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## Behavioral and Mechanical Particle Selectivity in a Suspension-Feeding Detritivorous Fish

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**Behavioral and Mechanical Particle Selectivity in a Suspension-Feeding  
Detritivorous Fish**

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**Manassas, Virginia**

**Bachelor of Science, The College of William and Mary, 2009**

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

**Department of Biology**

**The College of William and Mary  
August, 2011**

## APPROVAL PAGE

This Thesis is submitted in partial fulfillment of  
the requirements for the degree of

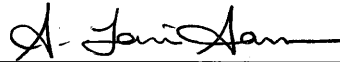
Master of Science



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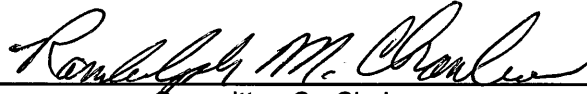
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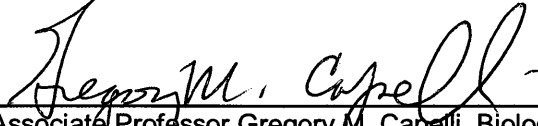
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## COMPLIANCE PAGE

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## ABSTRACT PAGE

Suspension-feeding detritivorous fish are an important link in aquatic food chains because they consume particles of decaying matter that are suspended in the water. Previous studies have suggested that fish such as gizzard shad (*Dorosoma cepedianum*, Clupeidae) can selectively ingest particles that are more nutrient-rich, but whether this selective ingestion results from behavioral selectivity within the environment or mechanical selectivity within the oropharyngeal cavity is unknown. Selectivity can be quantified by comparing the nutrient composition of the food in the environment vs. in the fish's foregut. We tested for mechanical selectivity within the oropharyngeal cavity by creating a homogeneous environment in which particles were not allowed to settle or stratify within the water column. The homogeneous environment was maintained by active particle stirring within the aquarium. When only one type of high-quality food particle was present in the environment, nutrient content of the gut was not significantly different from that of the aquarium water, demonstrating that any mucus contribution to foregut nutrient content was undetectable. I performed three experiments with varying levels of nutrient maintained in a homogeneous suspension within aquaria: (1) sediment, a low-quality food, (2) a 50/50 mixture of a high-quality food and sediment, and (3) a 25/75 mixture. A fourth experiment was performed using a non-homogeneous 50/50 mixture, allowing particles to settle. When the aquarium environment was homogeneous, foregut nutrient content was not significantly different from water nutrient content, indicating that gizzard shad do not have a mechanical mechanism for selection of high-quality particles within the oropharyngeal cavity. In contrast, when particles were allowed to settle in the aquarium, gizzard shad foreguts were significantly higher in nutrient content than samples from the water and from the bottom of the aquarium. Experiments were then conducted to investigate how particles stratify in a non-homogeneous environment in the absence of fish. I found that the nutrient content of particles followed no pattern among locations within the experimental aquaria. However, when fish movement was simulated along the bottom of the aquarium, strong movement caused the water directly above the substrate to rise in nutrient content, significantly greater than that in a vertical sample of the entire water column. Because swimming movements by the fish could create a nutrient gradient in the water above the substrate, gizzard shad behavior within the environment could result in selectivity for nutrient-rich particles.

## Table of Contents

	<b>Page</b>
<b>Acknowledgments</b>	<b>ii</b>
<b>List of Figures</b>	<b>iii</b>
<b>List of Tables</b>	<b>iv</b>
<b>Chapter 1:</b> Suspension feeding and particle selectivity overview	<b>1</b>
<b>References</b>	<b>19</b>
<b>Chapter 2:</b> Do gizzard shad select nutrient-rich particles within the oropharyngeal cavity?	<b>22</b>
<b>References</b>	<b>63</b>
<b>Appendix I:</b> Weighted average equation for multiple foregut samples	<b>65</b>
<b>Appendix II:</b> Scatter plots comparing dry mass of a sample to % nutrient	<b>66</b>
<b>Appendix III:</b> Contribution of mucus and high-quality food to foregut contents	<b>68</b>

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## List of Figures

<b>Figure</b>		<b>Page</b>
<b>1:</b>	%C food only 125-250 microns	<b>36</b>
<b>2:</b>	%N food only 125-250 microns	<b>36</b>
<b>3:</b>	%C food only 75-125 microns	<b>37</b>
<b>4:</b>	%N food only 75-125 microns	<b>37</b>
<b>5:</b>	%C sediment only 75-125 microns	<b>39</b>
<b>6:</b>	%N sediment only 75-125 microns	<b>39</b>
<b>7:</b>	%C 50/50 mix 75-125 microns	<b>40</b>
<b>8:</b>	%N 50/50 mix 75-125 microns	<b>40</b>
<b>9:</b>	%C 25/75 mix 75-125 microns	<b>42</b>
<b>10:</b>	%N 25/75 mix 75-125 microns	<b>42</b>
<b>11:</b>	% total C 50/50 mix not stirred 75-125 microns	<b>44</b>
<b>12:</b>	%N 50/50 mix not stirred 75-125 microns	<b>44</b>
<b>13:</b>	% organic C 50/50 mix not stirred 75-125 microns	<b>44</b>
<b>14:</b>	% total C 50/50 mix not stirred Stratification expt 75-125 microns	<b>47</b>
<b>15:</b>	%N 50/50 mix not stirred stratification expt 75-125 microns	<b>47</b>
<b>16:</b>	% organic C 50/50 mix not stirred Stratification expt 75-125 microns	<b>47</b>
<b>17:</b>	Scatter plot comparing sample dry mass and %C	<b>66</b>
<b>18:</b>	Scatter plot comparing sample dry mass and %N	<b>67</b>



