FEEDING MORPHOLOGY OF JUVENILE *UTTERBACKIA IMBECILLIS*  
(BIVALVIA: UNIONIDAE)

By

MATTHEW P. TRUMP

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Approved by:

Ronald V. Dimock, Jr., Ph.D., Advisor ________________________

Examining Committee:

Gerald W. Esch, Ph.D. ________________________

Anita K. McCauley, Ph.D. ________________________
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ABSTRACT

Matthew P. Trump

Feeding morphology of juvenile Utterbackia imbecillis (Bivalvia: Unionidae)

Thesis under the direction of Dr. Ronald V. Dimock, Jr., Thurman D. Kitchin Professor of Biology, Department of Biology, Wake Forest University

The ontogeny of the feeding morphology of juvenile U. imbecillis was examined to provide information about this relatively little studied stage of unionid mussel development. Glochidia larvae were induced to metamorphose to juveniles by using in vitro culture techniques. Juveniles were reared in a recirculating flow through trough system. Specimens were collected weekly from 0-130 days, and the progression of development of gill filaments, ciliation of the gills, foot and mantle, formation of the labial palps, and the ciliation of the oral field and mouth was followed with scanning electron microscopy. Newly metamorphosed juveniles possessed one pair of fully formed gill filaments with the full suite of lateral and frontal cilia and latero-frontal cirri, plus two pairs of gill buds – incompletely formed filaments. New filaments first appeared as buds at the posterior margin of the developing gill lamellae. As the juveniles grew, interfilamentary ciliary connections appeared at the tips of the filaments, to be followed by formal tissue connections. Subsequently the lamellae of each side of the animal joined at their ventral margins forming a basket-like array that effectively separated the ventral infrabranchial aspect of the mantle cavity from the dorsal suprabranchial space. This arrangement, coupled with the posterior-ward growth of the labial palps contacting the anterior of the paired lamellae, had the rudiments of a food groove that may enable juvenile mussels to filter feed prior to development of the
ascending lamellae and the outer demibranch characteristic of adult mussels. With this morphology of the gills and related pallial structures, juvenile *U. imbecillis* may efficiently sort particles much earlier than previously thought. The early onset of filter feeding could augment the commonly described behavior of deposit feeding through pedal sweeping by juveniles of this and other species of unionid mussels.
Anthropogenic factors such as irrigation, damming, fishing, and pollution have negatively impacted freshwater habitats across the globe since at least 5,000 BCE (Strayer and Dudgeon, 2010). Native freshwater mussel (Bivalvia: Unionidae) populations have been negatively affected by many of these uses. The greatest diversity of unionids occurs in North America where 297 of the known 621 species in the world have been identified (Bogan, 2008). About 200 of the identified 297 mussel species in North America are seriously imperiled, with a number of the 200 species thought to be either already extinct or nearly so (Lydeard et al., 2004). Recently the native unionids in the United States and Canada have been threatened by the 1985 introduction (Mackie, 1991) and subsequent transcontinental spread of the exotic zebra and quagga mussels, *Dreissena* spp. (Benson, 2010). Efforts to reestablish native mussel populations in areas within which they have become locally extinct have included raising juveniles in culture settings to be released into streams, lakes, and rivers. There has been little success with these freshwater mussel conservation efforts due in large part to the limited knowledge of the basic morphological development of post-metamorphic juvenile mussels.

Mussels constitute a primary biological filter in freshwater ecosystems. The filtration characteristics of their large lamellibranch gills are highly efficient at removing particles suspended in the water column ranging in size from 1 to 100 µm (Reid et al., 1992). Pathways of food uptake via the mussel’s foot and through the valve gape have also been documented (Nichols et al., 2005). While some research in regard to feeding has been performed on juvenile mussels (Lasee, 1991; Wright, 1995; Gatenby et al.,
1996; Uthaiwan et al., 2001; Beck and Neves, 2003; Kovitvadhi et al., 2006, Kovitvadhi et al., 2007), relatively little is known about the feeding morphology and pathways of particle uptake by unionid mussels from the time they drop off their fish host to the point that they are bona fide filter feeders with typical adult anatomy.

**Life Cycle**

The life cycle of the Unionidae is both unique and complex compared to many other bivalves. Most species in the family are dioecious, while some, including *Utterbackia (Anodonta) imbecillis*, are monoecious (Heard, 1975). In both sexually dimorphic and hermaphroditic species, males (or male hermaphrodites) typically release sperm into the water column that is taken into the mantle cavity of a female (or female hermaphrodite) mussel. There are, however, reports of *U. imbecillis* self-fertilizing (Johnston et al., 1998). Eggs are fertilized in the suprabranchial cavity, and embryos are stored in the water tubes of the outer demibranch of the female gill as they develop into glochidia larvae (Tankersley and Dimock, 1992).

Though there are reports that some unionid larvae may be capable of complete metamorphosis within the parental gill of the adult mussel (Sterki, 1898; Howard, 1914; Allen, 1924), glochidia within the water tubes, or brood chambers, of adult mussels are typically released into the water column where they enter an obligatory parasitic phase, attaching to the gills and/or fins of a host fish (Kat, 1984). After successful metamorphosis while encysted on an appropriate host fish (Rogers-Lowery and Dimock, 2006), mussels drop off their host to the benthos and begin life as free-living juveniles.
Embryonic mussels developing within the water tubes of the demibranchs of the adult mussels may gain nutrition from the adult as the larvae develop (Wood, 1974; Schwartz and Dimock, 2001), while larvae encysted on a host fish utilize their mantle cells to obtain and store nutrients from tissues and the blood vascular system of the host (Fisher and Dimock, 2002). These stored nutrients, including lipid droplets, may be vital for survival of newly metamorphosed mussels as they drop off their host fish and begin the juvenile phase of development as independent feeders (Lasee, 1991; Fisher and Dimock, 2002).

