoda and Heterobranchia. Adapted from Zapata et al. (2014).

While in some invertebrates the larval form is almost entirely destroyed during metamorphosis, with the post-metamorphic body developing separately, the larvae of gastropods are very much templates for the adult, where the post-metamorphic individual is mostly an elaboration of the larval form (Page 2009). Due to this style of development, the larval stage may impose many constraints on possible adult morphology. Therefore, the larval form must exist as a balanced compromise between the characteristics required for larval survival, and characteristics that will allow conversion to a successful juvenile (Page and Pedersen 1998). Ultimately, the main question emerges: how can novelties possibly arise in the larval or adult stages, without entirely compromising the intricate developmental framework or the requirements of either life stage?

This essential question certainly comes into play when addressing the specialization of adult feeding systems within the Caenogastropoda. Remarkably, the morphologically complex foregut of neogastropod predators has evolved within a life history that begins with a larval stage that feeds on microalgae. Subsequently, the larva undergoes metamorphosis to become a predatory juvenile, resulting in a transition from microherbivory to carnivorous predation. Metamorphosis in some gastropods has been found to be completed in as little as 24 hours or less (Hadfield and Strathmann 1996). Such a rapid transition can have serious implications for species that show a change in feeding mode between the larval and adult stages, which require different morphological structures. Due to the fact that the metamorphic period is so short, formation of many juvenile structures in marine invertebrates often precedes the metamorphic period (Hadfield et al. 2001). However, little is known about how this rapid transition and precocious development is accomplished in neogastropods that show extremely different feeding modes between life history stages.

Ultimately, two requirements must be met in order to ensure the survival of the organism: a) in order to fuel development throughout the larval stage, the larva must be able to maintain the conduction of microalgae from the mouth to the stomach, through the larval esophagus and b) the predatory definitive foregut of the juvenile must be ready for use shortly after metamorphosis so that the metamorphic transition can occur rapidly (Fretter 1969, Hadfield et al. 2001).

1.3 The role of modularity in the facilitation of caenogastropod evolvability

One developmental mechanism that can potentially explain the ability of biphasic animals to evolve is a modular organization of development. Modularity is an abstract concept of biological organization that describes the extent to which elements are connected to each other, with some elements being grouped into highly integrated subsets, which are relatively independent from other subsets. The elements within these subsets develop together, likely under the control of a discrete gene regulatory network (Wagner and Altenberg 1996, Wagner et al. 2007). The elements in a module can be anything from nucleotides in an RNA molecule, proteins in a cell, or morphological characters (Wagner et al. 2007). Therefore, although modularity can refer to very different kinds of elements, it retains its meaning, conveying the relative connectedness between elements (Wagner et al. 2007).

Studies to date have indicated that organismal development is modular, meaning that groups of traits that develop together in a highly integrated fashion constitute a module, and that modules develop mostly independently of other modules (von Dassow and Munro 1999, Bolker 2000, Wagner et al. 2007). The relative independence of developmental modules means that phenotypic variants can more easily be produced, without necessarily disrupting development as a whole (Page and Hookham 2017). A modular organization of development is a compelling concept, as it can provide an explanation for how adult morphological variants are generated in species that have a complex life cycle, where requirements of larval function might constrain the capacity of adults to evolve.

In addition to this, modularity can potentially add to explanations of asymmetric sister group diversification, which is classically interpreted by the Modern Synthesis as differences in exposure to ecological opportunity (Losos and Mahler 2010). However, this explanation on its own ignores the fact that ecological opportunity has little meaning if the developmental system is not capable of generating different phenotypes that can exploit new environmental opportunities. Modularity has been suggested to promote evolvability, the capacity of a developmental system to evolve, as it permits developmental change within any one module (leading to a change in phenotype),

without having deleterious effects on other modules (Raff 1996). Therefore, the presence of developmental modules may be an important requirement for clade diversification, because they allow the generation of heritable phenotypic variants that can exploit new environmental selective regimes (Gilbert et al. 1996, Erwin 2015). If developmental modules do in fact facilitate evolvability, then it can be hypothesized that highly diverse clades might possess a modular organization of development for those systems that are particularly derived.

1.4 Foregut development within the Caenogastropoda

Studies have suggested that the gastropod foregut consists of two different developmental modules (dorsal and ventral) and that temporal and spatial separation of these modules within caenogastropods may have facilitated the emergence of diverse, post-metamorphic foregut types within this clade (Fretter 1969, Page 2000, 2002, 2005, 2011, Parries and Page 2003, Hookham and Page 2016). While the dorsal module consists of the larval esophagus, the ventral module develops as an outpocketing of the ventral wall of the distal larval esophagus (Figure 4). It is from the ventral wall that most, if not all, of the adult feeding apparatus is generated in caenogastropods, including the buccal cavity, radular sac and salivary glands (D'Asaro 1965, Fretter 1969, Thiriot-Quiévreux 1974, 1969, Page 2000, 2002, 2005, 2011, Parries and Page 2003, Hookham and Page 2016, Page and Hookham 2017). In fact, it has even been found in some predatory neogastropod species, such as *Nassarius mendicus*, that the anterior esophagus has also been generated from the ventral module, and that the larval esophagus, along with the larval mouth, are completely destroyed at metamorphosis (Page 2000, 2005). Ultimately, the spatial separation of the dorsal developmental module (larval esophagus) and the ventral developmental module (post-metamorphic foregut) may have allowed for larval feeding and the development of the post-metamorphic foregut to occur simultaneously. In addition, foregut developmental modules may have enabled predators to evolve novel definitive foregut types because they were freed from larval constraints.

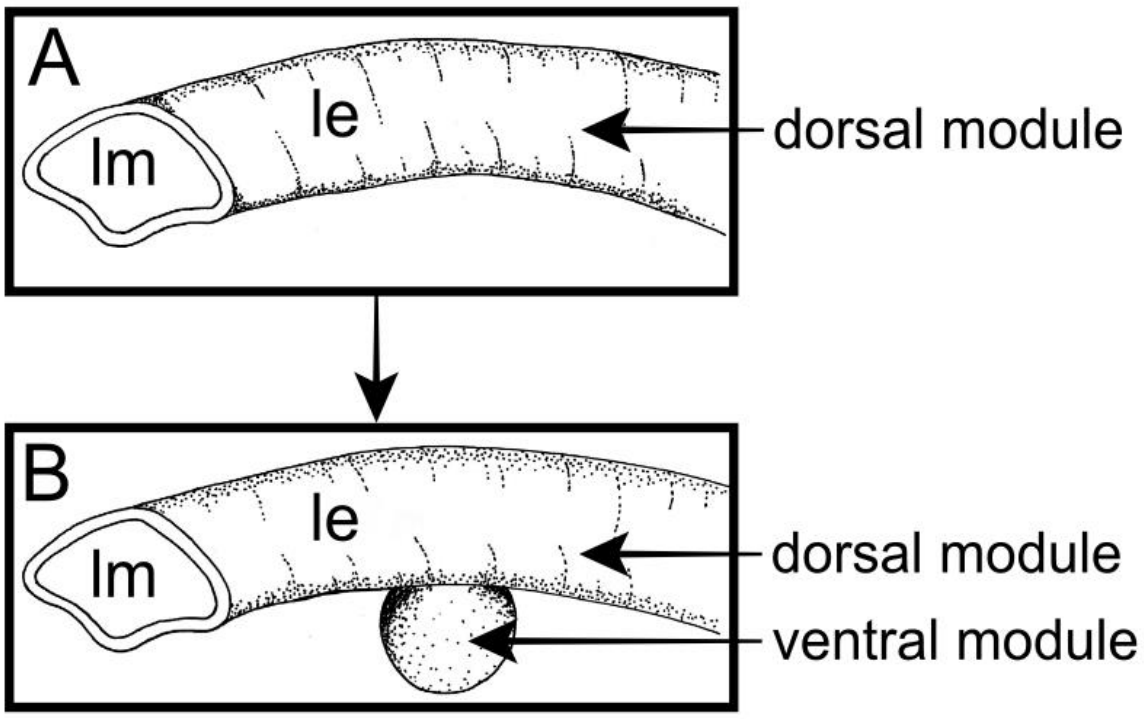

Figure 4. Sketches showing the initial differentiation of the dorsal and ventral modules in caenogastropods.

A. Distal foregut in hatching larvae. **B.** Initial outpocketing from the ventral wall of the larval foregut. Abbreviations: le=larval esophagus, lm=larval mouth. Adapted from Page (2000).

1.4.1 Foregut development in herbivorous caenogastropods

Although caenogastropods are well known for their diverse and sometimes very derived feeding systems, some extant caenogastropods have retained the herbivorous condition. One such herbivore is *Lacuna vincta* (Littorinoidea), in which foregut development was studied by Page (2000). Upon hatching, the foregut was a simple ciliated tube that was capable of transporting microalgae from the mouth to the stomach (dorsal module). When examined further, a small patch of enlarged cells were visible embedded in the ventral esophageal wall, which subsequently differentiated into a large hollow outpocketing by 20-30% completion of larval development. This hollow outpocketing was the anlage of the future post-metamorphic buccal cavity, salivary glands and radular sac (ventral module). This outpocketing would further elaborate into a hollow tube, with the anterior and posterior growths having different ontogenetic fates. While the posterior projection (the future radular sac) extended as a hollow tube separate from the overlying larval esophagus, the anterior region of the ventral outpocketing (the future buccal cavity), was connected along its length to the larval esophagus; the lumen was continuous with the lumen of the larval esophagus. By metamorphic competence, a pair of salivary glands had differentiated from the epithelium of the buccal cavity, the radular sac with a secreted ribbon of recurved radular teeth (radula) had elongated posteriorly, and a pair of radular cartilages had differentiated beneath the floor of the buccal cavity. During metamorphosis, the distal larval esophagus was retained; however, it became largely reduced (known as the dorsal food channel) due to cell loss.

Work has also been done on the herbivore *Trichotropis cancellata* (Capuloidea), which employs the novel feeding strategies of ctenidial suspension feeding, as well as kleptoparasitism, where food is stolen from suspension-feeding polychaetes (Pernet and Kohn 1998, Parries and Page 2003). In order to steal food, *T. cancellata* uses a "pseudoproboscis", which is an extension of the ciliated lower lip, to reach into the mouth of feeding polychaetes (Pernet and Kohn 1998). While *T. cancellata* employs these derived feeding techniques, results from Parries and Page (2003) illustrated that foregut development in *T. cancellata* shared major themes with *L. vincta*. In newly hatched larvae, the larval esophagus appeared as a uniformly ciliated tube (dorsal

module). However, the hollow outpocketing of the ventral wall of the distal larval esophagus that would form the post-metamorphic buccal cavity, salivary glands and radular sac (ventral module) was already present. Similar to *L. vincta*, the posterior projection (radular sac) of the outpocketing remained separate throughout larval development, whereas the anterior region (buccal cavity) remained continuous with the larval esophagus. At metamorphic competence the pseudoproboscis began to form, which appeared as an enlarged swelling of the lower lip, equipped with a ciliated strip along the midline. At metamorphosis, the larval esophagus underwent much cell loss, becoming the narrow, ciliated dorsal food channel. It was found that the juveniles were able to begin feeding anywhere from hours to a few days, immediately after metamorphosis.

Ultimately, because post-metamorphic foregut structures developed ventral to, and relatively isolated from the larval esophagus in both *L. vincta* and *T. cancellata*, larval feeding was not obstructed (Fretter 1969, Page 2000, Parries and Page 2003), and juvenile feeding was able to commence shortly after metamorphosis, due to precocious development during the larval stage.

1.4.2 Foregut development in predatory caenogastropods

In predatory caenogastropods, the larval foregut was quite similar to that of *L. vincta* and *T. cancellata* upon hatching; however, development of the ventral module and the metamorphic transformation were found to be quite derived. In *Euspira lewisii* (Naticoidea) and *Marsenina stearnsii* (Cypraeoidea), a simple ciliated esophagus was present in newly hatched larvae (dorsal module), which later gave rise to a hollow ventral outpocketing, which would form the future buccal cavity, salivary glands and radular sac (ventral module)(Page and Pedersen 1998, Page 2000, 2002). However, these two predatory species differed from the above-mentioned herbivorous caenogastropods, as the outpocketing bifurcated into two blind-ending projections (anterior and posterior) that remained separate from the overlying larval esophagus, with the exception of a narrow connection at the posterior end of the buccal cavity (the original outpocketing point)(Page and Pedersen 1998, Page 2000, 2002). At metamorphic competence, *E. lewisii* developed a pair of jaws at the anterior-end of the anterior projection of the buccal cavity (Page and

Pedersen 1998, Page 2000). During metamorphosis, the entire larval esophagus anterior to the modular connection point was destroyed, and the larval mouth was sealed shut by bordering epithelium (Page and Pedersen 1998, Page 2000). The jaws positioned at the anterior of the buccal cavity were then able to protrude from a new definitive mouth in the anterior body wall, ventral to the original larval mouth (Page and Pedersen 1998, Page 2000). This differed from *M. stearnsii,* where the larval mouth was retained (although it was remodeled); however, the larval esophagus anterior to the modular connection point was destroyed (Page 2002). In both species, feeding was reported within 3-6 days of metamorphic loss of the velar lobes, therefore the transition from herbivorous feeding to carnivorous predation was able to take place over a very short period of time (Page and Pedersen 1998, Page 2000, 2002).

In the neogastropod *Nassarius mendicus* (Buccinoidea; Nassariidae), foregut development was even more elaborate (Page 2000, 2005). The foregut of hatching neogastropod larvae was found to be like that of previously mentioned larvae: a simple, ciliated tube (dorsal module)(Page 2000, 2005). The future adult foregut then developed as a semi-isolated ventral outpocketing of the larval esophagus (ventral module), where the only connection was the narrow, original outpocketing point at the posterior of the buccal cavity (Page 2000, 2005). While the posterior projection gave rise to the radular sac, the anterior projection not only gave rise to the buccal cavity, but also the whole anterior esophagus of the post-metamorphic foregut (Page 2000, 2005). It was found that the proximal neck of the bifurcated outpocketing (at the posterior of the future buccal cavity) elongated to a great extent and was destined to become the future anterior esophagus (Page 2000, 2005). At the posterior of this anterior esophagus, a valve of Leiblein had formed, where the original outpocketing had connected to the larval esophagus (Page 2000, 2005). Once metamorphosis was initiated, the larval mouth was paved over with epithelial cells, and the whole of the larval esophagus anterior to the valve of Leiblein was destroyed (Page 2000, 2005). Following this, the buccal cavity ruptured through the anterior body wall, ventral to the original larval mouth, to create the definitive mouth (Page 2000, 2005).

Ultimately, in both herbivorous and predatory caenogastropods, the physical separation of the dorsal developmental module (larval esophagus) and the ventral

developmental module (post-metamorphic foregut) allowed for the co-occurrence of larval feeding and development of the post-metamorphic foregut. Although there were many similarities in initial development, the trajectories diverged after the initial outpocketing developed. In both *L. vincta* and *T. cancellata*, the anterior region of the ventral outpocketing was connected along its length to the larval esophagus, and the lumen was continuous. However, in the predatory caenogastropods, *E. lewisii*, *M. stearnsii*, and *N. mendicus*, both the anterior and posterior regions of the outpocketing were projections that had a high degree of isolation from the overlying larval esophagus, with the exception of a narrow connection at the original outpocketing point (the future valve of Leiblein). In addition to these differences, the fate of the larval esophagus also differed between the herbivores and predators. While in *L. vincta* and *T. cancellata* the distal larval esophagus underwent much cell loss, ultimately it was retained to form the dorsal food channel. In the predators *E. lewisii, M. stearnsii* and *N. mendicus*, the distal larval esophagus was entirely destroyed. Compared to the other predatory caenogastropods, the foregut of *N. mendicus* was especially derived, since an elongate anterior esophagus had also differentiated from the ventral module. Ultimately, these examples show that the organization of the foregut into modules may have enabled predators to evolve unconventional designs for the definitive foregut, free from larval constraints. However, it is not yet known whether foregut developmental modules are a widespread phenomenon within the Caenogastropoda, or even the Gastropoda.

Overall, little is known about how evolution occurs in biphasic lophotrochozoans without fatally disrupting development. While background studies have been conducted that show that there are dorsal and ventral modules in a few caenogastropod species (Table 1), further comparisons between less-derived herbivorous grazers and derived predatory gastropods are needed to discern whether foregut modularity is indeed a widespread phenomenon, and to gain further insight into the foregut morphogenesis process.

Table 1. Summary of studies that investigated foregut development in caenogastropod species.

Details of interest are included, such as whether the ventral module was found to be isolated from the larval esophagus, or connected along its anterior length, and whether the larval esophagus was entirely destroyed at metamorphosis.

Species and Superfamily	Life History	Adult Feeding Strategy	Outpocketing isolated or connected	Larval esophagus destroyed	Reference
Lacuna vincta, Littorinoidea	Indirect planktotrophic	Herbivorous grazing	Connected	No	Fretter, 1969; Page, 2000
Trichotropis cancellata, Capuloidea	Indirect planktotrophic	Ctenidial suspension feeder and kleptoparasite	Connected	No	Parries and Page, 2003
Euspira lewisii, Naticoidea	Indirect planktotrophic	Predator: bivalves	Isolated	Yes	Page and Pedersen, 1998; Page, 2000
Marsenina stearnsii, Lamellaroidea	Indirect planktotrophic	Predator: colonial ascidians	Isolated	Yes	Page, 2002
Nassarius mendicus, Buccinoidea	Indirect planktotrophic	Carnivore: scavenger	Isolated	Yes	Page, 2000, 2005
Conus lividus, Conoidea	Indirect planktotrophic	Predator	Isolated	Yes	Page, 2011
Nucella lamellosa, Muricoidea	Direct	Predator: bivalves, barnacles	Connected*	Yes	Hookham and Page, 2016
Nucella ostrina, Muricoidea	Direct adelphophagic	Predator: bivalves, barnacles	Connected	Yes	Hookham and Page, 2016

*Although the buccal cavity remained connected to the larval esophagus through much of larval development, just prior to metamorphosis, the larval esophagus and buccal cavity anterior to the valve of Leiblein were found to separate from each other to become two distinct tubes. After this the larval esophagus was destroyed.

1.5 Objective of present study

I have investigated foregut development in two caenogastropods: *Crepidula fornicata* (Linnaeus, 1758) and *Amphissa columbiana* Dall, 1916, in order to investigate possible evidence of foregut developmental modules within these species and if a modular organization could have facilitated a rapid morphological transition to the juvenile feeding system at metamorphosis. While *C. fornicata* is a herbivorous suspension feeder, *A. columbiana* is a predatory neogastropod that feeds using a highly derived proboscis. Overall, the comparison of these two species has the potential to reveal how development of foregut structures evolved to facilitate the transition from herbivory to predatory feeding within the Caenogastropoda, and to add to the body of knowledge that already exists on foregut development in gastropods.