## List of Tables

<b>Table</b>		<b>Page</b>
<b>1:</b>	Nutrient analysis of sediment used in laboratory experiments	<b>29</b>
<b>2:</b>	High-quality food only 125-250 $\mu\text{m}$	<b>35</b>
<b>3:</b>	High-quality food only 75-125 $\mu\text{m}$	<b>37</b>
<b>4:</b>	Low-quality food only (sediment) 75-125 $\mu\text{m}$	<b>38</b>
<b>5:</b>	50/50 mix of high-quality food and low-quality sediment by mass 75-125 $\mu\text{m}$	<b>40</b>
<b>6:</b>	25/75 mix of high-quality food and low-quality sediment by mass 75-125 $\mu\text{m}$	<b>41</b>
<b>7:</b>	50/50 mix by mass of high-quality food and low-quality sediment 75-125 $\mu\text{m}$ with no stirring of the aquarium	<b>43</b>
<b>8:</b>	50/50 mix of high-quality food and low-quality sediment in the 75-125 $\mu\text{m}$ size range with no stirring and no fish	<b>45</b>
<b>9:</b>	50/50 mix of high-quality food and low-quality sediment in the 75-125 $\mu\text{m}$ size range with no stirring and no fish, but with simulated fish movements	<b>45</b>
<b>10:</b>	Selectivity indices comparing LowF2 to water column values for total, inorganic, and organic C and total N	<b>47</b>
<b>11:</b>	Selectivity indices comparing LowF2 to bottom values for total, inorganic, and organic C and total N	<b>47</b>
<b>12:</b>	%C and %N of external mucus samples	<b>49</b>
<b>13:</b>	%C and %N of internal mucus samples	<b>49</b>
<b>14:</b>	Comparison of selectivity indices between our experiment 6, Higgins et al. (2006) field study, Mundahl and Wissing (1988) field study, and the two Mundahl and Wissing (1988) laboratory studies	<b>60</b>
<b>15:</b>	Food and mucus percentages by dry mass in the foregut and epibranchial organs calculated using % nitrogen values from experiments 1 and 2	<b>69</b>

## Chapter 1: Suspension Feeding and Particle Selectivity Overview

### Suspension Feeding

Filter or suspension feeding is a common strategy among many aquatic and marine organisms, including many invertebrates but is also present in aquatic and marine vertebrates such as whales, fish, and tadpoles, and even water fowl and flamingos (Sanderson and Wassersug 1993).

Suspension feeding by vertebrates involves the engulfment of large amounts of water into the oral cavity and is characterized by the non-selective capture of particulate matter that is too small to be selected for visually. However, the detailed mechanisms used in filter feeding by fish are relatively unknown but for five species (Hoogenboezem et al. 1991, Sanderson et al. 1996).

Fish suspension feeders comprise 21 families in 12 orders and have very important roles in nutrient cycling and translocation in their respective environments (Vanni 2002). Suspension feeding is considered to be among the most ancient strategies for feeding. It is hypothesized that the earliest vertebrates used suspension feeding as a feeding strategy before the appearance of cephalized sensory structures which allowed for active predation on larger organisms (Sanderson and Wassersug 1990). However, it is not the case that modern-day suspension feeders are primitive. Convergent evolution of suspension feeding in more derived organisms has allowed for a higher degree of specialization for particular food sources in a wide variety of concentrations and habitats (Sanderson and Wassersug 1990). For example, one of the most primitive suspension-feeding species still in existence today is the basking shark (*Cetorhinus maximus*), a member of the subclass Elasmobranchii which are the cartilaginous fishes along with skates and rays. The basking shark swims forward at a constant rate with its mouth open to sift through the water column, capturing zooplankton. More derived methods of

filtering involve specific movements of the head and jaw to draw water into the oral cavity, allowing for feeding bouts without expending energy to constantly swim, as seen in fish such as gizzard shad (*Dorosoma cepedianum*, Clupeidae) (Sanderson and Wassersug 1993).

### **Categories of Vertebrate Suspension Feeding**

Vertebrate suspension feeding can be separated into four categories: (1) continuous ram feeders, (2) intermittent ram feeders, (3) continuous suction feeders, and (4) intermittent suction feeders (Sanderson and Wassersug 1990). Ram filtration tends to be the more ancestral strategy as seen in fish as early as sharks, and suction feeders tend to be more derived.

However, fish that appear later in the fossil record such as sardines (*Sardina pilchardis*) and anchovies (Engraulidae) utilize ram filtration. Fish that appear earlier in the fossil record also use suction filtration, such as whale sharks (*Rhiniodon typus*) and mega-mouth sharks (*Megachasma pelagios*), so there is some argument as to which is the true origin of suspension feeding (Sanderson and Wassersug, 1990).

