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Mud and Mucus: Feeding Selectivity in a Suspension-Feeding Detritivorous Fish

Marie Louise Lammons
College of William & Mary - Arts & Sciences

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Mud and Mucus: Feeding Selectivity in a Suspension-Feeding Detritivorous Fish

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A Thesis presented to the Graduate Faculty
of the College of William and Mary in Candidacy for the Degree of
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Master of Science

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Aquatic detritivores, organisms that feed on dead and decaying organic matter, may be limited in body size by the relatively low nutrient content of their diet (Mundahl and Wissing 1987). Few studies have quantified whether detritivorous fish select for food particles that are high in nutrient content, and the fluid-dynamic and behavioral mechanisms that might enable such selectivity are unclear. The purpose of this study is to quantify internal, fluid-dynamic feeding selectivity based on nutrient content in the gizzard shad (Dorosoma cepedianum, Clupeidae), a detritivorous fish species native to Virginia waters. The detritus they consume is composed of small particles that are not engulfed individually. However, recent evidence suggests that these suspension-feeding may selectively ingest more nutrient-rich particles. By comparing the nutrient (carbon and nitrogen) content of suspended food particles available to gizzard shad in controlled laboratory experiments with the nutrient content of ingested food in the foregut, feeding selection can be quantified. Previous studies of nutrient-based feeding selectivity in detritivorous fish did not account for the possibility that secreted mucus may contribute to the nutrients quantified in fish foreguts. Thus, another objective of this study is to determine whether mucus associated with the interior of the mouth and foregut of gizzard shad accounts for a significant portion of the nutrients quantified in the ingested food. Nutrients derived from fish-secreted mucus may represent 40% of the nutrients quantified in gizzard shad foreguts. Understanding and quantifying feeding selectivity in gizzard shad is important, because detritivorous fish are a key link between benthic sediment and pelagic processes. This benthic-pelagic coupling influences economically important fisheries, food webs and nutrient cycling.
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CHAPTER 1: OVERVIEW OF SUSPENSION-FEEDING AND PARTICLE SELECTIVITY IN FISH

SUSPENSION FEEDING

Suspension (or filter) feeding is a widespread feeding strategy in which organisms filter water to capture small suspended particles such as phytoplankton (Sanderson and Wassersug 1993). Common among freshwater and marine invertebrate species, the morphology and physiology of suspension feeding in these groups has been well described (e.g., Jorgensen 1966, Vanderploeg 1990, Wotton 1990). However, the mechanisms explaining suspension feeding in vertebrates, a group including many whales, birds, tadpoles and fish, are less well understood (Sanderson and Wassersug 1993). Suspension-feeding fish belong to 21 families in 12 orders (Cheer et al. 2001), and are an important component of freshwater ecosystems, affecting their structure, function, species composition, and nutrient cycling (Northcote 1988, Vanni 2002).

While particulate-feeding fish visually select individual prey items, suspension-feeding fish do not seem to select prey visually (Gerking 1994). Instead, they engulf water containing multiple suspended food particles (Gerking 1994, Garrido et al. 2007). Suspension-feeding fish consume phytoplankton, zooplankton or detritus, retaining particles too small to be sensed and engulfed individually as water flows past their feeding structures. They filter prey between 5–3000μm from the large volumes of water that enter the mouth and exit the opercula (Sanderson and Wassersug 1993). Suspension feeding may include capturing particles already suspended in the water column, as well as benthic particles which become suspended in water by some action of the organism (Sanderson and Wassersug 1993). By feeding at low trophic levels, these fish are capable of accumulating substantial biomass; they may accumulate a large standing stock (e.g., clupeid species such as herring) or a large body size (e.g., basking and whale sharks) (Sanderson
and Wassersug 1993). To capture these prey particles, suspension-feeding fish employ two major feeding strategies: ram and pump suspension feeding.

**Modes of Suspension Feeding**

There are two predominant modes of suspension feeding in fish. Ram suspension feeders capture particles by swimming with their mouth and opercula open, so that water flows through the oropharyngeal cavity and exits through the opercular openings (Gerking 1994).

Pump suspension feeders (also called intermittent suction feeders) collect particles by using a rapid, aperiodic series of suctions not directed at specific particles (Drenner et al. 1982b, Gerking 1994). Sanderson et al. (2001) characterized the pattern of pumping for suspension-feeding Nile tilapia (*Oreochromis niloticus*, Cichlidae), and observed 30-40 second bouts of feeding interrupted by 5 second intervals of movements resembling prey processing. During feeding bouts, 2—3 pumps at a rate of 3 pumps s$^{-1}$ were followed by a flow reversal inside the mouth of these fish; after the reversal, the fish began pumping again (Sanderson et al. 1996). The pattern of pumping and pump rates are variable between different species of suspension-feeding fish. Another example of pump suspension-feeding fish, adult (16 cm SL) gizzard shad (*Dorosoma cepedianum*, Clupeidae), pump water at a rate of approximately 2.6 pumps s$^{-1}$ during feeding bouts; these feeding bouts are interrupted by “swallowing” movements during which the mouth is closed (Drenner et al. 1982b). During pumping bouts, water containing suspended particles enters the oropharyngeal cavity of suspension-feeding fish, where specialized structures filter food particles out of the suspended slurry for consumption.
Mouth Morphology

The oropharyngeal cavity of suspension-feeding fish contains specialized structures that are involved in the processing of suspended particles. These structures include the branchial arches and gill rakers. In teleost species, four or five paired branchial arches on the left and right sides of the head are moved back and forth by longitudinal muscles (Gerking 1994). The four anterior branchial arches in paddlefish and teleost suspension feeders each contain one or two rows of gill rakers, and the fifth arch typically contains one row of rakers (Sanderson and Wassersug 1993). Gill rakers have been shown to participate in the filtration mechanism used by fish to capture suspended particles by forming the filter surface and controlling fluid flow inside the oropharyngeal cavity (e.g., Sanderson et al. 2001, Smith and Sanderson 2008).

Gill rakers are comb-like, bony or cartilaginous structures muscularized at their attachment to the branchial arches (Sanderson and Wassersug 1993, Gerking 1994). The epithelium covering gill rakers may contain mucous cells (e.g., goblet cells), gustatory receptors or taste buds, and cuticle-secreting cells (Friedland 1985, Sibbing and Uribe 1985). In some species, the gill rakers have rows of spiny processes that may decrease the gap size between gill rakers (Gibson 1988). For example, in herring (Clupea harengus, Clupeidae), these processes may account for 2 percent of the total filtration area (Gibson 1988). Gill rakers form the mesh of the filter that fish use to capture suspended particles, and the gap size between gill rakers determines the filter mesh size. The orientation of gill rakers and raker processes may alter the sizes of the gaps between gill rakers, and as fish grow larger the length of gill rakers and the inter-raker gap sizes also increase (Gibson 1988). Fish may alter the position and orientation of branchial arches and gill rakers during suspension feeding, as well as the extent to which the
mouth is open (Gibson 1988). The function of gill rakers is contingent upon the filtering mechanisms each species employs, yet the extent to which gill rakers influence fluid-dynamic processes inside the oropharynx of suspension-feeding fish remains unclear.

**Filtration Mechanisms**

The fluid-dynamic mechanisms of vertebrate suspension feeding are not well described. Traditionally, biologists assumed that the filters in the oropharyngeal cavity of fish function as (1) dead-end sieves or (2) hydrosol filters with a sticky surface (Rubenstein and Koehl 1977, Sanderson and Wassersug 1993, Brainerd 2001). However, recent evidence suggests that some species utilize crossflow filtration (Sanderson et al. 2001).

**Dead-end Sieving**

In the dead-end sieve model for filtration, particles are forced against the filter surface by water flow perpendicular to the filter surface and either pass through pores in the filter mesh or are retained on the surface. Dead-end sieves trap particles too large to pass through the filter pores, while allowing filtrate and particles small enough to slip through the sieve mesh to be rejected (Rubenstein and Koehl 1977). Theoretically, a sieve with evenly spaced, uniform filter elements would retain every particle larger than the gap size and no smaller particles (Rubenstein and Koehl 1977). A problem associated with the dead-end sieve model is that particles retained on the gill rakers may clog the filter; however, in hydrosol and crossflow filtration particles, the filter elements are less prone to this problem (Brainerd 2001).
**Hydrosol Filtration**

During hydrosol filtration, particles brought into contact with the filtering elements by fluid mechanical processes and particle interactions may adhere to the filter surfaces (Rubenstein and Koehl 1977). In suspension-feeding fish, particles otherwise small enough to fit through the filter pores may adhere to sticky mucus (Northcott and Beveridge 1988, Sanderson *et al.* 1996). Therefore, hydrosol filtration allows suspension-feeding fish to trap particles too small to be retained by a non-adhesive, dead-end sieve.

Endoscopic video recordings of the interior of the oropharyngeal cavity of feeding Nile tilapia (*Oreochromis niloticus*, Cichlidae), a pump suspension-feeding fish species, revealed that particles impacting the filter surface became trapped in mucus (Sanderson *et al.* 1996). These mucus-bound particles then moved posteriorly in the oropharynx by either sliding along the arch surfaces or lifting off the surface of gill arches and traveling to the posterior of the oropharynx (Sanderson *et al.* 1996).

*O. niloticus* may alter mucus secretion in response to the particle size of available foods. While larger particles (3 mm or more in diameter) did not appear to stimulate mucus secretion, smaller particles (0.1-1.0 mm in diameter) did elicit the secretion of mucus, which then formed aggregates of mucus-bound particles on the gill arches (Sanderson *et al.* 1996). When *O. niloticus* fed on these smaller particles, Sanderson *et al.* observed mucus 97.9% of the time during feeding, while mucus was observed only 4.0% of the time during feeding on larger particles which might be captured by simple sieving alone (Sanderson *et al.* 1996).
Control over mucus secretion could play a role in feeding selectivity. Mucus entrapment of particles is common in both vertebrate and invertebrate suspension feeders, including other fish species (review in Sanderson and Wassersug 1993). In suspension-feeding fish, mucus entrapment may prevent particles from clogging filter pores. Crossflow filtration is another mechanism for suspension feeding in fish which may avoid the issues associated with particles blocking filter elements.

**Crossflow Filtration**

Some suspension-feeding fish capture prey using crossflow filtration, during which suspended particles are engulfed and travel parallel to the filter surface (Brainerd 2001, Sanderson et al. 2001). During crossflow filtration, mainstream flow (crossflow) transports particles posteriorly in the oropharyngeal cavity, while filtrate flow turns from the mainstream and exits between the filter elements (Sanderson et al. 2001). As particles travel posteriorly, they become more concentrated as the mainstream flow tends to remove particles from the area immediately adjacent to the filter surface and filtrate exits through filter pores (Brainerd 2001, Sanderson et al. 2001). Since particles are increasingly concentrated as they travel posteriorly in the oropharyngeal cavity, fish swallow very little water with their food (Brainerd 2001, Sanderson et al. 2001).

Because particles do not come into contact with the filter elements during crossflow filtration, but remain suspended, they do not clog the filter elements (Brainerd 2001). Endoscopic video of suspension-feeding gizzard shad (*Dorosoma cepedianum*, Clupeidae), goldfish (*Carassius auratus*, Cyprinidae) and ngege tilapia (*Oreochromis esculentus*, Cichlidae) showed that particles moved independently of one another and were not trapped in mucus, as
they were in *O. niloticus* (Sanderson et al. 1996, Goodrich et al. 2000, Sanderson et al. 2001). More than 95% of the particles observed by Sanderson et al. (2001) did not come into contact with any oropharyngeal surfaces, including the gill rakers, during crossflow filtration.

The exact fluid-dynamic forces maintaining particle suspension during crossflow filtration are unclear. Inertial lift may be one of the fluid dynamic forces that reduces the particle transport through the filter pores and concentrates particles in the crossflow. Inertial lift is the lateral migration of particles away from the filter surface as walls created by the filter elements result in a lift on the particles (Sethi and Wiesner 1997). Particles do not exit through filter pores or become trapped on the filter elements if the velocity of inertial lift is enough to compensate for the velocity of filtrate flow (Belfort et al. 1994). When modeled by Sanderson et al. (2001), inertial lift was at least an order of magnitude too low to adequately explain the lack of particle contact with gill rakers during crossflow filtration. Other fluid-dynamic processes may act concurrently with inertial lift to limit contact of particles with the filter surface.

During crossflow filtration in some species, mucus may be present on the gill arches and rakers but does not appear to trap particles (Sanderson et al. 2001, Callan and Sanderson 2003). Mucus present on the surface of gill rakers and arches during crossflow filtration may function to control water loss between filter elements, thereby increasing the speed of the mainstream flow (crossflow) and the inertial lift that may maintain particles in suspension (Sanderson et al. 2001, Smith and Sanderson 2007). Additionally, mucus may be used to aggregate food particles in the posterior pharynx (Callan and Sanderson 2003).

When suspension feeding, fish can adjust the patterns and velocity of flow within the oral cavity by altering oral gape (the openness of the mouth) and gill arch position (Sanderson et
suggesting that some kind of internal particle selection mechanism based on fluid
dynamics may exist.

**Particle Selectivity**

Since multiple particles are engulfed during each feeding bout and particles are not
selected individually during suspension feeding, the assumption has been made that
suspension-feeding organisms feed non-selectively (Jorgensen 1966, Sanderson and Wassersug
1993). Recent data indicate that suspension-feeding can be a selective process, but the
mechanisms of particle selectivity in fish are unknown (Higgins *et al.* 2006). Selective feeding, or
the preferential ingestion of certain prey items, may be based on several particle characteristics
such as size, density and nutrient content. Mechanisms allowing for feeding selectivity based on
particle characteristics may include (1) fluid-dynamic processes associated with feeding
(internal) and (2) fish behavior (external).

**Particle Characteristics**

The particles consumed by suspension-feeding fish may differ in a variety of physical or
chemical characteristics which may facilitate particle selection based on fluid-dynamic processes
or fish behavior. These characteristics include particle size, density and nutrient content.

Size-selective suspension feeding has been observed for a number of suspension-
feeding fish (e.g., Drenner *et al.* 1984, Gibson 1988, van der Lingen 1994, Garrido *et al.* 2007). In
a controlled aquarium environment, herring (*Clupea harengus*, Clupeidae) fed a mixture of
*Artemia* and *Balanus balanoides* nauplii retained a higher proportion of large (*Artemia*) to small
(*B. balanoides*) nauplii than was available, indicating selectivity for larger prey (Gibson 1988).
Drenner et al. (1984) observed that gizzard shad feeding in a controlled environment with a known concentration of zooplankton and microspheres across a broad size range (10-185.5 μm) selectively ingested particles 60 μm or larger.

Particle density is another physical property that may be a factor in determining selectivity for specific particle types by suspension-feeding fish. Smoot (1999) found that the foregut contents of gizzard shad, detritivorous suspension feeders, contained a disproportionately large amount of low-density matter relative to the particles available in sediment on which these fish were assumed to feed. Because organic detritus particles tend to be less dense and more nutrient-rich than inorganic sediment particles, it is possible that selective feeding for low-density particles leads to ingestion of higher-quality food particles in the case of fish that feed by re-suspending sediment detritus. Fish may benefit from preferentially ingesting particles that are high in biologically important nutrients such as organic carbon (C) and nitrogen (N). The nutritional and energetic quality of foods ingested by detritivorous fish are commonly quantified using C and N content (Mundahl and Wissing 1987). C and N are important components of macromolecules such as proteins and carbohydrates, which may be used for growth, maintenance, or energy.