The common slipper shell, *C. fornicata,* belongs to the superfamily Calyptraeoidea, and the family Calyptraeidae, and is a highly invasive caenogastropod that has spread from the east coast of North America to populate coastal bays of north-west USA, as well as European coastlines (McMillan 1938, Hoagland 1985, Blanchard 1997). Because of its availability, *C. fornicata* has quickly become a model species for molluscan developmental studies (Henry et al. 2006, 2010, Hejnol et al. 2007, Dean et al. 2009). Despite the large amount of embryological, developmental and genomic research on *C. fornicata,* information on foregut development is limited to a brief account by Werner (1955).

The herbivorous caenogastropod, *C. fornicata,* begins life as a planktotrophic larvae, but after metamorphosis utilizes the derived feeding method of suspension feeding on microalgae (Werner 1955), with the use of mucous nets arising from the gills (Orton 1912, Shumway et al. 2014). Adults of *C. fornicata* typically live in stacks consisting of two to six or more individuals that cling to each other using their adhesive foot (Werner 1953). Members of this species are protandrous hermaphrodites; the larger individuals within a stack are generally females and the smaller, more motile individuals are males (Coe 1953, Hoch and Cahill 2012). Fertilized eggs are deposited within thin-walled egg capsules, which are attached to the substrate in the area immediately beneath the opening into the mantle cavity of the sessile mother. After hatching, a short planktotrophic veliger

stage ensues, after which the individuals undergo metamorphosis to become suspension feeders (Werner 1955, Pechenik 1980). Therefore, larval foregut development might be similar to events seen in the herbivorous caenogastropods *L. vincta* (Page 2000) and *T. cancellata* (Parries and Page 2003), given that its derived feeding mode still utilizes particulate algal matter.

The wrinkled dove snail, *A. columbiana*, is an intertidal neogastropod that lives along the coastline of the Pacific North West of North America (Morris et al. 1980). This species belongs to the superfamily Buccinoidea, and the family Columbellidae. Members of the Buccinoidea are predators that feed with a highly extensible proboscis to prey on other gastropods, bivalves or polychaetes, or they may scavenge carrion (Taylor et al. 1980). Studies to date have found that buccinoidean post-metamorphic foreguts specifically feature an elongate anterior esophagus, and a valve of Leiblein of modest size (Fretter and Graham 1994, Kantor 1996, Simone 1996). *Amphissa columbiana* has a life history that begins with a feeding, planktotrophic larvae. As previously mentioned, metamorphosing larvae of the buccinoidean *N. mendicus* seal off the larval mouth and develop a new post-metamorphic mouth through which the new anterior foregut structures open (Page 2000, 2005). Due to the phylogenetic relationship between *A. columbiana* and *N. mendicus*, there is certainly a possibility that foregut development in *A. columbiana* resembles that of *N. mendicus*.

This study was conducted to determine if *C. fornicata* and *A. columbiana* show evidence of foregut modules and similar patterns of development to previously studied caenogastropod species. Specifically, the amount of isolation of the ventral esophageal outpocketing from the larval esophagus was examined, as well as the fate of the larval esophagus after metamorphosis. Due to the fact that evidence of modularity in foregut development has been found in at least five caenogastropod species studied to date, regardless of their feeding method and group within the clade Caenogastropoda, it can be hypothesized that a ventral module will be present in both species. In terms of how the ventral module will develop in relation to the larval esophagus, I would expect the anterior portion of the ventral module (the future buccal cavity) to be confluent with the larval esophagus in *C. fornicata*. However, I would expect the ventral module in *A. columbiana* to show a higher degree of isolation, where the two modules only connect at

the original outpocketing point. In addition, I would expect the distal larval esophagus to be retained through metamorphosis in a reduced form as the dorsal food channel in *C. fornicata*, but to be destroyed entirely in *A. columbiana*. These hypotheses are based on patterns of development that have been seen in herbivorous and carnivorous caenogastropods to date.

Overall, this study aimed to add to the available knowledge of foregut development in caenogastropods, and to help determine whether the patterns seen to date are prevalent throughout this clade. Additionally, the field of evolutionary developmental biology needs more model systems that will expose possible links between developmental modularity and evolvability. Specifically, my study of a neogastropod species that exhibits a derived ventral module will contribute to an understanding of how evolutionary change within and between developmental modules can explain differences in evolvability.

2.0 Materials and Methods

Developmental stages of both *Crepidula fornicata* and *Amphissa columbiana* were reared in the laboratory from embryo to post-metamorphic juveniles. Foregut development was investigated in both species with histological sectioning of multiple developmental stages, and sections were examined with a light microscope. Surface-rendered 3D reconstructions were generated for key developmental stages.

2.1 Specimen collection and culture

Crepidula fornicata

Adults of *C. fornicata* were hand-collected from the intertidal zone of Totten Inlet, Puget Sound, Washington, USA on July 7, 2017. Stacks of 3 to 8 adults were covered with damp kelp in plastic buckets and transported in coolers to the laboratory at the University of Victoria. Two to three adult stacks were placed in large glass jars or bowls with 4 L of coarse-filtered seawater collected from Ten Mile Point, Victoria, BC, an area of strong tidal mixing. Seawater in each bowl was continuously aerated by bubbling with air using an aquarium pump. The adults were kept at room temperature (20 °C) and the water was changed every 2 days. Within days of adult collection, larvae began to hatch and larval cultures were created.

Seawater for culturing larvae was collected twice weekly from Ten Mile Point and was stored at 12 °C in Nalgene carboys. This was coarse filtered under vacuum with a Pall Glass Fiber Filter (pore size 1 μm; Item # 61631) immediately prior to use. After hatching, larvae were reared in glass beakers, containing 500 mL of coarse filtered seawater, 50 μg/mL streptomycin (Sigma-Aldrich; Item # S6501) and 0.1 mM ethylenediaminotetraacetic acid, disodium salt (EDTA; ACP Item # E4320).

At the time of culture changes, the larvae were fed a mixture of the unicellular algae *Pavlova lutheri* (National Center for Marine Algae and Microbiota [NCMA]; Strain # CCMP1325) and *Isochrysis galbana* (NCMA; Strain# CCMP1323), at a density of 5 x 10^4 cells/mL, which was increased to 10^5 cells/mL after 6 days of culture. Algal cells were washed before they were added to the larval cultures by centrifuging aliquots of algal culture at approximately 1000 RPM for 10 minutes, discarding the supernatant, and

resuspending the algal pellet in coarse-filtered seawater. Density of algal cells was determined with a hemacytometer.

Young larvae were cultured at an initial density not exceeding one larva per 1 mL seawater, but this was gradually reduced by subdividing cultures and removing larvae until a density not exceeding one larva per 10 mL was reached by 50% completion of larval development. Larvae were transferred to fresh culture medium every 2 days by a combination of gentle sieving and hand pipetting. Larvae were cultured at 20 °C.

Once larvae of *C. fornicata* exhibited crawling behaviour, they were induced to metamorphose by exposure to 20 mM potassium chloride added to seawater. However, larvae that were older than 11 days post-hatching frequently underwent spontaneous metamorphosis without the addition of potassium chloride.

Amphissa columbiana

Adults of *A. columbiana* were hand-collected from the Ogden Point Breakwater, Victoria, BC, Canada, on October 18, 2016. The snails were found on the sides of large boulders in the intertidal zone at low tide. Once brought back to the laboratory at the University of Victoria, the adults were introduced to the re-circulating seawater system of the University of Victoria's Aquatics Facility, where they were maintained at 12 °C.

The collected *A. columbiana* were placed in four small aquaria, and were provided with flowing seawater and pebbles and small cobbles collected from the intertidal zone. From October 18, 2016 to January 13, 2017 the adults deposited egg capsules on the underside of the collected rocks. After oviposition, the rocks with egg capsules were removed from the aquaria and placed in a separate glass dish with flowing seawater, and were kept at 12 °C.

Near the completion of the embryonation period (approximately 41 days), the rocks with the encapsulated, fully developed embryos were placed in one glass beaker each with 500 mL of aerated seawater until hatching.

Larval culture for *A. columbiana* was as described above for *C. fornicata*, with a few exceptions. All cultures were maintained at 12 °C. They were fed a mixture of *Isochrysis galbana* and *Pavlova lutheri* at a concentration of 4×10^4 cells/mL.

Additionally, EDTA was not used in these cultures, as it had not yet been added to the protocol.

To induce metamorphosis by larvae of *A. columbiana*, larvae that were capable of crawling behaviour were placed in small bowls containing 100 mL seawater and small pebbles freshly collected from the rocky intertidal zone of Ogden Point. The pebbles were covered with an organic surface film and had attached spirorbid polychaetes and bryozoan colonies, along with assorted small polychaetes, nematodes, amphipods, and copepods. It was not known which component of the pebble substrate promoted metamorphosis of *A. columbiana*.

2.2 Preparation of specimens for histological sectioning

Multiple developmental stages of *C. fornicata* and *A. columbiana* were anesthetized and fixed following hatching.

The larval period to metamorphic competence of *C. fornicata* was approximately 11 days. Therefore, in order to capture key developmental stages, larvae were fixed at 8 different ages relative to the time of hatching (Table 2): newly hatched, 2 days post-hatching, 4 days post-hatching, 6 days post-hatching, 8 days post-hatching, 10 days post-hatching, metamorphic competence (minimum 11 days post-hatching) and post-metamorphic juveniles at 36 to 48 hours after loss of the velar lobes. The fixed ages relative to time of hatching were then organized into developmental stages (Table 2), which are explained in the Results. Metamorphic competency was defined as the point at which the larvae gained the ability to crawl with a fully formed propodium. These juveniles were confirmed to be capable of post-metamorphic feeding at the time of fixation, therefore I could be sure of full foregut differentiation.

The larval period of *A. columbiana* to the stage of crawling ability is approximately 45 days, which was considerably longer than that of *C. fornicata*. Therefore, the larvae were fixed at the following 8 time points relative to hatching (Table 3): newly hatched, 6 days post-hatching, 11 days post-hatching, 16 days post-hatching, 20 days post-hatching, 27 days post-hatching, metamorphic competence (minimum 45 days post-hatching), and 1-4 days after metamorphic loss of the ciliated velar lobes (young

juveniles). The fixed ages relative to time of hatching were later organized into developmental stages, which are explained in the Results (Table 3).

Table 2. Summary of the larval and juvenile ages of *Crepidula fornicata* that were fixed for histological sectioning.

Days post-hatching (DPH)	Assigned developmental stage	Number of individuals sectioned
Newly Hatched	Stage 1	2
2 DPH	Stage 1	2
4 DPH	Stage 1	2
6 DPH	Stage 2	2
8 DPH	Stage 2	2
10 DPH	Stage 3	2
Metamorphic competence (minimum 11 DPH)	Stage 3	2
Young juvenile (36-48 hours after velum loss)	Stage 4	2

Table 3. Summary of the larval and juvenile ages of *Amphissa columbiana* that were fixed for histological sectioning.

Days post-hatching (DPH)	Assigned developmental stage	Number of individuals sectioned
Newly Hatched	Stage 1	2
6 DPH	Stage 1	4
11 DPH	Stage 2	3
16 DPH	Stage 2	3
20 DPH	Stage 3	2
27 DPH	Stage 4	5
Metamorphic competence (minimum 45 DPH)	Stage 5	8
Young juvenile (1-4 days after velum loss)	Stage 6	4

The fixation protocol was the same for all fixed ages of both species, unless otherwise mentioned. Larvae to be fixed were placed in an 8 mL glass vial for processing. Larvae were anesthetized to prevent muscle contraction and withdrawal into the shell. Anesthesia was accomplished by gradually replacing seawater in the fixation vial with an artificial seawater solution containing high Mg^{2+} and low Ca^{2+} concentrations (Audesirk and Audesirk 1980). Every 15 to 20 minutes for a total of 3 hours, 2 to 4 mL of the seawater in the vial was removed and replaced with high Mg^{2+}/low Ca^{2+} seawater. During anesthesia, the vials with *A. columbiana* larvae were placed in a petri dish with scant ice to maintain the temperature at 12 °C, whereas the *C. fornicata* larvae were kept at 20 °C.

Once the larvae were sufficiently relaxed, a more robust anesthetic was used. The volume of fluid in each vial was reduced to 1 mL and three drops of chlorotone were added to the vial and swirled vigorously to mix the solution. This was repeated six times at 1.5 minute intervals with the solution in the vial maintained at just above 0 °C (more ice was added to the petri dish for developmental stages of both species). Afterwards, the anesthetizing solution was replaced with a glutaraldehyde primary fixative consisting of 2.5% glutaraldehyde, 0.2 M phosphate buffer (pH 7.6) and 0.14 M sodium chloride (Cloney and Florey 1968). The fixative was then removed and replaced with a second dose of the same 2.5% glutaraldehyde fixative. Specimens remained in the primary fixative for a minimum of 12 hours and a maximum of 7 days at 8 °C.

To decalcify the larval shells, the primary fixative was replaced with a 1:1 solution of 2.5% glutaraldehyde fixative and 10% EDTA. The decalcifying solution in the vial was occasionally replaced during a total decalcification period of 2 to 8 hours, depending on the size and thickness of the shells.

After the completion of decalcification, specimens were rinsed in 2.5% sodium bicarbonate ($NaHCO_3$) buffer (pH 7.2) three times for 15 minutes each at room temperature. The larvae were then post-fixed in a 1:1 solution of 2.5% $NaHCO_3$ and 4% osmium tetroxide (OsO_4) at room temperature. After an hour of post-fixation, the solution was removed and the larvae were briefly rinsed with distilled water.

Specimens were dehydrated with a graded acetone dilution series (30%, 50%, 70%, 90%, 95%, and 3 x 100%). The specimens were left in each acetone dilution for 20

minutes. Over a period of 24 hours, increasing concentrations of Embed 812 resin (an Epon 812 substitute; Electron Microscopy Sciences) diluted with 100% acetone was infiltrated into the tissues of the specimens. Finally, the specimens were embedded in the Embed 812 resin and put in an oven at 60 °C for 2 days to polymerize the resin.

2.3 Histological sectioning

Major stages of foregut development in *C. fornicata* and *A. columbiana* were identified by sectioning through the foregut of multiple ages in various orientations and viewing serial sections with a light microscope. Histological sections were cut at 1 µm thickness using glass knives or a DiATOME diamond histoknife on a Leica Ultracut UCT microtome. Sections were dried onto glass slides and the tissues were stained with methylene blue and azure II (Richardson et al. 1960). Glass cover slips were applied to the slides with Permount to protect the sections. Serial sections were photographed in their appropriate sequence using a Zeiss Axioskop compound light microscope with an attached Retiga 200T digital camera; the computer software used was QCapture Pro 5.1 (QImaging). Brightness, contrast and sharpness of images were adjusted with Adobe Photoshop CS6.

2.4 Surface-rendered 3D reconstructions of the foregut

Surface-rendered 3D reconstructions of the foregut at multiple ages through larval and juvenile development of both *C. fornicata* and *A. columbiana* were produced using Reconstruct (v. 1.1.00)(Fiala 2005). Images of serial sections through the foregut were ordered from anterior to posterior and imported into Reconstruct. These images were then size-calibrated to represent the appropriate thickness *i.e.* 1 µm. Every second section cut was used to produce the 3D reconstructions. Sections were aligned and specific structures of the foregut were manually traced using a graphics tablet. Important components of the foregut, *e.g.* the larval esophagus, anterior esophagus, buccal cavity, radular apparatus and salivary glands, were traced in separate profiles and are shown in different colours. Stacks of each profile were reconstructed and fit together to represent the 3D morphology of the foregut. The reconstructed profiles were surface rendered using a Boissant

surfacing algorithm. Images of the 3D reconstructions at various orientations were taken and imported into Adobe Photoshop CS6 for minor surface smoothing.

3.0 Results

3.1 *Crepidula fornicata*: overview of larval stage

Veliger larvae of *C. fornicata* developed over a period of approximately 11 days under laboratory culture at 20 °C before crawling behaviour was observed. Throughout larval development, the larvae used ciliated velar lobes to swim within the water column and to feed. Two bands of cilia along the periphery of each velar lobe allowed the capture and consumption of the microalgae that fueled larval growth and development.

Examination of young larvae that were pipetted out of larval culture, mounted on a glass slide and viewed through a compound light microscope revealed that the mouth led to the larval esophagus, a simple ciliated tube that conducted ingested microalgae to the stomach. The stomach was regionally differentiated into gastric shield and style sac regions and was connected to a large left and small right digestive gland, as is typical for planktotrophic larvae of other species of caenogastropods (Werner 1955, Fretter and Montgomery 1968). An intestine led from the stomach to the anus, which opened into the dorso-lateral right side of the mantle cavity.

Once larvae of *C. fornicata* exhibited crawling behaviour, they could be induced to metamorphose. During metamorphosis, the velar lobes were destroyed; a process that began with the sloughing of the large velar ciliated cells.

Two to three days after metamorphic loss of the velar lobes, the young juveniles of *C. fornicata* began capturing and ingesting microalgae using the elongate ctenidial filaments and radular apparatus. These had differentiated to an advanced stage in larvae, prior to actual metamorphic loss of the velar lobes.

3.2 *Amphissa columbiana*: overview of larval stage

Veliger larvae of *A. columbiana* developed over a period of approximately 45 days under laboratory culture at 12 °C before crawling behaviour was observed. Much like larvae of *C. fornicata*, those of *A. columbiana* used ciliated velar lobes to swim within the water column and to feed on microalgae.

The basic features of the digestive tract of young larvae of *A. columbiana*, as seen during microscopic observation of live specimens, were similar to those of young larvae of *C. fornicata*.

Once larvae of *A. columbiana* exhibited crawling behaviour, they could be induced to metamorphose. Metamorphosis could be recognized externally by loss of the ciliated velar lobes. Once metamorphosis was completed, the adult feeding apparatus was highly differentiated; however, carnivorous predation was not yet observed in the juveniles that were fixed (1-4 days after loss of the velar lobes).

3.3 Foregut development in *Crepidula fornicata*

Although development is continuous, my description of foregut development in *C. fornicata* organizes the process into four stages (Figure 5). These stages are based on study of histological sections of specimens fixed at eight sequential time points between larval hatching and young juveniles at 36-48 hours after metamorphosis (Table 2). At stage 1, sections showed that hatching veligers had a simple, ciliated esophagus; however, a ventral hollow outpocketing (the anlage of the buccal mass and radular apparatus) was already present and had begun to bifurcate anteriorly and posteriorly. At stage 2, the ventral outpocketing began to differentiate and proliferate anteriorly and posteriorly to produce the future buccal cavity and future radular sac, respectively. At stage 3, when individuals were competent or nearly competent to metamorphose, all components of the future juvenile foregut had almost fully differentiated, and were located ventral to the larval esophagus. Finally, at stage 4, sections of young juveniles after metamorphosis showed that the mouth led directly into the buccal cavity and the larval esophagus had undergone much cell-loss distally, resulting in a very narrow channel (dorsal food channel) to be formed in the mid-dorsal wall of the buccal cavity. These stages are described in detail below.