Continuous ram feeders swim forward in the water column with their mouths open, allowing large volumes of water to pass into the mouth and out of designated openings at the back of the oral cavity that exit to the exterior of the body (gill slits, opercula, etc.) (Sanderson and Wassersug 1993). They perform this action for an extended duration of time at a consistent speed that prevents plowing while keeping their mouths open in the water column. This leaves species that use this mode of suspension feeding unable to capture larger, more evasive prey. Thus, these species can only capture and use small non-evasive prey such as zooplankton (Tomilin 1967). Continuous ram feeders must rely on full body motion that causes them to be

propelled forward in the water column in order to obtain food, which elicits a tight relationship between movement patterns and resource acquisition.

Intermittent ram feeders employ the same strategy as continuous ram feeders in that they must use body motion to propel forward to capture prey items. However, it is performed in shorter bursts toward prey items instead of swimming continuously. Species that use this strategy rely on high internal forces caused by rapid opening of a very large mouth so that pressure caused by swimming forward does not push away water and food, but rather it quickly enters the mouth (Hain et al. 1982). This mode of feeding can only be utilized by the largest of all animals, such as blue whales (*Balaenoptera musculus*) and fin whales (*Balaenoptera physalus*) that feed mainly on euphausiacean krill, because of the forces needed by the opening of the mouth while swimming forward. For this reason, no sharks or teleost fish can use this strategy for suspension feeding (Sanderson and Wassersug 1993). Because this type of feeding is seen only in the largest of mammals it can be considered a more advanced form of ram feeding.

Continuous suction feeders comprise an unlikely grouping of animals due to the highly specialized mechanisms needed for this strategy. These organisms use an oscillating buccopharyngeal pump to generate a current that runs through the oropharyngeal cavity to create the water flow necessary to filter feed. Thus, these organisms do not need to move their body in order to feed. The term “continuous” does not imply that these organisms never stop feeding; it means that while feeding the flow of water through the mouth is continuous at a high velocity caused by a specialized pump. In organisms that have gill slits and utilize this strategy, the pharyngeal arches and the musculature associated with them form the piston for the pump

(tadpoles). For those animals that lack gill slits (ducks and flamingos), the tongue and hyoid serve as the pump (Sanderson and Wassersug 1993).

Intermittent suction feeding is a strategy thus far found only in teleost fish, making it a more derived trait, but as mentioned before it is argued that the mega-mouth shark and whale shark use this method (Compagno 1990). This strategy is characterized by the generation of suction by creating aperiodic pulses using the specialized mouth and head morphological features. Organisms that use this strategy do not typically alter their swimming pattern or direction in order to visually find and focus on particular food items. Rather, they are able to sense the presence of food (in certain densities) and begin generating suction by opening the mouth and other head parts to create a pulse of water that runs into the mouth and out a designated exit at the back of the oral cavity (Drenner 1977). This strategy allows the organism to be more specific about when to feed without having to move its entire body to do so.

### **Fish Suspension-Feeding Morphology and Mechanisms**

Within the oropharyngeal cavity of teleost fish are sets of gill arches composed of bone and/or cartilage. These arches are paired along the midline of the oropharyngeal cavity and are usually found in four to five pairs. The posterior portion of each arch is where gill filaments used for respiration are attached and the anterior portion is where comb-like projections of bone or cartilage called gill rakers are attached that extend toward the opening of the mouth, forming pores between each raker element. These projections are thought to have two functions: (1) protecting the delicate gill filaments from prey that is taken into the mouth and (2) preventing the escape or loss of prey once it is in the mouth (Lagler et al. 1962). The shape and size of the projections are thought to be related to feeding habit. Fish that tend to capture and eat large

evasive prey tend to have fewer, short, thick gill rakers for handling prey, while fish that have multiple, longer, thinner gill rakers tend to be filter feeders (Magnuson and Heitz 1971). In a comparison of Miocene salmon (*Oncorhynchus*) fossils and current extant salmon species such as sockeye salmon (*Oncorhynchus nerka*) and chum salmon (*Oncorhynchus keta*), key differences were found in gill raker number and size. Miocene species of salmon had gill rakers that were thin, more finely spaced, and more numerous than extant species. This finding agrees with previous studies' oceanographic evidence for much higher plankton productivity and abundance in the North Pacific Ocean during the middle Miocene. These results also demonstrate the evolution from one gill raker type designated for filtering plankton to another raker type in a fish that is known today for its predation on other fish (Etting and Smith 2007).

The numerous, long, thin gill rakers found in many suspension-feeding fish are thought to act as sieves in separating their food source from water that is drawn into the mouth. However, how exactly the gill rakers achieve this function is still an active area of investigation (Higgins et al. 2006). Three mechanisms for separating food from water have been described in organisms: (1) dead-end sieving, (2) hydrosol filtration, and (3) crossflow filtration (Sanderson and Wassersug 1990, Sanderson et al. 2001). Dead-end sieving is the simplest and most easily recognized method, but is also the most prone to complications. In dead-end sieving, water runs perpendicular to a porous surface, forcing particles against the filter surface and the pores associated with it. The sieve will simply retain particles that are larger than the pore size while those that are smaller will pass through and not be retained (Rubenstein and Koehl 1977). This method is hypothesized not to be used by fish because sieves are prone to rapid clogging. This method still does not describe how particles are moved to the back of the oral cavity for consumption. Also, particles that are smaller than the pore size created by gill rakers can be

retained by certain fish (Brainerd 2001). Hydrosol and crossflow filtration provide solutions to these problems (Brainerd 2001).