**Particle Selectivity Based on Fluid-Dynamic Mechanisms**

Fluid-dynamic processes inside the oropharyngeal cavity of suspension-feeding fish may lead to selectivity for certain particle characteristics, such as size or density. Although the mode of filtration used by fish could be an important factor in size-selective feeding, the mechanisms for size selectivity remain unclear. Assuming that the gill rakers function as a simple, non-sticky dead-end sieve, particle retention is explained by the sizes of the inter-raker spaces (Rubenstein
and Koehl 1977, Gibson 1988). Theoretically, all of the particles larger than the inter-raker gaps would be retained by fish, while every particle smaller than the pore size would be rejected. Therefore, researchers who model particle retention given a non-adhesive dead-end sieve typically plot a cumulative size-frequency distribution of the inter-raker gaps (Drenner et al. 1984, Gibson 1988). However, particles smaller than the inter-raker gaps may be captured by hydrosol filtration, since they might adhere to sticky mucus on the surface of gill rakers (Rubenstein and Koehl 1977). Similarly, during crossflow filtration, particles smaller than the pore size of the filter may be retained, since this mechanism of particle capture does not depend upon physical encounter of the particles with the sieve (Sanderson et al. 2001).

Gibson (1988) found that suspension-feeding herring (Clupea harengus, Clupeidae) were much more efficient at retaining large Artemia nauplii when feeding in an aquarium containing both Artemia and smaller B. balanoides nauplii. However, projections for feeding efficiency based on cumulative frequency plots of inter-raker distances did not accurately predict the feeding efficiency of herring, substantially over-estimating the observed feeding efficiencies for smaller B. balanoides nauplii (Gibson 1988). Therefore, the simple, non-adhesive sieve model for suspension feeding does not explain the filtering efficiency for prey particles of different sizes in herring (Gibson 1988). Herring may capture particles using crossflow filtration, since the inter-raker gap sizes do not necessarily function as limits for prey size retention during crossflow filtration (Sanderson et al. 2001).

The proportion of suspended particles in various size classes removed by gizzard shad feeding on a mixture of zooplankton and microspheres (10-185 μm size range) in the laboratory increased with particle size class, reaching a maximum and asymptote at 60 μm (Drenner et al. 10)
1984). Using a cumulative size-frequency distribution model of gizzard shad inter-raker spaces, Drenner et al. found that the observed removal rates of suspension-feeding gizzard shad correlated with predicted removal rates. Interestingly, gizzard shad use crossflow filtration rather than dead-end sieving to capture prey (Sanderson et al. 2001). The results of Drenner et al. are not inconsistent with crossflow filtration, since the magnitude of inertial lift is dependent on particle size (Sethi and Wiesner 1997, Sanderson et al. 2001).

In addition to particle size, suspension-feeding fish may select food particles based on particle density. Using endoscopic video recordings during crossflow filtration in suspension-feeding carp (Cyprinus carpio, Cyprinidae), Callan and Sanderson (2003) observed that food particles were filtered and retained, while inorganic sand particles sank ventral to the slurry of food particles and were either spat anteriorly out of the mouth or left the oropharynx through gaps between the gill arches. Food particles were smaller and less dense than the inorganic sand particles. It is possible that inertial lift and other fluid-dynamic forces responsible for maintaining particle suspension in the crossflow were not high enough to keep the inorganic particles suspended inside the oropharyngeal cavity of the carp.

**Particle Selectivity Based on Behavioral Mechanisms**

Aside from the fluid-dynamic processes inside the oropharyngeal cavity associated with suspension feeding after particles have been engulfed, feeding behavior may explain some of the selectivity observed in suspension-feeding fish. For example, fish that ingest zooplankton by suspension-feeding may feed on less evasive prey when compared to particulate-feeding fish that prey on individual zooplankton (Drenner et al. 1982a, Michaletz et al. 1987).
When ingesting benthic detritus, fish such as gizzard shad might selectively ingest low-density detritus by agitating the sediment when they feed and filtering filter more nutritious particles from the water once dense, inorganic particles settle (Mundahl and Wissing 1987, Smoot 1999). Since high-density particles sink before low-density particles, the low-density (more nutrient-rich) particles remain suspended above the sediment-water interface as gizzard shad feed (Mundahl and Wissing 1987, Smoot 1999). This behavioral explanation for selective feeding does not preclude selectivity based on the fluid-dynamic mechanisms of filtration within the oropharynx of suspension-feeding fish after particles have been engulfed.

Suspension-feeding fish that ingest benthic detritus may choose to feed in locations with more nutrient-rich sediment. Low-energy areas with slow currents accumulate more fine particulate detritus, since the velocity of current flow dictates the size and density of particles which settle out of suspension (Bowen 1983). Once entrained, small, low-density particles remain suspended at lower flow velocities than do large particles (Bowen 1983).

**Selectivity for Nutrient Content by Suspension-Feeding Detritivorous Fish**

Suspension-feeding detritivorous fish consume benthic detritus particles, defined as dead and decaying organic matter, as well as live epibenthic algae and bacteria that may be associated with detritus (Bowen 1979, Smoot 1999). These fish consume benthic detritus by ingesting sediment with water or by disturbing the sediment-water interface and ingesting newly suspended particles (Sanderson and Wassersug 1993, Smoot 1999). The detritus food chain is an important link for nutrient and energy cycling. Detritivores convert a substantial amount of plant and algal biomass into animal biomass, representing as much as 90% of secondary production in some ecosystems (Bowen 1983). Detritus is readily available and may...
accumulate in large quantities in aquatic environments. However, detritus has low nutrient quality relative to other food sources, and there is evidence that the growth rate and adult body condition of detritivorous fish may be limited by the nutrient availability in their food (Bowen 1983, Mundahl and Wissing 1987, Ahlgren 1990, Bowen et al. 1995, Higgins et al. 2006).

For example, the nutritional value of organic detritus in gizzard shad diets (quality), as well as the proportion of the diet that is made of detritus (quantity), influence the growth and body condition of gizzard shad (Mundahl and Wissing 1987). The balance between detritus and other food sources may depend on availability in the environment, and organic detritus is typically abundant. Gizzard shad populations feeding more heavily on detritus tend to have lower body condition factors and growth rates than gizzard shad that consume a larger proportion of live foods (Mundahl and Wissing 1987). Mundahl and Wissing found that growth and body condition of gizzard shad improved when zooplankton were available to supplement a detritivorous diet.

A similar association between the quantity of detritus consumed and body condition was observed by Ahlgren (1990) in omnivorous juvenile white sucker (Catostomus commersoni, Catostomidae). When juvenile C. commersoni were fed only detritus ad libitum, they lost weight, though they grew rapidly when fed Artemia ad libitum (Ahlgren 1990). Though a diet of detritus alone was associated with a decline in body condition, when offered a limited diet of invertebrates supplemented by detritus, C. commersoni grew and gained weight. This suggests that detritus is a valuable source of nutrients for this fish (Ahlgren 1990). The amino acid content of detritus is lower than that of invertebrate larvae, and, since energy and amino acid or
protein content may be crucial determinants of food quality, fish feeding on detritus exclusively may become limited by the availability of essential amino acids (Bowen 1980, Ahlgren 1990).

The amino acid and protein content of detritus may limit fish growth because 65-75% of the dry weight of teleost tissue is composed of protein (Evans and Claiborne 2006). However, the protein and non-protein amino acid content of detritus is variable. This variability may explain why fish of the same species are differentially productive in different habitats (Bowen 1979). The need to extract nutrients from an energy- and nutrient-depleted food source has led to several adaptations to detritivory.

Numerous adaptations allow detritivorous fish to increase absorption of nutrients. Detritivorous fish exhibit important morphological adaptations of the digestive system that allow them to successfully exploit detritus as a food resource (Bowen 1983). For example, some species (e.g., Prochilodus platensis, Citharinus sp., Mugil sp., D. cepedianum) have a nearly rigid pyloric stomach to grind ingested food and sand particles together, aiding in mechanical digestion of detritus (Kapoor et al. 1975, Bowen 1983). Grinding in the pyloric stomach reduces detritus particle size and increases the uniformity of particle size, thereby increasing the surface area available for interactions between enzymes and substrates (Schmitz and Baker 1969, Bowen 1983). Many detritivorous fish have elongated digestive tracts to allow for enough time for the digestion and assimilation of detritus (Schmitz and Baker 1969, Smoot and Findlay 2000). Gizzard shad have elevated gut enzyme activity to obtain sufficient nutrients from detritus (Smoot and Findlay 2000).

In addition to the nutrient limitations of detritus itself, benthic detritus is often mixed with inorganic sediment, such as sand. Therefore, in addition to adaptations for more complete
digestion and assimilation of detritus, detritivores may selectively feed on the organic portion of sediments. Since detritus is nutritionally dilute, there may be an advantage to preferentially ingesting biologically important nutrients, which include the organic carbon (C) and nitrogen (N). There is some evidence that detritivorous suspension-feeding fish choose particles based on nutrient content (Bowen 1983, Mundahl and Wissing 1988, Ahlgren 1996, Lemke and Bowen 1998, Smoot 1999, Higgins et al. 2006). However, researchers understand very little about the behavioral and functional morphological mechanisms through which these organisms select for nutrient-rich foods. Bowen (1983) concluded that consuming detritus relatively rich in proteins is advantageous and proposed mechanisms for selection through a behavioral preference for detritus in different locations. Detritivorous fish feeding in shallow, low-energy backwater areas may prefer these areas where more small, organic particles settle out of suspension (Bowen 1983). Smoot (1999) found that the low density portion of benthic sediment is likely an important source of organic N for gizzard shad and other sediment-feeding detritivores, since it is rich in microbial biomass and other proteins (Smoot 1999). Gizzard shad may selectively ingest the low-density portion of the sediment, since low-density sediment is higher in protein content, microbial biomass, and possibly plant debris than whole sediment (Smoot 1999).

Data suggest that gizzard shad may feed selectively on particles of high nutrient content relative to those available in their food source, benthic sediment (Drenner et al. 1982a, Jenkins and Burkhead 1994, Higgins et al. 2006). Mundahl and Wissing (1988) found that the gizzard (pyloric stomach) contents of gizzard shad feeding on sediment detritus contained higher percentages of C, N and organic matter than were available in surface sediments. While unable to explain this selectivity, Mundhal and Wissing proposed that particle size or gustatory preferences based on chemoreception might play a role in feeding selectivity. The expulsion of
some particles from the mouth, a potential avenue for rejecting unwanted particles, was observed (Mundahl and Wissing 1988). Also, Higgins et al. (2006) compared the nutrient content of sediments in three lakes to foregut contents from gizzard shad caught in each lake. Since nutrient analyses revealed higher percentages of carbon, nitrogen and phosphorus in fish foreguts relative to sediment samples, they concluded that gizzard shad selected for a relatively nutrient-rich portion of the available sediment in each lake (Higgins et al. 2006). However, it is unclear whether the sediment sampled was representative of the sediment that gizzard shad consumed, since gizzard shad may have fed in many areas of the reservoir. Additionally, the study by Higgins et al. (2006) did not consider the influence that fish-secreted substances such as mucus might have on nutrients quantified in the gut.

Mucus

Several functions of fish mucus have been proposed, including ionic and osmotic regulation, nest building and protection, respiration, reproduction, disease resistance, excretion, communication, gas exchange, locomotion, and feeding (Shephard 1994). How mucus is involved in these processes, however, is poorly described and understood (Shephard 1994).

Occurrence and Function of Fish Mucus

Mucus is produced by different forms of mucous cells (e.g., goblet cells and other epithelial cells) that secrete glycoproteins, called mucins, that interact with each other and surrounding water to form a continuous gel (Shephard 1994). Mucins may be neutral or they may contain sialic acid or sulphated monosaccharides which make them acidic (Kapoor et al. 1975, Shephard 1994). While a higher content of acidic mucins may be associated with more
viscous mucus, the relationship between mucus chemical content and physical properties is debated (Northcott and Beveridge 1988, Shephard 1994). Distinct types of mucous cells may produce different mucins, and this chemical diversity likely has a physiological role that has not been explained (Kapoor et al. 1975). Some connections between mucus composition and function have been made in gastropods. The adhesive mucus used by marsh periwinkle snails (*Littorina irrorata*, Littorinidae) to hold onto substrates contains 2.7 times more protein than the trail mucus these snails use for locomotion (Smith and Morin 2002). This difference in protein content, explained by the presence of two proteins in the adhesive mucus which are absent from trail mucus, may result in the functional difference between the two mucus types, since adhesive mucus is more tenacious than trail mucus (Smith and Morin 2002). Similarly, the adhesive mucus used by the limpet *Lottia limatula* (Lottiidae) to form glue-like attachments to substrate has a protein content 2.1 times and a carbohydrate content 1.9 times greater than non-adhesive mucus (Smith et al. 1999).

In common carp (*Cyprinus carpio*, Cyprinidae), morphologically distinct mucous cells in different areas of the oropharynx produce chemically distinct mucins; sulfomucines are produced in the posterior portion of the oropharynx, while sialomucines are produced in the anterior portion (Sibbing and Uribe 1985). This distinction may have functional ramifications. Mouthbreeding *Tilapia mossambica* (Cichlidae) produce a variety of chemically distinct mucins which vary seasonally with their breeding cycle (Varute and Jirge 1971). While it is unclear why these different mucins are produced, antibacterial, nutritive, and other hypotheses have been tested (Varute and Jirge 1971).
Goblet cells are found on the surface of skin and gills, and are also associated with the esophagus and gut lining of fish (Shephard 1994). The lining of the entire digestive tract in teleost fishes is covered with mucus which lubricates food and other materials passing through the digestive tract (Kapoor et al. 1975, Heinrichs 1982, Evans and Claiborne 2006). This mucus may also protect gut epithelia from physical and chemical damage (Kapoor et al. 1975). In gizzard shad, goblet cells are present throughout the mucosa of the digestive tract, and are abundant in the epithelium of the posterior pharynx and in the epibranchial organs (Heinrichs 1982).

Mucus present on surfaces in the oropharyngeal cavity may play an important role in suspension feeding. In hydrosol filtration, mucus on the surface of gill arches and rakers can capture and aggregate small particles that might be otherwise lost through the filter mesh (Sanderson et al. 1996). Hoogenboezem and van den Boogaart (1993) observed large numbers of zooplankton contained in mucus boluses in the oropharyngeal cavity of suspension-feeding bream (Abramis brama, Cyprinidae). Each bolus was composed of a conglomerate of particles, and each particle was enveloped in a thin mucus layer (Hoogenboezem and van den Boogaart 1993). They hypothesized that mucus was an important component in the accumulation, storage and transport of food particles (Hoogenboezem and van den Boogaart 1993).

Similarly, Drenner (1982a) found plankton bound in mucus in gizzard shad epibranchial organs. However, the plankton were probably not captured in mucus, since gizzard shad were not observed to use mucus to trap particles in the anterior portion of the oropharynx during crossflow filtration (Sanderson et al. 2001). Gizzard shad intraoral mucus may have another function, such as aggregating particles in the posterior oropharyngeal cavity or increasing the
surface for inertial lift during crossflow filtration (Sanderson et al. 2001). Further research is needed to investigate the proposed functional differences associated with chemically distinct mucines and to explain the fluid-dynamic role of mucus in the oropharynx of fish.