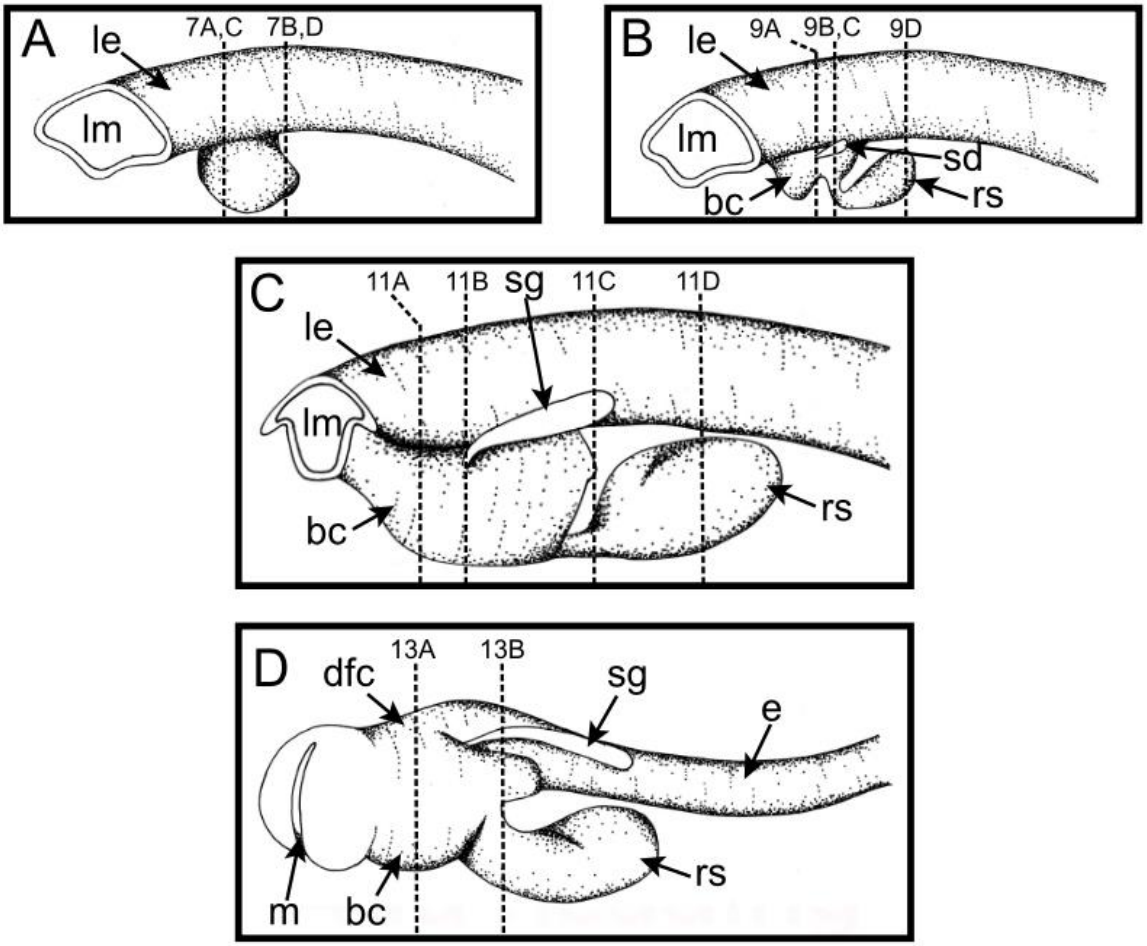

Figure 5. Schematic summarizing four stages (**A-D**) of distal foregut morphogenesis in *Crepidula fornicata.*

Radular cartilages and musculature not shown. Dashed vertical lines indicate locations of cross sections presented in the following figures; associated figure numbers are provided for reference. **A.** Stage 1: Distal foregut with initial outpocketing from the ventral wall in hatching larvae. **B.** Stage 2: Regional differentiation and proliferation of the original outpocketing **C.** Stage 3: Metamorphically competent larva. **D.** Stage 4: Post-metamorphic juvenile. Abbreviations: bc= buccal cavity, dfc=dorsal food channel, e= esophagus, le= larval esophagus, lm= larval mouth, m= mouth, rs= radular sac, sd= salivary duct, sg= salivary gland.

3.3.1 Stage 1: Outpocketing and bifurcation of future post-metamorphic foregut

Stage 1 of distal foregut development was characterized by the bifurcation of the ventral outpocketing of the distal larval esophagus (Figures 6 and 7).

The foregut of hatching *C. fornicata* larvae consisted of an esophagus with a ciliated epithelium that was narrowed mid-ventrally. The lumen of the esophagus was small and collapsed (Figure 7A and B). A ventral hollow outpocketing (the anlage of the future buccal cavity and radular apparatus) of the distal larval esophagus was evident upon hatching, located at the level of the statocysts (Figure 6). Based on the apicobasal polarity of the foregut epithelium, this outpocketing is actually an epithelial invagination. The ventral outpocketing was distinct from the epithelial cells of the larval esophagus because it consisted of columnar cells that gave rise to apical microvilli, but not cilia (Figure 7A). The anlage of the future radular sac extended slightly as a posterior projection from the outpocketing, separate from the overlying larval esophagus (Figures 6 and 7B). The radular rudiment terminated at the posterior-most point of the statocysts (Figure 6).

As stage 1 progressed, the outpocketing had begun to form an anterior growth that was connected along its length to the larval esophagus; the lumen was continuous with the lumen of the larval esophagus (Figure 7C). However, evidence of cell differentiation was not yet visible. The radular sac had extended further posteriorly, terminating at the posterior-most part of the statocysts; the statocysts were also located more posterior than previous. At 2 days post-hatching (20% completion of larval development), the radula was beginning to form radular teeth (Figure 7D). Little change in development was seen from mid- to late stage 1.

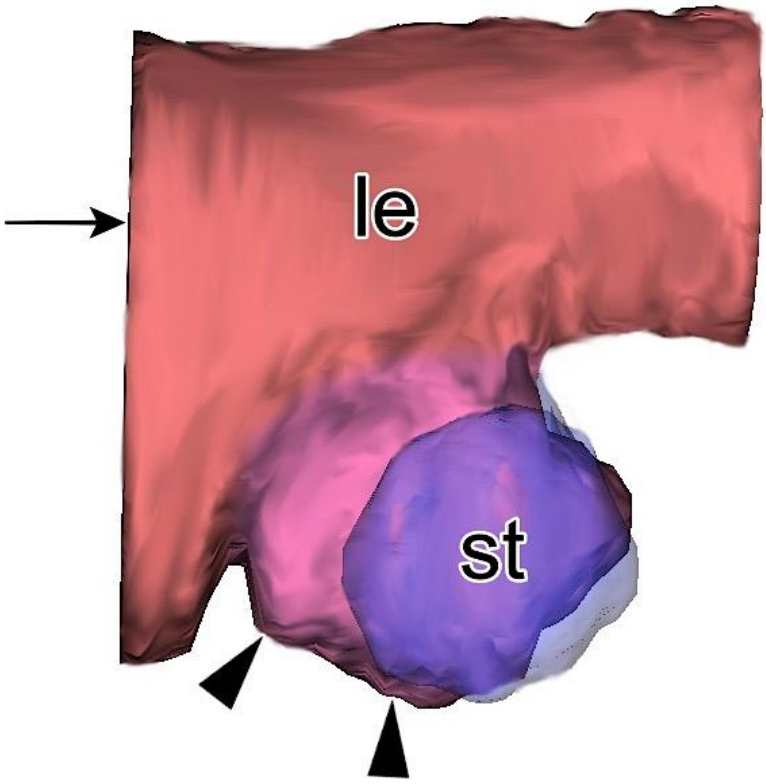

Figure 6. Surface-rendered 3D reconstruction of the foregut of *Crepidula fornicata* during stage 1 (newly hatched larva) in left lateral view.

The anlage of the buccal cavity and radular apparatus was present upon hatching as a ventral outpocketing of the distal larval esophagus (arrowheads). Arrow indicates the location of the larval mouth. Abbreviations: le= larval esophagus, st= statocyst.

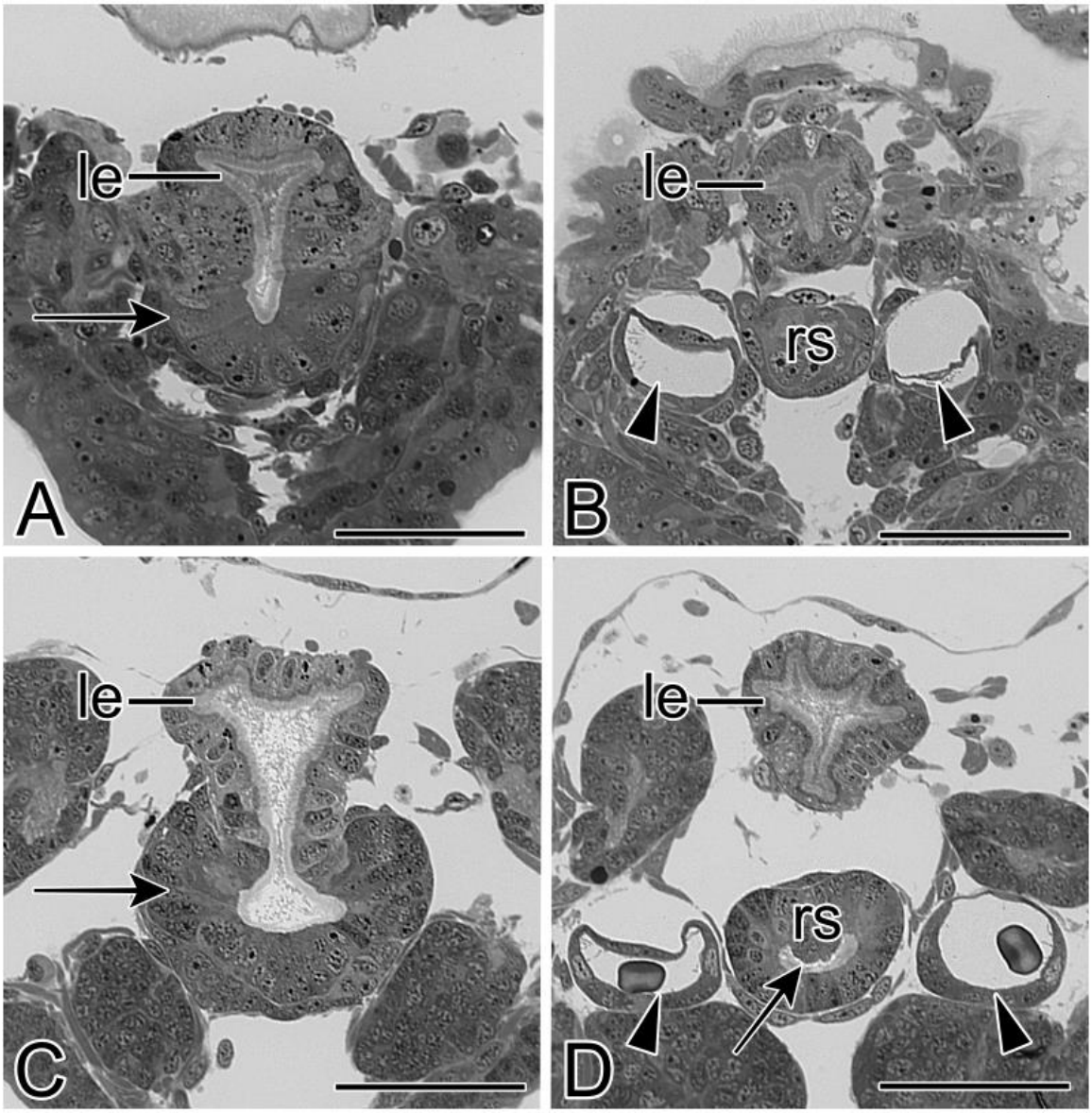

Figure 7. Histological transverse sections through the distal foregut of *Crepidula fornicata* during stage 1.

Scale bars= 50 μm. **A.** Onset of stage 1 (newly hatched larva) showing the ciliated larval esophagus with a ventral outpocketing of non-ciliated epithelium, marking the anlage of the buccal cavity and radular sac (arrow). **B.** Onset of stage 1 (newly hatched larva) showing the posterior projection of the outpocketing separate from the overlying larval esophagus, located at the level of the statocysts (arrowheads). **C.** Mid-stage 1 (2 days post-hatching larva) showing the widened ventral outpocketing of the distal larval esophagus (arrow). **D.** Mid stage 1 (2 days post-hatching larva) showing the developing

radula and radular teeth (arrow) within the radular sac, located at the level of the statocysts (arrowheads). Abbreviations: le= larval esophagus, rs= radular sac.

3.3.2 Stage 2: Regional differentiation and proliferation of post-metamorphic foregut

Stage 2 was characterized by the differentiation and proliferation of the anterior and posterior regions of the ventral outpocketing.

During stage 2, larvae fixed at 6 and 8 days post-hatching (55% and 72% completion of larval development, respectively) showed anterior proliferation of the buccal cavity to just posterior of the larval mouth (Figure 8). The length of the lumen of the buccal cavity remained continuous with the lumen of the larval esophagus, although it was flattened (Figure 9A). The buccal cavity received ducts of the salivary glands (Figure 9A); however they led to blind endings where the salivary glands had not yet developed (Figures 8, 9B and 9C). The radula that had begun developing at the posterior of the radular sac had now developed further anteriorly within the radular sac, almost extending up into the buccal cavity (Figure 9B and C). The radular teeth were numerous (Figure 9B and C) and a pair of radular cartilages were developing on either side of the anterior radula (Figure 9A,B and C). The radular sac extended posteriorly to the level of the statocysts (Figures 8 and 9D); however, the statocysts in stage 2 were much more posterior than in stage 1 (compare Figure 6 to Figure 8). Therefore, the radula had indeed undergone posterior growth.

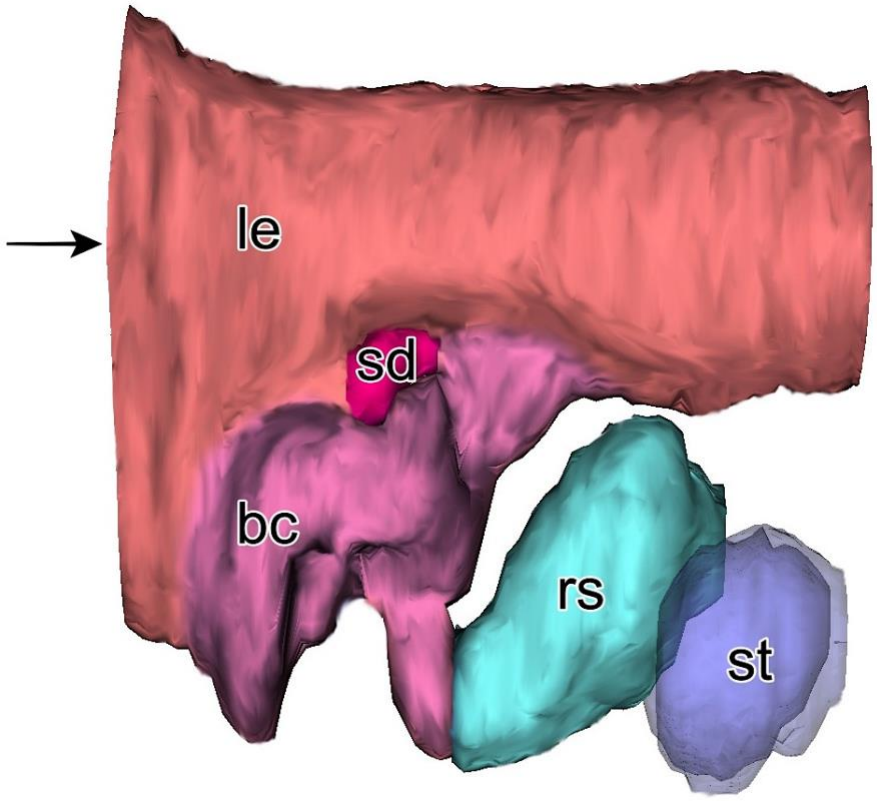

Figure 8. Surface-rendered 3D reconstruction of the distal foregut of *Crepidula fornicata* during early stage 2 (6 days post-hatching larva) in left lateral view.
The ventral outpocketing has experienced further growth and differentiation. The buccal cavity has proliferated further anteriorly, while the radular sac has extended posteriorly, terminating at the level of the statocysts. Salivary ducts connect to the buccal cavity; however they have blind-endings. Arrow indicates the location of the larval mouth. Abbreviations: bc= buccal cavity, le= larval esophagus, rs= radular sac, sd= salivary duct, st= statocyst.

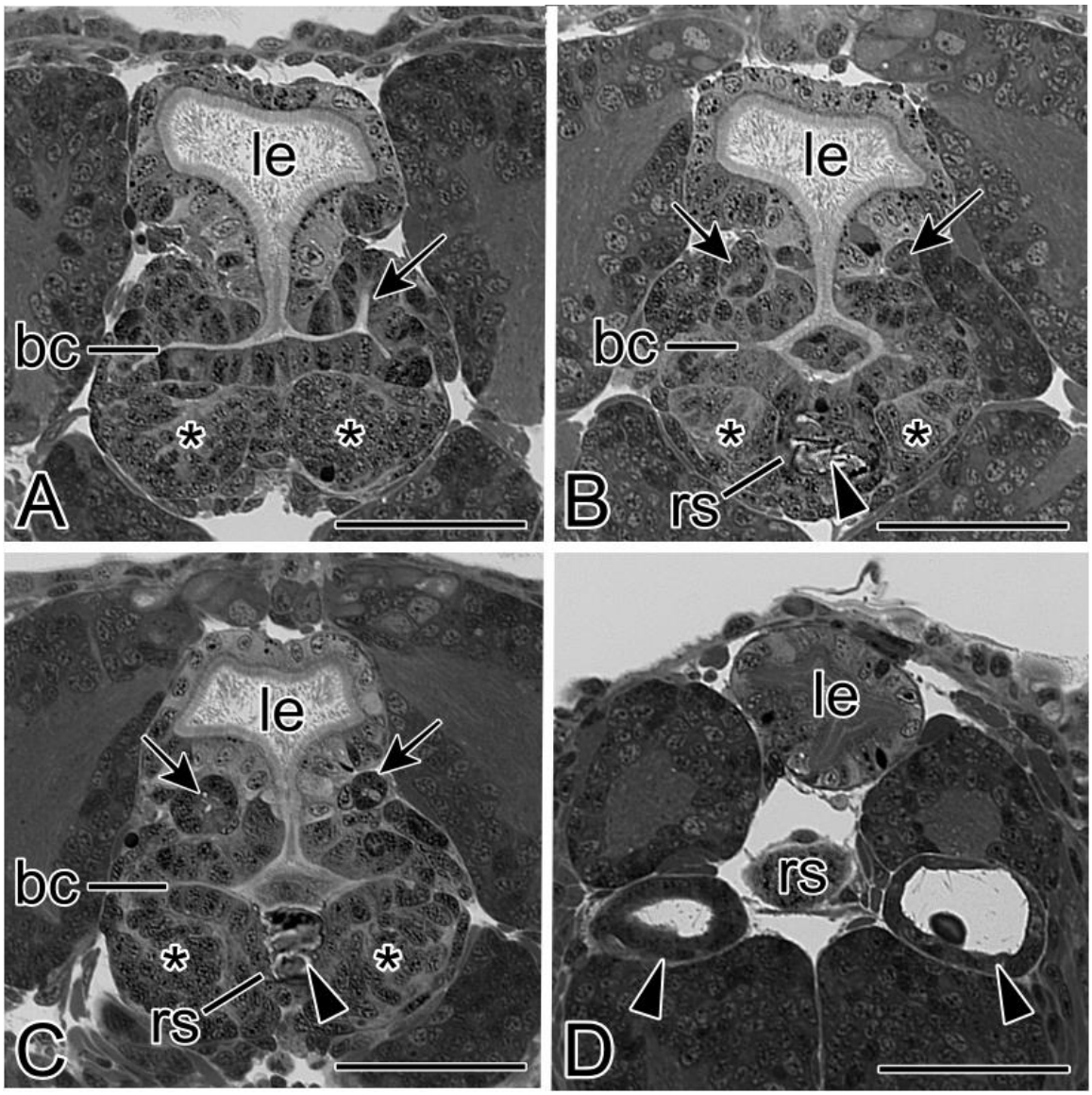

Figure 9. Histological transverse sections through the distal foregut of *Crepidula fornicata* during stage 2.