Hydrosol filtration is characterized by the parallel or perpendicular flow of water past gill raker elements and relies on physical mechanisms between the gill rakers and the particles being captured. Using this method of filtration allows for water to pass through pores created by gill rakers, while capturing and retaining particles on the gill raker even if the particles are smaller than the pore size. There are three physical mechanisms on which this method relies: (1) direct interception, (2) inertial impaction, and (3) gravitational deposition (Rubenstein and Koehl 1977). Direct interception is characterized by sticky surfaces that capture particles as they follow the stream of water over the gill raker elements. Inertial impaction occurs when water is diverted around a gill raker element and the particle, due to its own inertia, deviates from the flow of water and is captured by the raker as the particle collides with it. Gravitational deposition occurs when particles that are more dense than water fall out of suspension in the flow of water onto gill raker elements or other oropharyngeal structures, and are thereby captured (Rubenstein and Koehl 1977). Hydrosol filtration may solve the problem of retaining smaller particles than the pore size and reducing clogging, but does not explain how particles are moved to the back of the mouth for consumption. In a study performed by Sanderson et al. (1996), tilapia (*Oreochromis niloticus*) were shown to use hydrosol filtration by capturing food particles on their gill rakers which had a layer of mucus on them to make them “sticky”. Once enough food was captured on the gill rakers, the fish would reverse the flow of water in their oropharyngeal cavity in short bursts towards the anterior end to lift the captured particles up into the stream of water and then again suck water posteriorly towards the back of the oral cavity where food was concentrated and consumed (Sanderson et al. 1996).

Crossflow filtration is the mainstream flow of suspended particles parallel to the filter surface, while inertial lift and other fluid dynamic mechanisms maintain particles in suspension. This method of filtration is commonly used in industry by companies that need to purify water and concentrate juices, since it is least prone to clogging (Sanderson et al. 2001, Brainerd 2001). In fish, the food particles travel toward the back of the oral cavity and become more concentrated as filtrate exits through gaps between the gill rakers (Sanderson et al. 2001). The main structures that control fluid flow and crossflow filtration are the rows of gill rakers (Sanderson et al. 2001). The gill rakers form filter pores through which particles and water pass while particles that might be selected for are retained by inertial lift due to varying densities of particles. Mucus produced by fish using crossflow filtration to coat gill rakers is thought to help regulate fluid flow and aid in the inertial lift needed to retain food particles (Sanderson et al. 2001). Using video endoscopy crossflow filtration was observed in gizzard shad (*Dorosoma cepedianum*) (Sanderson et al. 2001), by tracking particles as they entered the mouth and traveled posteriorly through the oropharyngeal cavity. Very few of the particles came in contact with the gill raker elements. Most of the particles continued with the flow of water toward the back of the mouth (Sanderson et al. 2001).

### **Feeding Selectivity**

Predatory fish that feed on large, visible prey items can use visual cues to help select for the prey item that will allow for greatest net energy gain. Visual predators can use size and distance cues to choose prey that will grant the greatest output for their efforts in capturing and handling prey. However, it is thought that suspension-feeding fish do not select for individual particles within the water column but simply engulf many particles at once with each bout of feeding



(Sanderson and Wassersug 1990). Though suspension-feeding fish do not select for individual particles, they are still thought to have some degree of selectivity when feeding. There are two hypothesized mechanisms of selectivity in suspension-feeding fish: (1) behavioral mechanisms and (2) mechanical mechanisms within the oropharyngeal cavity (Bowen 1983).

Behavioral selectivity is based on how suspension-feeding fish react to their environment in relation to feeding. In each environment, food particles in the water column can vary in size from 5 to > 3000  $\mu\text{m}$  (Sanderson et al. 1996) and vary in density. More dense particles are thought to contain a higher proportion of inorganic carbon and silica which are unusable by suspension-feeding fish from a metabolic standpoint. Less dense particles are thought to contain a higher proportion of organic carbon, nitrogen, and phosphorus, key elements in protein synthesis, growth, repair, and other metabolism and are the particles that are the most valuable (Bowen 1980, Bowen 1983, Smoot 1999). In the water column, more dense particles tend to settle to the bottom more readily than particles that are less dense. This is dependent on water velocity and flow in particular parts of the water body in question (Bowen 1983). In high flow areas all particles other than the most dense tend to stay suspended in the water column. In low flow areas such as backwaters, high density particles settle more readily than less dense particles, leaving the water column more nutrient-rich (containing more carbon, nitrogen, and phosphorus rich particles) than the bottom sediment where heavy inorganic particles are settling out of suspension (Bowen 1983). Where water flow is intermediate, the water column can exhibit a stratification where less dense more nutrient-rich particles are found at the top portion of the water column and more dense less nutrient-rich particles are found in the bottom portion of the water column (Bowen 1983). These physical characteristics of potential food particles and how they interact with water can allow suspension-feeding fish to

select for more nutrient-rich particles behaviorally. Depending on water flow, suspension-feeding fish can position themselves in the water column to engulf the most nutrient-rich particles (Bowen 1983). They can also simply swim to low flow areas where high density particles have settled out and take advantage of the more nutrient-rich particles that are not settling (Bowen 1983). Certain suspension-feeding fish such as gizzard shad have developed a very particular way of behaviorally selecting for more nutrient-rich particles at the sediment-water interface. These fish can disturb the interface, causing particles to enter the water column and can then allow denser particles (nutrient-poor) to settle while engulfing nutrient-rich particles that are still suspended (Smoot 1999).