**Nutrient and Energy Content of Mucus**

Nutrients quantified in fish foreguts and attributed to food may, in fact, include nutrients from mucus ingested with food particles or mucus secreted into the foregut. Despite the prevalence of mucus in the alimentary tract of fish, the contributions of mucus to the nutrient or energy content of feces are traditionally considered minor and have not been factored into calculations of absorption efficiency (Jobling 1994). This assumption that fish-secreted substances have a negligible influence on gut nutrient content is also common in studies of feeding selectivity. Since mucus secreted in fish oropharyngeal cavities and alimentary canals contains nutrients, researchers may attribute to food the nutrients that are derived from mucus, thereby forming incorrect conclusions regarding the selective abilities of fish. One estimate from unpublished data suggests that mucus and enzyme secretions associated with the foregut lining contributed <5% of the organic content in the gut of juvenile white sucker (*Catostomus commersoni*, Catostomidae) (Ahlgren 1996). However, there is no published estimate for mucus contributions to fish gut contents and other studies do not account for the contribution of mucus to the nutrients quantified in fish guts.

The only study that examined the nutrients in fish mucus found significant, species-specific differences in the C and N content of body mucus of four saltwater fish species; the range of mean %C was 14.6-35.2% by dry weight, while the range of mean %N for the same four species was 3.4-8.8% by dry weight (Gorlick 1980). This variation may be a result of
differences in the mucins secreted by these species. The C:N ratio quantified in mucus from these four fish species ranged from 3.8 to 4.3 (Gorlick 1980). This range of values is lower than the C:N ratio of 8-14 quantified for three coral species (*Acropora* spp.), indicating that fish mucus may be more nitrogen-rich than coral mucus (Wild *et al.* 2005).

Since many unanswered questions remain about mucus histochemistry and function, the physiological cost of mucus production is not independently considered in energy budgets, and gut mucus is assumed to be a negligible portion of fecal losses in fish and marine mollusks as well as other organisms (Davies *et al.* 1990, Jobling 1994). However, future studies should take mucus contributions into consideration because in gastropods mucus may account for 9-23% of energy consumption (Denny 1980, Davies *et al.* 1990). Additionally, the nutrients contained in mucus may be an attractive food source for pelagic microbes, since oxygen consumption increased 7-fold in seawater amended with mucus from coldwater corals (Wild *et al.* 2008). Understanding fish bioenergetics and the relationship between assimilation efficiencies and environmental factors has important applications in understanding growth and production in fish populations and in aquaculture (Jobling 1994).

**GIZZARD SHAD, DOROSOMA CEPIDIANUM (CLUPEIDAE)**

A well-studied example of a suspension-feeding detritivorous fish is the gizzard shad (Drenner *et al.* 1982a, Heinrichs 1982, Smoot 1999, Vanni *et al.* 2005, Higgins *et al.* 2006). Gizzard shad are members of the family Clupeidae, which includes herrings, menhaden and sardines. Because of their ability to feed at low trophic levels and their abundance, clupeid fish are important links in food webs (Moyle and Cech 2004). In the lentic fish communities of the central and southeastern United States, clupeid fish such as gizzard shad and threadfin shad
(Dorosoma petenense) may represent 45% of the ichthyomass (Jenkins 1968). Native to the interior drainage of Eastern and Central North America, as well as the Gulf and Atlantic slope watersheds, gizzard shad are pelagic, schooling fish found in a variety of habitats (lakes, rivers, streams, estuaries, swamps and reservoirs) (Jenkins and Burkhead 1994).

As larvae, gizzard shad are particulate feeders and consume primarily zooplankton, while as they grow these fish shift from particulate- to suspension-feeding strategies and consume increasing amounts of algae and detritus relative to zooplankton (Michaletz et al. 1987). Suspension-feeding gizzard shad are pump suspension feeders and use crossflow filtration to capture food particles (Sanderson et al. 2001). While mucus is present in the oropharynx of gizzard shad, particles were not seen to become trapped in mucus in intra-oral endoscopic videotapes recorded during suspension feeding (Sanderson et al. 2001).

As they transition from particulate-feeding larvae to suspension-feeding juveniles and adults, young-of-year gizzard shad (between 2.5 and 3.0 cm SL) develop morphological features such as a subterminal mouth, elongated intestinal tract, and muscular gizzard which allow them to feed on benthic detritus (Heinrichs 1982). Additionally, gizzard shad have epibranchial organs, paired organs located above the gills and supported by the fourth and fifth branchial arches (Kapoor et al. 1975). These organs, which contain an entrance canal leading to a blind sac, are hypothesized to consolidate food particles, which are then released into the esophagus (Schmitz and Baker 1969). However, the details of the function of epibranchial organs are not known (Schmitz and Baker 1969, Kapoor et al. 1975).

Suspension-feeding gizzard shad consume live foods (zooplankton, phytoplankton) when they are abundant, and rely heavily on benthic detritus when zooplankton and
phytoplankton are unavailable (Mundahl and Wissing 1988). In gut analyses of gizzard shad, Mundahl and Wissing (1988) did not find sufficiently abundant zooplankton for providing a reliable food source, and rarely found particles from sources other than the sediment. As detritivores, gizzard shad may be limited by nutrient availability in their food (Mundahl and Wissing 1987). There is evidence that gizzard shad selectively ingest the more nutrient-rich portion of sediment (Higgins et al. 2006), particles >60 μm in size (Drenner et al. 1984), or the low-density fraction of the sediment (Smoot 1999). However, the mechanisms which explain this feeding selectivity are unclear.

As detritivorous fish, gizzard shad play a key part in nutrient cycling in their ecosystems (Higgins et al. 2006). Nutrient cycling is critical for ecosystem maintenance and nitrogen and phosphorus cycling is of particular interest as these nutrients limit primary production (Vanni 2002). Gizzard shad both recycle (release nutrients into the habitat in which they originated) and translocate (move nutrients between habitats and ecosystems) nutrients such as nitrogen and phosphorus which might be otherwise unavailable to primary producers (Schaus et al. 1997, Vanni 2002, Vanni et al. 2005, Higgins et al. 2006). Based on a simple model for animals as a source of new nutrients within ecosystems, nutrient translocation by gizzard shad significantly influences primary production (Vanni 2002).

Gizzard shad are a key link in the trophic webs in their ecosystems. As an important prey species in North America, gizzard shad have been widely stocked and stocking programs are responsible for populations of gizzard shad in inland reservoirs (Mundahl and Wissing 1988, Jenkins and Burkhead 1994). In reservoirs in the midwestern and southeastern US, gizzard shad have been heavily stocked to support populations of commercially and recreationally valuable
predator species, such as largemouth bass (*Micropterus salmoides*) (Bremigan and Stein 2001). Recruitment success after gizzard shad stocking varies; these fish may dominate hypereutrophic reservoirs, and larval survival and hatch abundance are directly correlated with total phosphorus concentrations (Bremigan and Stein 2001). Gizzard shad biomass is positively correlated with productivity in Florida, where there is a large standing crop of gizzard shad in eutrophic and hypereutrophic lakes, and gizzard shad are rarely found in oligo- and mesotrophic lakes (Bachmann et al. 1996). Perhaps, in meso- and oligotrophic systems, gizzard shad are limited by nutrient availability, since they consume nutritionally dilute detritus. This idea is supported by the observation that significant reductions in the organic material, phosphorus, and crude protein in gizzard shad diets were associated with a decline in body condition (Gido and Matthews 2001).

Explaining selective feeding will elucidate further the ways that detritivorous fish, such as gizzard shad, interact with their environment since the relationship between the body nutrients of these fish and the nutrients available in sediments is not well understood (Higgins et al. 2006). Additionally, the intra-oral fluid-dynamic processes occurring in suspension-feeding fish are not well understood and studies of feeding selectivity may elucidate the mechanisms used by these fish to capture prey.
LITERATURE CITED


Ahlgren, M. O. 1996. Selective ingestion of detritus by a north temperate omnivorous fish, the juvenile white sucker, Catostomus commersoni. Environmental Biology of Fishes 46:375-381.


Chapter 2: Nutrients Quantified in the Foregut and Oropharyngeal Cavity Mucus of Fish

Abstract

Suspension-feeding detritivorous fish consume detritus particles (dead and decaying organic matter) that are not engulfed individually. Detritus is low-quality relative to other food sources, and the condition of detritivorous fish may be limited by the nutrient availability in detritus (Mundahl and Wissing 1987). To compensate for the nutrient-limitation of their food source, fish such as gizzard shad (*Dorosoma cepedianum*, Clupeidae) may preferentially ingest particles that are high in biologically important nutrients such as organic carbon (C) and nitrogen (N). Feeding selectivity may be quantified by comparing the nutrient content of fish foreguts to the nutrients in an available food source. However, previous studies of nutrient-based feeding selectivity in detritivorous fish do not account for the possibility that secreted mucus may contribute to the nutrients quantified in fish foreguts. The purpose of this study is to determine whether mucus associated with the interior of the oropharyngeal cavity and foregut of gizzard shad accounts for a significant portion of the nutrients quantified in the ingested food. The C and N content of gizzard shad foreguts, food available in feeding trials, and mucus samples taken from the oropharyngeal cavity of gizzard shad were compared to determine whether mucus associated contributed to the nutrients quantified in foregut contents. Mucus collected from the oropharyngeal surfaces of gizzard shad was 49.8%C ± 5.7 and 11.1%N ± 1.7 by dry mass. Nutrients derived from fish-secreted mucus may represent 46% ± 15 of the nutrients quantified in gizzard shad foreguts. By considering the contribution of mucus to the nutrients in gizzard shad foreguts, it is possible to more accurately describe the abilities of detritivorous fish to selectively ingest certain food particles.
INTRODUCTION

Unlike particulate-feeding fish that visually select a single prey item, suspension-feeding fish engulf water containing suspended food particles that are too small to be sensed and engulfed individually (e.g., Garrido et al. 2007). Inside the oropharyngeal cavity, rows of tufted or comb-like gill rakers attached to the branchial arches serve as filter elements to retain food particles as water exits posteriorly from the oropharyngeal cavity (Sanderson et al. 2001).

Suspension feeding detritivorous fish consume benthic detritus particles (dead and decaying organic matter), that they can suspend by ingesting sediment with water or by disturbing the sediment-water interface and ingesting newly suspended particles (Sanderson and Wassersug 1993, Smoot 1999). Detritus is low in nutrient quality relative to other food sources, and the growth rate and adult body condition of detritivorous fish may be limited by the nutrient availability in detritus (Bowen 1983, Mundahl and Wissing 1987, Bowen et al. 1995, Higgins et al. 2006). Consequently, detritivorous fish may benefit from preferentially ingesting particles that are high in biologically important nutrients such as organic carbon (C) and nitrogen (N). Feeding selectivity may be based on nutrient content in suspension-feeding detritivorous fish, but researchers understand very little about the behavioral and functional morphological mechanisms through which these organisms may select for nutrient-rich foods (Bowen 1983, Mundahl and Wissing 1988, Ahlgren 1996, Lemke and Bowen 1998, Smoot 1999, Higgins et al. 2006).

Since these fish do not select particles individually, but instead engulf multiple particles simultaneously, mechanisms of selectivity are not known. It is possible that a behavioral mechanism is responsible for feeding selectivity. For example, fish may re-suspend sediment at
the sediment-water interface and filter more nutritious particles from the water once dense, inorganic particles settle, as described by Smoot (1999), or fish may feed in locations with more nutrient-rich sediment. Alternatively, particles may be sorted by the intra-oral, fluid-dynamic mechanisms that suspension-feeding fish employ to capture particles.

Many suspension-feeding fish filter particles using crossflow filtration, in which suspended particles are engulfed and travel in the mainstream flow parallel to the filter surface (Brainerd 2001, Sanderson et al. 2001). As particles travel posteriorly, fluid-dynamic forces (e.g., inertial lift) keep particles suspended and particles become more concentrated as filtrate exits through gaps between the gill rakers (filter pores) (Brainerd 2001, Sanderson et al. 2001). Since particles are increasingly concentrated as they travel posteriorly in the oropharyngeal cavity, fish swallow very little water with their food (Brainerd 2001, Sanderson et al. 2001). Rows of gill rakers attached to the branchial arches in the oropharyngeal cavity of suspension-feeding fish form the filter surface and are involved in the control of fluid flow inside the oropharyngeal cavity (Sanderson et al. 2001, Smith and Sanderson 2008). Mucus present on the surface of gill rakers and arches during crossflow filtration may function to regulate water loss between filter elements, thereby affecting the speed of the mainstream flow and the inertial lift that is involved in maintaining particle suspension (Sanderson et al. 2001, Callan and Sanderson 2003, Smith and Sanderson 2007). Additionally, mucus may be used to aggregate food particles in the posterior pharynx (Callan and Sanderson 2003).

Recent studies suggest that gizzard shad, *Dorosoma cepedianum* (Clupeidae), suspension-feeding detritivorous fish native to Virginia waters, feed on particles of high nutrient content selected from benthic sediment (Drenner et al. 1982, Jenkins and Burkhead 1994, Higgins et al.
Specifically, Higgins et al. (2006) quantitatively compared the nutrient content of sediments in three lakes to foregut contents from gizzard shad caught in each lake. Since nutrient analyses revealed higher percentages of carbon, nitrogen and phosphorus in fish foreguts relative to sediment samples, they concluded that gizzard shad selected for a relatively nutrient-rich portion of the available sediment in each lake (Higgins et al. 2006). A mechanism by which gizzard shad might ingest particles of high nutrient content has not been established.

Higgins et al. (2006) did not consider the influence that fish-secreted substances such as mucus might have on quantified gut nutrients. In gizzard shad, mucus is present on surfaces in the oropharyngeal cavity and is associated with gut mucosa (Heinrichs 1982). Drenner (1982) found plankton bound in mucus in the epibranchial organs, accessory digestive organs of gizzard shad at the posterior of the oropharyngeal cavity. Despite the prevalence of mucus in the alimentary tract, the contribution of mucus to the nutrient or energy content of feces is traditionally considered minor in fish and has not been factored into calculations of absorption efficiency (Jobling 1994). The assumption that fish-secreted substances have a negligible influence on gut nutrient content is also common in studies of feeding selectivity. Since mucus secreted in fish oropharyngeal cavities and alimentary canals contains nutrients, researchers may attribute nutrients from mucus to food, thereby reaching incorrect conclusions regarding selective abilities during feeding. One estimate from unpublished data suggests that mucus and enzyme secretions associated with the foregut lining contributed <5% of the organic content in the gut of juvenile white sucker (Catostomus commersoni, Catostomidae) (Ahlgren 1996). However, there is no published estimate for mucus contributions to fish gut contents and studies do not account for the contribution of mucus to the nutrients quantified in fish guts. The purpose of
this study is to determine the contribution of mucus to the nutrient content quantified in food from the foregut (considered the esophagus and gizzard) of gizzard shad.

METHODS

Gizzard Shad Collection: Gizzard shad were collected using electroshocking and seine-netting from waters in the Virginia coastal plain, specifically, College Creek, Waller Mill Reservoir, Chickahominy Lake and Little Creek Reservoir. Gizzard shad were maintained at 19-21°C in glass aquaria with external bio-ball filtration. Fish were fed TetraMin® flake food daily and pH and ammonia were monitored in the aquaria. A nitrofurazone anti-fungal agent was used as a prophylaxis in holding aquaria (but was not added to experimental aquaria) when fish were brought into the lab, and fish collected from different reservoirs were kept in separate aquaria so that any communicable diseases would not be passed between fish populations. Gizzard shad were allowed to adjust to laboratory conditions for at least 10 days before inclusion in experiments.

Mucus Collection: The C and N content of gizzard shad mucus was quantified to determine the amount of mucus associated with the ingested food in fish foreguts. To collect data regarding the nutrient content of mucus, adult gizzard shad (150-260 mm standard length, SL) were euthanized using an overdose of MS-222 and subsequent pithing (see Appendix I for influence of MS-222 on quantified nutrients in fish mucus). These large adult gizzard shad were used for mucus collections to obtain a sufficient volume for nutrient analyses. Mucus samples were taken by sliding a rubber-tipped probe over surfaces within the oropharyngeal cavity (palatal, gill raker and branchial arch surfaces). Mucus samples were viewed under a microscope to qualitatively observe whether cells and tissue debris were present in samples (Appendix II).
Mucus samples were also taken from the external, lateral surfaces of adult gizzard shad for comparison with intra-oral mucus (Appendix III).