**Juvenile Stage**

The term “juvenile”, in regard to freshwater mussels, has been used very loosely. Howard (1914) described a juvenile as a mussel that has successfully metamorphosed. Successful metamorphosis is generally described as the transition from a glochidium with a single adductor muscle to an individual with both anterior and posterior adductor muscles and a well-developed foot. Juveniles could also be described as individuals prior to sexual maturity. The age at which mussels reach sexual maturity varies among species and has been reported to range from 2-12 years (Shiver, 2002). Kovitvadhi et al. (2007), however, reported observing 360-day-old *Hyriopsis myersiana* with adult reproductive anatomy containing mature gametes. Juveniles have also been referenced as mussels lacking typical adult feeding structures. However, the point at which adult feeding morphology is fully developed is not well documented and likely varies among species. Some malacologists also ignore any visceral development or stages of sexual maturity and simply refer to mussels that are smaller than a typical adult as juveniles. While the
onset of the juvenile stage seems to be well defined, the terminus of this developmental stage and subsequent transition into an adult form lacks such definition.

In Vivo vs. In Vitro Culturing

Following the pioneering work of Hudson and Isom (1982), in vitro techniques are now available to take glochidia larvae developing in the demibranchs of adult freshwater mussels through the process of metamorphosis in artificial culture, eliminating the need for a live fish host. However, studies have reported that juvenile mussels reared in vitro were not as robust as those reared in vivo on appropriate host fish. Fisher and Dimock (2002) reported that U. imbecillis reared in vitro had significantly less glycogen at the completion of metamorphosis than individuals reared in vivo. Wright (1995) found that 1-week-old juvenile U. imbecillis cultured in vitro were shorter than those cultured in vivo and that the diameter of the openings of the digestive glands of in vitro individuals was smaller. Thus, in vitro cultured juveniles were limited to digesting smaller and fewer potential food items and were thus at a nutritional disadvantage compared to similar aged individuals that had metamorphosed in vivo (Wright, 1995).

Juvenile Feeding

Attempts have been made to describe the mechanics of juvenile bivalve feeding strategies. Reid et al. (1992) described 4 types of “pedal feeding” in juvenile Tridacna and Patinopecten: (1) locomotory pedal feeding; (2) pedal probe-feeding; (3) pedal sweep-feeding; and (4) interstitial pedal feeding. All of these methods involve using the relatively well developed foot of juvenile bivalves to collect food prior to the full
development of ciliated gills that create the typical posterior inhalant current of filter feeding adults. Yeager et al. (1994) described feeding behaviors of juvenile rainbow mussels, *Villosa iris*, as pedal locomotory when in sediment and pedal sweeping when in the presence of algal cells and no sediment. Beck and Neves (2003) supported the findings of Yeager et al. and went further to state that there was very little difference in the selectivity of particles based on size among juvenile and adult mussels. Pedal sweep feeding has become one of the most cited methods of particle acquisition by juvenile bivalves (Reid et al., 1992; Wright, 1995; Gatenby et al., 1996).

*Villosa iris* has been observed to pedal feed for 140 days post metamorphosis and develop siphons to begin filtering water only after 272 days (Gatenby et al., 1996). Feeding behaviors and the transition between these 2 time frames were not reported. Pedal feeding has been described in the adult bivalves *Corbicula fluminea* and *Mysella bidentata* (Reid et al., 1992) which are relatively small compared to many adult unionids and have thus been compared to the juvenile periods of larger mussels. However, Reid et al. (1992) compared juvenile unionid bivalves of lengths of roughly 250-470 µm to *C. fluminea* which commonly reach lengths of 25 mm (Gottfried and Osborne, 1982), and to *M. bidentata*, which is closer in size to recently metamorphosed juvenile unionids, but still 3 mm long (Passos et al., 2005) and thus 10-fold larger than post-metamorphosis unionids.

Due to the differences in size and the concept of the Reynolds number, small animals such as newly metamorphosed juvenile unionids have an inertial relationship with water that differs markedly from that of *C. fluminea* or *M. bidentata*. This
difference may warrant different feeding regimes. Nichols et al. (2005) reported that as adults many species of both large and small bivalves utilize the foot for particle capture.

Kovitvadhi et al. (2006) reported that juvenile *H. myersiana* (0- to 40-days-old) utilized not only the ciliated foot, but also cilia on the mantle and gills to transport phytoplankton to the mouth for ingestion. They rejected the term “pedal feeding” and proposed replacing it with “pedal-mantle-gill-feeding” (Kovitvadhi et al., 2006), a mode of feeding that has not been reported for other species. Thus, while the inhalant siphon may be the primary portal for particle procurement via a flow of water originating from the beat of lateral cilia on gill filaments in adult mussels, there may be other avenues for particle acquisition among younger age classes of bivalve species. Furthermore, there are quite possibly differences among particle uptake strategies among species that could fill unique feeding niches in a given habitat occupied by multiple species of bivalves.

**Bivalve Gill Development**

Beninger et al. (1994) described the development of the gill of the scallop *Pecten maximus* (Bivalvia: Pectinidae) through the first 58 days post metamorphosis. Four stages of gill organogenesis were reported that directly related to shell length (reported in parentheses): (1) gill buds (240 µm); (2) homorhabdic unreflected gill filaments (250-900 µm); (3) homorhabdic reflected filaments (900-4000 µm), and (4) heterorhabdic gill (>4000 µm). The successive stages of gill development were increasingly effective at particle capture; however it was reported that until the development of adult heterorhabdic gills, potential food particles were not captured at a rate necessary for growth and survival. Thus, it was hypothesized that juvenile scallops may rely on stored
nutritional reserves, absorption of dissolved organic matter, or alternative particle capture mechanisms such as pedal probe feeding, to obtain essential nutrients during the stages of adult gill development (Beninger et al., 1994).

Early development of the heterorhabdic gills of the Pectinidae may share some similarities with the development of the eulamellibranch gills of the Unionidae. The hypothesis concerning alternative methods of nutrient acquisition is supported in a study of the unionid Anodontia cygnea in the first 15 days post metamorphosis (Lima et al., 2006) in that the gill structure of A. cygnea was not yet capable of efficient particle capture. There may be common characteristics of bivalve gill development among various taxa until a point at which differentiation occurs leading to the various gill morphologies seen in the adult stages.