Scale bars= 50 μm. **A.** Onset of stage 2 (6 days post-hatching larva) showing the ciliated lumen of the larval esophagus connected to the flattened lumen of the buccal cavity, with the left salivary duct opening into the buccal cavity (arrow); note radular cartilages within the ventral portion of the outpocketing (asterisks). **B.** Onset of stage 2 (6 days post-hatching larva) showing the larval esophagus, buccal cavity and blind-endings of the salivary ducts (arrows); radular teeth (arrowhead) are evident within the radular sac, flanked by radular cartilages (asterisks). **C.** Late stage 2 (8 days post-hatching larva) showing the ciliated larval esophagus connected to the buccal cavity, with associated

salivary ducts; note radular cartilages (asterisks) and radular teeth (arrowhead). **D.** Late stage 2 (8 days post-hatching larva) showing the posterior projection of the radular sac, which terminates at the level of the statocysts (arrowheads). Abbreviations: ae= anterior esophagus, bc= buccal cavity, le= larval esophagus, rs= radular sac.

3.3.3 Stage 3: Elongation and further differentiation leading to a competent larva

Stage 3 marked the onset of metamorphic competence (minimum 10-11 days post-hatching). Larvae that were 10 days post-hatching or 11 days post-hatching showed a similar level of differentiation; however the post-metamorphic foregut of larvae that were 11 days post-hatching showed that marked elongation had occurred in the span of one day.

At metamorphic competence, the distal foregut had reached an advanced level of differentiation (Figure 10). The buccal cavity now extended anteriorly to the level of the larval mouth. The larval mouth itself had lost some of its cells and had become smaller. The lumen of the buccal cavity remained continuous with the larval esophagus along its length (Figures 10, 11A and B). The buccal cavity received ducts from the small salivary glands that had begun to accumulate secretory granules (Figure 11B and C). The ribbon of radular teeth had elongated to extend dorsally from the radular sac onto the floor of the buccal cavity (Figure 11A and B). The radular teeth were extremely well-developed along the length of the radula (Figure 11). The radular cartilages were enlarged anteriorly, but only extended along the anterior-most half of the radular sac (Figure 11B and C). The radular sac had proliferated further posteriorly (Figure 11D); however it no longer reached the statocysts, which appeared further posteriorly than in earlier larval stages (compare Figures 8 and 10).

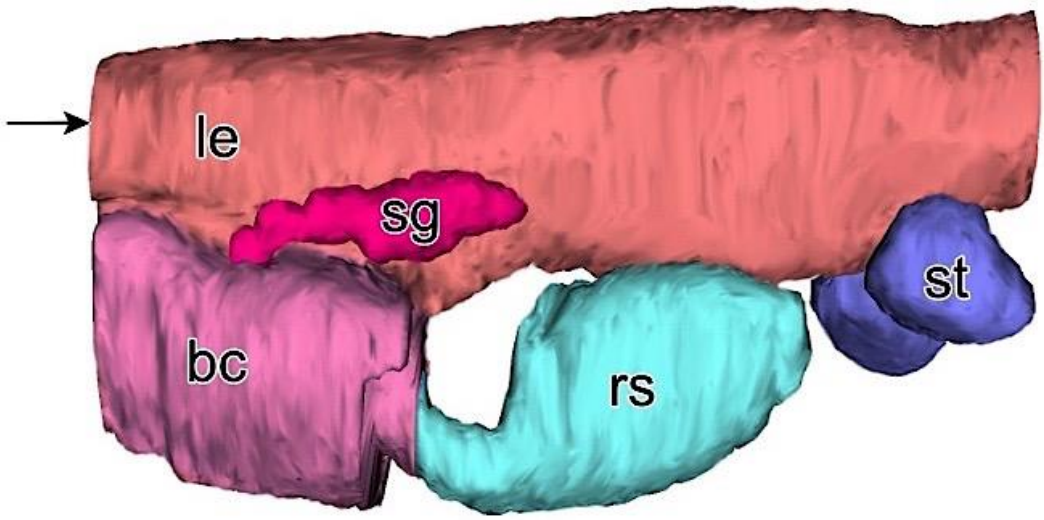

Figure 10. Surface-rendered 3D reconstruction of the distal foregut of *Crepidula fornicata* during stage 3 (metamorphic competence) in left lateral view.
All components of the post-metamorphic foregut have differentiated beneath the larval esophagus; salivary ducts lead from the buccal cavity to small salivary glands. Note the radular sac no longer reaches the statocysts, which appear further posteriorly than in earlier stages. Arrow indicates the location of the larval mouth. Abbreviations: bc= buccal cavity, le= larval esophagus, rs= radular sac, sg= salivary gland, st= statocyst.

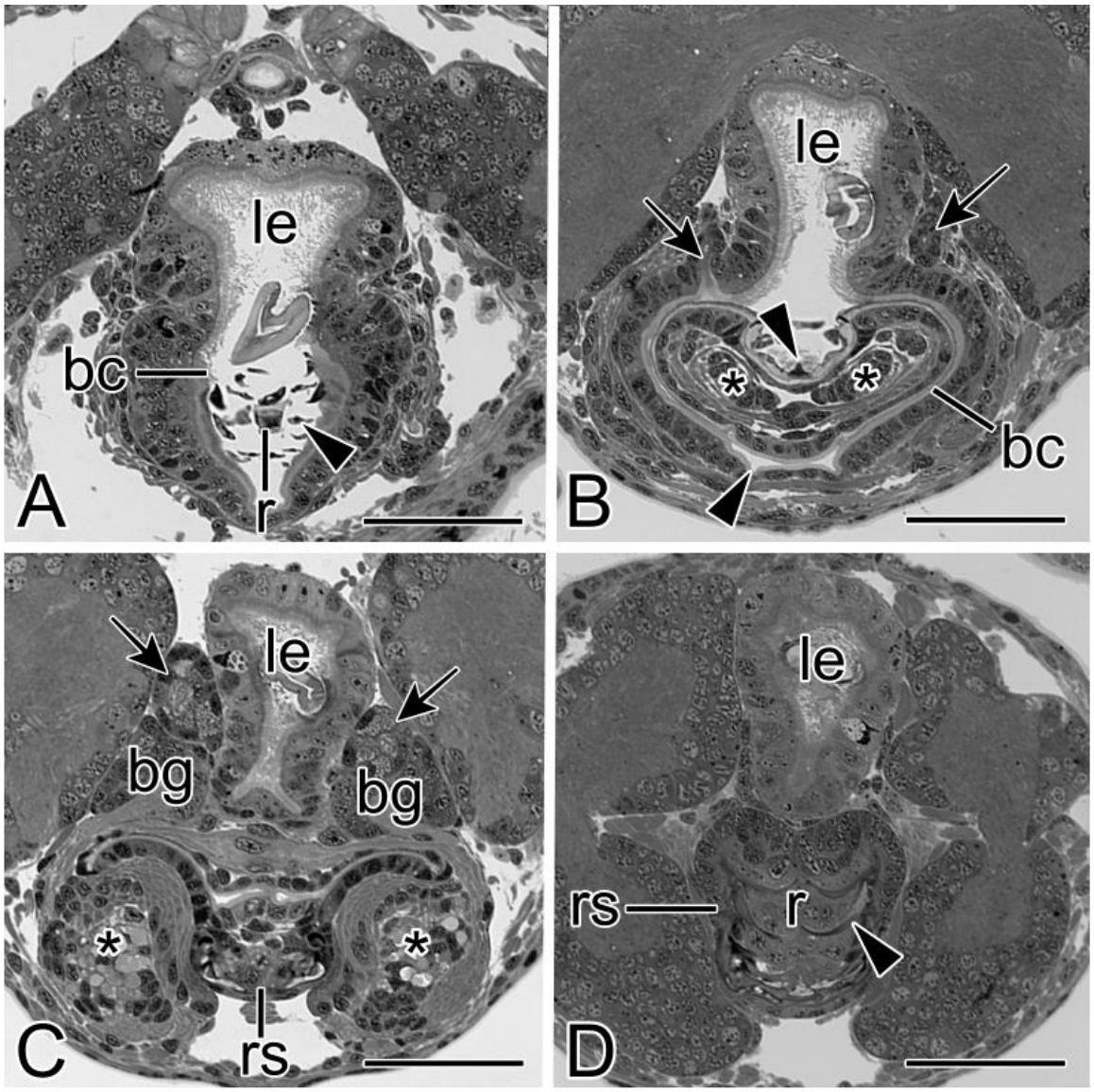

Figure 11. Histological transverse sections through progressively more posterior levels of the distal foregut of *Crepidula fornicata* during stage 3 (metamorphic competence). Scale bars= 50 μm. **A.** Section through the anterior extremity of the larval esophagus with the ventrally connected post-metamorphic buccal cavity, containing the tip of the radula with radular teeth (arrowhead). **B.** More posterior section of the larval esophagus and buccal cavity, where the right salivary duct can be seen connecting to the buccal cavity, whereas the left salivary duct is shown posterior to the connection to the buccal cavity (arrows); note the obvious dorsal and ventral teeth of the radula(arrowheads), and the radular cartilages (asterisks). **C.** Section at the level of the buccal ganglia, posterior to the buccal cavity; note the developed salivary glands flanking the larval esophagus

(arrows) and the radula sitting within the radular sac, with enlarged radular cartilages (asterisks). **D.** Section of the posterior larval esophagus and radula with secreted radular teeth (arrowhead). Abbreviations: ae= anterior esophagus, bc= buccal cavity, bg= buccal ganglion, le= larval esophagus, r= radula, rs= radular sac.

3.3.4 Stage 4: Post-metamorphosis

At 36-48 hours after metamorphic loss of the velar lobes, changes had occurred to the pre-metamorphic foregut. The mouth connected immediately to the buccal cavity (Figure 12). The larval esophagus had undergone much cell-loss distally, but had not been completely destroyed, resulting in a very narrow channel to be formed in the mid-dorsal wall of the buccal cavity, known as the dorsal food channel (Figure 13A). The dorsal food channel led posteriorly into the esophagus. The salivary ducts opened into the buccal cavity, and connected to small salivary glands, posteriorly (Figures 12 and 13B). The secreted ribbon of radular teeth extended up from the radular sac into the buccal cavity, flanked by well-developed radular cartilages (Figure 13A). A relatively short radular sac extended from the posterior of the buccal cavity (Figure 12). The statocysts resided far posterior to the distal foregut, in extreme contrast to the previous larval stages (compare Figures 6, 8, 10, 12).

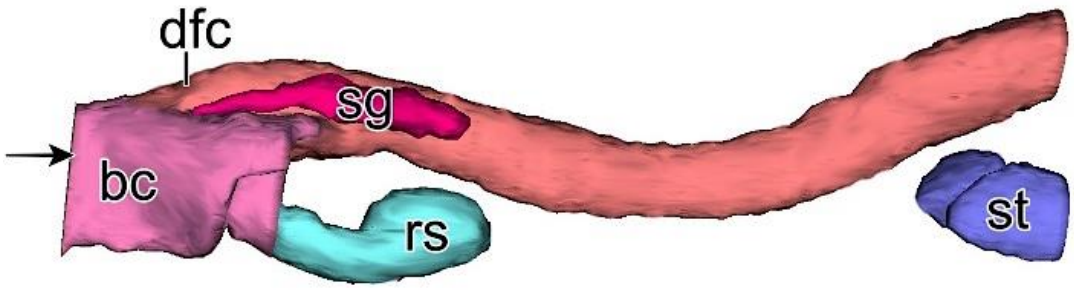

Figure 12. Surface-rendered 3D reconstruction of the distal foregut of a post-metamorphic juvenile of *Crepidula fornicata* in left lateral view. Rearrangements have occurred in the distal foregut including the buccal cavity forming the whole mouth and the distal larval esophagus losing most of its cells to form the dorsal food channel. The statocysts appear very posteriorly in the juvenile, but are still associated with the head. Arrow indicates the location of the mouth. Abbreviations: bc= buccal cavity, dfc= dorsal food channel, rs= radular sac, sg= salivary gland, st= statocyst.

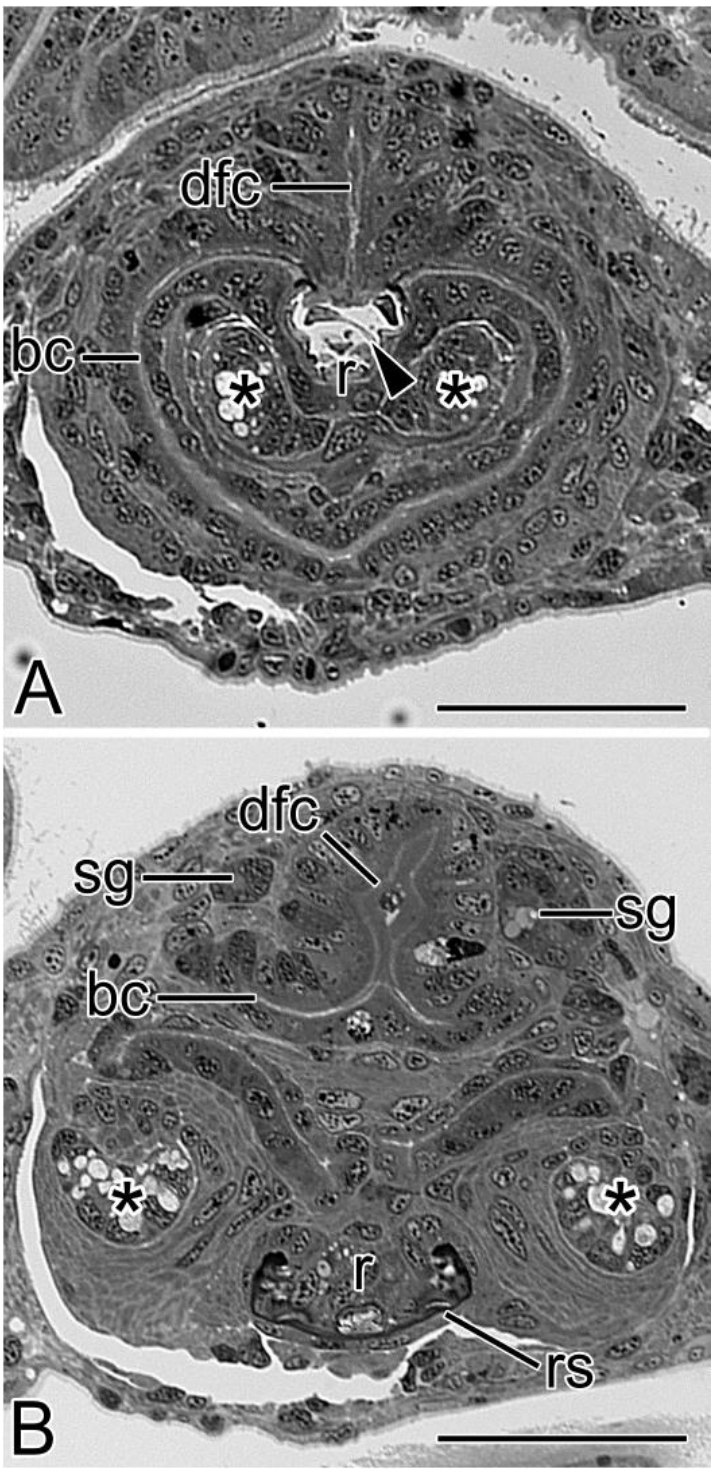

Figure 13. Histological transverse sections through the foregut of *Crepidula fornicata* at 36-48 hours after metamorphic loss of the velar lobes.

Scale bars= 50 μm. **A.** Anterior definitive foregut showing the newly formed dorsal food channel connected to the buccal cavity, into which the radula with radular teeth

(arrowhead) extends, flanked by radular cartilages (asterisks). **B.** More posterior section showing the dorsal food channel and buccal cavity remaining continuous, while the radula is shown within the radular sac, ventrally, flanked by radular cartilages (asterisks). Abbreviations: bc= buccal cavity, dfc= dorsal food channel, r= radula, rs= radular sac, sg= salivary gland.

3.4 Foregut development in *Amphissa columbiana*

In order to illustrate the major developmental events of the foregut during the larval and metamorphic period of *A. columbiana*, 6 stages were chosen (Figure 14). These stages are based on study of histological sections of specimens fixed at eight sequential time points between larval hatching and young juveniles at 1-4 days after metamorphosis (Table 3). At stage 1, sections showed that veligers had a simple, ciliated esophagus that led from the mouth to the stomach, although cells of the ventral wall of the larval esophagus were enlarged. At stage 2, the ventral wall of the larval esophagus developed into a ventral hollow outpocketing. At stage 3, the ventral outpocketing began to bifurcate into anterior and posterior regions, which demarcated the future buccal cavity and anterior esophagus projecting anteriorly, and the future radular sac projecting posteriorly. At stage 4, much differentiation had occurred and the future buccal cavity and anterior esophagus of the juvenile continued to grow anteriorly, while the radular sac experienced much growth posteriorly. Notably, the lumen of the anterior projection was not confluent with the overlying larval esophagus. At stage 5, when individuals were competent or nearly competent to metamorphose, all components of the future juvenile foregut, except the definitive mouth, had almost fully differentiated and these coexisted with the ciliated larval esophagus. Finally, at stage 6, sections of young juveniles after metamorphosis showed radical foregut rearrangements that accommodated the formation of an extensible proboscis but eliminated the distal larval esophagus. These stages are described in detail below.