**Feeding Trials:** Before experiments were conducted, three gizzard shad were dissected 24 hours after feeding to confirm that 24 hours is sufficient time for the foregut to empty, since the presence of food in gizzard shad foreguts at the beginning of experiments could affect the quantification of nutrient content. Because their foreguts were devoid of food, 24 hours was deemed an appropriate period of food deprivation. Gizzard shad were moved to the experimental aquarium 24 hours prior to each trial, during which time they were not fed and were allowed to acclimate to tank conditions. Samples were taken from the interior of empty foreguts using the method described above for mucus collection, but sample volumes were not sufficiently large to allow for nutrient analyses.

For feeding trials, gizzard shad (65-95 mm SL) were placed in groups of three (for social purposes) in a 110L aquarium filled with 70L of water. Big Strike® brand fish pellets were ground using a Black and Decker® electric coffee grinder (model CBM205) and sifted to a size range of 125-250μm using Dual Manufacturing Co.© market grade sieves with mesh no. 120 (125 μm) and 60 (250 μm). Trials began with the addition of 10.00 g dry food particles to the aquarium. In addition to air stones along the bottom of the aquarium, four Little Giant® model PE-A submersible water pumps (150 liters-hour⁻¹) attached to perforated tygon tubing were used to maintain a homogeneous mixture of food within the water column during trials. These pumps prevented particles from settling to the bottom of the aquarium, where they might sort by differences in physical characteristics such as density.
Fish were allowed to feed for one hour. During that time, three water column samples were taken, 0, 30 and 60 minutes after the particles were added to the water, as a measure of the food available to gizzard shad. Water column samples were taken by moving a tube (2.5 cm diameter) vertically through the water column onto a randomly placed rubber stopper resting at the bottom of the aquarium, retaining a 120 ml sample of the particles present in the water along the entire height (31 cm) of the water in the aquarium.

At the end of one hour, one randomly chosen fish was sacrificed using the method described for mucus collection. Only one fish was sacrificed for foregut analysis in each trial to avoid pseudoreplication in the form of multiple non-independent samples. The fish was dissected immediately (within 5-7 minutes of capture) to extract contents of the foregut, considered to be the esophagus and gizzard. The foregut is commonly used in gut content analysis and feeding selectivity studies in gizzard shad (Mundahl and Wissing 1988, Higgins et al. 2006). Samples were also collected from the epibranchial organs of gizzard shad, when possible (Appendix IV).

**Elemental Analysis:** The entire contents of gizzard shad foreguts (the esophagus and gizzard), entire water column samples of food, and mucus samples were filtered onto tared 25mm glass Whatman® GF/C microfiber filters for total C and N analysis. Filtered samples were kept in a drying oven at 60°C for 24 hours before they were weighed on a Perkin-Elmer AD6 balance to determine dry mass. A Perkin-Elmer 2400 Analyzer was used to determine the percent total C and N by dry mass of foregut, water column and mucus samples (Higgins et al. 2006, Wach and Chambers 2007).

Since fish do not assimilate inorganic C, during preliminary trials organic C content was quantified differently than total C in water column food samples and mucus samples (Higgins et
Samples of food particles and mucus were filtered using the method described above and placed in a muffle furnace at 450°C for 3 hours to burn off organic matter. Inorganic C was then measured using the elemental analyzer and subtracted from the total C yield to determine the amount of organic C in each substance (Higgins et al. 2006). These preliminary results showed inorganic C was not detectable in mucus (n=3) and represented less than one standard deviation of the mean total C quantified in food samples (0.7% inorganic C ± 0.2, mean ± SD, n=9). Therefore, total C was used for all analyses.

**Statistical Analysis:** Since three water column samples were taken during each trial (at t=0, 30 and 60 minutes after the beginning of each trial), differences in concentrations of C and N were compared across the three time points using repeated-measures ANOVAs and Tukey-Kramer HSD tests (p<0.05). Differences in concentrations of each nutrient (C and N) were compared between the three sample types (mucus, water-column food samples, and foregut contents) using one-way ANOVAs and Tukey-Kramer HSD tests (p<0.05). The Shapiro-Wilk test showed that the residuals were normal (P<0.05). Log-transformation did not affect the results of statistical tests, so data were not transformed for analysis.

**Contribution of Mucus and Food to Foregut Contents:** A system of equations was developed to calculate the percent contribution by dry mass of mucus and food in the foregut contents taken from gizzard shad in experimental trials, assuming that only mucus and food are present in foregut contents (Equations 1 and 2, Appendix V). Assuming that the mass of the foregut contents is 1.0g, substituting Equation 2 into Equation 1 and solving for \( W_{food} \), the dry mass of food in the entire foregut, yields Equation 3. See Appendix V for the entire derivation of Equation 3.
Equation 1 \[ (\%N_{mucus})(Wt_{mucus}) + (\%N_{food})(Wt_{food}) = (\%N_{gut})(Wt_{gut}) \]

Equation 2 \[ (Wt_{mucus}) = (Wt_{gut}) - (Wt_{food}) \]

Equation 3 \[ (Wt_{food}) = ([(\%N_{gut}) - (\%N_{mucus})]/[(\%N_{food}) - (\%N_{mucus})]] \]

Equations 1, 2 and 3. Contribution of Mucus and Food to Foregut Contents.

Known variables:
- \( (\%N_{mucus}) \) = % nutrient per g dry mass in mucus
- \( (\%N_{food}) \) = % nutrient per g dry mass in food
- \( (\%N_{gut}) \) = % nutrient per g dry mass in the foregut
- \( (Wt_{gut}) \) = dry mass of the foregut

Unknown variables:
- \( (Wt_{mucus}) \) = dry mass of mucus in the foregut
- \( (Wt_{food}) \) = dry mass of food in the foregut

RESULTS

Gizzard Shad Feeding Behavior: When particles were added to the experimental aquarium at the beginning of each trial, gizzard shad changed from typical respiratory motions to the series of rapid suctions associated with pump suspension-feeding. During the first five minutes of feeding trials, gizzard shad pumped at a mean rate of 2.8 pumps·s\(^{-1}\) ± 0.2 (mean ± SE, n=4; Armstrong 2008, unpublished). Gizzard shad continued to exhibit feeding behavior and fed from the water column, rather than the bottom of the aquarium, for the duration of trials.

Water Column Samples: Water column sample of the food available to gizzard shad were taken at t=0 (n=5), 30 (n=4) and 60 (n=5) minutes after the start of each trial. While six trials were completed, some of the water column samples were lost during processing and there are not six water column samples from each time point. Repeated-measures ANOVAs showed that the slight declines in C and N content over time were non-significant (p=0.19 for %C, p=0.06 for %N, Figure 2.1).
Since the water column samples were not significantly different at t=0, 30, and 60 minutes, the mean of the three values from each trial was used in analyses to represent the particles available to gizzard shad during experiments.

**Carbon Content Comparison:** One-way ANOVA comparing the %C by dry mass in foregut (50.9±4.6, n=6), mucus (49.8±5.7, n=10) and food (45.2±0.8, n=14) samples was significant (mean ± SD, p=0.005, Figure 2.2). Tukey-Kramer HSD multiple comparisons analysis showed that food samples were significantly different from foregut (p=0.01) and mucus (p=0.02) samples. Mucus and foregut samples were not significantly different (p=0.9).

**Nitrogen Content Comparison:** One-way ANOVA comparing the %N by dry mass in foregut, mucus and food samples showed a significant difference between groups (p<0.001). Tukey-Kramer HSD multiple comparisons analysis showed that the %N by dry mass of mucus (11.1%±1.7) was significantly higher than both foregut (p<0.001) and food nitrogen content (p<0.001, Figure 2.2). Tukey-Kramer analyses also showed that the %N quantified in foregut contents was significantly higher than the %N by dry mass in food alone (8.5%±0.7 and 6.5%±0.1, respectively; p<0.001, mean ± SD, Figure 2.2).

**Contribution of Mucus and Food to Foreguts:** Calculations to determine the proportion of foregut contents that were food vs. mucus by dry mass were made for each foregut sample (n=6) using the mean %N per g dry mass in mucus and food samples (Equation 3). The result of calculations showed that each gram of foregut sample is 54%±15 food (range=31%-70%) and 46%±15 (range=30%-69%) mucus by dry mass (mean ± SD, n=6). Equation 3 is not applicable to the C content data because there is no significant different between the %C quantified in gizzard shad foreguts and mucus samples (p=0.9) and because the %C quantified in foregut samples is
higher than the %C quantified in food samples. The %C quantified in gizzard shad foreguts may be higher than the %C quantified in gizzard shad mucus due to experimental error in the elemental analyses or to the presence of enzymes or tissue in foregut samples.

**Discussion**

Foregut contents from gizzard shad were significantly higher in both C and N composition than the available food (Figure 2.2). The food particles available to gizzard shad were from a single known, quantified source, in a narrow size range, and were distributed homogeneously in the aquarium water. This experimental design does not allow for external, behavioral particle selectivity, since the particles were evenly distributed in the water column. However, it is possible that slight variation in the available food allowed particle sorting inside the oropharyngeal cavity once gizzard shad engulfed particles, and the experimental design does not eliminate the possibility of internal selectivity. The lack of statistical significance in Figure 2.1 suggests that gizzard shad did not selectively ingest particles, though this method of water column sampling may not be sensitive enough to detect changes in particle composition in the water that might result from selective ingestion. However, mucus secretions (which contained significantly more C and N than the food), rather than internal particle selectivity, can account for the difference between foregut and food nutrient content.

Mucus is produced by different forms of mucous cells (goblet cells) which secrete glycoproteins, called mucins, that interact with each other and surrounding water to form a continuous gel (Shephard 1994). Mucous and goblet cells are found on the surface of fish skin and gills, and are also associated with the gut lining of fish (Shephard 1994). Mucus lining the esophagus of fish lubricates food and other materials passing through the digestive tract (Heinrichs 1982, Evans and Claiborne 2006). In gizzard shad, goblet cells are present throughout the mucosal lining of
the gut, and are abundant in the epithelium of the posterior pharynx and in the epibranchial organs (Heinrichs 1982). Since mucus is closely associated with feeding structures, nutrients attributed to mucus are expected to be present in food samples taken from fish foreguts.

While mucus secretions present in the foreguts of gizzard shad may account for the observed differences in nutrient composition between the available food and gizzard shad foreguts, enzymes secreted in gizzard shad foreguts may also contribute to the nutrients quantified therein. Pepsin, lipase, amylase and rennin have been qualitatively documented in the gizzard of gizzard shad (Bodola 1966). However, no quantitative analysis of the digestive enzymes present in the foregut of gizzard shad has been made. Smoot and Findlay (2000) characterized the enzyme and surfactant activity in gizzard shad guts by extracting gut fluid from each region (esophagus, gizzard and four intestinal sections) of the digestive tract. However, due to small volumes of material found in the esophagus and gizzard (foregut) of gizzard shad, these regions of the gut were not sampled for enzyme activity (Smoot and Findlay 2000). Although fish-secreted enzymes and surfactants may affect the observed nutrient content of the foregut of gizzard shad, these secretions were not considered in this study; rather, the foregut was assumed to contain only food particles and mucus.

To quantify the proportion of foregut contents composed of mucus, assuming that foregut contents were comprised solely of food and mucus, Equation 3 was applied to data collected from gizzard shad feeding trials. Mucus was calculated to constitute 46% ± 15 of foregut contents by dry mass, a much higher value than Ahlgren’s (1996) statement without supporting data that mucus represents 5% of the organic mass of gut contents of *Catostomus commersoni* (Catostomidae). However, since mass does not scale uniformly with volume, food of different
densities may compose a different proportion of the dry mass in a given gram of foregut sample, and the food sources in these experiments were different (Ahlgren 1996). Also, Ahlgren’s (1996) data were collected using *Catostomus commersoni* (Catostomidae), which may produce mucus with a different chemical composition than the mucus of gizzard shad.

Gorlick (1980) found significant, species-specific differences in the nutrient (C and N) content of body mucus of four saltwater fish species. The range of mean %C by dry mass was 14.6-35.2% C, while the range of mean %N for the same four species was 3.4-8.8% N (Gorlick 1980). Nutrients quantified in mucus from gizzard shad oropharyngeal cavities were higher in both C and N than any of these species. Mouthbrooding *Tilapia mossambica* (Cichlidae) produce a variety of chemically distinct mucins which vary seasonally with their breeding cycle (Varute and Jirge 1971). Mucins may be neutral or they may contain sialic acid or sulphated monosaccharides which make them acidic (Shephard 1994). While a higher content of acidic mucins may be associated with more viscous mucus, the exact relationship between mucus chemical content and physical properties is debated (Northcott and Beveridge 1988, Shephard 1994).

Since there are many unanswered questions about mucus histochemistry and function, the physiological cost of mucus production is not considered independently in vertebrate energy budgets and assimilation efficiencies, and gut mucus is assumed to be a negligible portion of fecal energy losses (Mundahl and Wissing 1988, Davies *et al.* 1990, Jobling 1994). However, future studies should take mucus contributions into consideration, since mucus may represent a substantial portion of an organism’s energy use. For example, in gastropods mucus may account for 9-23% of caloric consumption (Denny 1980, Davies *et al.* 1990). Understanding fish bioenergetics and the relationship between assimilation efficiencies and environmental factors...
has important applications in understanding growth and production in fish populations and in aquaculture (Jobling 1994).

Additionally, it is important to consider mucus when studying feeding selectivity in detritivorous fish. For example, nutrients shown here to be present in fish secreted mucus were excluded from calculations of gizzard shad feeding selectivity by Higgins et al. (2006). Using the values of nitrogen content that Higgins et al. (2006) quantified in sediment (1.6 mg N/g dry mass sample, or = 0.16%) and gizzard shad foreguts (20.1 mg N/g dry mass sample, or 2.01%) from one reservoir (Burr Oak) and using the mean value of 11.1% N by dry mass of gizzard shad oral mucus (Figure 2.2), the output from Equation 3 shows that foregut contents from Burr Oak can be estimated as 83% sediment and 17% mucus by dry mass if gizzard shad fed non-selectively on the sediment. Similarly, foreguts of gizzard shad sampled by Higgins et al. (2006) at Pleasant Hill and Acton reservoirs can be estimated as 93% and 89% sediment and 7% and 11% mucus by dry mass, respectively.

In addition to calculations using %N, the C content quantified in gizzard shad foreguts and sediment samples by Higgins et al. 2006 were used in Equation 3 to calculate the proportion of gizzard shad foreguts composed of food vs. sediment. Calculations using %C were not feasible using the data collected for this thesis because the %C quantified in gizzard shad foreguts was higher than the %C quantified in food and there was no significant difference between the %C quantified in mucus and foregut samples (p=0.9, Figure 2.2). However, the %C quantified by Higgins et al. (2006) in gizzard shad foreguts and the sediment available for gizzard shad consumption were substantially different from each other, and it is reasonable to calculate the
proportion of gizzard shad foreguts comprised of mucus vs. sediment using data collected by Higgins et al. 2006.