Lasee (1991) described the morphological changes in feeding and digestive structures of juvenile Lampsilis ventricosa (Unionidae) from encapsulation on host fish through 56 days post release from the host. Description of gill organogenesis was limited to the number of filaments observed and cilia patterns on gill filaments. One interesting difference from the scallop gill development described by Beninger et al. (1994) is that juvenile Lampsilis ventricosa had both frontal cilia and laterofrontal cirri (Lasee, 1991), whereas similarly aged Pecten maximus had neither of these types of cilia but developed them later in life.

Wright (1995) described the development of juvenile U. imbecillis feeding morphology from immediately post metamorphosis to 10 days. Similar stages of gill organogenesis were described as those in P. maximus by Beninger (1994), with gill buds and initial filaments forming at similar overall sizes in both species of study (shell length
of ≈340 µm). Ciliary cross connections were described linking opposing filaments, and a suite of frontal and lateral cilia as well as latero-frontal cirri were reported in juvenile *U. imbecillis* that were less than 2-weeks-old (Wright, 1995).

It is a commonly held notion that bivalve gills do not function as effective filtering mechanisms until the point at which gill filaments (and eventually lamellae) reflect by means of folding back on themselves and begin to grow dorsally forming the beginnings of the infra- and suprabranchial chambers (Yonge, 1947; Beninger, 1994). Gill reflection was not described in *U. imbecillis* at 10-days-old (Wright, 1995). There was no mention of gill reflection in the description of juvenile *L. ventricosa* to 56 days post metamorphosis (Lasee, 1991). Herbers (1914) reported that gill reflection occurred in *Anodonta cellensis* (now *A. cygnea*) at 7 weeks post metamorphosis. Lasee (1991) reported a broadening of the distal ends of gill filaments in *L. ventricosa* at 56-days-old (≈890 µm shell length). Beninger (1994) similarly reported the broadening and general inflation of the ventral tips of gill filaments in developing *P. maximus* at 300-900 µm shell length. The gills at this stage also formed a curved “basket” that was hypothesized to be a more efficient particle capturing precursor to the reflected gill than the initial gill buds. Kovitvadhi et al. (2007) found that the development of *H. myersiana* gills up to day 30 displayed the previously described curved nature of the descending lamella of the inner demibranch, which is reported to develop prior to the outer demibranch of *H. myersiana*. At day 50 of development, ascending lamellae are described indicating that gill reflection took place sometime between days 30 and 50 of development of *H. myersiana*. 

The filter feeding behavior of adult bivalves is well documented. Information on gill ciliary structure and function, filtration rate, particle selection and retention, and even the feeding structure and function of marsupial gills, is readily available (Gibbons, 1961; Coughlan, 1969; Kryger and Riisgard, 1988; Tankersley and Dimock, 1993). As reported by Galbraith et al. (2009), different unionid bivalve species differ markedly in feeding morphologies as adults in relation to parameters such as density of gill cilia and total gill surface area. This led to the conclusion that different species, though occurring in close proximity, could occupy different niches in regard to feeding. Thus, it would be advantageous to describe the organogenesis of gill structures in various unionid species to determine if separate feeding niches may occur among juvenile stages. Kovitvadhi et al. (2006, 2007) and Uthaiwan (2001) have made observations of development of feeding structures of *H. myersiana* through 360 days, while Lasee (1991) has reported development in *L. ventricosa* through 56 days, but information regarding feeding in juvenile *U. imbecillis* beyond 10 days post-metamorphosis (Wright, 1995) does not exist.

The aim of the present study was to describe the development of the feeding morphology of juvenile *U. imbecillis* from immediately post metamorphosis to the point at which the juvenile gills resemble those of adults. Though, development through 130 days post metamorphosis was achieved, adult-like gills did not fully develop. In describing the anatomy of juvenile mussels at various stages of development, feeding strategies of the juveniles may be better understood and defined. As a result, efforts in raising imperiled unionid mussels in aquaculture may become more successful in that design of diet and feeding processes can be optimized for a given age class and, with future studies of a variety of species, taxon of mussel.
MATERIALS AND METHODS

Collection and Maintenance of Adult U. imbecillis

Adult mussels (5-8 cm shell length) were collected from Lake Rockingham (Reidsville, North Carolina). Mussels were maintained in the lab in glass aquaria with about 5 cm of sand and approximately 20 L of aerated tap water. Adults were fed daily about 2 ml each of Shellfish Diet (25% Isochrysis, 20% Pavlova, 20% Tetraselmis, 30% Thalassiosira weissflogii, and 5% Nannochloropsis) and 100% Nannochloropsis algal solutions from Reed Mariculture Inc. (San Jose, California). The total volume of water in the tank was replaced weekly with aged (1 week) and aerated tap water.

Culturing and Maintenance of Juvenile U. imbecillis

Juvenile mussels were obtained by making slight modifications to previously described in vitro culture techniques (Hudson and Isom, 1984; Dimock and Wright, 1993; C. Owen, pers. comm.). A sample of glochidia from the outer demibranch of a gravid U. imbecillis was taken from the gill with a Pasteur pipette. Viability of larvae was determined by first observing repeated adduction of glochidial shells, then by the response of larvae to 5 drops of concentrated KCl in a petri dish with 5 ml of aquarium water. If the glochidia rapidly closed their shells and remained closed, the remaining larvae in the parental mussel were assumed to be viable (Fisher and Dimock, 2000).

After selection of a donor gravid adult U. imbecillis, great effort was made to keep instruments, containers, measurement devices, and workspaces as clean and sterile as possible to prevent infection of the subsequent cultures. In addition to autoclaving...
materials and washing workspaces, instruments were rinsed in Zephiran® Chloride germicide and disinfectant.

The gravid adult mussel was dissected by manually forcing the valves to slightly gape and severing both the anterior and posterior adductor muscles. Both outer demibranchs were removed and immediately placed into a volume of 0.2-µm sterile filtered Medium 199 in a sterile 450-ml beaker. The gills were teased apart until all glochidia were removed from the demibranch, and all gill tissue was then carefully removed from the beaker.