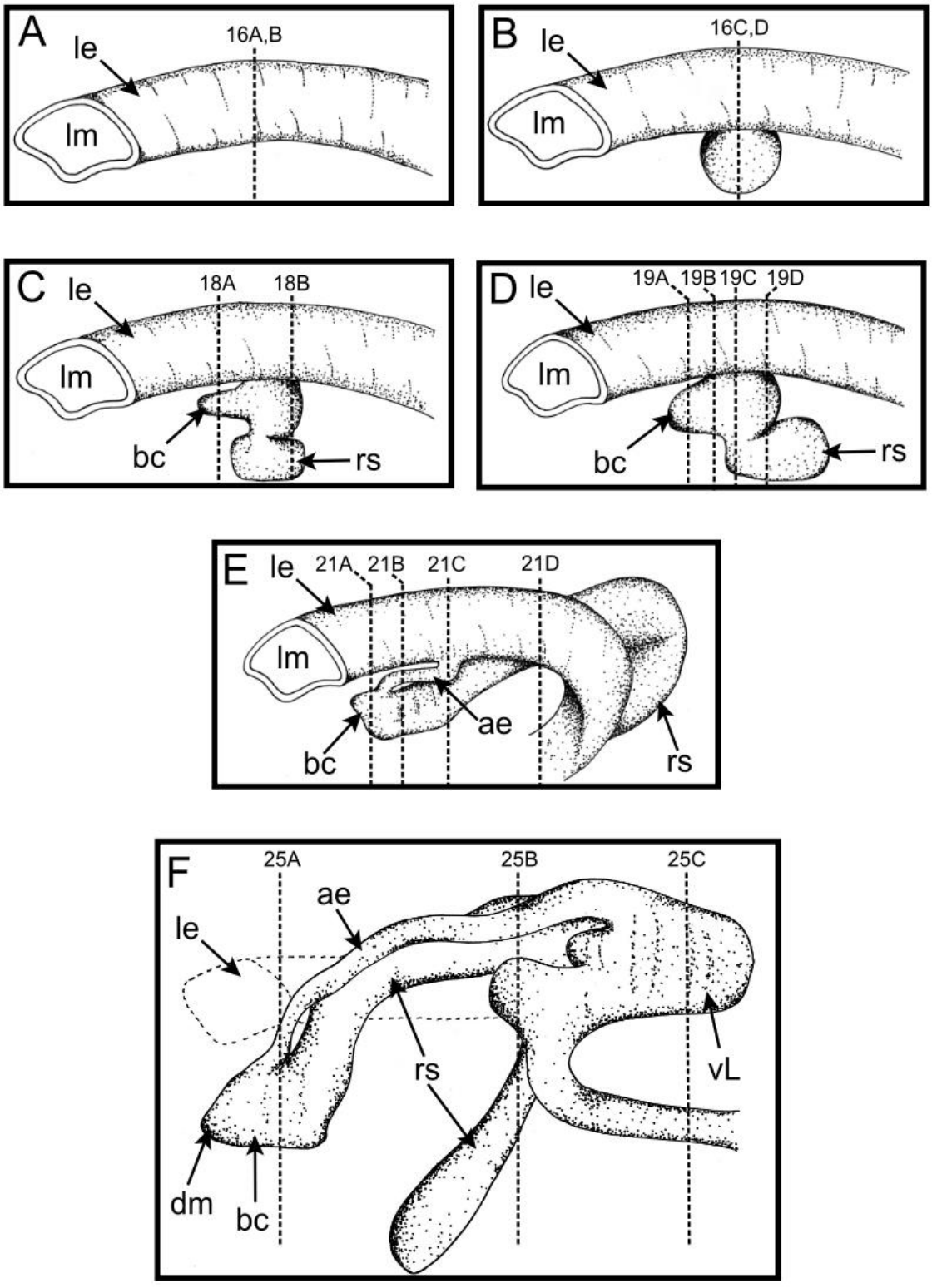

Figure 14. Schematic summarizing six stages (**A**-**F**) of distal foregut morphogenesis in *Amphissa columbiana.*

Radular cartilages and musculature not shown. Salivary ducts and glands have been removed from view, so as not to obstruct other structures. Dashed vertical lines indicate locations of cross sections presented in the following figures; associated figure numbers are provided for reference.

A. Stage 1: Distal foregut in hatching larva consisting of larval mouth and esophagus only. **B.** Stage 2: Initial outpocketing from the ventral wall of the larval esophagus. **C.** Stage 3: Bifurcation of the outpocketing. **D.** Stage 4: Elongation and further differentiation of the outpocketing. **E.** Stage 5: Metamorphically competent larva. **F.** Stage 6: Post-metamorphic juvenile; dashed larval esophagus indicates that it has been destroyed; note new definitive mouth opening. Abbreviations: ae= anterior esophagus, bc= buccal cavity, dm= definitive mouth, le= larval esophagus, lm= larval mouth, rs= radular sac, vL= valve of Leiblein.

3.4.1 Stage 1: Larval esophagus in young larvae

The foregut of hatching larvae of *A. columbiana* consisted of an esophagus that was a simple, cylindrical tube (Figure 15) of ciliated epithelium (Figure 16A), which characterized stage 1. The cells that subsequently proliferated to give rise to the ventral outpocketing (the anlage of the definitive adult foregut) could be seen as a slight thickening of the ventral esophageal wall, at the level of the statocysts. Within a week of hatching, this area of the larval esophagus had become narrowed mid-ventrally (Figure 16B).

3.4.2 Stage 2: Development of ventral outpocketing

Stage 2 of distal foregut development was characterized by the appearance of a ventral outpocketing of the distal larval esophagus (Figure 16C), which was first seen in larvae fixed at 11 days post-hatching (25% completion of larval development). This outpocketing is actually an epithelial invagination, based on the apicobasal polarity of the foregut epithelium. The wall of the ventral outpocketing consisted of columnar cells that gave rise to apical microvilli but not cilia, making them distinct from the epithelial cells of the larval esophagus (Figure 16C). The connection between the ventral outpocketing and the larval esophagus was very narrow, although the outpocketing itself bulged laterally. As stage 2 progressed, the lumen of the ventral outpocketing of the distal larval esophagus widened; however, evidence of cell differentiation was not yet visible (Figure 16D).

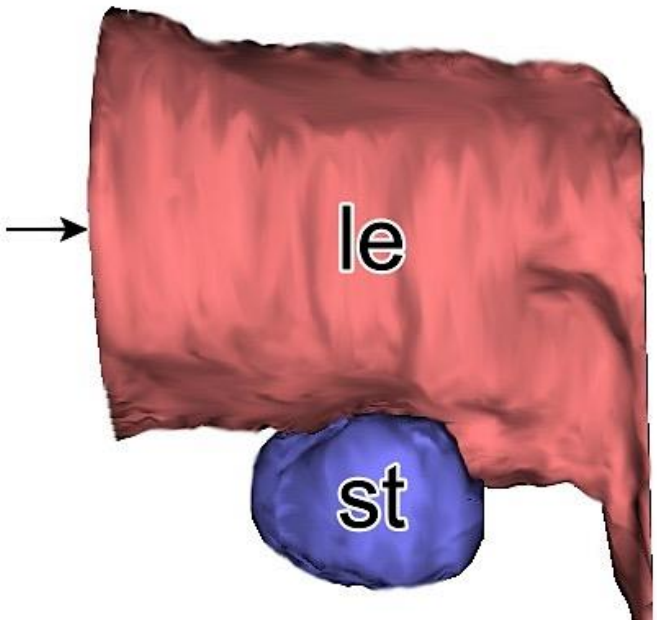

Figure 15. Surface-rendered 3D reconstruction of the simple foregut of *Amphissa columbiana* during stage 1 (newly hatched larva) in left lateral view.
Arrow indicates the location of the larval mouth. Abbreviations: le= larval esophagus, st= statocyst.

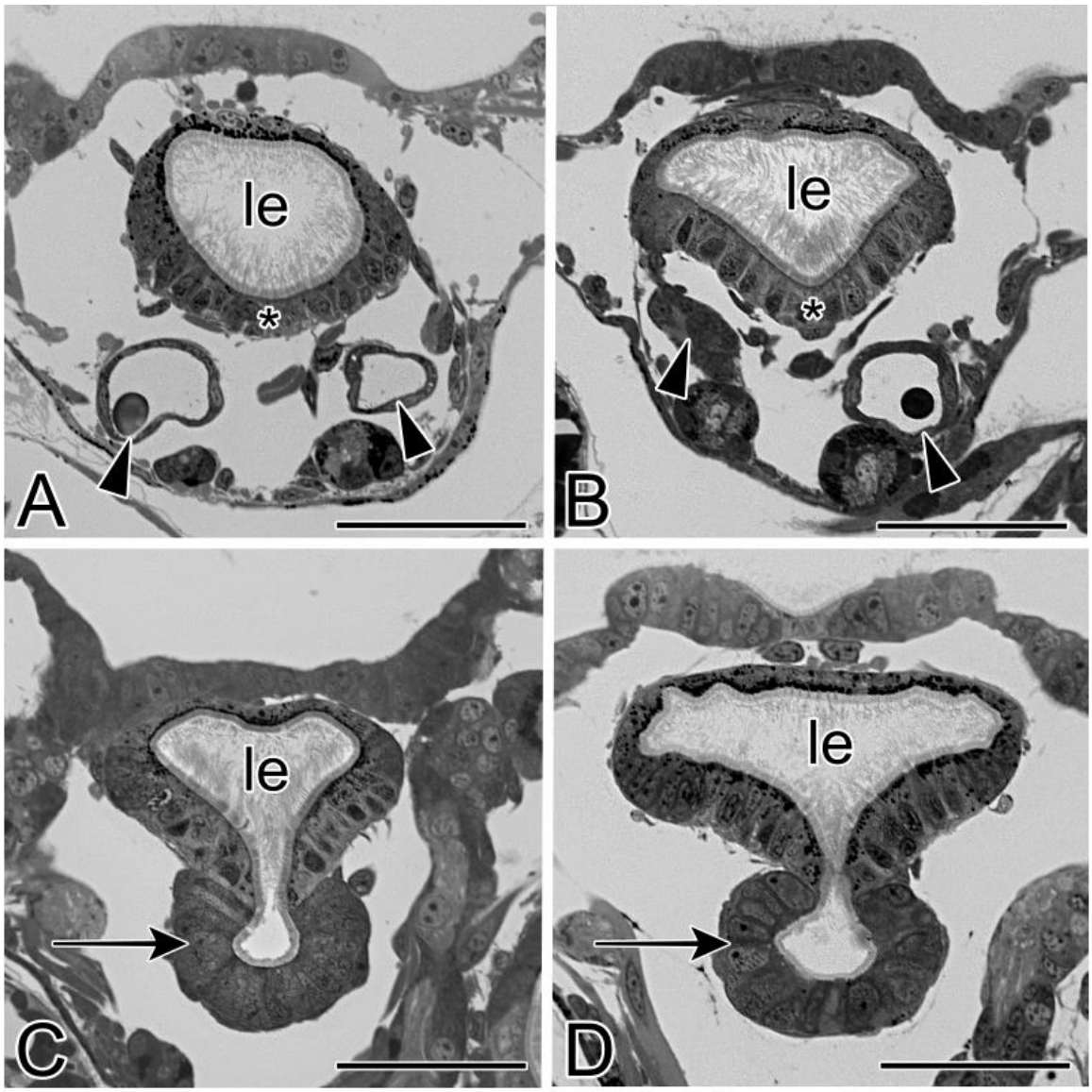

Figure 16. Histological transverse sections through the distal foregut of *Amphissa columbiana* during stages 1 and 2.

Scale bars= 50 μm. **A.** Onset of stage 1 (newly hatched larva) showing the ciliated larval esophagus with thickened cells forming the ventral wall (asterisk) at the level of the statocysts (arrowheads). **B.** Late stage 1 (6 days post-hatching larva) showing the mid-ventrally narrowed larval esophagus with the enlarged cells in the ventral esophageal wall (asterisk). **C.** Onset of stage 2 (11 days post-hatching larva) showing the ventral outpocketing of non-ciliated epithelium, marking the anlage of the definitive adult foregut (arrow). **D.** Late stage 2 (16 days post-hatching), showing widening of the ventral

outpocketing (arrow), although cell differentiation had not yet occurred. Abbreviations: le= larval esophagus.

3.4.3 Stage 3: Bifurcation of ventral outpocketing

Stage 3 was characterized by the bifurcation of the anlage of the definitive adult foregut, as was evident in larvae fixed at 20 days post-hatching (45% completion of larval development). The ventral outpocketing became subdivided into anterior and posterior projections (Figure 17). These anterior and posterior projections were blind-ending channels that were separate from the overlying larval esophagus, with the exception of a narrow connection to the larval esophagus. Regional differentiation of the definitive adult foregut had become evident, with the rudiments of the future buccal cavity and anterior esophagus present in the anterior projection and the radular sac in the posterior projection (Figure 18).

Histological sections of the anterior projection, just posterior to the mouth, illustrated that the cells of the future buccal cavity and anterior esophagus, ventral to the larval esophagus, had not yet begun to differentiate (Figure 18A), although the lumen of the buccal cavity was quite wide (Figure 18B). In sections of the posterior projection, the radular rudiment was present; however radular teeth had not yet begun to develop (Figure 18B).

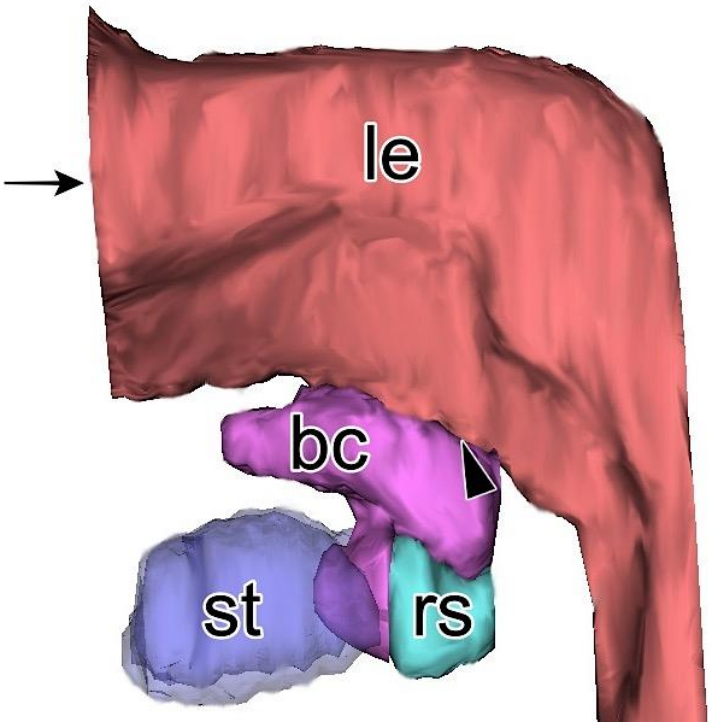

Figure 17. Surface-rendered 3D reconstruction of the distal foregut of *Amphissa columbiana* during stage 3 (20 days post-hatching larva) in left lateral view. The ventral outpocketing has now bifurcated into anterior and posterior projections, which correspond to the buccal cavity and the radular sac, respectively. Arrow indicates the location of the larval mouth; arrowhead indicates the point of connection between the larval esophagus and the ventral anlage of the definitive foregut. Abbreviations: bc= buccal cavity, le= larval esophagus, rs= radular sac, st= statocyst.

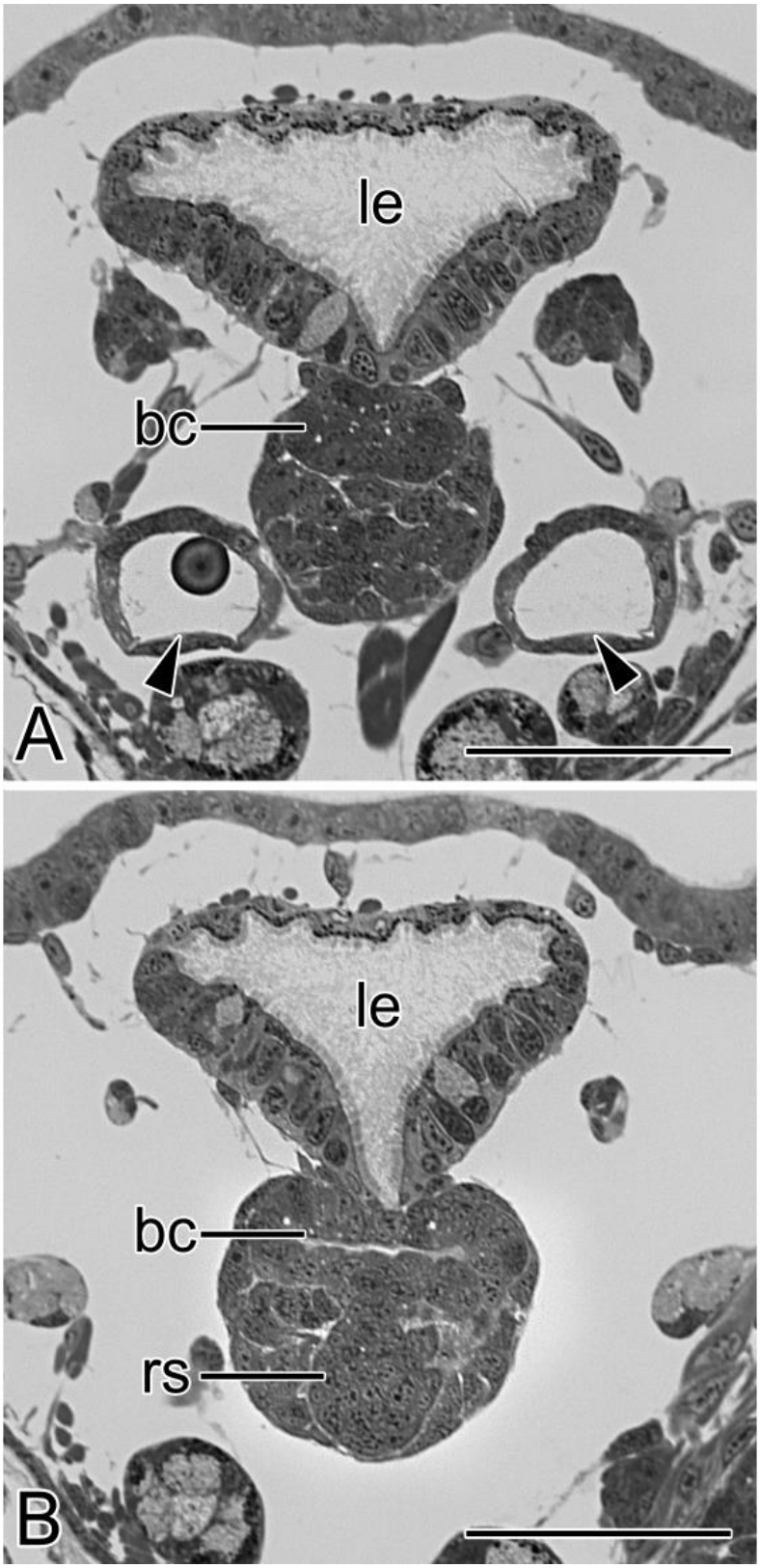

Figure 18. Histological transverse sections through the distal foregut of *Amphissa columbiana* during stage 3 (20 days post-hatching larva).