Using the values of carbon content that Higgins et al. (2006) quantified in sediment and gizzard shad foreguts from Burr Oak (1.8% and 10.5% C, respectively), and using the mean value of 49.8% C by dry mass of gizzard shad oral mucus (Figure 2.2), the output from Equation 3 shows that the foregut contents of gizzard shad collected at Burr Oak are 82% sediment and 18% mucus by dry mass if gizzard shad fed non-selectively on the sediment. Similarly, the foreguts of gizzard shad sampled at Pleasant Hill can be estimated as 92% sediment and 8% mucus by dry mass, and the foreguts of gizzard shad sampled at Acton can be estimated at 89% sediment and 11% mucus by dry mass. The calculations of the proportion of gizzard shad foreguts comprised of sediment vs. mucus completed using both C and N content quantified by Higgins et al. 2006 are very similar. Therefore, nutrients from mucus present in gizzard shad foreguts can account for the difference in nutrient content between the sediment and gizzard shad foreguts quantified by Higgins et al. (2006). This result is a strong indication that mucus can be the source of the higher C and N content in the foregut observed by Higgins et al. 2006, rather than feeding selectivity.

The proportion of gizzard shad foregut contents composed of mucus calculated using data from this thesis (46% ± 15 mucus) was higher than the result of calculations using data collected by Higgins et al. (Range: 7-18% mucus) by dry mass. Given that these data are based on the dry mass of mucus and food, rather than volume or density, the difference in the food particles available for gizzard shad between these two studies may explain the difference in quantified proportion of mucus. The particles available for gizzard shad consumption in this thesis were
ground Big Strike® pellet, while the gizzard shad in the study by Higgins et al. (2006) consumed sediment detritus. If the sediment particles consumed during the study by Higgins et al. were more dense than the Big Strike® food particles used in this thesis, an equal volume of mucus may represent a larger proportion, by dry mass, of the foregut contents of gizzard shad consuming food particles, assuming that mucus production in the foregut of gizzard shad is constant. Alternatively, mucus production might vary based on satiation, gizzard shad body condition, or the nutrient quality of food may affect the rate of mucus secretion within the oropharyngeal cavity and foregut of gizzard shad.

The overarching objective of this study is to begin to explain mechanisms of selectivity for more nutritious particles by asking whether gizzard shad can select nutritious particles inside the oropharynx once particles have been engulfed, rather than behaviorally. Behavioral explanations for particle sorting include fish feeding in locations with more nutrient-rich benthic sediment. Also, as gizzard shad disturb the sediment-water interface while feeding, they may filter the lower-density particles out of the water column while higher-density particles sink (Bowen 1983, Mundahl and Wissing 1988, Smoot 1999). In a future study, fish will be introduced into a controlled environment where particles of varying nutrient content are homogeneously mixed and are of a limited and consistent size range, thus eliminating opportunities for behaviorally selection of particles. After correcting for the influence mucus has on nutrients quantified in the foregut, it will be possible to determine whether gizzard shad select for particles based on nutrient content alone.

Explaining selective feeding will elucidate further the ways detritivorous fish, like gizzard shad, interact with their environment since the stoichiometric relationship between these fish and
sediments is not well understood (Higgins et al. 2006). Detritivorous fish play an integral role in nutrient cycling processes that are critical for ecosystem maintenance. Gizzard shad both recycle (release nutrients into the habitat in which they originated) and translocate (move nutrients between habitats and ecosystems) nutrients such as nitrogen and phosphorus that might be otherwise unavailable to primary producers (Schaus et al. 1997, Vanni 2002, Vanni et al. 2005, Higgins et al. 2006). Additionally, the fluid-dynamic processes behind suspension feeding in fish are not well understood. Evidence that fish might use crossflow filtration to select for nutritious particles may offer insights into potential mechanisms controlling flow in the oropharyngeal cavity of suspension-feeding fish.
Ahlgren, M. O. 1996. Selective ingestion of detritus by a north temperate omnivorous fish, the juvenile white sucker, Catostomus commersoni. Environmental Biology of Fishes 46:375-381.


Figure 2.1. Percent of each nutrient (C, N) by dry mass (mean ± standard deviation) in water column samples taken 0 (n=5), 30 (n=4) and 60 (n=5) minutes after the beginning of each trial. There is no significant difference between samples taken at different times throughout the trials.
Figure 1.2. Percent of each nutrient (C, N) by dry mass (mean ± standard deviation). Bars with the same letter within each nutrient graph are not significantly different (p>0.05). Bars represent:
Foregut=foregut contents from experimental fish (n=6)
Mucus=mucus samples from the oral cavity of gizzard shad (n=9), and
Food= ground pellet food sampled from the water column (n=14)
CHAPTER 3: PARTICLE SELECTIVITY IN A SUSPENSION-FEEDING DETRITIVOROUS FISH

ABSTRACT

Suspension-feeding detritivorous fish engulf water containing numerous suspended food particles, ingesting detritus (dead and decaying organic matter) and other potential food as water flows past their feeding structures. Recent evidence suggests that these suspension-feeding fish may preferentially ingest particles that are high in biologically important nutrients such as organic carbon (C) and nitrogen (N), which may be beneficial because detritus is low-quality relative to other food sources, and the condition of detritivorous fish may be limited by the nutrient availability in detritus (e.g., Bowen et al. 1995). Feeding selectivity may be based on fish behavior or the internal, fluid-dynamic processes associated with suspension feeding. The purpose of this study is to quantify feeding selectivity for nutrient-rich particles based on internal, fluid-dynamic mechanisms in gizzard shad (*Dorosoma cepedianum*, Clupeidae), a detritivorous fish species native to Virginia waters. By comparing the nutrient (C and N) content of suspended food particles available to gizzard shad in controlled laboratory experiments with the nutrient content of ingested food in the foregut, feeding selectivity can be quantified. Experiments using particles in the 125-250 μm size class did not offer evidence for selective ingestion of nutrient-rich particles by gizzard shad. However, particles in the 125-250 μm size settled to the bottom of the aquarium, and it is not feasible to use particles in the 125-250 μm size class in experiments designed to quantify feeding selectivity in gizzard shad. Food and sediment particles in the 75-125 μm size class did remain in suspension during preliminary trials, and will be used for proposed feeding selectivity experiments. Understanding the mechanisms...
by which detritivorous fish obtain nutrients helps to clarify their role in ecosystem dynamics, including nutrient recycling and linking benthic and pelagic processes.

**INTRODUCTION**

Suspension-feeding fish engulf water containing various suspended food particles, ingesting phytoplankton, zooplankton or detritus as water flows past their feeding structures (Gerking 1994, Garrido *et al*. 2007). They filter items between 5–3000μm from the large volumes of water that enter the mouth and exit the opercula (Sanderson and Wassersug 1993). Suspension feeding may include capturing particles already suspended in the water column, as well as benthic particles that become suspended in water by some action of the organism (Sanderson and Wassersug 1993). The oropharyngeal cavity of suspension-feeding fish contains specialized structures that allow for the processing of suspended particles. In teleost species, four or five paired branchial arches on the left and right sides of the head contain one or two rows of gill rakers, tufted or comb-like bony or cartilaginous structures that have been shown to participate in the filtration mechanism used by fish to capture particles (Sanderson and Wassersug 1993, Sanderson *et al*. 2001, Smith and Sanderson 2008). Gill rakers form the filter surface and may control fluid flow inside the oropharyngeal cavity, though the extent to which gill rakers influence fluid-dynamic processes inside the oropharynx of suspension-feeding fish remains unclear (Sanderson *et al*. 2001, Smith and Sanderson 2008).

The fluid-dynamic mechanisms of vertebrate suspension feeding are not well described. Some species of suspension-feeding fish use crossflow filtration to capture particles (Sanderson *et al*. 2001). During crossflow filtration, mainstream flow (crossflow) transports particles posteriorly in the oropharyngeal cavity (downstream), while filtrate flow turns from the mainstream and exits...
through the filter elements (Sanderson et al. 2001). Suspended particles that are engulfed travel parallel to the filter surface (Sanderson et al. 2001). As particles travel posteriorly, fluid-dynamic forces (e.g., inertial lift) keep particles suspended and particles become more concentrated as filtrate exits through gaps between the gill rakers (Sanderson et al. 2001).

Since numerous particles are engulfed during each feeding bout, suspension-feeding organisms have been assumed to feed non-selectively (Jorgensen 1966, Sanderson and Wassersug 1993). Recent data indicate suspension-feeding can be a selective process, but the mechanisms of particle selectivity in fish are unknown (Higgins et al. 2006). Selective feeding, or the preferential ingestion of certain prey items, may be based on several particle characteristics such as size, density and nutrient content. Mechanisms allowing for feeding selectivity based on particle characteristics may include (1) fluid-dynamic processes associated with feeding (internal), and (2) fish behavior (external).

Fish behavior might lead to selective ingestion of certain types of particles. For example, fish such as gizzard shad might re-suspend sediment and filter more nutritious particles from the water once dense, inorganic particles settle (Mundahl and Wissing 1987, Smoot 1999). Suspension-feeding fish that ingest benthic detritus may choose to feed in locations with more nutrient-rich sediment (Bowen 1983).

The fluid-dynamic processes associated with crossflow filtration may also lead to particle selectivity by suspension-feeding fish. When suspension feeding, fish can adjust the patterns and velocity of flow within the oral cavity by altering oral gape (the openness of the mouth) and gill arch position, suggesting some kind of internal particle selection mechanism based on fluid dynamics may exist (Sanderson et al. 2001). During crossflow filtration, particles smaller than
the pore size of the filter may be retained, since this mechanism of particle capture does not depend upon physical encounter of the particles with the filter (Sanderson et al. 2001).

Detritivorous suspension-feeders also may choose particles based on nutrient content (Bowen 1983, Mundahl and Wissing 1988, Ahlgren 1996, Lemke and Bowen 1998, Smoot 1999, Higgins et al. 2006). However, researchers understand very little about the behavioral and functional morphological mechanisms for selection of nutrient-rich foods.

Suspension-feeding detritivorous fish consume benthic detritus particles, defined as dead and decaying organic matter, as well as live epibenthic algae and bacteria that may be associated with detritus (Bowen 1979, Smoot 1999). Detritus is readily available and may accumulate in large quantities in aquatic environments. However, detritus has low nutrient quality relative to other food sources, and the growth rate and adult body condition of detritivorous fish may be limited by the nutrient availability in their food (Bowen 1983, Mundahl and Wissing 1987, Ahlgren 1990, Bowen et al. 1995, Higgins et al. 2006). For example, gizzard shad populations feeding more heavily on detritus tend to have lower body condition indices and growth rates than gizzard shad that consume a larger proportion of live foods (Mundahl and Wissing 1987). Mundahl and Wissing (1987) found that growth and body condition of gizzard shad improved when zooplankton were available to supplement a detritivorous diet. In addition to the nutrient limitations of detritus itself, benthic detritus often is mixed with inorganic sediment, which is generally more dense than more nutrient-rich organic particles. Since detritus is nutritionally dilute, there may be an advantage to the preferential ingestion of biologically important nutrients, which include the elements carbon (C) and nitrogen (N).
Gizzard shad, *Dorosoma cepedianum* (Clupeidae), suspension-feeding detritivorous fish native to Virginia waters, may feed selectively on particles of high nutrient content derived from benthic sediment (Drenner *et al.* 1982a, Jenkins and Burkhead 1994, Higgins *et al.* 2006). In the lentic fish communities of the central and southeastern United States, clupeid fish such as gizzard shad may represent 45% of the ichthyomass (Jenkins 1968). Gizzard shad are pelagic, schooling fish found in a variety of habitats (lakes, rivers, streams, estuaries, swamps and reservoirs) (Jenkins and Burkhead 1994).

Suspension-feeding gizzard shad are pump suspension feeders, collecting particles by using a rapid, aperiodic series of suctions not directed at specific particles (Drenner *et al.* 1982b, Gerking 1994). They use crossflow filtration to retain food particles (Sanderson *et al.* 2001). As they transition from particulate-feeding larvae to suspension-feeding juveniles and adults, young-of-year gizzard shad (between 2.5 and 3.0 cm SL) develop morphological features including subterminal mouths, elongated intestinal tracts, and muscular gizzards that allow them to feed on benthic detritus (Heinrichs 1982). Additionally, gizzard shad have paired epibranchial organs accessory to the digestive system that are hypothesized to consolidate and amass food particles, directed into the esophagus to be swallowed (Schmitz and Baker 1969, Kapoor *et al.* 1975). Suspension-feeding gizzard shad consume live foods (zooplankton, phytoplankton) when they are abundant, and rely on benthic detritus when zooplankton and phytoplankton are unavailable (Mundahl and Wissing 1988). Gizzard shad may selectively ingest the more nutrient-rich portion of sediment (Higgins *et al.* 2006), larger particles (Drenner *et al.* 1984), or the low-density fraction of the sediment (Smoot 1999). However, the mechanisms that describe this feeding selectivity are unclear.
Mundahl and Wissing (1988) found that gizzard (pyloric stomach) contents of gizzard shad feeding on sediment detritus contained higher percentages of C, N and organic matter than were available in surface sediments. While unable to explain this selectivity, Mundhal and Wissing proposed that particle size or gustatory preferences based on chemoreception might play a role in feeding selectivity. The expulsion of some particles from the mouth, a potential avenue for rejecting ‘undesirable’ particles, was observed (Mundahl and Wissing 1988). Also, Higgins et al. (2006) compared the nutrient content of sediments in three lakes to foregut (esophagus and gizzard) contents from gizzard shad caught in each lake. Since nutrient analyses revealed a higher percentage of carbon, nitrogen and phosphorus in fish foreguts relative to sediment samples, they concluded that gizzard shad selected for a relatively nutrient-rich portion of the available sediment in each lake (Higgins et al. 2006). However, the sediment sampled may not have been an exact depiction of the sediment that gizzard shad consumed, since gizzard shad may have fed in many benthic. Additionally, Higgins et al. (2006) did not test the influence of fish-secreted substances such as mucus on quantified foregut nutrients.

Mucus may be a component of the foregut contents of gizzard shad, since mucus-secreting goblet cells line gizzard shad gut epithelia and are present in the epithelial covering of gill rakers (Heinrichs 1982). Drenner (1982a) found plankton bound in mucus in gizzard shad epibranchial organs. However, the plankton probably were not captured in mucus, since gizzard shad were not observed to use mucus to trap particles in the anterior portion of the pharynx during crossflow filtration (Sanderson et al. 2001). Instead, mucus may be used to aggregate particles in the posterior pharynx before swallowing (Sanderson et al. 2001). Nutrients quantified in fish foreguts and attributed to food may, in fact, include nutrients from mucus ingested with food particles or mucus secreted into the foregut. The nutrients in mucus taken from the
oropharyngeal cavity of adult gizzard shad may represent 46% ± 15 of quantified foregut nutrients (Chapter 2).

The purpose of this study was to determine whether gizzard shad selectively ingest particles of high nutrient content based on internal, fluid-dynamic processes. Previous research that quantified the nutrients of mucus from the oropharyngeal cavity of gizzard shad will be used to correct for the likely presence of mucus in gizzard shad foreguts (Chapter 2).

**METHODS**

**Gizzard shad collection:** Gizzard shad were collected using electroshocking and seine-netting from waters in the Virginia coastal plain, specifically, College Creek, Waller Mill Reservoir, Chickahominy Lake and Little Creek Reservoir. Gizzard shad were maintained at 19–21°C in glass aquaria with external bio-ball filtration. Fish were fed TetraMin® flake food daily and pH and ammonia were monitored in the aquaria. A nitrofurazone anti-fungal agent was used as a prophylaxis in holding aquaria (but was not added to experimental aquaria) when fish were brought into the laboratory, and fish collected from different reservoirs were kept in separate aquaria so that any communicable diseases would not be passed between fish populations. Gizzard shad were allowed to adjust to laboratory conditions for at least 10 days before inclusion in experiments.