Mechanical selectivity is determined by a combination of particle characteristics (size and density) and hydrodynamic forces within the oropharyngeal cavity and associated structures. In the dead-end sieving method previously mentioned, all particles that are larger than the pore sizes created by the gill raker elements within the oropharyngeal cavity will be selected for retention while particles that are smaller than the pore size will pass through the pores along with the filtrate (Rubenstein and Koehl 1977). Using hydrosol filtration with sticky gill raker elements, particles with sizes that are smaller than the pore size can be captured and retained through inertial impaction and direct interception, as mentioned earlier, as well as particles of particular densities. Less dense particles are retained more easily than those of higher densities. However, particles that are too small will still pass through the pores and cannot be acted upon by these physical mechanisms (Rubenstein and Koehl 1977). The physical mechanism of gravitational deposition can work in the same fashion, selecting against particles that are denser and nutrient-poor by allowing them to settle more readily within the oral cavity followed by ejection through the opercula or spitting. In a study performed by Callan and Sanderson (2003)

video endoscopy was used in the oropharyngeal cavity of a suspension-feeding fish, the carp (*Cyprinus carpio*, Cyprinidae). Small less dense particles were retained in suspension while denser particles settled to the bottom of the oropharyngeal cavity and were spat out of the mouth or allowed to sink through gaps between the gill arches and out the opercula.

In crossflow filtration, since particles do not interact directly with gill raker elements and are carried posteriorly within the oropharyngeal cavity along with the flow of water, density and size of particles are the primary characteristics for mechanical selection within the oral cavity (Sanderson et al. 2001). Depending on how a particular suspension-feeding fish utilizing crossflow filtration regulates flow speed over the gill rakers, as water flows through the oropharyngeal cavity less dense more nutrient-rich particles will tend to stay suspended within the flow due to inertial lift as denser particles sink out of suspension and out of the oropharyngeal cavity through the opercula. As this occurs, water exits through pores in the gill rakers and out of the opercula as well, concentrating food particles in the back of the oropharyngeal cavity (Sanderson et al. 2001, Brainerd 2001).

### **Detritivory**

Most suspension-feeding fish feed on small organisms suspended within the water column such as zooplankton, phytoplankton, and bacteria. However, for some suspension-feeding fish, the majority of their diet is comprised of detritus. These fish are categorized as detritivores.

Detritus is characterized by small particles of decaying matter that are found either suspended in the water column or at the sediment-water interface (Bowen 1983). Detritus is relatively lower in nutrient content than other food sources such as plankton. Mundahl and Wissing (1987) observed that gizzard shad (*Dorosoma cepedianum*) in natural environments containing

more abundant zooplankton have much faster growth rates than those in environments that contain more detritus. Fish that are not regularly detritivorous do turn to detritus when the availability of a preferred food source is low, but prove to be unable to sustain themselves on detritus for an extended period of time (Lowe-McConnell 1975).

Detritivorous fish tend to display common adaptations for feeding on detritus and utilizing it efficiently to sustain regular metabolic demands. One important adaptation is specialized oropharyngeal structures (gill rakers, palatal organs, etc.) that function in the separation of denser less nutrient-rich inorganic particles (sand, mud, etc.) from less dense more nutrient-rich organic particles for consumption (Bowen 1983). Another adaptation is behavioral selectivity for more nutrient-rich organic particles in the water column and at the sediment-water interface (Bowen 1983). The third and very important adaptations are morphological differences in the digestive tract. Many detritivorous fish species possess a pair of epibranchial organs which are two blind sacs, usually modified from the fifth gill arch, located dorsally and anteriorly to the entrance of the esophagus (Bowen 1983). Food is collected here first after retention inside the oropharyngeal cavity, and then squeezed out of these blind sacs to enter into the esophagus.

The epibranchial organs are thought to produce mucus to form a bolus of food particles to aid in passage through the esophagus and possibly aid in nutrient absorption (Angelescu and Gneri 1949). However, gut content examined visually in fish that possess epibranchial organs does not contain noticeably more mucus than in species that do not possess them (Bowen 1983). After exiting the epibranchial organs, food boluses enter the esophagus. In gizzard shad, for example, the esophagus is highly folded, creating what are called rugae that are thought to provide

increased surface area and aid in the initial breakdown of food particles in the bolus (Schmitz and Baker 1969).