Scale bars= 50 μm. **A.** Stage 3 showing the ciliated larval esophagus and the anterior projection of the ventral anlage that represents the rudiment of the buccal cavity and anterior esophagus, located at the level of the statocysts (arrowheads). **B.** Stage 3 showing the ciliated larval esophagus, the flattened lumen of the buccal cavity and the rudiment of the radular sac. Abbreviations: bc= buccal cavity, le= larval esophagus, rs= radular sac.

3.4.4 Stage 4: Further elongation and differentiation of post-metamorphic foregut

Stage 4 was characterized by the differentiation and proliferation of the cells of the anterior and posterior projections of the definitive adult foregut.

In larvae fixed at 27 days post-hatching (60% completion of larval development), additional proliferation at the anterior end of the bifurcated outpocketing was evident, as the buccal cavity had extended further anteriorly, remaining separate from the larval esophagus (Figure 19A). Ducts of the salivary glands had proliferated posteriorly from the buccal cavity (Figure 19B); however the glands themselves were not fully formed and had blind-endings that were located on either side of the larval esophagus (Figure 19C). Posterior to the entrance of the salivary ducts, the future anterior esophagus had also begun to proliferate posteriorly from the buccal cavity, although it was very short and had clearly just begun to develop. The narrow connection to the larval esophagus was located at the posterior of the future anterior esophagus and just anterior to the commissure of the buccal ganglia (Figure 19D).

The developing radular sac was posteroventral to the anterior projection, which contained the radula. The radular teeth had just begun to develop, and the radular sac had lengthened posteriorly (Figure 19D). The radular cartilages had also begun to develop, and were located on either side of the radula at the level of the statocysts, ventral to the larval esophagus and buccal cavity (Figure 19B).

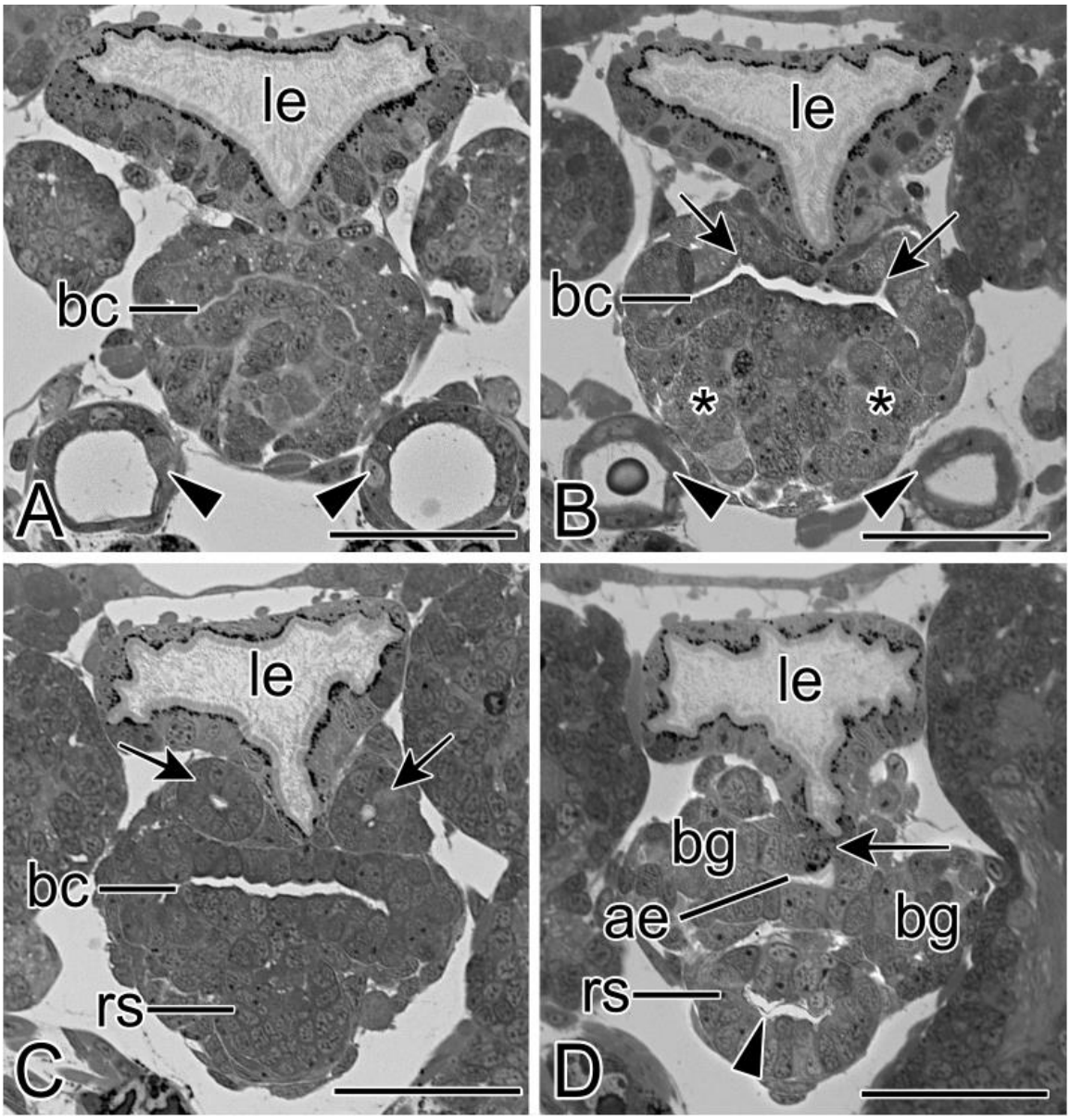

Figure 19. Histological transverse sections through progressively more posterior levels of the distal foregut of *Amphissa columbiana* during stage 4 (27 days post-hatching larva).

Scale bars= 50 μm. **A.** Stage 4 showing the ciliated larval esophagus and the anterior projection of the buccal cavity, at the level of the statocysts (arrowheads). **B.** Stage 4 showing the ciliated larval esophagus and flattened lumen of the buccal cavity; salivary ducts connect to the buccal cavity (arrows) at the level of the statocysts (arrowheads); note the radular cartilages have also begun to develop (asterisks). **C.** Stage 4 showing the ciliated larval esophagus and the flattened lumen of the buccal cavity; salivary ducts are

located on either side of the larval esophagus (arrows); note radular sac ventral to the buccal cavity. **D.** Stage 4 showing the ciliated larval esophagus and the differentiated components of the ventral outpocketing; note the lumen of the future anterior esophagus is about to connect to the lumen of the larval esophagus (arrow) and teeth are now visible on the radula (arrowhead). Abbreviations: ae= anterior esophagus, bc= buccal cavity, bg= buccal ganglion, le= larval esophagus, rs= radular sac, sg= salivary gland.

3.4.5 Stage 5: Post-metamorphic foregut at metamorphic competence

Stage 5 marked the onset of metamorphic competence (minimum of 45 days post-hatching).

All components of the definitive adult foregut (such as the buccal cavity and radula), except the definitive mouth, showed advanced differentiation at this stage (Figures 20 and 21). The buccal cavity now extended very far anteriorly, just short of the larval mouth (Figures 20 and 22A). The radula with many radular teeth, extended from the radular sac to rest on the floor of the buccal cavity (Figures 21A and 22A). The salivary ducts led from the buccal cavity to well-developed salivary glands, posteriorly (Figures 20A, 21B and 21C). Cells of the salivary glands contained abundant secretory granules (Figure 21C). Posterior to the buccal cavity extended the future anterior esophagus (Figures 20B, 21B and 22A), which connected to the larval esophagus (Figure 21C) just anterior to the commissure of the buccal ganglia. This point of connection became the future valve of Leiblein.

The radular sac underwent much growth, as its length at stage 5 extended beyond where the larval esophagus bent ventrally to connect to the stomach (Figure 20). The larval esophagus and radular sac were found to twist around each other so that they ended up side-by-side, with the radula terminating on the right side of the larval esophagus (Figures 20, 21D and 22B). The radular cartilages had also become very prominent, encircling the radula within the radular sac in a horseshoe shape (Figures 21C, 21D and 22A).

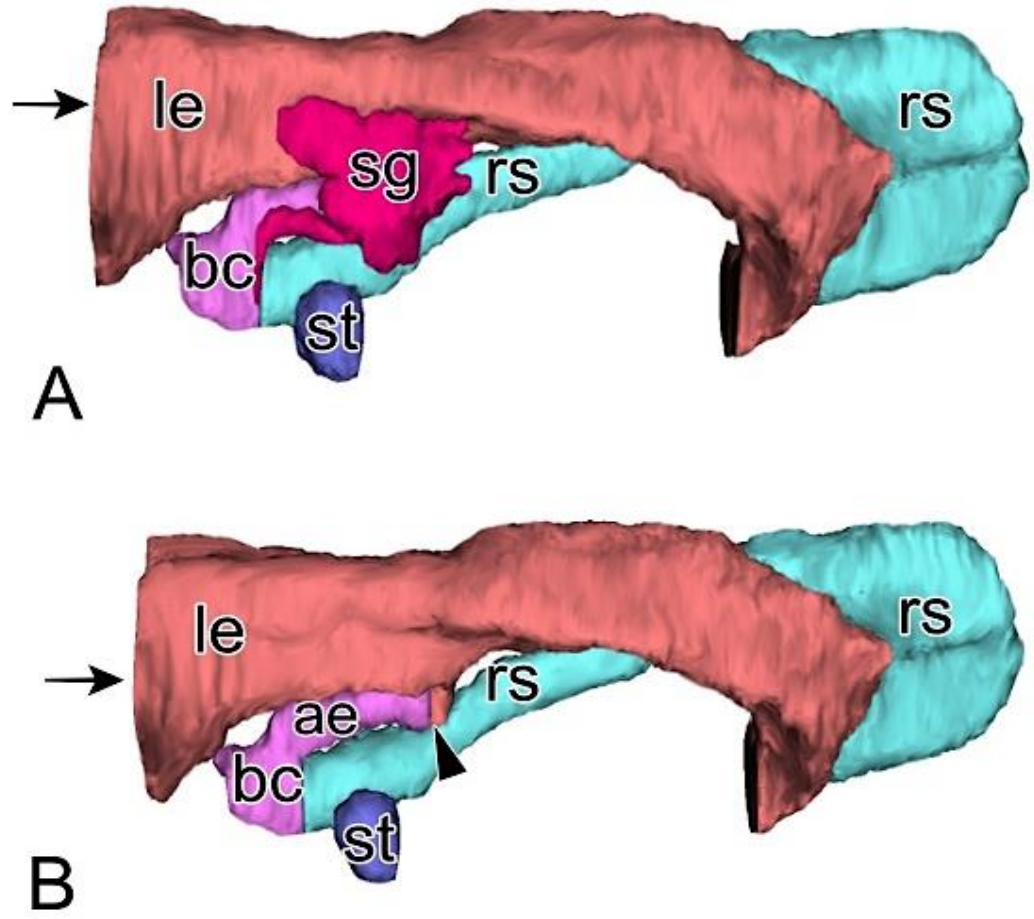

Figure 20. Surface-rendered 3D reconstructions of the distal foregut of *Amphissa columbiana* during stage 5 (metamorphic competence) in left lateral view. Salivary gland omitted in **B**. Arrow indicates the location of the larval mouth. **A.** All components of the post-metamorphic foregut have differentiated beneath the larval esophagus; salivary ducts lead from the buccal cavity to well-developed salivary glands. **B.** With the salivary ducts and glands removed from view, the narrow connection between the post-metamorphic anterior esophagus and the larval esophagus is visible (arrowhead). Abbreviations: ae= anterior esophagus, bc= buccal cavity, le= larval esophagus, rs= radular sac, sg= salivary gland, st= statocyst.

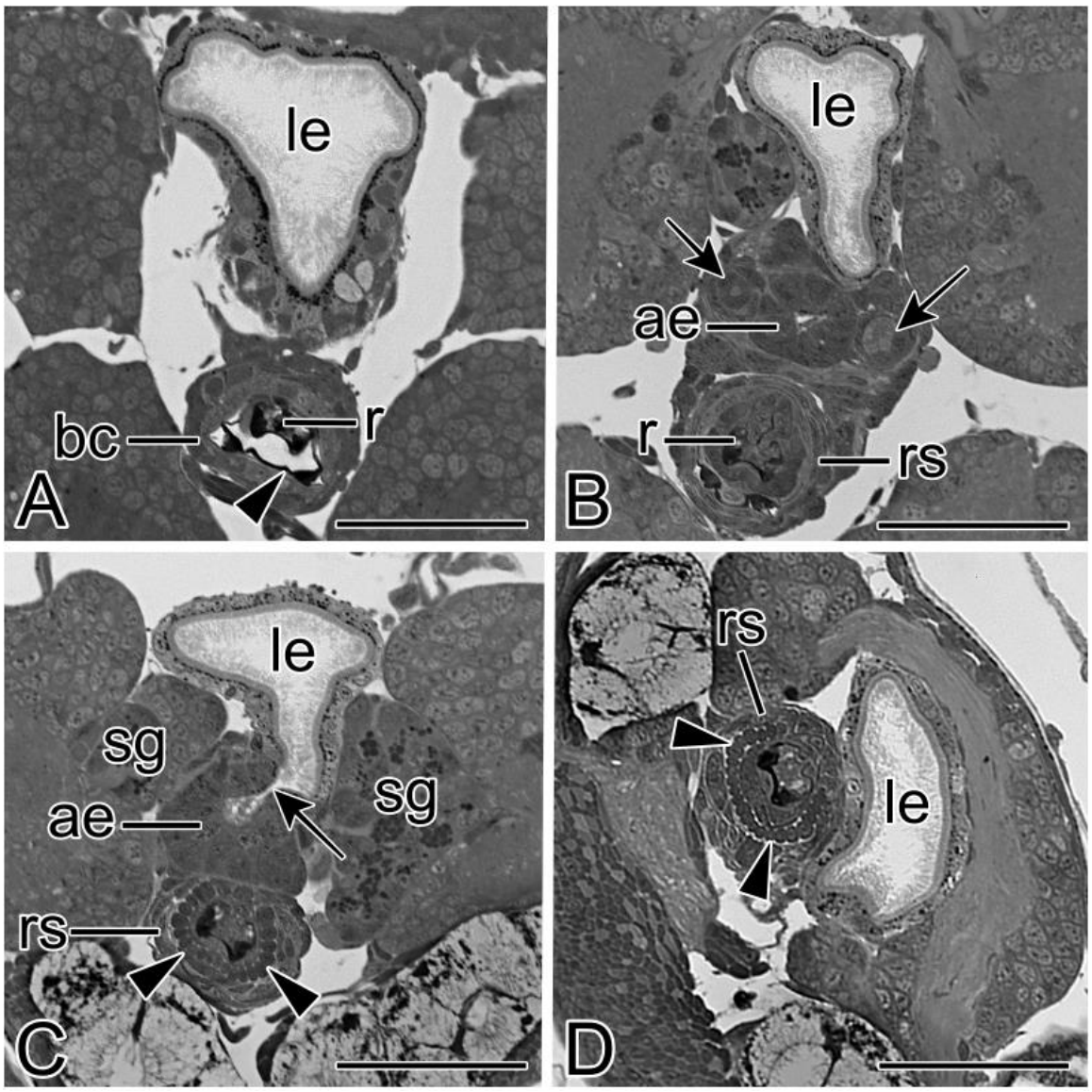

Figure 21. Histological transverse sections through progressively more posterior levels of the distal foregut of *Amphissa columbiana* during stage 5 (metamorphic competence). Scale bars= 50 μm**. A.** Section through the anterior extremity of the larval esophagus overlying the future post-metamorphic buccal cavity containing the radula with radular teeth (arrowhead). **B.** More posterior section showing the larval esophagus overlying the future anterior esophagus flanked by two salivary ducts (arrows); note radula housed within radular sac, ventrally. **C.** Section at the level at which the anterior esophagus connects to the larval esophagus (arrow); note the salivary glands and horseshoe-shaped array of radular cartilage (arrowheads). **D.** Section through the larval esophagus and the posterior projection of the radular sac with radular cartilage (arrowheads); note that the

larval esophagus and radular sac appear side-by-side instead of dorsal and ventral. Abbreviations: ae= anterior esophagus, bc= buccal cavity, le= larval esophagus, r= radula, rs= radular sac, sg= salivary gland.

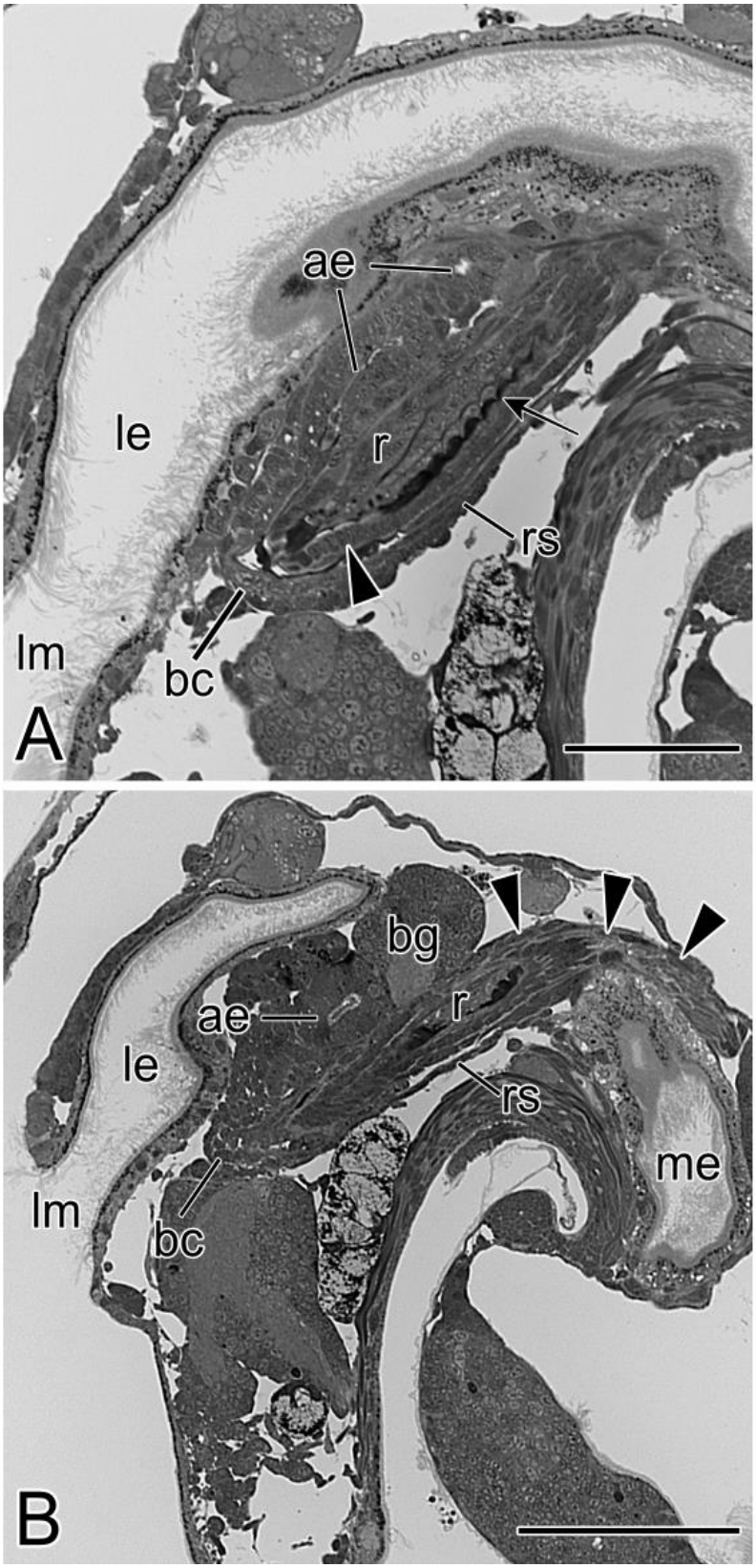

Figure 22. Histological longitudinal sections of *Amphissa columbiana* during stage 5 (metamorphic competence).