The antibiotics Gentamicin, Rifampicin, and Amphotericin B were added to a culture solution of Medium 199 and rabbit serum while being vacuum filtered through a sterile 0.2-µm filter. The volumetric ratio of the mixture of Medium 199, rabbit serum, Gentamicin (150 µg/ml), Rifampicin (50 µg/ml), and Amphotericin B (2 µg/ml) was 75:25:3:3:2.5:1, respectively. Chemicals were from Sigma-Aldrich (St. Louis, Missouri).

Under a sterile hood, ten 60x15 mm petri dishes were each filled with 3.0 ml of the above described filtered culture medium. The extracted and cleaned glochidia were distributed among the 10 dishes at about 200 individuals/dish. The glochidial cultures were kept in a temperature controlled CO2 incubator at 20±1°C with 5% CO2 for 7 days. A 1.0 ml aliquot of the culture medium in each dish was replaced with fresh medium on days 3 and 5 of incubation under sterile conditions.

Culture dishes were removed from the CO2 incubator on day 7 and received 3.0 ml of 0.2-µm filtered and aerated Litcher’s Pond Water (LPW, Lewisville, North Carolina). After 10 min, about 80% of the medium was replaced with filtered LPW while ensuring that the juveniles were immersed in water at all times. Three additional
changes of LPW were performed at 10-min intervals to remove any remaining culture medium. The juveniles were then held in filtered LPW for about 15 hr.

Live and fully metamorphosed juvenile mussels were identified by the presence of anterior and posterior adductor mussels as well as a developed and active foot. Metamorphosed mussels were transferred to a re-circulating flow-through trough system with a 3-mm layer of screened (0.18 mm) Litcher’s Pond silt. The mussels were fed a medley of Shellfish Diet (see above for constituents), *Nannochloropsis*, and live cultured *Neochloris* algae. The system was kept at 21±1°C with a 12:12 hr light:dark cycle. Ninety percent of the volume of water in the system was replaced weekly with 10-µm filtered LPW.

**Microscopy**

Individual juvenile *U. imbecillis* (n=5-30) were collected weekly from the rearing trough apparatus described above and were separated from the sediment either by filtering a volume of sediment through Nitex mesh or by sifting through a small volume of sediment in a glass finger bowl. Mussels were isolated in 6-8 ml of clean 10-µm filtered LPW in a 60-mm petri dish. Individuals were relaxed by slowly adding crystals of MgCl₂ to the dish until the larval valves were open and the larvae failed to respond to gentle agitation. Roughly half of the individuals were then forcibly pried open, tearing the adductor muscles, so as to allow unencumbered optical access to the gill structures, foot, and visceral mass.

All juveniles were fixed in 2.0% glutaraldehyde in 0.05 M Sorensen’s buffer (pH 7.2) for at least 3 hr. They were rinsed twice (10 min per rinse) in 0.05 M Sorensen’s
phosphate buffer (pH 7.0) and then were passed through a graded alcohol series (30%, 50%, and 70%, 10 min each). Samples were stored in 70% ETOH until further preparation and imaging.

The fixed individuals were examined in 70% ETOH with a Leica MZ16 FA stereomicroscope. The numbers of paired gill filaments were counted, and digital images were taken with a QImaging Retiga 4000R camera. The maximum anterior to posterior shell length of each individual was measured from the images using ImageJ software.

Individuals were prepared for scanning electron microscopy by dehydration with 80%, 90%, 95%, and 2x100% ETOH. They were then dried via 1 of 2 methods. Some individuals were prepared with a conventional Tousimis Samdri-PVT-3D critical point drier. Other samples were dehydrated by immersion in Hexamethyldisilazane (HMDS) as follows: juvenile mussels were placed in a 10-ml glass beaker, and the 100% ETOH was replaced with a sufficient volume of HMDS to cover the specimens. The beaker was sealed with Parafilm and allowed to soak for 30 min with gentle manual agitation at 10 and 20 min. This process was repeated for a second 30 min soak in HMDS. The samples were then placed on filter paper in a Petri dish and air-dried for 30 min. After drying with either the critical point drier or HMDS, the specimens were mounted on aluminum stubs using carbon conductive tabs and were sputter coated with gold using a Cressington Model 108 Sputter Coater. Samples (n=5-10 at each age class) were then viewed using an Amray 1810 scanning electron microscope.
RESULTS

Gills

Post-metamorphic *U. imbecillis* at 3-days-old had 3 paired gill filament structures. The most anterior of these paired structures contained a full suite of lateral and frontal cilia as well as laterofrontal cirri and were considered fully developed filaments (Fig. 1). The posterior 2 pairs of filament structures contained sparse cilia lacking the organization of the anterior-most pair and were considered gill filament buds (Fig. 1). Gill filaments developed from the 2 post-metamorphic buds, and additional filaments budded on the posterior portion of the developing gill lamellae as the mussels increased in length (Figs. 12, 15).

After 7 days of development ciliary cross connections between adjacent filaments as well as between opposite filaments on the right and left developing lamellae (Fig. 2) were evident in specimens that were not pried open during preparation. These ciliary connections remained present in all subsequent age classes (Figs. 4-13). By 18 days post metamorphosis, the ciliary cross connections between adjacent filaments became more substantial, and the tissue of the distal end of the filaments became broadened in an anterior to posterior direction (Fig. 3). The anterior most filaments at 21 days of development were long enough and were oriented to physically reach the area of the developing labial palps. Around 51 days post metamorphosis, the ventral tips of the gill filaments began to bend slightly in a dorsal direction, but no growth of new tissue was observed in this direction. At 62 days post metamorphosis, the newest gill buds (most posterior) on the left and right developing gill lamellae were nearly barren of cilia.
compared to more mature filaments, as was always the case with the 2-3 newest posterior buds, but they were connected from the right to the left via ciliary cross connections with the small number of cilia on their ventral tips (Figs. 4-6). The ciliary cross connections between the developing lamellae on the left and right of the mussel joined the 2 lamellae together and formed a barrier separating the mantle cavity into infrabranchial and suprabranchial chambers (Figs. 5, 6). The ventral side of the barrier was the ciliated surface of the lamellae, and the dorsal side lacked cilia (Fig. 9). This barrier was established as early as 28 days post metamorphosis. By 85 days of development, cilia were present on the medial face of the dorsally folded ventral tips of the developing filaments which took on a relatively more structured lamellar organization (Fig. 7). By 113 days, these folds were more densely ciliated and consisted of interfilamentary gill tissue rather than simply ciliary connections (Figs. 8, 9). Also present at 113 days was an individual thicker compound cilium among normal frontal cilia on the ventral tip of some, but not all of the developing filaments (Figs. 10, 11). These thicker cilia were also observed on the relatively cilia-free posterior-most gill buds at 130 days of age (Fig. 13).