From the esophagus, the food bolus then enters a stomach that is usually modified into two sections. In gizzard shad, this modified stomach is called the gizzard and is characterized by a sac-like cardiac region where food enters and a pyloric section (Schmitz and Baker 1969). The esophagus and both portions of the gizzard are collectively referred to as the foregut. The pyloric section of the gizzard is characterized by high muscularization and two internal folds which slide past one another when the gizzard contracts. This morphology allows for a high amount of mechanical breakdown along with chemical breakdown to prepare food for absorption (Schmitz and Baker 1969).

From the pyloric section of the gizzard, food particles, now extensively physically broken down, enter into the first segment of the intestine which is characterized by hundreds of small finger-like projections called caeca extending off the main tract. These caeca again increase surface area and secrete proteins to help aid absorption (Schmitz and Baker 1969). Once food has passed through the first segment it enters into the rest of the intestine which is highly elongated for increased absorption. The extreme length of the intestine is used to assimilate non-protein amino acids which occurs gradually as food moves along the full length of the intestine (Bowen 1980). This extensive digestive system has been a key adaptation in the evolution of detritivorous fish along with the other adaptations mentioned, because it has allowed these fish to take advantage of a food source that is extremely abundant but relatively low in quality (low carbon, nitrogen, and phosphorus) and be able to sustain regular metabolism and growth (Mundahl and Wissing 1987).

An important component of the digestive tract in detritivorous fish is mucus. Mucus lines the surface of oropharyngeal structures and may aid in the regulation of water flow (Sanderson et al. 2001, Shepherd 1994). Mucus also lines the rest of the digestive tract to aid in the passage of food boluses, especially in the foregut. Mucus associated with the capture and transport of food is subsequently swallowed and digested.

### **Mucus: Composition**

Mucus is produced by many organisms and has many different functions throughout the animal kingdom. Mucus is comprised of large protein-polysaccharide complexes (Denny 1983). In a study of marsh periwinkle snails (*Littorina littorea*), the composition of mucus used for locomotion (trail mucus) and mucus used for adhesion to surfaces (adhesive mucus) were compared (Smith and Morin 2002). Trail mucus consisted primarily of large carbohydrate-rich molecules with smaller proteins, creating a less viscous gel that allowed for reduced friction and more efficient locomotion. Adhesive mucus was found to have 2.7 times more protein than trail mucus but no difference in carbohydrate concentration. The increase in protein was attributed to the presence of two large proteins in particular that were absent in the trail mucus. This composition produced a more viscous, sticky gel used to anchor the organism in place and seal the opening of the shell to a surface when the conditions were dry (Smith and Morin 2002).

The cleaning wrasse (*Labroides phthirophagus*) is a coral reef fish that feeds on ectoparasites found on other larger coral reef fishes. When they feed they tend to ingest mucus that is present on the surface of fish. Gorlik (1980) tested cleaning wrasse preference to determine from which host, if any, they preferred to feed. It was found that preference was strongly correlated with hosts that produced the “best” mucus, measured as calories per square

centimeter (Gorlik 1980). These two experiments illustrate the value of mucus and demonstrate that it does contain a substantial amount of nutrient that is both costly to produce and has inherent metabolic value.

### **Mucus: Function**

Mucus has numerous functions in fish, such as coating the body to increase swimming efficiency, ionic balance, and respiration (Shepherd 1994). In suspension-feeding fish it has an extremely important role within the oropharyngeal cavity in regulating food intake. For suspension-feeding fish that utilize hydrosol filtration, mucus is critical. These fishes' gill rakers, gill arches, and palatal organs are coated with mucus in which food particles are trapped using the physical mechanism of direct interception (Sanderson et al. 1996). Mucus is also thought to be involved in crossflow filtration. However, few food particles have been observed to be retained on the gill rakers and gill arches of fish during crossflow filtration (Smith and Sanderson 2007). Instead, mucus on the gill rakers may control water loss through the gaps between rakers. This in turn could increase the speed of the water flow in the oropharyngeal cavity, thus increasing inertial lift on food particles and keeping them in suspension (Sanderson et al. 2001). Since mucus is produced in the oropharyngeal cavity and is closely associated with feeding, it is subsequently swallowed and can be recycled to contribute nutrients used in metabolism.

### **Gizzard Shad (*Dorosoma cepedianum*, Clupeidae)**

Gizzard shad are detritivorous suspension-feeding, intermittent suction-feeding fish, which utilize crossflow filtration. They are a member of the family Clupeidae which includes other

suspension-feeding fish such as menhaden, sardines, and herring. Being detritivorous, gizzard shad are able to feed at low trophic levels and take advantage of a resource that is predominantly untapped by other vertebrates. Consequently, they are capable of reaching large population sizes and can grow to approximately 60 cm in total body length. They are also an important part of the food web, connecting a slowly decaying resource that might only be utilized by bacteria, and making it available to other organisms in higher trophic levels (Moyle and Cech 2004). Gizzard shad can be found in the interior drainage of the Eastern and Midwestern United States as well as the Gulf and Atlantic slopes. They form schools (especially in juvenile stages) in the pelagic sections of many different types of bodies of water such as lakes, reservoirs, ponds, swamps, and rivers. Gizzard shad are primarily freshwater fish but can also be found in brackish water such as that in estuaries (Jenkins and Burkhead 1994).