**Food collection:** The objective of this study was to determine whether gizzard shad can select more nutritious particles from those available in the environment based on internal, fluid-dynamic mechanisms. Therefore, during feeding trials, gizzard shad were presented with a mixture of two particle classes of different nutrient content. The “high quality” particles (with a relatively high C and N content) were ground Big Strike® brand fish pellets. The “low quality”
particles were benthic sediment composed of both detritus and inorganic particles. Benthic sediment was collected in the main channel of Lake Matoaka near the western bank in 2.5-3.0 meters of water on the campus of the College of William and Mary in Williamsburg, VA using an Ekman Grab sampler. The top 2 cm of sediment were collected, since the uppermost layer of benthic sediment is most likely to be consumed by gizzard shad feeding in a natural setting. The sediment was dried completely in an oven at 60 °C. Sediment and food particles were ground using a Waring® commercial blender and sifted to a uniform size range using Dual Manufacturing Co.© market grade sieves with mesh no. 200 (75 μm), 120 (125 μm) and 60 (250 μm). Preliminary experiments were run using two particle size ranges: 125-250 μm and 75-125 μm. These size ranges were used because gizzard shad were shown not to select for particle (microspheres and zooplankton) sizes above 60 μm (Drenner et al. 1984).

To verify that gizzard shad would consume both sediment and food particles during experimental trials, sediment particles and food particles were introduced on separate occasions to holding aquaria. When either particle type was added to the aquaria, gizzard shad changed from typical respiratory motions to series of rapid suctions associated with pump suspension feeding.

**Preliminary Trials**

**Feeding Trials using 125-250 μm Particles:** During a first round of preliminary trials, a mixture of 5.00 g Big Strike food and 5.00 g sediment particles in the 125-250 μm size range was used.

Since the presence of food in gizzard shad foreguts at the beginning of experiments could affect the quantification of nutrient content, before experiments were conducted, three gizzard shad were dissected 24 hours after feeding to confirm that 24 hours is sufficient time for the foregut
to empty. Because their foreguts were devoid of food, 24 hours was deemed an appropriate period of food deprivation. Gizzard shad were moved to the experimental aquarium 24 hours prior to each trial, during which time they were not fed and were allowed to acclimate to aquarium conditions.

 Trials began with the addition of particles into a 110L aquarium containing 70L tap water. In addition to air stones along the bottom of the aquarium, four Little Giant® model PE-A submersible water pumps (150 liters-hour⁻¹) attached to perforated tygon tubing were used to maintain a homogeneous mixture of food and sediment within the water column during trials. These pumps were used to prevent particles from settling to the bottom of the aquarium, where they might sort by differences in physical characteristics such as density.

 Gizzard shad (65-95 mm SL) were placed in groups of three (for social purposes) in the aquarium. Fish were allowed to feed for one hour. During that time, water column samples were taken 0, 30 and 60 minutes after the beginning of each trial as a measure of the particles suspended in the aquarium. Water column samples were taken by moving a tube (2.5 cm diameter) vertically through the water column onto a randomly placed rubber stopper resting at the bottom of the aquarium, retaining a 120 ml sample of the particles present in the water along the entire height (31 cm) of the aquarium.

 At the end of one hour, one randomly chosen fish was sacrificed for foregut analysis. Only one fish was sacrificed for gut analysis each trial to avoid pseudoreplication in the form of multiple non-independent samples. The fish was dissected immediately (within 5-7 minutes of capture) to extract contents of the foregut, considered to be the esophagus and gizzard. The foregut is commonly used in gut content analysis and feeding selectivity studies in gizzard shad (Mundahl
and Wissing 1988, Higgins et al. 2006). All fish in this study were sacrificed using an overdose of MS-222 and subsequent pithing.

To verify whether the mixture of 5.00 g food and 5.00 g sediment particles (125-250 μm particles) remained suspended and evenly mixed in the experimental aquarium during trials, samples of food and sediment only were analyzed for C and N content. In the absence of fish, 10.00g food particles (125-250 μm particles) were introduced into the experimental aquarium and water column samples were collected 0, 30 and 60 minutes after food particles were introduced. Additionally, the C and N content was quantified from dry sediment samples (125-250 μm particles) not introduced into the aquarium.

**Elemental analysis:** The entire contents of gizzard shad foreguts and entire water column samples of available particles were filtered onto tared 25mm Whatman® GF/C glass microfiber filters for total C and N analysis. Filtered samples were kept in a drying oven at 60°C for 24 hours before they were weighed on a Perkin-Elmer AD6 balance to determine dry mass. A Perkin-Elmer 2400 Analyzer was used to determine the percent total C and N by dry mass of foregut and water column samples (Higgins et al. 2006, Wach and Chambers 2007).

**Preliminary Trials using 75-125 μm Particles:** The results of preliminary experiments using particles in the size range 125-250 μm showed that the pumps and airstones used in the experimental aquarium were not sufficient to maintain a homogeneous suspension of food and sediment particles in the water column, since sediment particles were likely sinking and the mixture of particles in the water column did not contain equal proportions of food and sediment (see Results, Figures 3.1, 3.2). Therefore, preliminary trials were conducted using 75-125 μm particles in the absence of gizzard shad.
Trials using 75-125 μm particles were completed using 10.00 g sediment only, 10.00 g food only, and a mix of 5.00 g sediment and 5.00 g food. Trials ran for one hour. During that time, three replicate water column samples were taken at each of five time periods, 0, 5, 15, 30 and 60 minutes after the beginning of each trial as a measure of the particles suspended in the aquarium.

**Proposed Methods for Feeding Trials Using 75-125 μm Particles:**

Due to limited gizzard shad availability, feeding trials using 75-125 μm particles were not completed. For feeding trials, gizzard shad (65-95 mm SL) will be placed in groups of three (for social purposes) in the same 110L aquarium containing 70L water used in preliminary experiments. Trials will begin with the addition of 5.00 g sediment and 5.00 g food particles (75-125 μm size range) to the aquarium, and particles will be suspended by submersible water pumps and air stones along the bottom of the aquarium. Fish will be allowed to feed for one hour. During that time, water column samples will be taken using the same method as in preliminary trials, 0, 5, 15, 30 and 60 minutes after the particles are added to the water, as a measure of the food available to gizzard shad.

**Statistical analysis:** Differences in concentrations of each nutrient (C and N) were compared between the foregut contents and water column samples taken 0, 30, and 60 minutes after the beginning of feeding trials using 125-250 μm particles using one-way ANOVA and Tukey-Kramer HSD tests (p<0.05).

**Contribution of Sediment vs. Food in Water Column Samples:** A system of equations was developed to calculate the percent contribution by dry mass of sediment and food in the water
column samples taken during trials which use a mixture of food and sediment particles (Equations 1 and 2). Assuming that the mass of the water column samples is 1.0, substituting Equation 2 into Equation 1 and solving for $W_{food}$, the dry mass of food in the entire water sample, yields Equation 3.

**Equation 1**

$$\left(\%N_{sed}\right)W_{sed} + \left(\%N_{food}\right)W_{food} = \left(\%N_{mix}\right)W_{mix}$$

**Equation 2**

$$W_{sed} = W_{mix} - W_{food}$$

**Equation 3**

$$W_{food} = \left[\left(\%N_{mix}\right) - \left(\%N_{sed}\right)\right]/\left[\left(\%N_{food}\right) - \left(\%N_{sed}\right)\right]$$

**Equations 1, 2, and 3.** Contribution of sediment and food to water column samples taken during trials using a mixture of food and sediment particles.

**Known Variables:**

- $\left(\%N_{food}\right)$: % nutrient per g dry mass in food
- $\left(\%N_{sed}\right)$: % nutrient per g dry mass in sediment
- $\left(\%N_{mix}\right)$: % nutrient per g dry mass in the water sample

**Unknown variables:**

- $W_{mix}$: dry mass of the water sample
- $W_{food}$: dry mass of food in the foregut
- $W_{sed}$: dry mass of sediment in the foregut

**Contribution of Sediment vs. Food in Foregut Contents:** A system of equations was developed to define the proportion, by dry weight, that both food and sediment particles contribute to the entire foregut content, assuming that each foregut sample contains 46% ± 15 mucus by dry mass (Equations 4 and 5, Chapter 2). Assuming that the mass of the foregut contents is 1.0, substituting Equation 5 into Equation 4 and solving for $W_{sed}$, the dry mass of sediment in the entire foregut, yields Equation 6. See Appendix VI for the entire derivation of Equation 6.
Equation 4 \[ \frac{\%N_{\text{mucus}}(W_{\text{mucus}})}{\%N_{\text{gut}}(W_{\text{gut}})} + \frac{\%N_{\text{food}}(W_{\text{food}})}{\%N_{\text{food}}(W_{\text{food}})} + \frac{\%N_{\text{sed}}(W_{\text{sed}})}{\%N_{\text{sed}}(W_{\text{sed}})} = 1. \]

Equation 5 \[ (W_{\text{gut}}) = (W_{\text{mucus}}) + (W_{\text{food}}) + (W_{\text{sed}}). \]

Equation 6 \[ (W_{\text{sed}}) = \frac{(\%N_{\text{gut}}) - (\%N_{\text{mucus}})(W_{\text{mucus}}) - (\%N_{\text{food}}) - (\%N_{\text{food}})(W_{\text{mucus}})}{(\%N_{\text{sed}}) - (\%N_{\text{food}})}. \]

Equations 4, 5, and 6. Contribution of sediment and food to foregut contents of gizzard shad, assuming that foregut contents are 46% mucus by dry mass.

**Known Variables:**
- \(\%N_{\text{mucus}}\) = nutrient per g dry mass in mucus
- \(\%N_{\text{food}}\) = nutrient per g dry mass in food
- \(\%N_{\text{sed}}\) = nutrient per g dry mass in sediment
- \(W_{\text{gut}}\) = dry mass of the foregut sample
- \(W_{\text{mucus}}\) = dry mass of mucus in the foregut

**Unknown variables**
- \(W_{\text{food}}\) = dry mass of food in the foregut
- \(W_{\text{sed}}\) = dry mass of sediment in the foregut

**RESULTS**

**Preliminary Trials**

**Trials Using 125-250 \(\mu\)m Particles:** Nutrients (both C and N) quantified in water column samples during trials using 125-250 \(\mu\)m particles (5.00 g food and 5.00 g sediment) tended to increase as time progressed (Figures 3.1, 3.2). The result of a repeated-measures ANOVA comparing the %C of water column samples taken 0, 30 and 60 minutes after the beginning of each trial was significant (p<0.001). Results of Tukey-Kramer pairwise comparisons indicated that the mean %C by dry weight in water column samples was significantly different from the beginning of each trial (t=0, 33.4% ± 1.1) to samples taken 30 (41.7% ±1.2, p<0.001) and 60 minutes after the beginning of each trial (41.48% ± 2.2, p<0.001, mean ± SD, n=4, Figures 3.1, 3.2). Samples taken at t= 30 and t=60 minutes were not significantly different (p=0.99). The increase in %N from t=0 (4.9% ± 0.6) to t= 30 (6.5% ± 1.1) and t=60 minutes (7.61% ± 3.1) was not significant (repeated measures ANOVA, p=0.2, mean ± SD, n=4, Figures 3.1, 3.2).
The C and N quantified in water column samples at t=30 and 60 minutes was very similar to the mean C and N content of water column samples taken using 10.00 g of food particles in the absence of fish at t=0 (45.1%C ± 0.3, 6.2%N ± 0.03), t=30 (45.3%C ± 0.4, 6.13%N ± 0.01), and t=60 (44.9%C ± 0.2, 6.17%N ± 0.04, n=3) minutes, and was higher than the C and N content of dry sediment samples (9.6%C ± 0.2, 0.4%N ± 0.04, n=4, Figure 3.2). This similarity between the nutrients quantified in trials using a mixture of food and sediment and trials using only food particles (125-250 µm) may indicate that the pumps and airstones used to maintain particles in suspension were insufficient to continuously suspend the more dense, inorganic sediment particles which may have had a lower nutritional quality. Sediment particles, which were darker in color and thus distinguished from food particles, were visible on the bottom of the experimental aquarium during feeding trials conducted using 125-250 µm particles. The C and N quantified in water column samples appeared stable at t=30 and t=60 minutes, indicating that the particles in the aquarium may have reached equilibrium within the first 30 minutes of experimental trials (Figures 3.1, 3.2).

The result of an ANOVA comparing the %C quantified in gizzard shad foreguts to water column samples from feeding trials using 125-250 µm particles was significant (p<0.001). Tukey-Kramer pairwise comparisons showed that the %C (42.4% ± 4.2) quantified in gizzard shad foreguts from trials using 125-250 µm particles was significantly different than the nutrients quantified in water column samples taken at t=0 minutes (p=0.001), and was not significantly different from nutrients quantified in water column samples taken at t=30 (p=0.97) or t=60 (p=0.94) minutes (Figure 3.1, mean ± SD, n=4). The result of an ANOVA comparing the %N quantified in water column samples and gizzard shad foreguts (7.6% ± 1.6) from feeding trials using 125-250 µm particles was not statistically significant (p=0.32, Figure 3.1, mean ± SD, n=4).
The fact that there are no statistically significant differences in the %C and %N quantified in water column samples of available food taken at t=30 and t=60 minutes after the beginning of each trial and gizzard shad foreguts may indicate that gizzard shad did not selectively ingest nutrient-rich particles. However, it is unclear whether gizzard shad may have selectively ingested high-quality particles during the first 30 minutes of feeding trials before the nutrient levels in the aquarium stabilized.

**Trials Using 75-125 μm Particles:** Results from preliminary experiments showed a drop in nutrient content for trials with mixtures of food and sediment particles (75-125 μm) between t=0 and t=5 minutes, before the nutrients quantified in water column samples stabilized (Figure 3.3). This drop was likely due to the particles being poorly mixed with the aquarium water when they were introduced into the water column. Water column samples may have collected clumps of food rather than a homogeneous mixture. Between t=5 and t=60, the C and N content of water column samples were stable (Figure 3.3). This trend also was seen in the trials using sediment alone (Figure 3.3). Sediment particles were not observed settling in large quantities on the bottom of the experimental aquarium as they did in preliminary trials using 125-250 μm particles.

To calculate the proportion of food vs. sediment in the water column and for comparison with fish foreguts in actual experiments, the nutrient content of the water column 5 minutes after the beginning of each trial was used. These samples most accurately represent the nutrients available to gizzard shad from the beginning of experiments because they represent the water column nutrient content after particles have become homogenized in the aquarium and before
any large changes in nutrient content in the water column owing to gizzard shad feeding could have occurred.

The proportion of water column samples comprised of food vs. sediment was calculated based on the %C in samples taken from trials (75-125 μm) using food particles only (44.7% ± 0.4), sediment particles only (9.5 % ± 0.3) and a mix of food and sediment (26.7% ± 0.5). Based on C content, water column samples from trials using this mixture of 5.00 g food and 5.00 g sediment particles (75-125 μm) were 48.7 ± 0.01% food and 51.3 ± 0.01% sediment by dry mass (Equations 2 and 3, mean ± SD).

The proportion of water column samples comprised of food vs. sediment also was calculated based on the %N in samples taken from trials (75-125 μm) using food particles only (6.2% ± 0.1), sediment particles only (1.0% ± 0.4) and a mix of 5.00 g food and 5.00 g sediment (3.60 ± 0.4 %C). Based on N content, water column samples from trials using a mixture of food and sediment particles were 50.1 ± 0.03% food and 49.9 ± 0.03% sediment by dry mass (Equations 2 and 3, mean ± SD).