**Mouth/Labial Palps**

As early as 3 days post metamorphosis, cilia were observed around the mouth and initial small folds of labial palp tissue located medially anterior to the base of the foot (Fig. 1). At 21 days, growth of developing labial palp tissue forming around the mouth was more evident in all samples. By 62 days of development, the mouth and folds of tissue surrounding it were heavily ciliated (Fig. 4), but lacked any sort of ridges and grooves as observed in adult labial palps. Around 113 days the tissue folds surrounding
the mouth extended in a posterior direction and reached the anterior-most gill filaments (Fig. 8). At 130 days of development, the labial palps surrounded the heavily ciliated mouth and anterior portion of the foot and began to take on a ridge and groove morphology (Fig. 14).

**Foot**

At 3-days-old the distal portion of the post-metamorphic foot of juvenile *U. imbecillis* had a ring of cilia encircling an area devoid of cilia (Fig. 1). The remaining portion of the foot extending dorsally to the junction with the mantle was not ciliated. At 21-days-old, the ring of cilia expanded to cover a greater portion of the ventral tip of the foot, while the central bare spot remained. A sparse number of individual cilia appeared on the anterior base of the foot at 62 days. By 113 days, the dorsal third of the anterior and lateral portions of the foot was covered in tufts of cilia while the ventral tip remained covered in dense simple cilia (Figs. 8, 14).

**Mantle**

The perimeter of the mantle near the valve margin was lined with cilia in newly metamorphosed individuals (Fig. 1). This perimeter of cilia remained present in individuals for all age classes observed. The remainder of the interior surface of the mantle was devoid of cilia in juvenile mussels immediately post-metamorphosis. At 44-days-old, tufts of cilia began to appear first on the anterior medial surface of the mantle. These tufts, consisting of 20-80 individual cilia, became more numerous as the juveniles developed. At 62 days, the cilia of the mantle margin in the posterior portion of the
mantle increased in an anterior direction at the location of the developing siphons (Figs. 4, 6). At 113 days post metamorphosis, there were thicker compound cilia between the developing buds of opposite gill lamellae around the area of the siphons. By 130 days, compound cilia were present among the tufts of cilia on the inner surface of the mantle (Figs. 12, 14, 15). These thicker ciliary structures consisted of about 10 individual cilia fused together.

Siphons were present in individuals by 175 days post metamorphosis and a total shell length of about 3 mm (Fig. 18). These siphons appeared to be similar to those of adult mussels. However the sample size observed in this age class was small, and observations were performed on live organisms leading to descriptions less accurate than SEM specimen preparation allowed.

Shell Length and Number of Gill Filaments

Immediately post metamorphosis, juvenile *U. imbecillis* reared in an *in vitro* culture system were 320±10 µm long with 2 paired gill buds and 1 pair of gill filaments. Individuals increased in length by the addition of new shell material starting at 3-5 days post metamorphosis. This addition of new shell occurred in anterior, posterior, and ventral directions from the glochidial shell (Fig. 2). Early growth rings were added in a rounded and uniform distribution around all edges of the glochidial shell excluding the dorsal hinge. Around 150 days post metamorphosis at a length of about 2 mm, new shell growth on the dorsal shell margin began to take on a flattened distribution (Fig. 17). This growth pattern marked the beginning of the stage of shell growth at which the umbo was
flush with the hinge line which is a diagnostic shell characteristic of adult *U. imbecillis* (Williams et al., 2008).

Mussels from separate cultures that were grown in identical conditions had growth rates that were significantly different from one another at specific age classes (Fig. 19). Most cultures of *U. imbecillis* did not survive more than 50-70 days post metamorphosis. A small sample of juvenile *U. imbecillis*, however, survived through 280 days. After linear growth from 0-50 days post metamorphosis, relatively little increase in length occurred from 50-100 days. From 100-280 days, the surviving juveniles exhibited exponential growth (Fig. 20).
DISCUSSION

Freshwater unionid mussels serve several important ecosystem functions and are a key component to the diversity of many lentic and lotic freshwater habitats. Unfortunately unionid mussels are one of the most endangered taxa worldwide. There is currently an effort to culture threatened mussels in the laboratory in hopes of reestablishing dwindling populations in natural habitats. In light of current efforts to rear freshwater mussels in aquaculture, it has become increasingly important to understand the development of the feeding morphology of post-metamorphosis juveniles. An understanding of this stage of development will allow optimal feeding regimes and culture environments to be utilized in such efforts leading to higher percent survival and more rapid growth of cultured mussels.

In comparison to the quantity of published research on the glochidial stage of unionid reproduction and development, research with the juvenile life stage is limited. Most of the information available in regard to juvenile unionid morphological development is limited to within the first 2-3 weeks (Tucker, 1927; Wright, 1995; Uthaiwan et al., 2001; Lima et al., 2006) to the first 56 days post-metamorphosis (Lasee, 1991). However, Kovitvadhi et al. (2007) succeeded in describing the development of *Hyriopsis myersiana* through 360 days of development. This description of *H. myersiana* serves both for comparison and contrast in the current study describing the development of feeding structures in juvenile *Utterbackia imbecillis* through 130 days post metamorphosis.
**Gills**

Although post metamorphic *U. imbecillis* begin their juvenile life with 2 pairs of gill buds and 1 pair of bona fide gill filaments with a full suite of lateral and frontal cilia as well as laterofrontal cirri, the development of gills in *U. imbecillis* occurs much more slowly than in *H. myersiana* (Kovitvadhi et al., 2007). That mussel is reported to have had 1 pair of gill buds post-metamorphosis, but quickly adds filaments to the developing lamellae of the inner demibranchs. Within 50 days at a total shell length of about 2.1 mm, *H. myersiana* begins the formation of the outer demibranch (Kovitvadhi et al., 2007). In the present study at 50 days post-metamorphosis, *U. imbecillis* were only about 0.8 mm long, and even at 130 days post metamorphosis had not shown any signs of developing an outer demibranch.