As juveniles and adults, gizzard shad express the morphological traits that are seen in most detritivorous suspension-feeding fish in order to utilize a nutrient-poor source. However, as larvae they do not display the same visually non-selective behavior that the juveniles and adults do during feeding. Larval gizzard shad are zooplanktivorous and select individual prey items visually (Baker and Schmitz 1971). Larval gizzard shad also possess digestive morphology that is very different than that of juveniles and adults. The larval mouth is in a supra-terminal position, designed for directional prey capture, as opposed to a sub-terminal position seen in adults. The larval pharynx is not designed for the straining of particles from the water column. They do not possess many goblet cells used for secreting mucus or taste buds in the oropharyngeal cavity (Heinrichs 1982). But the most obvious and arguably the most important difference is in the digestive tract. The larval gizzard does not possess a highly muscularized wall but is more sac-like and the intestine is markedly reduced in length in comparison to more mature individuals



(Heinrichs 1982). These observations support the idea that the previously described morphologies associated with detritivorous suspension-feeding fish are important in utilizing detritus as a sustainable food source.

Gizzard shad can have a very high impact on nutrient content and availability within their respective habitats. They feed primarily on detritus and are an important nutrient link between decaying matter and organisms in higher trophic levels (Vanni et al. 2006). Gizzard shad are important in nutrient recycling, the cycling of nutrients within a habitat. They are also important in nutrient translocation, where nutrients are taken up in one habitat and moved to or released in another (Vanni 2002). Gizzard shad feed on sediment detritus near the bottom of a body of water and absorb the associated nutrients (carbon, nitrogen, phosphorus, etc.). Through excretion, gizzard shad deposit nutrients such as nitrogen and phosphorus into the water column, making these nutrients available to primary producers, most importantly phytoplankton. In other words, they translocate nutrients from bottom sediment to the water column and pelagic areas of particular bodies of water. Gizzard shad can be found in great numbers in many fresh and brackish bodies of water. Therefore, nutrients can be translocated at relatively high rates, having a substantial impact on phytoplankton populations and the basis of the food web in a body of freshwater (Vanni 2002, Vanni et al 2006).

The actual impact that gizzard shad have on primary production compared to other sources was investigated by Vanni et al. (2006). It was found that in lakes that were surrounded by agricultural watersheds, thus having nutrient runoff, 51% of phytoplankton primary production was due to gizzard shad translocation. In contrast, in lakes with few agricultural watersheds and little runoff, gizzard shad only supported 18% of phytoplankton primary production (Vanni et al.

2006). With this evidence displaying how important gizzard shad are for primary production, it is important that we fully understand how they feed on sediment detritus and whether they are capable of selecting for particulate matter with higher nutrient content and subsequently excreting higher nutrient content.

### **Feeding Selectivity in Gizzard Shad**

Higgins et al. (2006) took sediment cores from the top two centimeters of shallow pools in the upstream region of three separate reservoirs in Ohio and compared the nitrogen, carbon, and phosphorus content of the sediment with the foregut contents of adult gizzard shad that lived in each of the same regions. They found that the foreguts had significantly higher percentages of nitrogen, carbon, and phosphorus than what was found in the sediment from the upstream portion of the respective lakes. This finding led to the conclusion that gizzard shad selected for portions of the sediment that were nutrient-rich, but the selection mechanism used is unknown (Higgins et al. 2006).

Mundahl and Wissing (1988) performed a similar study in which they collected sediment from the bottom of Acton Lake, Ohio by placing Plexiglas discs on the lake bottom for 18-20 hours to capture freshly settling sediment. They then compared its nutrient content to that collected from adult gizzard shad foreguts and found results similar to those of Higgins et al. (2006).

Mundahl and Wissing (1988) also performed a laboratory study in which they placed gizzard shad in aquaria and fed them two prepared food sources, a high-quality and a low-quality food source, and allowed the food to sink. They then compared the food source to foregut content and found that nutrient content was significantly higher in the foregut than in the food source when fed the low-quality food, similar to the field studies. When fed the high-quality food

source, foregut content nearly matched that of the food. However, Higgins et al. (2006) and Mundahl and Wissing (1988) did not account for mucus produced by the fish itself in the oropharyngeal cavity that may have been swallowed along with food particles. The nutrient content of this mucus could cause the nutrient content of the foregut to be higher than that of the food source. Unpublished data mentioned in Ahlgren (1996) estimated that <5% of the organic content in the gut of the white sucker (*Catostomus commersoni*, Catostomidae) was attributable to mucus from the oropharyngeal cavity and gut mucosa in her studies. However, this has not been quantified experimentally. Lammons (2009) conducted experiments to discover how much nutrient content in the foregut is attributable to mucus from the oropharyngeal cavity and gut mucosa and found that 30% to 69% of the foregut content was mucus by dry mass (W&M Biology Master's thesis, August 2009). With this information and additional experimentation it can be better understood whether gizzard shad can somehow select for particles that have a higher nutrient value.

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## Chapter 2: Do gizzard shad select nutrient-rich particles within the oropharyngeal cavity?