A. Section showing the larval mouth leading into the larval esophagus, overlying the future post-metamorphic buccal cavity containing the radula, radular teeth (arrow) and cartilages (arrowhead); note the thin lumen of the anterior esophagus leading posterodorsally from the buccal cavity; scale bar= 50 μm**. B.** Deeper section showing the radular sac curving around the right side of the larval esophagus (arrowheads); scale bar= 100 μm. Abbreviations: ae= anterior esophagus, bc= buccal cavity, bg= buccal ganglion, le= larval esophagus, lm= larval mouth, me= mid-esophagus, r= radula, rs= radular sac.

3.4.6 Stage 6: Post-metamorphosis

At 1-4 days after metamorphic loss of the velar lobes, a major reorganization of the foregut components had clearly taken place (compare Figure 20 to Figures 23 and 24).

Individuals sectioned revealed that the whole of the adult foregut sat within an extensible proboscis, which was contained within a proboscis sac (Figure 25A). Additionally, the larval esophagus distal to the connection of the anterior esophagus with the larval esophagus had been almost completely destroyed, including the larval mouth (Figures 23 and 24). Instead, the anterior-most area of the buccal cavity had ruptured through the anterior body wall, creating a new, definitive mouth.

From the definitive mouth the buccal cavity extended posteriorly. The radula was extended from the radular sac and resting on the floor of the buccal cavity, with well-developed radular cartilages and teeth present on the dorsal and ventral sides of the anterior radula (Figure 25A). The ducts of the salivary glands entered the buccal cavity just anterior to where the radula entered from the radular sac (Figure 23). The salivary ducts and glands were elaborate, with the glands being extremely large (Figure 23). Surprisingly, the left salivary duct was found to loop under the remaining vestige of the larval esophagus that had yet to be destroyed, to connect to the left salivary gland (Figures 23A and 25B). The remaining larval esophagus was connected to the valve of Leiblein, and was filled with sloughed off cells from the already destroyed anterior section of the larval esophagus (Figure 25B).

A very elongate anterior esophagus was connected to the posterior of the buccal cavity, which connected to the valve of Leiblein. The valve of Leiblein was very bulbous (Figure 25C), and connected to the mid-esophagus (Figure 24).

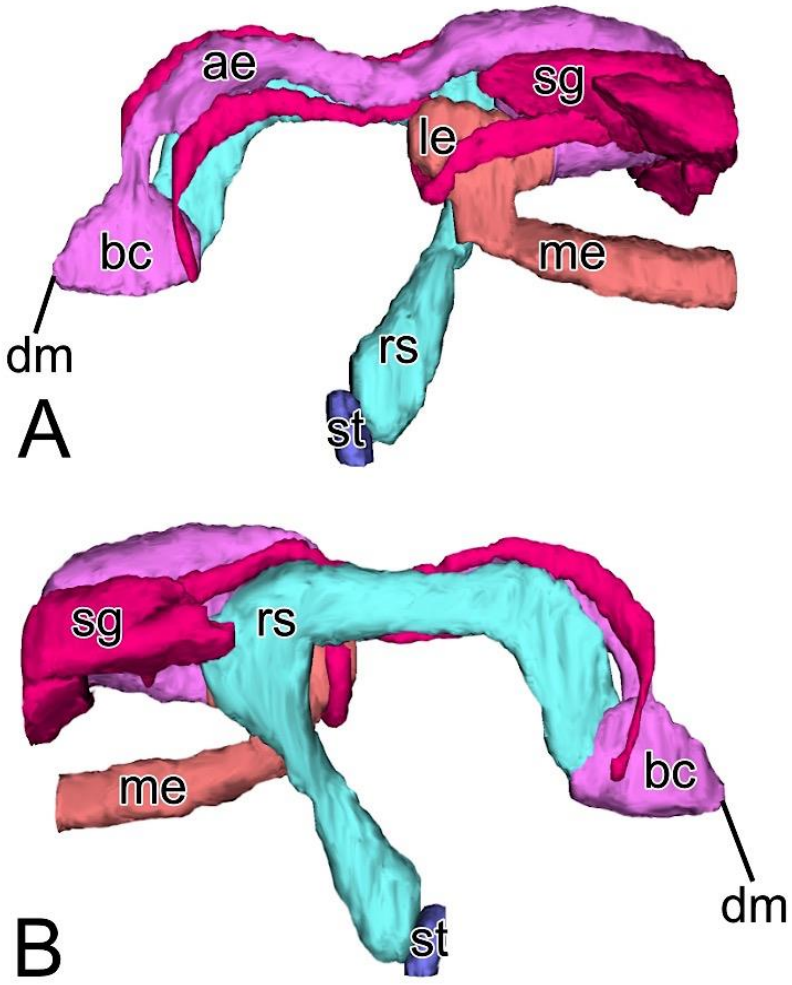

Figure 23. Surface-rendered 3D reconstructions of the distal foregut of a post-metamorphic juvenile of *Amphissa columbiana*.

The ventral anlage of the larval foregut has now become the functional adult feeding apparatus and the larval esophagus is in the process of being destroyed.

A. Left lateral view showing the absence of the larval mouth with a new definitive mouth located at the anterior of the buccal cavity; only a remnant of the distal larval esophagus remains. **B.** Right lateral view showing the elongate radular sac on the right side of the anterior esophagus. Abbreviations: ae= anterior esophagus, bc= buccal cavity, dm= definitive mouth, le= larval esophagus, me= mid-esophagus, rs= radular sac, sg= salivary gland, st= statocyst.

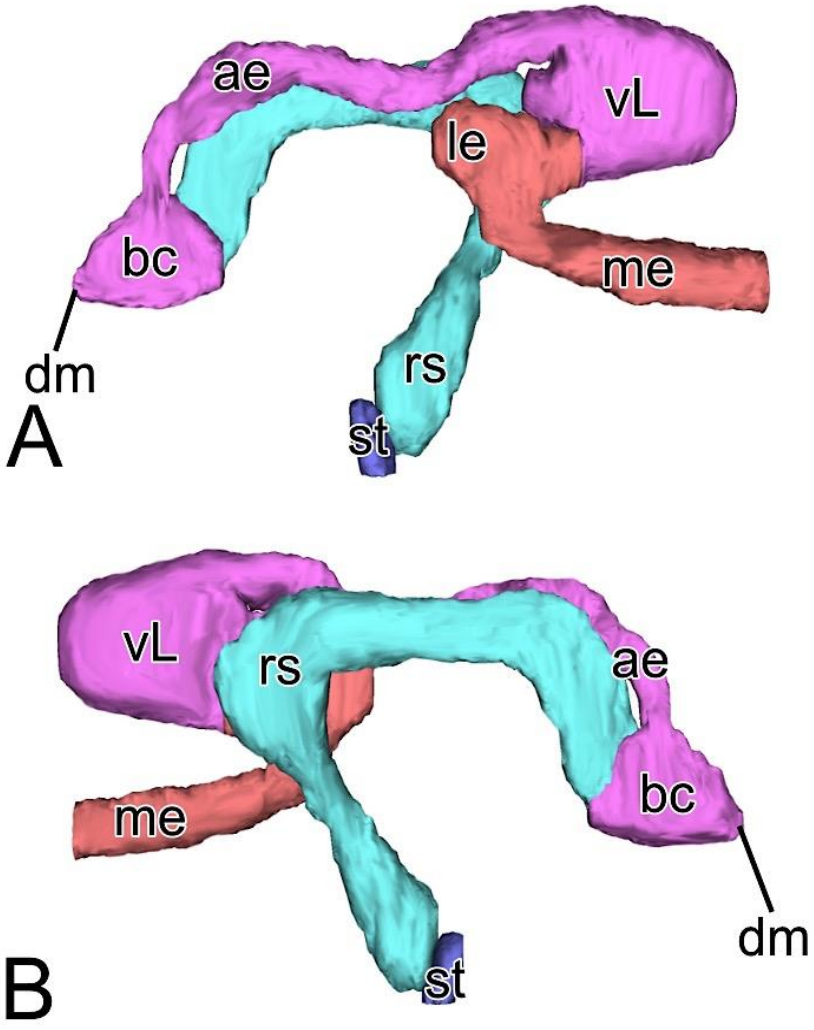

Figure 24. Surface-rendered 3D reconstructions of the distal foregut of a post-metamorphic juvenile of *Amphissa columbiana*.
The salivary ducts and glands have been removed from view.
A. Left lateral view showing the extremely long anterior esophagus leading to the bulbous valve of Leiblein, which was the original point of connection between the larval esophagus and anterior esophagus; a remnant of the larval esophagus can still be seen connecting to the valve of Leiblein. **B.** Right lateral view showing that the anterior esophagus remains on the left side of the specimen, while the radula extends posteriorly and then ventrally on the right side. Abbreviations: ae= anterior esophagus, bc= buccal cavity, dm= definitive mouth, le= larval esophagus, me= mid-esophagus, rs= radular sac, st= statocyst, vL= valve of Leiblein.

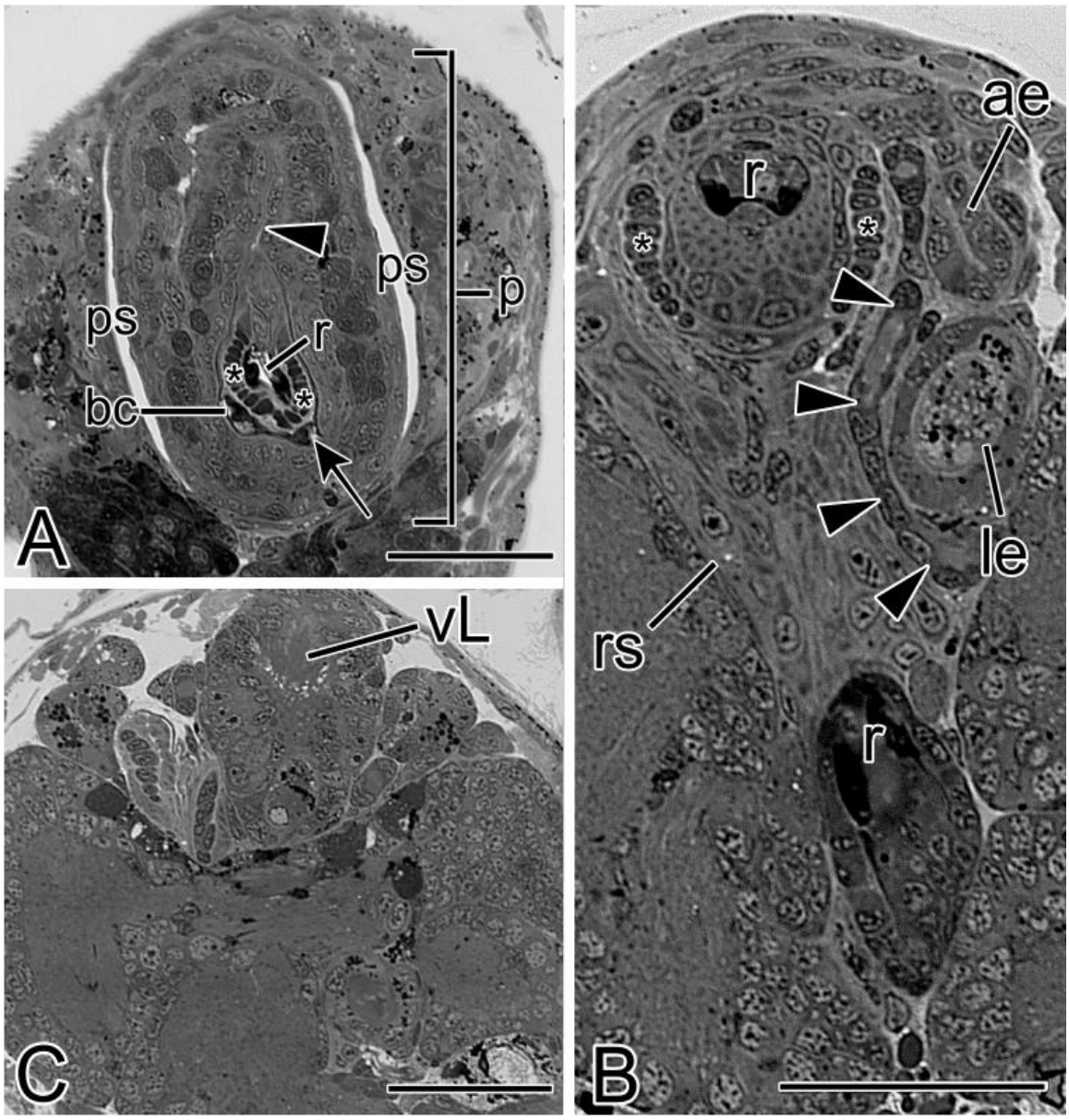

Figure 25. Histological transverse sections through the foregut of *Amphissa columbiana* at 1-4 days after metamorphic loss of the velar lobes.

Scale bars= 50 μm. **A.** Anterior definitive foregut showing the buccal cavity within the proboscis of the adult feeding apparatus; radular teeth and cartilages (asterisk) are present within the buccal capsule; the anterior esophagus branches off from the buccal cavity dorsally (the lumen is collapsed and appears as a thin white stripe; arrowhead); note the larval esophagus is no longer present this far anteriorly. **B.** More posterior section passing through the curved radular sac in two places (radular cartilages indicated by asterisks), the anterior esophagus and the duct of the left salivary gland (arrowheads); note the larval

esophagus is filled with sloughed off cells. **C.** Section through the valve of Leiblein, which is where the anterior esophagus connects with the remainder of the larval esophagus. Abbreviations: ae= anterior esophagus, bc= buccal cavity, le= larval esophagus, p= proboscis, ps= proboscis sac, r= radula, rs= radular sac, vL= valve of Leiblein.

4.0 Discussion

While the feeding systems found within the Caenogastropoda are diverse, the emergence of predatory feeding with a muscular proboscis and a derived foregut may have been an exceptional evolutionary innovation within the Neogastropoda that allowed this clade to undergo a rapid rate of speciation during the Cretaceous (Ponder 1973, Taylor et al. 1980, Kohn 1983, Ponder et al. 2008). However, because the morphologically complex foregut of neogastropod predators has evolved within a life history that begins with a herbivorous larval stage, constraints may have been imposed on the evolution of derived adult feeding morphology. When the larva undergoes metamorphosis to become an adult, a rapid switch from herbivory to carnivorous predation occurs, which raises interesting questions about how this is accomplished given that the two feeding modes require profoundly different morphological structures. Overall, two requirements must be met to ensure survival of the organism: 1) the larva must be able to maintain feeding throughout larval development and 2) the predatory definitive foregut must be ready for use shortly after the rapid metamorphic transition.

This study addressed three overarching questions arising from the ontogenetic change in feeding behaviour in *Crepidula fornicata* and *Amphissa columbiana*. These are: 1) do developmental modules exist in these species, 2) how does the adult feeding apparatus of *A. columbiana* differentiate without obstructing larval feeding, and 3) how does larval herbivorous feeding switch over to carnivorous predatory feeding within a short metamorphic period?

My observations on the columbellid neogastropod *A. columbiana* indicate that the process of transformation is similar to that previously described in the nassariid neogastropod, *N. mendicus* (Page 2000, 2005). This result helps to strengthen the hypothesis that the developing caenogastropod foregut consists of a dorsal and ventral module that are each developmentally self-contained. Furthermore, it suggests that the anterior esophagus of buccinoidean neogastropods is not homologous to what is called the anterior esophagus in caenogastropods outside of the Neogastropoda, but rather it is a posterior extension of the buccal cavity.

4.1 Foregut developmental modules in *Crepidula fornicata* and *Amphissa columbiana*

This investigation of foregut development in *C. fornicata* and *A. columbiana* has revealed evidence of the presence of developmental modules during the planktotrophic larval life history stage. In both of these species, most or all of the post-metamorphic feeding apparatus differentiated from an outpocketing of cells (the ventral module) arising from the ventral wall of the larval esophagus (the dorsal module). These results agree with previous descriptions given for caenogastropods with indirect development, where the developing distal foregut appeared to have dorsal and ventral components (Werner 1955, D'Asaro 1965, Fretter 1969, Thiriot-Quiévreux 1969, 1974, Page 2000, 2002, 2005, 2011, Parries and Page 2003). Additionally, similar developmental patterns and metamorphic fates were observed between *C. fornicata* and two previously studied herbivorous caenogastropods (Page 2000, Parries and Page 2003), and between *A. columbiana* and two predatory caenogastropods (Page 2000, 2005) that are in line with my initial hypotheses. In larvae of *C. fornicata*, the anterior portion of the ventral module was attached along its entire length to the distal larval esophagus. The larval esophagus was retained through metamorphosis but it underwent much cell loss to become a narrow ciliated channel on the roof of the buccal cavity, known as the dorsal food channel. However, in *A. columbiana*, both anterior and posterior projections of the ventral module were isolated from the larval esophagus, with the exception of a narrow connection at the original point of outpocketing. The isolated anterior projection gave rise to not only the buccal cavity and salivary glands, but also to an elongate anterior esophagus and valve of Leiblein. At metamorphosis, the larval esophagus distal to the ventral module connection was destroyed, including the larval mouth. Subsequently, the anterior tip of the buccal cavity broke through to the exterior to form a new mouth.