**Anticipated Results from Feeding Experiments using 75-125 μm Particles**

Due to limited gizzard shad availability, feeding selectivity trials in the presence of fish using 75-125 μm particles were not conducted. However, results from these proposed experiments will allow for calculations of the proportion of gizzard shad foregut contents composed of food and sediment, assuming foreguts are 46% ± 15 mucus. If the proportion of particles in gizzard shad foreguts composed of food is higher than the proportion of food available in the water column, then the hypothesis that gizzard shad selectively ingest more nutritious particles will be supported.
DISCUSSION

Preliminary trials using 125-250 μm particles indicated that the airstones and water pumps used to suspend particles were not sufficient to prevent sediment particles from settling to the bottom of the aquarium (Figures 3.1, 3.2). This was likely due to an increased proportion of high-density inorganic sediment particles in the 125-250 μm fraction of the sediment. There was no statistically significant difference in the quantified C and N content of water column samples of available food taken at t=30 and t=60 minutes after the beginning of each trial and gizzard shad foreguts, indicating that gizzard shad may not have selectively ingested nutrient-rich particles (Figure 3.1). However, it is unclear whether gizzard shad may have selectively ingested high-quality particles during the first 30 minutes of feeding trials before the nutrient levels in the aquarium stabilized. Because the water column food availability was not consistent during trials using particles in the 125-250 μm size range, it is not reasonable to use these data to quantify feeding selectivity in gizzard shad. Therefore, proposed feeding selectivity trials will use sediment and food particles in the size range of 75-125 μm.

The 75-125 μm sediment particles remained in suspension during preliminary trials, suggesting that the density of these sediment particles is more similar to the density of food particles in the same size range (Figure 3.3). The 75-125 μm sediment and food particles available to gizzard shad during proposed feeding trials will be well mixed in the water column, of a homogeneous size range, and of a reasonably similar range of densities. Therefore, any observed feeding selectivity may be related to the nutrient content of the available particles. Due to limited gizzard shad availability, feeding selectivity experiments using particles in the 75-125 μm size range were not conducted. Once data have been collected from feeding selectivity experiments
using gizzard shad, it will be possible to assess whether gizzard shad selectively ingest the more nutrient-rich portion of the particles available in experimental aquaria.

Documented explanations for internal, fluid-dynamic particle selectivity during crossflow filtration in suspension-feeding fish include particle size and density (Drenner et al. 1984, Callan and Sanderson 2003). For example, the proportion of particles in various size classes suspended in a pool and removed by gizzard shad feeding on a mixture of zooplankton (78-185.5 μm size range) and microspheres (10-80 μm size range) increased with particle size class, reaching a maximum and asymptote at 60 μm (Drenner et al. 1984). Callan and Sanderson (2003) observed that, during crossflow filtration in suspension-feeding carp (Cyprinus carpio, Cyprinidae), low-density food particles were filtered and retained, while more dense, inorganic sand particles sank ventral to the slurry of food particles and either were spat anteriorly out of the mouth or left the oropharynx through gaps between the gill arches.

Because the available particles in the gizzard shad selectivity experiments using 5.00 g sediment and 5.00 g food in the 75-125 μm particle size range will be at least qualitatively similar based on previously hypothesized mechanisms of particle selectivity (size, density), it is unlikely that feeding selectivity by gizzard shad will be observed. Rather, observations of feeding selectivity based on nutrient content from previous studies (e.g., Higgins et al. 2006, Mundahl and Wissing 1988) may be explained by (1) fluid-dynamic selectivity for physical particle characteristics or (2) fish behavior.

Suspension-feeding fish that ingest benthic detritus may choose to feed in locations with more nutrient-rich sediment. Low-energy areas with slow currents accumulate more fine particulate detritus, since the velocity of current flow dictates the size and density of particles which settle
out of suspension (Bowen 1983). Additionally, fish might choose their feeding location on a smaller scale, engulfing particles in localized areas defined by variations in benthic topography where more or less dense particles may settle. For example, desirable particles may settle in troughs or depressions in the benthic surface.

Additionally, fish such as gizzard shad might re-suspend sediment and filter more nutritious particles from the water once dense, inorganic particles settle (Mundahl and Wissing 1987, Smoot 1999). Gizzard shad may selectively ingest low-density detritus by agitating the sediment when they feed, thereby disturbing the sediment-water interface and suspending detrital particles (Mundahl and Wissing 1987, Smoot 1999). Since high-density particles sink before low-density particles, the low-density particles may remain suspended above the sediment-water interface as gizzard shad feed (Mundahl and Wissing 1987, Smoot 1999). Because organic detritus particles tend to be smaller and less dense than inorganic sediment particles, gizzard shad that selectively ingest low-density particles may also selectively ingest more nutrient-rich particles (Smoot 1999). Previous studies which characterized gizzard shad feeding selectivity for more nutritious particles did not differentiate between behavioral and fluid-dynamic mechanisms for feeding selectivity (Mundahl and Wissing 1988, Higgins et al. 2006).

If feeding experiments result in a difference between the nutrient content of particles in gizzard shad foreguts and the nutrient content of particles available in the water column, the hypothesis that gizzard shad may selectively ingest more nutritious particles using internal, fluid-dynamic mechanisms within the oropharyngeal cavity will be supported. Gizzard shad feeding selectivity may be explained by nutrient-based selectivity as a result of chemical cues from particles or by shortcomings in the experimental design which could allow for some difference in
the physical particle properties, such as particle density, between the food and sediment particles available to gizzard shad.

Gizzard shad may be able to selectively ingest more nutritious particles based on nutrient content alone. There are chemosensory cells and taste buds in the entrance canals to the epibranchial organs and sparsely throughout the epibranchial organs of gizzard shad (Schmitz and Baker 1969, Heinrichs 1982). It is reasonable that gizzard shad might reject a large number of particles by expelling the contents of the epibranchial organs and not swallowing those particles. However, the mechanism by which gizzard shad might physically sort particles on an individual particle basis or other small scale is unclear. In carp, protrusions of tissue from the palatal organ can retain food particles and likely serve a chemosensory function (Callan and Sanderson 2003). However, gizzard shad are not known to exhibit these palatal protrusions.

Mundahl and Wissing (1988) suggested that there may be some threshold of nutrient content or food quality, below which gizzard shad choose to selectively ingest certain particles, and above which they do not feed selectively. This hypothesis is rooted in the observation that gizzard shad in the laboratory selectively ingested high-quality particles when feeding on a low-quality diet (based on C and N content), but did not feed selectively when a high-quality diet was available (Mundahl and Wissing 1988). The high-quality diet they used was composed of trout pellets, while the low-quality diet was a mix of aufwuchs (algae scraped from rocks) and sediment. The selectivity observed in gizzard shad feeding on the low-quality diet may be the result of gizzard shad selecting the aufwuchs particles, which were likely less dense than sediment particles (Mundahl and Wissing 1988). Conversely, the high-quality diet available for gizzard shad was derived from a single source (ground trout pellets), and may not have presented differences in
particle nutrient content or density. Additionally, Mundahl and Wissing (1988) did not account for fish-secreted mucus, which might influence the nutrients quantified in food ingested by gizzard shad. The range of mean N content quantified by Mundhal and Wissing in gizzard shad foregut contents (7.4-8.1) was higher than the range of mean N content quantified in the available laboratory diet of high-quality particles (6.5-6.8), indicating that gizzard shad likely ingested mucus in conjunction with food particles.

In addition to the possibility that gizzard shad use chemosensory cues to ingest certain particles, if feeding selectivity is observed during experiments, there may be some physical differences in particle types that were not controlled by the experimental design. For example, the extent to which particle shape may affect retention by suspension-feeding fish is not known. Additionally, it was not feasible to completely control differences in density of food and sediment particles, beyond ensuring that both remained suspended in the water column for the duration of trials. The fluid-dynamic forces explaining why particles remain suspended in the mainstream flow during crossflow filtration are not completely understood (Sanderson et al. 2001). The limit for the magnitudes of particle size and density that may remain suspended has not been quantified. It is possible that some particle size selectivity could occur within the 75-125 µm size range during crossflow filtration. However, this particle size range was chosen because gizzard shad were shown not to select for particle (microspheres and zooplankton) sizes above 60 µm (Drenner et al. 1984). Despite these possibilities for slight differences in particle characteristics (shape, size and density), the experimental design for the proposed feeding selectivity experiments is highly controlled. Because the external, fish behavior component for particle selectivity is eliminated, any selectivity observed can be related to fluid-dynamic processes in the oropharyngeal cavity of gizzard shad.
In conclusion, experiments using particles in the 125-250 µm size class did not offer evidence for selective ingestion of nutrient-rich particles by gizzard shad (Figure 3.1). However, the airstones and water pumps used to suspend particles were not sufficient to prevent sediment particles in the 125-250 µm size class from settling to the bottom of the aquarium (Figures 3.1, 3.2), and it is not feasible to use particles in the 125-250 µm size class in experiments designed to quantify feeding selectivity in gizzard shad. Food and sediment particles in the 75-125 µm size class did remain in suspension during preliminary trials, and will be used for proposed feeding selectivity experiments (Figure 3.3). It is not expected that gizzard shad will selectively ingest food or sediment particles during feeding experiments using particles in the 75-125 µm size range. By allowing fish to feed in a highly controlled environment, it may be possible to distinguish the cues by which suspension-feeding detritivorous fish selectively ingest particles in nature, or whether they are selective at all.

Ahlgren, M. O. 1996. Selective ingestion of detritus by a north temperate omnivorous fish, the juvenile white sucker, Catostomus commersoni. Environmental Biology of Fishes 46:375-381.


Figure 3.2. Percent of each nutrient (C, N) by dry mass (mean ± standard deviation) in water column samples taken 0, 30 and 60 minutes after the beginning of each trial, and in foregut contents from fish taken at the end of each experiment. Trials used 125-250 μm size particles (n=4). Bars with the same letter within each nutrient graph are not significantly different (p>0.05).
Figure 3.3. Percent of each nutrient (C, N) by dry mass (mean ± standard deviation) in trials using 125-250 μm particles. Samples represent: (1) water column samples taken 0, 30 and 60 minutes after the beginning of trials with gizzard shad using a mix of 5.00g sediment and 5.00g food (n=4) (2) water column samples taken 0, 30 and 60 minutes after the beginning of experimental trials using 10.00g food particles in the absence of fish (n=3), and (3) sediment samples before being added to aquaria (n=4).
Figure 4.3. Percent of each nutrient (C, N) by dry mass (mean ± standard deviation) in water column samples from preliminary trials without fish using particles in the 75-125μm size range. Water column samples were taken 0, 5, 15, 30 and 60 minutes after the beginning of each trial (n=3).
APPENDIX I.

INFLUENCE OF MS-222 ANESTHETIC ON NUTRIENTS QUANTIFIED IN GIZZARD SHAD MUCUS

MS-222 (Tricaine Methane Sulphonate, C_{10}H_{15}NO_{5}S) is a commonly used organic compound used to anesthetize and euthanize fish. In this study, gizzard shad were euthanized using an overdose of MS-222 and subsequent pithing. To determine whether nutrients from MS-222 may have altered the nutrients quantified in fish mucus, the C and N content of mucus collected by scraping using a rubber-tipped probe from fish that were anesthetized with MS-222 was compared to fish that had not been anesthetized. Mucus was collected using the same method from non-anesthetized gizzard shad that died in transit from field collection sites to the laboratory, and mucus was collected within 45 minutes of death. All samples were analyzed for C and N content using the method described in Chapter 2. Comparisons between the two mucus types were made using Welch’s t-test. Additionally, samples of MS-222 were analyzed for C and N content.

There was no significant difference in the %C by dry weight in mucus samples collected from anesthetized fish (52.2 % ± 4.7, n=3) and non-anesthetized fish (48.8% ± 6.2, n=7, p=0.4, mean ± SD, Figure A.1). There also was no significant difference in the %N by dry weight in mucus collected from anesthetized (11.4% ± 1.0) and non-anesthetized (11.0% ± 2.0) fish (p=0.7, mean ± SD, Figure A.1). The C and N content of MS-222 alone is lower (45.37%C ± 0.1; 5.44%N ± 0.0) than the nutrients quantified in gizzard shad mucus (n=3, mean ± SD).

While there is a slight trend showing that the nutrients quantified in fish anesthetized using MS-222 are higher than fish that were not anesthetized, this trend is not statistically significant and
mucus collected using both anesthetized and non-anesthetized fish was used in analyses of mucus-derived nutrients found in fish foreguts.
Figure A.1. Carbon and Nitrogen quantified in mucus collected from the oropharyngeal cavity of gizzard shad with (n=3) and without (n=7) the use of MS-222 as an anesthetic. Differences in C and N content are not statistically significant (p>0.05, mean ± standard deviation).
APPENDIX II.

MUCUS COLLECTION METHODS AND

MICROSCOPIC QUALITATIVE ANALYSIS OF MUCUS FROM THE OROPHARYNGEAL CAVITY OF GIZZARD SHAD

MUCUS COLLECTION METHODS

Two alternate methods were used in the collection of mucus samples from the oropharyngeal cavity of large adult gizzard shad (150-260 mm SL). In the first method, a rubber-tipped probe was used to gently scrape the surfaces of gill rakers and gill arches within the oropharyngeal cavity of gizzard shad that were either euthanized using an overdose of MS-222 and subsequent pithing or that died in transit to the laboratory from the field. Mucus was collected by scraping within 5-7 minutes of euthanasia or within 45 minutes of death. The second mucus collection method was a modification of Gorlick’s (1980) method for collecting body mucus from marine fish. Deionized water was heated to 50°C. Fish euthanized using an overdose of MS-222 were suspended over a beaker and a syringe was used to direct a gentle stream of heated water against the branchial arches and gill rakers within five minutes of euthanasia. Consistent with Gorlick’s (1980) observation, 50°C was sufficient to dislodge surface mucus, which was then collected in the beaker below.

Mucus subsamples were filtered onto tared 25mm Whatman® glass microfiber GF/C filters for total C and N analysis. The volume of mucus that comprised a subsample was dictated by the point at which the filter paper saturated. Filtered samples were kept in a drying oven at 60°C for 24 hours before they were weighed on a Perkin-Elmer AD6 balance to determine dry mass. A Perkin-Elmer 2400 Analyzer was used to determine the percent total C and N by dry mass of mucus samples (Higgins et al. 2006, Wach and Chambers 2007).
Samples were collected by rinsing from five adult gizzard shad; however, only one fish yielded mucus samples of sufficient weight for nutrient analysis. While the total mucus sample collected might not vary substantially between fish, the samples collected by rinsing were more likely to saturate filter papers so a smaller sample was retained. This is because samples obtained by rinsing were distributed through approximately 60mL water, while scraped samples were more likely to remain in a globular form. The scraped mucus tends to remain on the surface of the filter paper in discrete conglomerates, while filters became saturated quickly with the more diffuse rinsed samples. The mucus samples collected by rinsing represent 3 replicate samples from one gizzard shad that yielded large mucus samples. Comparisons between the two mucus sampling methods were made using Welch’s t-test.

The %C from rinsed mucus samples (56.0% ± 1.3) was significantly higher than the %C from scraped mucus samples (49.8% ± 5.7, p= 0.009, mean ± SD, Figure A.2). Similarly, the %N from rinsed mucus samples (12.6% ± 3.4) was significantly higher than the %N from scraped samples (11.1% ± 1.7, p=0.03, mean ± SD, Figure A.2).

However, because the only successful samples of mucus collected by rinsing of the intra-oral surfaces were all from the same fish, it is possible that the higher nutrient content quantified in these mucus samples is an artifact of the content of the fish, rather than the sampling method. Therefore, for analysis of the mucus and food contributions to the foregut of gizzard shad, only mucus samples collected by scraping were used in the final analysis of food and mucus contributions to gizzard shad foregut contents (Chapter 2).
MICROSCOPIC QUALITATIVE ANALYSIS OF MUCUS

To qualitatively assess whether epithelial cells were present in mucus samples, some collected mucus was reserved on-ice for observation under a microscope within one hour of collection. Mucus collected by both scraping (Figure A.3) and rising (Figure A.4, A.5) was observed. Epithelial cells were observed in mucus samples. Cells were typically clumped together, and may have been aggregated by mucus.