Gill reflection began in *U. imbecillis* at about 113 days at which point the developing lamellae consist of about 15 filaments. These data are consistent with the report of Kaestner (1967) that gill reflection occurs in *Anodonta* sp. when the number of gill filaments reaches 18. If gill buds were included in my count, then reflection in *U. imbecillis* would have occurred also with 18 filaments. Unfortunately, Kaestner did not provide shell length data. However, in the current study, the number of gill filaments was more closely related to total length than age of individual *U. imbecillis*. Thus, it could be assumed that Kaestner’s (1967) reported development of *Anodonta* was on par with that observed in the present study, where individuals were about 1.3 mm long at the onset of gill reflection.

Hudson and Isom (1984) reported 74-day-old *Anodonta (Utterbackia) imbecillis* at 5.1 mm with “at least” 23 paired gill filaments. This complicates the hypothesis that
gill development is more closely related to length than age, since some juveniles in the current study had 23 paired filaments at only 2.1 mm and 158 days. However, Hudson and Isom (1984) provided no detail about measurement techniques. In using simple light microscopy, as evidenced by images in their publication, it is possible that some gill filaments and buds could have been overlooked.

The gill lamellae on the 2 sides of *U. imbecillus* connect to one another at an early stage of development via ciliary cross connections (Figs. 2, 3). This connection effectively separates a juvenile infrabranchial chamber from a juvenile suprabranchial chamber. The cilia on the ventral fold of tissue on these filaments, present in individuals around 113 days, may serve as a preliminary food groove directing captured particles toward the labial palps and mouth. Thus, it may be possible for developing juveniles to capture particles effectively with gills that have not developed the ascending lamellae of the inner demibranchs. Such suspension feeding could augment or even replace pedal feeding.

*Labial Palps*

The ciliated labial palps of juvenile *U. imbecillus* begin to form early in development and most likely create increased movement of water and potential food toward the heavily ciliated mouth (Figs. 1, 4). The labial palps of adult mussels have a complex series of ciliated ridges and grooves, but lack laterofrontal cirri (Tankersley, 1996). Apparently, these ridges and grooves form as the palps enlarge and project increasingly posteriorly, eventually contacting the anterior filaments of the developing gills (Fig. 8). The ciliary tufts that develop on the base of the foot were in close
proximity to the junction of the labial palps and the most anterior gill filaments (Figs. 8, 14). These tufts may aid in the transfer of particles from the preliminary food groove around the foot to the labial palps and mouth.

*Circulation of Water in the Mantle Cavity*

The foot of juvenile *U. imbecillis* is relatively well developed and has dense ciliation immediately post metamorphosis. This structure is clearly useful to the juvenile for locomotion, which in young mussels involves very active gross extension and contraction of the foot. Pedal feeding hypotheses cite the foot as the primary method of particle transport into the mantle cavity (Reid et al., 1992). As observed in live juveniles, the flow of water through the mantle cavity is initially unidirectional from the anterior to the posterior. It is unclear whether or not this flow is generated by cilia of the foot, gills, mantle, labial palps, or mouth. This preliminary unidirectional flow may result from a combination of the beating cilia of all of these structures due to the lack of lateral cilia on gill filaments in newly metamorphosed animals. However, by 178 days, after the development of the inhalant and exhalant siphons, the flow of water into and out of the mantle cavity mirrors the bidirectional posterior ventral to dorsal flow of adults. At this stage of development, a significant amount of lateral cilia may have formed on the filaments of the gill lamellae so that the flow of water into and out of the mantle cavity would be primarily a result of the lateral cilia rather than the cilia of the mouth, palps, and mantle.
**Growth Rate**

Comparing growth rates of mussels among various studies poses many challenges. There are clearly temporal differences in organogenesis and overall growth rates of various taxa of bivalves. In comparing 2 species of unionids, by 160 days, *H. myersiana* were about 24 mm long (Kovitvadhi, 2007), while *U. imbecillis* at 160 days in the present study were 2.1 mm. Growth rates of mussel cultures within the same genus and species differ as well (Fig. 21). Differences in growth rates may be due to varying abiotic factors such as temperature, water chemistry, and the nature of the sediment, or lack thereof, in a culture facility or laboratory. Differences in growth rates may also be a result of unique diets available to the juvenile mussels in different culture systems. In the current study, however, significantly different growth rates were observed in separate cultures of *U. imbecillis* in which identical culture techniques were practiced (Fig. 19). The season or state of glochidial maturity at the point of removal from the parental mussel as well as the general condition of the brood stock may have had a significant effect on the growth of the subsequent juveniles.

In the cultures described above with significantly different growth rates, the numbers of gill filaments in mussels of similar lengths were not significantly different (data not shown). Thus, the development of feeding anatomy of the mussels was a result of increases in overall size of the animals rather than strictly a function of age. Though certain rearing techniques may keep mussels alive, organismal development is likely retarded in some instances as a result of less than ideal abiotic and biotic culture conditions. This is a significant challenge in comparing studies concerning feeding
behaviors of juvenile mussels among cultures of individual species as well as various bivalve taxa.

Microscopy Techniques

To accurately describe the development of feeding morphology in juvenile mussels, scanning electron microscopy was a valuable tool. During sample preparation it was important to preserve the ciliary structures of the animals including the connections between opposite gill filaments as observed in live specimens. The 2 different techniques utilized for drying produced samples of differing quality. Samples prepared in HMDS (Fig. 4) suffered less physical damage than those dried in the critical point drier (Fig. 7). The process of drying with HMDS did not require as much physical manipulation and transfer of specimens from container to container and container to mounting stub as compared to conventional critical point drying and thus resulted in less physical damage.