4.1.1 Evidence of foregut developmental modules

The dorsal and ventral regions of the developing foregut of caenogastropods are suggested to be organized into two developmental modules because they have different developmental trajectories and begin differentiation at different times. Developmental

modules exist as discrete subunits of the developing organism that show independent organization (Raff 1996). By virtue of the autonomous nature of modules, they permit developmental change without having deleterious effects on other modules (Raff 1996). Developmental modules can therefore be dissociable both temporally and spatially. Indeed, the most convincing evidence for the presence of developmental modules comes from demonstration of temporal and spatial dissociability of different components during development, resulting in the widespread phenomenon of heterochrony (Raff 1996, Friedman and Williams 2003). This was observed in the dorsal and ventral regions of the developing foregut in *C. fornicata and A. columbiana.*

Sections of newly hatched larvae of *C. fornicata* during stage 1 revealed that the ventral outpocketing had begun to form prior to hatching from the larval esophagus at this stage. However, this ventral outpocketing had not yet begun to differentiate, therefore the developmental timing of this ventral outpocketing was offset from the fully-formed larval esophagus. Because this was the earliest stage of development sectioned for *C. fornicata*, it is unknown whether there was a temporal difference in the time of onset of the development of the ventral outpocketing in comparison to the larval esophagus. Overall, there was an offset for development as a whole between the two modules, as the larval esophagus was already fully functional, whereas the ventral outpocketing was just beginning to proliferate and differentiate.

The presence of the ventral outpocketing upon hatching in stage 1 was somewhat surprising, as this had not been reported in most of the caenogastropods studied to date. In the herbivore *Lacuna vincta*, and the carnivores *Euspira lewisii* and *Nassarius mendicus*, only the thickened epithelium of the ventral wall of the larval esophagus was visible until approximately 20-30% completion of larval development (Page 2000). This difference in the apparent onset and rate of development of the ventral outpocketing could be predetermined by the length of the larval stage. *Crepidula fornicata* had a larval stage of approximately 11 days before reaching metamorphic competence, which is relatively short for a planktotrophic larva. However, *L. vincta, E. lewisii and N. mendicus* required at least 4-6 weeks to reach metamorphic competence (Page 2000). This discrepancy in larval developmental rate can be explained by differences in maternal provisioning, as well as the temperature at which the larvae were raised. Due to the fact

that *C. fornicata* did have such a short larval stage, accelerated foregut development during the larval stage and even prior to hatching, would be reasonable.

While it is unknown whether a delay in the onset of development between modules occurs in *C. fornicata*, a clear delay was evident in *A. columbiana.* Upon hatching, the larval esophagus of *A. columbiana* had a wide, ciliated lumen. The epithelium of the ventral esophageal wall was thickened, but there was no evidence of a ventral outpocketing until later in development. At stage 2 (25% completion of larval development), the outpocketing had begun to form through an invagination of the epithelial wall of the fully functional distal larval esophagus. This shift in developmental timing is a clear indicator of the presence of separate developmental processes. Initial evidence for a temporal uncoupling of developing foregut components in caenogastropods was first identified by Fretter (1969), who reported that the radular apparatus of caenogastropods with feeding larvae appeared to begin development later than the radular apparatus of patellogastropods (Smith 1935) and vetigastropods (Crofts 1937), which have non-feeding larvae. These results are also congruent with what has been seen in four other caenogastropod species where foregut development was studied in detail (Page 2000, 2002, 2005). In feeding larvae of caenogastropods that become herbivorous adults (*L. vincta*) or predatory adults (*E. lewisii*, *M. stearnsii* and *N. mendicus*), a delay was seen in the development of the ventral outpocketing. The outpocketing did not begin to form until approximately 20% to 30% completion of larval development (Page 2000, 2002, 2005).

Another indicator of a modular organization of development is when comparative studies show that homologues among related species show varying amounts of spatial separation from other components of a complex system. For example, the origin of the vertebrate jaw may have depended on the disassociation of the nasohypophyseal complex from other branchial arch derivatives during development of early vertebrates (Kuratani et al. 2001). A spatial separation of the ventral module from the dorsal module was seen in both *C. fornicata* and *A. columbiana,* to varying degrees. Although initial development of the outpocketing appeared very similar in *C. fornicata* and *A. columbiana* (despite differences in the onset of development), the developmental trajectory began to diverge as the outpocketing underwent cellular proliferation and differentiation. During stages 2

and 3 in *C. fornicata*, proliferation occurred anteriorly and posteriorly, forming the buccal cavity and future radular sac, respectively. While the ventral outpocketing proliferated posteriorly in the form of an isolated projection (separate from the overlying larval esophagus), the cells that proliferated anteriorly remained connected to the larval esophagus; the lumens were continuous. After metamorphosis at stage 4, the larval esophagus of *C. fornicata* was reduced through cellular loss to form a narrow ciliated groove (the dorsal food channel) that extended down the midline of the roof of the buccal cavity and anterior part of the esophagus. However, in *A. columbiana* during stages 3-5, the anterior proliferation of the ventral outpocketing, which formed the buccal cavity and anterior esophagus of the post-metamorphic foregut, was an isolated projection that was separate from the overlying larval esophagus. After metamorphosis in stage 6, the larval esophagus and mouth of *A. columbiana* distal to the valve of Leiblein were clearly in the process of being destroyed, and the buccal cavity of the definitive adult foregut opened a new mouth through the anterior body wall.

This difference in the degree of isolation of the ventral outpocketing from the larval esophagus between *C. fornicata* and *A. columbiana* is consistent with other studies that examined the developing definitive foregut of caenogastropods in detail, depending on whether the species was herbivorous or predatory. In the two herbivores *L. vincta* (Page 2000) and *T. cancellata* (Parries and Page 2003), the anterior region of the ventral outpocketing (buccal cavity) was intimately connected to the larval esophagus, and remained that way through metamorphosis. During metamorphosis, the size of the larval esophagus was reduced to a very narrow channel that lined the roof of the buccal cavity and anterior part of the esophagus (Page 2000, Parries and Page 2003). However, in both of the predatory caenogastropods *E. lewisii* and *N. mendicus*, the anterior region of the ventral outpocketing was entirely separate from the overlying larval esophagus (Page 2000, 2005). At metamorphosis, the distal larval esophagus was completely destroyed including the larval mouth, and a new mouth was formed in the anterior body wall that connected to the definitive adult foregut (Page 2000, 2005).

Results of this comparative study show clear evidence of temporal and spatial dissociation between the dorsal and ventral regions of the foregut of *C. fornicata* and *A. columbiana*, suggesting the presence of developmental modules. These results are

consistent with reports on the foregut development of at least 5 other species of caenogastropods. Although the dissociability of different developing components are good indicators of a modular organization of development, this does not explain the potential functional significance.

4.1.2 Functional significance of differences in timing

While an overall delay in development occurs between the dorsal and ventral modules of the caenogastropod foregut, the difference in the onset of development between the modules (as seen in *A. columbiana*) may have its own functional significance. Upon hatching, a major role of the larva must be to capture and digest microalgae within the water column to fuel development (Page and Pedersen 1998). The environmental transition from egg capsule to water column is likely complex on many levels; therefore an adjustment period may exist, where food energy is dedicated to essential processes before more complex developmental processes can take place. During the first two weeks of larval development, *A. columbiana* increased noticeably in size which may have contributed positively to the larva's ability to capture food. Overall, this time delay may allow the larva to establish maximal feeding efficiency before it begins to develop the definitive feeding structures for the post-metamorphic stage.

An overall delay in the development of the definitive foregut may also reflect differences in the amount of time structures require to develop. At the onset of the larval stage, the dorsal module is fully functional, as the planktotrophic larva needs to be able to feed immediately upon hatching. However, the ventral module often does not begin developing until after hatching, and continues to develop throughout the larval stage. As the anlage of the definitive foregut of the adult, the ventral module is not required to be functional until metamorphosis has been completed. Therefore, development does not need to begin at the same time as it does in other structures that are needed immediately for the larval lifestyle. Depending on the species, the ventral module may vary in the time required for development. While four species of caenogastropods begin the development of the ventral module at 20-30% completion of larval development (Page 2000, 2005), *Trichotropis cancellata* (Parries and Page 2003) and *C. fornicata* (this study) begin development earlier, whereas the neogastropod *Conus lividus* begins development later in

the larval stage (Page 2011). In fact, the development of the definitive foregut of *C. lividus* does not begin until approximately two-thirds completion of larval development (Page 2011). Regardless of the time of onset, many caenogastropods clearly show that precocious development of the definitive foregut occurs, likely to allow the metamorphic transition and the onset of adult feeding to occur rapidly.

One of the main questions regarding the life history of predatory neogastropods is to answer how the switch from herbivory to carnivory occurs so quickly. Due to the fact that metamorphosis in predatory neogastropods results in a dramatic alteration of the feeding apparatus, feeding clearly cannot occur during the metamorphic period. Therefore, the metamorphic transition must proceed rapidly so as to allow juvenile feeding as soon as possible. While precocious development of structures within gastropod larvae have been reported, the extent to which this happens varies between lineages and species. With the current data available and the results of this study, it is clear that precocious development of the definitive adult foregut occurs to facilitate the rate at which metamorphosis occurs. After metamorphic competence has been reached, remodeling can occur rapidly through cell loss, which proceeds at a much faster rate than cell proliferation, growth and differentiation.

4.1.3 Functional significance of spatial separation

As proposed by Page (2000), the separation of the distal larval foregut into dorsal and ventral modules was likely selected based on the need to maintain larval feeding while concurrently developing an elaborate post-metamorphic foregut in species with indirect planktotrophic development. However, a side-effect of this adaptation, particularly the provision to create a *de novo* post-metamorphic mouth, may have been the opening of an opportunity to create diverse foregut types. The isolation of the ventral module from the dorsal module during foregut development may have enabled the post-metamorphic foregut components of predators to specialize and diversify, free from the morphological constraints of larval feeding. While larval feeding occurred unobstructed throughout larval development, precocious development of the adult feeding apparatus was accomplished, so that the definitive foregut would be ready when the larva reached metamorphic competence.

In both *A. columbiana* (this study) and *N. mendicus* (Page 2000, 2005), the anterior projection of the ventral outpocketing also gives rise to a very elongate anterior esophagus. The proliferation of such a complex post-metamorphic foregut would likely not have been allowed to occur, had the dorsal and ventral modules not been isolated, as larval feeding may likely have been obstructed.

This isolation, however, likely necessitated another novelty: the creation of a *de novo* mouth. While in *C. fornicata* the mouth was retained, in *A. columbiana* the mouth that led to the larval esophagus was destroyed, and a new mouth formed ventrally that led into the buccal cavity. From the individuals sectioned, it was not obvious how the destruction of the mouth occurred; however, this process has been observed in *E. lewisii* and *N. mendicus* (Page 2000, 2005). In both species, the larval mouth was sealed shut by fusion of the bordering epithelium, where subsequently the larval esophagus was destroyed (Page 2000, 2005).

4.2 Homology of post-metamorphic foregut components in caenogastropods

A question of homology is prompted by the similar pattern of the initial stages of foregut development seen in herbivorous and predatory caenogastropods. Although the stage prior to outpocketing was apparently missed in *C. fornicata*, it can still be discussed with regards to larvae of *A. columbiana*. In the larval foregut of *A. columbiana*, the outpocketing of the ventral esophageal wall (which would form the whole of the adult foregut) was preceded by a cellular thickening of this wall. Similar enlarged cells were seen in herbivorous and predatory caenogastropods alike; however, these studies also reported the presence of a patch of non-ciliated cells in the ventral wall (Fretter 1969, Page 2000, 2002, 2005, Parries and Page 2003). These progenitor cells have been termed "set-aside cells" because they have delayed development compared to the cells that are already fully functional at the onset of the larval stage, and they give rise to post-metamorphic structures (Davidson et al. 1995, Peterson et al. 1997). Studies of caenogastropods and heterobranchs with planktotrophic larvae suggest that these progenitor cells may be widespread throughout gastropod groups with a planktotrophic larval stage because they give rise to the adult foregut (Kriegstein 1977, Bickell and Chia

1979, Bickell et al. 1981, Little et al. 1985, Page 2000, 2005, Parries and Page 2003). However, developmental patterns differ depending on the species after the initial outpocketing stage, as was seen in this study. Throughout stage 1 of *A. columbiana*, all cells of the larval esophagus appeared ciliated; however, the loss of cilia or the appearance of cells without cilia may have been missed due to the ages of larvae that were sectioned. Because development is continuous and discrete ages were chosen for analysis, details of development that occur quickly can be missed. However, because the thickened cells of the ventral wall proliferated to form a similar non-ciliated ventral outpocketing in the five species mentioned previously, which ultimately gave rise to the adult foregut, these cells are likely homologous within caenogastropods, based on their location and ontogenetic fates. Regardless, a more detailed ultrastructural study is required to confirm hypotheses about this homology.

In the herbivores *L. vincta* (Page 2000), *T. cancellata* (Parries and Page 2003) and *C. fornicata* (this study), the direct transformation of the larval esophagus into the dorsal food channel was evident upon metamorphosis, which strongly suggests that these two structures are developmentally homologous. During metamorphosis, the cross-sectional diameter of the ciliated larval foregut was dramatically reduced by loss of cells from the lining epithelium. This resulted in the formation of a small ciliated channel extending down the mid-dorsal wall of the buccal cavity and anterior part of the esophagus. The lack of a dorsal food channel in neogastropod predators may be the result of an upregulation of this cell loss process that is observed in herbivorous caenogastropods (Page 2000). During metamorphosis the larval esophagus in *E. lewisii*, *N. mendicus* (Page 2000) and *A. columbiana* (this study) is completely destroyed, which explains why predatory neogastropods do not appear to have a dorsal food channel. One study by Graham (1941) identified a putative dorsal food channel in the neogastropod predator *Nucella lapillus* and this interpretation was corroborated by a developmental study of this species (Ball 2002). However, in a developmental study on the conspecifics *Nucella lamellosa* and *N. ostrina*, which involved histological and ultrastructural analysis of many more stages of late larval and metamorphic development, it was shown that the homologue of the distal larval esophagus was entirely destroyed at metamorphosis (Hookham 2014, Hookham and Page 2016). This result illustrates the value of detailed

developmental data for identifying homologous structures, because comparative morphological data alone can sometimes lead to misleading conclusions.

This study adds to clear evidence that the "anterior esophagus" of buccinoidean predators is not homologous to the "anterior esophagus" of herbivorous caenogastropods, but is instead a posterior extension of the buccal cavity. This was especially obvious in stage 6 individuals of *A. columbiana*, where both the anterior esophagus and the dissociating larval esophagus were still present as clear, distinct structures. It has been hypothesized that the anterior esophagus of predatory neogastropods can be interpreted as a posterior extension of the buccal cavity (Ponder 1973). Ponder (1973) noted that the buccal ganglia lie just beneath the junction of the buccal cavity and the esophagus in most gastropods (as seen in *C. fornicata*); however, in buccinoidean neogastropods the buccal ganglia and connecting commissure lie beneath the junction of the valve of Leiblein and the mid-esophagus. Therefore, in buccinoidean neogastropods the buccal ganglia appear to have been pushed posteriorly. These notes closely approximate what was seen in *A. columbiana*, where cells at the posterior end of the developing buccal cavity proliferated to form the future anterior esophagus and valve of Leiblein.

The proboscis of both *A. columbiana* and *N. mendicus* is very long, which presumably may have been selected to increase access to the number of food items available. Therefore, an elongation of the foregut would have been required during evolution, which may have occurred through Graham's (1941) terminal addition hypothesis. Predatory feeding with an increasingly elongate foregut in predators likely necessitated a valve of Leiblein to prevent the regurgitation of ingested food during proboscis elongation (Amaudrut 1898, Graham 1941, Fretter and Graham 1994, Kantor 1996). However, additional functions could include the retention of digestive secretions from the gland of Leiblein (Ponder 1973) or to separate liquid from solid food for transport to the gland of Leiblein (Andrews and Thorogood 2005). While the valve of Leiblein has been described to be much reduced in buccinoideans like *N. mendicus*, this structure was quite bulbous in *A. columbiana*. However, because the larval esophagus had not yet been completely destroyed, there is a possibility that the size of the valve of Leiblein was overaccentuated where the two connected. Further cell reduction leading up to, and possibly including parts of the valve of Leiblein was likely imminent, however

older specimens of *A. columbiana* that have fully completed metamorphosis would need to be sectioned to determine this.

An excellent group that can be used to compare feeding morphology is the Heterobranchia, the sister group to the Caenogastropoda. Interestingly, predator feeding is prevalent in this group; however, the specialized foregut of predatory heterobranchs does not necessarily require the destruction of the larval mouth and esophagus in planktotrophic veligers. For example, in the dorid nudibranch *Doridella steinbergae*, the future adult foregut develops as an isolated outpocketing from the ciliated larval esophagus, similar to caenogastropods (Bickell and Chia 1979, Bickell et al. 1981). While this outpocketing gives rise to the radular apparatus during larval development, the larval esophagus and mouth were remodeled after metamorphosis to form the specialized buccal pump, which is used to prey on bryozoans (Bickell and Chia 1979, Bickell et al. 1981). Overall, it is hypothesized that larval planktotrophy arose once in the ancestor to the Neritomorpha, Caenogastropoda and Heterobranchia; therefore the larval esophagus and the separation of components into dorsal and ventral modules is hypothesized to be homologous throughout these groups. However, the ventral module has become further elaborated in caenogastropods compared to heterobranchs, producing not only the radular apparatus, but also the buccal cavity and various other specialized structures. Clearly, ventral module specialization has been an important factor in caenogastropod foregut evolution.

4.3 Summary

Through histological sectioning and 3D reconstructions, the present study has demonstrated that development of the post-metamorphic foregut in *Crepidula fornicata* and *Amphissa columbiana* involved developmental modules that may have been selected based on the need to maintain larval feeding while concurrently developing the post-metamorphic foregut in species with indirect planktotrophic development. In both species, the larval foregut was composed of dorsal and ventral modules that had different ontogenetic fates and showed temporal and spatial dissociation. Histological sections revealed that the post-metamorphic buccal cavity and radula of both species form as a ventral outpocketing (ventral module) from the larval esophagus (dorsal module).

However, in *A. columbiana* the ventral outpocketing is semi-isolated from the larval esophagus and also produces an "anterior esophagus" that is not developmentally homologous to the "anterior esophagus" of herbivorous caenogastropods. At metamorphosis, the larval esophagus and mouth of *A. columbiana* is destroyed, which allows the post-metamorphic foregut to be slotted into place with a new definitive mouth.

Overall, this research adds to the available knowledge of foregut development in caenogastropods, and lends support to the hypothesis that foregut developmental modules may be prevalent throughout this clade. Although increased spatial separation between foregut developmental modules in neogastropod predators may have been initially selected based on the need to maintain larval feeding while simultaneously developing the post-metamorphic foregut, it is this spatial uncoupling that may have facilitated the extensive diversification of post-metamorphic foreguts among predatory neogastropods. In essence, morphological variants of the post-metamorphic foregut that might arise during development could be tested directly within the selective environment of the juvenile habitat, without first being compatible with the larval feeding system.

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