It is possible that the cells observed in mucus samples were present as a result of dislodging epithelial tissues along with mucus during mucus collection. However, cells were observed in samples collected using both scraping and rinsing, and it is likely that the mucus lining the intra-oral surfaces in fish naturally contains cells. Sloughed epithelial cells and cellular debris may be found in fish mucus, particularly in the layer of macromolecular gel (mucus) covering fish epidermal surfaces called the cuticle (Shephard 1994).
Figure A.2. Carbon and Nitrogen content by dry mass of mucus collected from the oropharyngeal cavity of fish. Mucus was collected by rinsing (n=3) and scraping (n=10). Bars with different letters within each nutrient graph are significantly different (mean ± standard deviation, p>0.05).
Figure A.3. Cells visible in mucus collected by scraping the intra-oral surfaces of gizzard shad (400x).
Figure A.4. Cells visible in mucus collected by scraping the intra-oral surfaces of gizzard shad (400x).
Figure A.5. Cells visible in mucus collected by rinsing the intra-oral surfaces of gizzard shad (400x).
APPENDIX III.

COMPARISON OF MUCUS TAKEN FROM INTRA-ORAL AND LATERAL SURFACES OF GIZZARD SHAD

In addition to samples taken from the oropharyngeal cavity of gizzard shad (“oral mucus”), mucus scrapings were taken from the external, lateral surfaces of large adult gizzard shad (“external mucus”, 150-260 mm SL). The C and N content of gizzard shad oral and external mucus was quantified using the methods described in Chapter 2. The nutrients (C and N) in oral cavity and lateral, external mucus were then compared to determine whether the nutrient content of mucus varies among different regions of the fish. Comparisons between the two mucus types were made using Welch’s t-test.

There was no significant difference between the %C by dry weight in external (54.4% ± 5.8) and oral (49.8% ± 5.7) mucus (p=0.1, mean ± SD, Figure A.6). Similarly, the %N quantified in external (12.2% ± 1.5) and oral (11.1% ± 1.7) mucus were not significantly different (p=0.2, mean ± SD, Figure A.6). However, the content of C and N quantified in mucus scraped from the external surfaces of gizzard shad was slightly higher than mucus collected from oral cavity surfaces.
Figure A.6. Carbon and Nitrogen content of mucus collected from the external and oral cavity surfaces of gizzard shad (n=10, mean ± standard deviation).
APPENDIX IV.

NUTRIENT CONTENT OF SAMPLES FROM THE EPIBRANCHIAL ORGANS OF GIZZARD SHAD

In addition to samples taken from gizzard shad foreguts for nutrient analysis during experiments, samples were taken from the epibranchial organs when possible.

The epibranchial organs are paired organs located above the gills and supported by the fourth and fifth branchial arches (Kapoor et al. 1975). These organs, which contain an entrance canal leading to a blind sac, are hypothesized to consolidate and amass food particles that are then released into the esophagus (Schmitz and Baker 1969). However, the details of the function of epibranchial organs are not understood (Schmitz and Baker 1969, Kapoor et al. 1975). The mucosal lining of the epibranchial organs contains goblet cells, and Drenner et al. (1982a) found particles enveloped in mucus in the epibranchial organs of gizzard shad. There has been no study quantifying the nutrients in the epibranchial organs of feeding gizzard shad.

Out of 6 trials, the epibranchial organs of 4 fish contained food. Because the volume of particles in epibranchial organs was small (0.24-0.71 mg) relative to esophagus and gizzard contents (0.30-3.20 mg), only 2 samples were successfully analyzed for C and N content.

Carbon Content Comparison: ANOVA comparing the %C by dry weight in the epibranchial organs (50.8% ± 3.7, n=2), foregut contents (50.9% ± 4.6, n=6), mucus (49.8% ± 5.7, n=10), and water column samples of food (45.2% ± 0.8, n=14) was statistically significant (p=0.009, mean ± SD Figure A.7). Tukey-Kramer HSD pairwise comparisons show that the %C quantified in the epibranchial organs was not significantly different from foregut (p=0.9), mucus (p=0.9) or water samples (p=0.2, Figure A.7). Consistent with the results in Chapter 2, water column samples of
food were significantly different than foregut contents (p=0.03) and mucus samples (p=0.04). Mucus and foregut samples were not significantly different (p=0.9).

**Nitrogen Content Comparison**: ANOVA comparing the %N by dry weight in epibranchial organ (8.6% ± 0.2, n=2), foregut (8.5% ± 0.7, n=6), mucus (11.1% ± 1.7, n=10), and water column samples (6.4% ± 0.2, n=14) was statistically significant (p<0.001, mean ± SD, Figure A.7). Tukey-Kramer HSD pairwise analyses show that the nutrients quantified in the epibranchial organs were significantly different then mucus (p=0.01) and water-column food samples (p=0.03), but were not statistically different from foregut samples (p=.99, Figure A.7). Consistent with the results of Chapter 2, water column samples were significantly different from foregut contents (p=0.001) and mucus (p<0.001). The N quantified in gizzard shad mucus and foregut contents was also significantly different (p<0.001).

The similarities between foregut and epibranchial organ samples indicate that food particles may enter the epibranchial organs and become aggregated in mucus before being released to the esophagus, as hypothesized by Schmitz and Baker (1969). It is possible that the mucus present in food sampled from gizzard shad foreguts is secreted in the epibranchial organs to conglomerate particles. Epibranchial organs have been linked to feeding in many species of microphagous fish, including Osteoglossiformes, Cypriniformes, Gonorhychiformes and Clupeiformes, but the exact function of the epibranchial organs of gizzard shad and other species is still unknown (Schmitz and Baker 1969, Kapoor et al. 1975).
Figure A.7. Percent of each nutrient (C, N) by dry mass (mean ± standard deviation). Bars with the same letter within each nutrient graph are not significantly different ($p>0.05$). Bars represent:
EP Organs=epibranchial organ contents taken from experimental fish (n=2)
Foregut=foregut contents taken from experimental fish (n=6)
Mucus=mucus samples from the oral cavity of gizzard shad (n=10), and
Food=ground pellet food sampled from the water column (n=14)
A system of equations was developed to define the proportion, by dry Mass, that mucus contributes to the entire foregut content, assuming that only mucus and food are present in foregut contents (Equations 1 and 2). The equations use the relationship between the mass and nutrient content of each foregut component (food particles and mucus) to determine the portion of foregut contents comprised of each component, assuming the mass of foregut contents is 1.0g.

**Known Variables:**
- \( %N_{\text{mucus}} \) = % nutrient per g dry mass in mucus
- \( %N_{\text{food}} \) = % nutrient per g dry mass in food
- \( %N_{\text{gut}} \) = % nutrient per g dry mass in the foregut

**Unknown variables**
- \( W_{\text{gut}} \) = dry mass of the foregut
- \( W_{\text{mucus}} \) = dry mass of mucus in the foregut
- \( W_{\text{food}} \) = dry mass of food in the foregut

**Equation 1**
\[
(\%N_{\text{mucus}})(W_{\text{mucus}}) + (\%N_{\text{food}})(W_{\text{food}}) = (\%N_{\text{gut}})(W_{\text{gut}})
\]

**Equation 2**
\[
W_{\text{mucus}} = W_{\text{gut}} - W_{\text{food}}
\]

Equations 1 and 2 are the system of equations which defines the relationship between % Nitrogen and dry mass of each foregut component (food, mucus, and the entire foregut).

**Equation 3**
\[
(\%N_{\text{mucus}})(W_{\text{gut}} - W_{\text{food}}) + (\%N_{\text{food}})(W_{\text{food}}) = (\%N_{\text{gut}})(W_{\text{gut}})
\]

Equation 3 is the result of substituting Equation 2 into Equation 1.

**Equation 4**
\[
(\%N_{\text{mucus}})(W_{\text{gut}}) - (\%N_{\text{mucus}})(W_{\text{food}}) + (\%N_{\text{food}})(W_{\text{food}}) = (\%N_{\text{gut}})(W_{\text{gut}})
\]

**Equation 5**
\[
[(\%N_{\text{food}}) - (\%N_{\text{mucus}})](W_{\text{food}}) = [(\%N_{\text{gut}}) - (\%N_{\text{mucus}})](W_{\text{gut}})
\]
Equations 4 and 5 are the result of simplifying Equation 3 by solving for $W_{tfood}$.

**Equation 5.5**  
Set $(W_{tgut}) = 1.0$

Equation 5.5 defines that the dry mass of the foregut $(W_{tgut}) = 1.0g$. By assuming $W_{tgut} =1.0$, it is possible to determine the proportion of the foregut comprised of mucus vs. food.

**Equation 6**  
$(W_{tfood}) = \frac{[\%N_{gut} - (\%N_{mucus})]}{[\%N_{food} - (\%N_{mucus})]}$

Equation 6 is the result of Equation 5.5, and yields the proportion of the foregut sample comprised of food by dry mass. Substituting this value $(W_{tfood})$ into Equation 2 yields the proportion of the foregut sample comprised of mucus by dry mass.
By modifying the system of equations developed to explain the contribution of mucus and food to foregut content, it is possible to determine, once correcting for the amount of mucus in the foregut, how much of each available food type gizzard shad ingested.

**Known Variables:**

\(\%N_{\text{mucus}}\) = \% nutrient per g dry mass in mucus

\(\%N_{\text{food}}\) = \% nutrient per g dry mass in food

\(\%N_{\text{sed}}\) = \% nutrient per g dry mass in sediment

\(\%N_{\text{gut}}\) = \% nutrient per g dry mass in the foregut

\(W_{\text{mucus}}\) = dry mass of mucus in the foregut

\(W_{\text{food}}\) = dry mass of food in the foregut

\(W_{\text{sed}}\) = dry mass of sediment in the foregut

**Unknown variables**

\(W_{\text{food}}\) = dry mass of food in the foregut

\(W_{\text{sed}}\) = dry mass of sediment in the foregut

---

**Equations 1 and 2**

**Equation 1**

\[
\frac{\%N_{\text{mucus}}}{\%N_{\text{gut}}} (W_{\text{mucus}}) + \frac{\%N_{\text{food}}}{\%N_{\text{gut}}} (W_{\text{food}}) + \frac{\%N_{\text{sed}}}{\%N_{\text{gut}}} (W_{\text{sed}}) = \frac{\%N_{\text{gut}}}{\%N_{\text{gut}}} (W_{\text{gut}})
\]

**Equation 2**

\[
W_{\text{gut}} = (W_{\text{mucus}}) + (W_{\text{food}}) + (W_{\text{sed}})
\]

Equations 1 and 2 are the system of equations which defines the relationship between \% Nitrogen and dry mass of each foregut component (food, sediment, mucus, and the entire foregut).

**Equation 3**

\[
(W_{\text{food}}) = (W_{\text{gut}}) - (W_{\text{mucus}}) - (W_{\text{sed}})
\]

Equation 3 is the result of rearranging Equation 2 so it can be substituted into Equation 1.

**Equation 4**
\[
\left(\%N_{\text{gut}}\right)\left(W_{\text{gut}}\right) = \left(\%N_{\text{mucus}}\right)\left(W_{\text{mucus}}\right) + \left(\%N_{\text{food}}\right)\left(W_{\text{gut}}\right) - \left(\%N_{\text{food}}\right)\left(W_{\text{mucus}}\right) + \left(\%N_{\text{sed}}\right)\left(W_{\text{sed}}\right)
\]

Equation 4 is the result of substituting Equation 3 into Equation 1.

**Equation 5**
\[
\left(\%N_{\text{gut}}\right)\left(W_{\text{gut}}\right) = \left(\%N_{\text{mucus}}\right)\left(W_{\text{mucus}}\right) + \left(\%N_{\text{food}}\right)\left(W_{\text{gut}}\right) - \left(\%N_{\text{food}}\right)\left(W_{\text{mucus}}\right) - \left(\%N_{\text{sed}}\right)\left(W_{\text{sed}}\right)
\]

**Equation 6**
\[
\left(\%N_{\text{gut}}\right)\left(W_{\text{gut}}\right) = \left(\%N_{\text{mucus}}\right)\left(W_{\text{mucus}}\right) + \left(\%N_{\text{food}}\right)\left(W_{\text{gut}}\right) - \left(\%N_{\text{food}}\right)\left(W_{\text{mucus}}\right) + \left(\%N_{\text{sed}}\right)\left(W_{\text{sed}}\right)[\left(\%N_{\text{sed}}\right) - \left(\%N_{\text{food}}\right)]
\]

**Equation 7**
\[
\left(W_{\text{sed}}\right)[\left(\%N_{\text{sed}}\right) - \left(\%N_{\text{food}}\right)] = \left(\%N_{\text{gut}}\right)\left(W_{\text{gut}}\right) - \left(\%N_{\text{mucus}}\right)\left(W_{\text{mucus}}\right) - \left(\%N_{\text{food}}\right)\left(W_{\text{gut}}\right) + \left(\%N_{\text{food}}\right)\left(W_{\text{mucus}}\right)
\]

**Equation 8**
\[
\left(W_{\text{sed}}\right) = \frac{\left(\%N_{\text{gut}}\right)\left(W_{\text{gut}}\right) - \left(\%N_{\text{mucus}}\right)\left(W_{\text{mucus}}\right) - \left(\%N_{\text{food}}\right)\left(W_{\text{gut}}\right) + \left(\%N_{\text{food}}\right)\left(W_{\text{mucus}}\right)}{\left(\%N_{\text{sed}}\right) - \left(\%N_{\text{food}}\right)}
\]

Equations 5, 6, 7 and 8 are the result of simplifying Equation 4 by solving for \(W_{\text{sed}}\).

**Equation 8.5** \(\left(W_{\text{gut}}\right) = 1.0\)

Equation 8.5 defines the dry mass of the foregut \(W_{\text{gut}}\). By assuming \(W_{\text{gut}} = 1.0\)g, it is possible to determine the proportion of the foregut comprised of sediment vs. food.

**Equation 9**
\[
\left(W_{\text{sed}}\right) = \frac{\left(\%N_{\text{gut}}\right) - \left(\%N_{\text{mucus}}\right)\left(W_{\text{mucus}}\right) - \left(\%N_{\text{food}}\right) - \left(\%N_{\text{food}}\right)\left(W_{\text{mucus}}\right)}{\left(\%N_{\text{sed}}\right) - \left(\%N_{\text{food}}\right)}
\]

Equation 9 yields the proportion of the sample comprised of sediment by dry mass. Substituting this value \(W_{\text{sed}}\) into Equation 2 yields the proportion of the foregut sample composed of food by dry weight.
VITA

Marie Louise Lammons

M. Louise Lammons was born in New Orleans, Louisiana on November 22, 1985. She graduated Cum Laude from Memorial Senior High School in Houston, Texas in 2003. Louise attended Wake Forest University in Winston-Salem, NC, where she graduated Cum Laude in 2007 with a B.S. in Biology and a minor in Spanish.

In the fall of 2007, Louise began graduate studies in the Department of Biology at the College of William and Mary. During her time as a graduate student, Louise presented her research at the William and Mary Graduate Research Symposium, and in 2009 was awarded for Excellence in Scholarship in the Natural and Computational Sciences. As a graduate student, Louise also attended the Summer Institute in Statistical Genetics in Seattle, Washington. She defended the work presented in this thesis on July 13, 2009.