The current project describes the morphological development of feeding structures and growth rates of juvenile *U. imbecillis*. If feeding niches based on characteristics such as particle size selection exist in adult freshwater bivalves (Galbraith et al., 2009), similar niches may exist in juveniles due to the development of gill structures that allow for particle sorting prior to the development of complete adult gills. By providing more knowledge concerning the feeding structures and growth rates of juvenile unionid mussels, more effective feeding regimes and conservation strategies may be implemented in conservation efforts of what is one of the most endangered taxa of organisms in the world (Neves and Widlak, 1987; Lasee, 1991; Lydeard et. al., 2004).
Fig. 1: Scanning electron micrograph (SEM) of ventral view of 3-day-old *Utterbackia imbecillis*. Anterior is to the right. Present are the glochidial shell (GS), gill buds (GB), gill filament (GF), foot (F), and labial palps (LP) obscuring the view of the mouth. Arrows indicate ciliated mantle margins of the otherwise cilia-free mantle.

Fig. 2: SEM of 7-day-old *U. imbecillis* showing the foot (F), glochidial shell (GS), new shell growth (NS), and the ciliated mantle margin (CMM). Arrows indicate ciliary cross connections both between adjacent gill buds and filaments and between developing gill buds on opposite sides of the visceral mass. Anterior is to the right.
Fig. 3: SEM of posterior view of gills of 18-day-old *U. imbecillis*. Indicated are the foot (F), new shell (NS), outer surface of the mantle (M) with shell removed, and the mantle margin cilia (MMC). On the developing gills are the lateral cilia (LC), laterofrontal cirri (LFC), frontal cilia (FC), and ciliary cross connections (CC) joining opposite gill filaments.

Fig. 4: SEM of 62-day-old juvenile *U. imbecillis*. Glochidial shell (GS) with intact hooks and new shell (NS) with irregular ridges on posterior are labeled along with the foot (F). Ciliary connections between developing right and left gill (G) lamellae and between adjacent gill filaments were present. The mantle (M) remains ciliated at the margin and shows a medial growth in the posterior region (near the label, M) in the location of the developing siphons.
Fig. 5: SEM of posterior view of 62-day-old *U. imbecillis* showing the glochidial shell (GS) and pitted new shell (NS) growth. Ciliary cross connections exist between the right and left developing gill lamellae forming an oval shaped orifice.

Fig. 6: Close up of Fig. 5, SEM of posterior view of 62-day-old *U. imbecillis* showing new shell (NS) growth, the ciliated mantle (M), posterior base of the foot (F), ciliary cross connections between opposite developing lamellae, and the ciliated anus (A) located on the dorsal portion of the mantle posterior to the foot.
Fig. 7: SEM of 85-day-old *U. imbecillis*. Anterior is to the left. The glochidial shell is visible at the top of the image with the remnant of the glochidial hook still present. The ciliary tufts on the mantle as well as the interfilamentous ciliary connections forming lamellae were present. The tears in the mantle in the top half of the mussel are a result of sample preparation with conventional critical point drying.

Fig. 8: SEM of visceral mass of 113-day-old *U. imbecillis*. Posterior is to the bottom, left of the image. Shell material was broken off in this preparation, but intact glochidial shell (GS) is visible in the upper left corner of the frame. The outer, or shell side, (top left M) and medial side (bottom right M) of the mantle are visible. The ciliated labial palp (LP) is sufficiently large to make contact with the anterior-most gill filaments. Arrows indicate ciliary tufts on the foot (F). The ciliated ventral fold (VF) of the gill lamella is visible posterior to the foot.
Fig. 9: Close-up of SEM of posterior gill filaments of 113-day-old *U. imbecillis* as shown in Fig. 8. Posterior is to the bottom, left of the image. The foot (F) with a posterior ciliary tuft and the mantle (M) are labeled. The ventral fold (VF) of the gill lamella is covered with simple cilia. The medial surfaces of the filaments of the lamella lack cilia.

Fig. 10: SEM of posterior gill of 113-day-old *U. imbecillis*. The three posterior-most (left of the frame) gill structures are gill buds (GB). The fourth and fifth filaments contain the full suite of lateral cilia (LC), frontal cilia (FC), and laterofrontal cirri (LFC). A compound cilium at the ventral portion of the first developed filament is indicated by an arrow. There is a large amount of cilia on the ventral portion (top of the frame) of the developing gill buds. The ciliated mantle (M) is in the foreground, out of focus.
Fig. 11: SEM of ventral portion of gill filaments of 113-day-old *U. imbecillis*. Dorsal is to the bottom left of the frame. These filaments have lateral cilia (LC), laterofrontal cirri (LFC), and frontal cilia (not labeled). Arrows indicate the compound cilia found on the lateroventral tip of the filaments. Simple cilia cover the medial ventral fold of the developing lamella.

Fig. 12: SEM of 130-day-old *U. imbecillis*. Anterior is to the right of the frame. The broken valve is due to prying open the specimen in preparation. Interfilamentary tissue connections form structured gill lamellae. Numerous ciliary tufts cover the mantle especially in the anterior of the mussel.
Fig. 13: SEM of posterior gill and mantle (M) of 130-day-old *U. imbecillis* as shown in Fig. 12. Posterior is to the left. The three posterior-most gill units are gill buds (GB) showing increasing degrees of development with laterofrontal cirri (LFC) on the anterior portion of the anterior-most bud. The fourth-most posterior unit of gill tissue is a developed gill filament (GF) with lateral cilia (LC) and frontal cilia (FC). This filament as well as the most developed gill bud each have a lateroventral compound cilium indicated by the arrows.

Fig. 14: SEM of region anterior to the foot of 130-day-old *U. imbecillis* as shown in Fig. 12. The heavily ciliated mouth (MO) is located medially anterior to the foot (F) which is covered in ciliary tufts. The labial palp (LP) laterally surrounds the anterior portion of the foot as well as the mouth. The mantle (M) has tufts of cilia and is torn in the bottom of the frame due to specimen preparation.
Fig. 15: SEM of mantle of 130-day-old *U. imbecillis* as shown in Fig. 12. The several compound cilia in the left of the frame appear thicker than the individual, simple cilia of the ciliary tufts.

Fig. 16: SEM of the mantle of 130-day-old *U. imbecillis* as shown in Fig. 14. Among the ciliary tufts are compound cilia as well as what appear to be open pores, however they may be sites of missing cilia.
Fig. 17: Image of 158-day-old *U. imbecillis* fixed in glutaraldehyde and stored in 70% ETOH. On either side of the small, rounded glochidial shell (GS) is early rounded shell growth, and later growth (distal to glochidial shell) is flattened. The gill (G) is ventral to the posterior adductor muscle (PA) and the dark, tubular intestine (IN) that empties into the mantle cavity dorsal to the gill. The foot (F) is fixed in the anterior portion of the mantle cavity ventral to the anterior adductor muscle (AA).

Fig. 18: Image of live 200-day-old *U. imbecillis* showing the inhalant siphon (IS) and exhalant siphon (ES) extended from the posterior valve gape. The inhalant siphon has papillae and directs water into the infrabranchial chamber, ventral to the gills (G). The exhalant siphon lacks papillae and directs water out of the suprabranchial chamber, dorsal to the gills. Note the intestine (IN) emptying contents into the suprabranchial chamber.
Fig. 19: The growth of two separately reared cultures of *U. imbecillis* from 10-56 days post metamorphosis. Significantly different linear regressions are shown. Regression ANOVA resulted in a single $R^2=0.993$ and an intercept $P=5.16E^{-4}$. $n=5-30$ individuals for each data point.

Fig. 20: The growth of *U. imbecillis* from 7-280 days post metamorphosis. $n=5-30$ individuals for each data point with error bars displaying standard deviation. The last three points, 200, 223, and 280 days are represented by 4, 3, and 1 individual, respectively. Exponential regression is shown with $R^2=0.956$. 
Fig. 21: Lengths of juvenile *Anodonta* and *Utterbackia* species from multiple studies (Herber, 1913 [*Anodonta cellensis*], Kaestner, 1967 [*Anodonta sp.*], Hudson and Isom, 1984 [*Anodonta (Utterbackia) imbecillis*], Wright, 1995 [*Utterbackia imbecillis*], and Trump, 2010 [*U. imbecillis*]). *Utterbackia* and *Pyganodon* were elevated from subgenera within *Anodonta* to generic status (Hoeh, 1990). Thus early studies of *Utterbackia imbecillis* referred to the species as *Anodonta imbecillis*. These various studies reported similar sized mussels through 25-30 days post metamorphosis with varying growth rates thereafter. There was a significant delay in growth greater than 1 mm in the Trump (2010) study versus the Herbers (1914) and Hudson and Isom (1984) studies.
CONCLUSIONS

This study described the previously undocumented development of the feeding morphology of juvenile *U. imbecillis* from immediately post metamorphosis to 130-days-old. In so doing, it has highlighted aspects of a stage of development that could be important in aquaculture efforts to rear freshwater mussels.

Mussels grew to a length of about 1.4 mm in 130 days post metamorphosis. Growth rates differed significantly among cultures reared in identical conditions. Overall rates of growth also differed substantially from those of other published studies of the same species. It is clear that differences in culture and husbandry techniques result in markedly different growth rates.

The gills of 130-day-old mussels do not resemble fully adult eulamellibranch gill morphology but instead consist only of the descending lamellae of the inner demibranchs. The ventral edge of each developed lamella at 130 days has a ciliated fold of tissue that may serve as a preliminary food groove transporting particles to the mouth. Ciliary cross connections link gill lamellae on opposite sides of the visceral mass. This connection effectively separates the mantle cavity into an infrabranchial and a suprabranchial chamber. The separation of the mantle cavity and development of a preliminary food groove may allow juvenile mussels to filter feed prior to development of the ascending lamellae and the outer demibranch. Early development of this morphology may enable juvenile *U. imbecillis* to capture and perhaps efficiently sort particles much earlier than previously thought. This filter or suspension feeding could augment the described behavior of deposit feeding through pedal sweeping.
RECOMMENDED FUTURE RESEARCH

The post-metamorphic juvenile stage of freshwater mussels remains a too little investigated area of molluscan research. Many future directions and topics of research have arisen from the present study. These include:

1) Describing the morphological development of juvenile *U. imbecillis* through the formation of the typical adult eulamellibranch anatomy.

2) Comparisons of growth rates and morphological development between laboratories and aquaculture facilities rearing identical species of juvenile freshwater mussels.

3) Describing the development of juvenile feeding morphologies in a variety of bivalve species in order to determine if there may be differences among species leading to distinct feeding niches in the juvenile stage.

4) Documenting particle selection and ingestion among various age and size classes of juvenile mussels.

5) Live imaging of juveniles tracing the path of water and particles into and out of the mantle cavity at various developmental stages, and the entrapment and ultimate ingestion of some fraction of those particles.
LITERATURE CITED


SCHOLASTIC VITA

Matthew P. Trump

BORN: August 19, 1984, York, Pennsylvania

UNDERGRADUATE STUDY:
Wake Forest University
Winston-Salem, North Carolina
B.S., Biology, 2005

GRADUATE STUDY:
Wake Forest University
Winston-Salem, North Carolina
M.S., Biology, 2010
Thesis title: Feeding morphology of juvenile Utterbackia imbecillis (Bivalvia: Unionidae)

SCHOLASTIC AND PROFESSIONAL EXPERIENCE

Undergraduate Researcher, Wake Forest University, 2004-2005

Educational Product Development, Carolina Biological Supply Company, 2006

Environmental Educator, Chesapeake Bay Foundation, 2006-2008

Graduate Teaching Assistant, Wake Forest University, 2008-2010

PROFESSIONAL ORGANIZATIONS

Association of Southeastern Biologists, 2010

PRESENTATIONS


Trump, M.P. 2010. Feeding morphology of juvenile Utterbackia imbecillis. Departmental Seminar, Department of Biology, Wake Forest University.