### Introduction

Gizzard Shad (*Dorosoma cepedianum*, Clupeidae) are detritivorous suspension-feeding, intermittent suction-feeding fish, which utilize crossflow filtration (Bowen 1983, Sanderson et al. 2001, Sanderson and Wassersug 1993). They are a member of the family Clupeidae which includes other suspension-feeding fish such as menhaden, sardines, and herring. Gizzard shad feed at low trophic levels predominantly on detritus which is characterized by small particles of dead decaying matter that are found either suspended in the water column or at the sediment-water interface (Bowen 1983). Gizzard shad can have a very high impact on nutrient content and availability within their respective habitats. They are an important nutrient link between decaying matter and organisms in higher trophic levels such as piscivorous fish and birds. Gizzard shad also feed on sediment detritus near the bottom of a body of water and absorb the associated nutrients (carbon, nitrogen, phosphorus, etc.). Through excretion, gizzard shad then deposit these nutrients into the water column, making nutrients available to primary producers, most importantly phytoplankton. (Vanni et al. 2002, Vanni et al. 2005). Previous studies have concluded that gizzard shad are capable of selectivity when feeding. These conclusions could have a major impact on our understanding of how nutrients cycle within an aquatic environment.

Higgins et al. (2006) captured gizzard shad from three separate reservoirs in Ohio and compared the nitrogen, carbon, and phosphorus content of food in the foregut to that which was available to the fish in shallow pools in the upstream regions. They found that gizzard shad foregut contents had significantly higher percentages of nitrogen, carbon, and phosphorus than were

available in the environment. Mundahl and Wissing (1988) performed a similar study in which they compared gizzard shad foregut content to settled detritus collected from the bottom in Acton Lake, Ohio. They too found that gizzard shad foreguts had significantly higher percentages of nitrogen, carbon, and phosphorus. Mundahl and Wissing (1988) also performed a laboratory study in a controlled environment where gizzard shad were fed either a high-quality or a low-quality diet. Gizzard shad were then dissected and the foregut contents were analyzed. When fed a low-quality diet, consisting of a 5:1 mixture of dried sediment and aufwuchs, nutrient content of the foregut was higher than the diet. When fed a high-quality diet, foregut content nearly matched the diet. These findings led to the conclusion that gizzard shad are being selective for particles that are nutrient-rich, but the selection mechanism used is unknown (Higgins et al. 2006, Mundahl and Wissing 1988).

Although suspension-feeding fish do not select for individual particles, they are still thought to have some degree of selectivity when feeding (Bowen 1983). There are two hypothesized mechanisms of selectivity in suspension-feeding fish: (1) behavioral and (2) mechanical mechanisms within the oropharyngeal cavity (Bowen 1983). Behaviorally, a fish can choose to feed in a more nutrient-rich environment or in a more nutrient-rich area of a particular environment by moving to an area where denser less nutrient-rich particles settle out of suspension more readily, leaving less dense more nutrient-rich particles in suspension and available for feeding (Bowen 1983). Fish can also disturb the sediment-water interface, causing particles to enter the water column, and can then allow denser particles (nutrient-poor) to settle while engulfing nutrient-rich particles that are still suspended (Smoot 1999). Mechanically, gizzard shad could possibly use crossflow filtration within the oropharyngeal cavity to select for and ingest only the most nutrient-rich particles. Since particles are sieved by



gill raker elements and are carried posteriorly within the oropharyngeal cavity along with the flow of water, density and size of particles are the primary characteristics for mechanical selection within the oropharyngeal cavity (Sanderson et al. 2001).

The purpose of this study was to place gizzard shad into a controlled laboratory environment and investigate whether the selectivity seen in the Higgins et al. (2006) and the Mundahl and Wissing (1988) studies was behavioral or mechanical. Gizzard shad were placed in a feeding environment where food particles were continuously mixed, making the environment homogeneous and thereby removing the potential for behavioral selection of particles. This is in contrast to previously published laboratory experiments in which the environment was heterogeneous. Different particle sizes and nutrient concentrations were used to investigate the effects of these variables on feeding while in a homogeneous environment. Gizzard shad were fed and were then dissected soon after to extract foregut contents and compare with the food available to them. A separate experiment was conducted in which the environment was not mixed homogeneously, allowing food particles to settle and stratify, and foregut content was compared to the environment.

Two hypotheses were tested: (1) when in a homogeneous environment gizzard shad will not show any sign of selection, and foregut content will closely match that of the environment and (2) when in a heterogeneous environment there will be evidence of selection, and foregut content will have higher nutrient content than that of the environment. Support for these hypotheses would indicate that gizzard shad cannot sort particles within the oropharyngeal cavity and would provide evidence for behavioral selectivity. For there to be evidence of

mechanical selection inside the oropharyngeal cavity, foregut nutrient contents would have to be higher than that of the environment in a homogenized environment.

## **Methods**

**Gizzard Shad Collection:** Gizzard shad were collected from the waters of the Virginia Coastal plain using electroshock fishing techniques in association with the Virginia Department of Game and Inland Fisheries. The bodies of water sampled were Chickahominy Lake, Kent Lake, Armistead Pond, and Diascund Reservoir. Gizzard shad (range 15-30 cm SL;  $22.8 \pm 0.6(37)$  cm SL, Mean  $\pm$  SE(n)) were caught and transported in 125 L tubs with aerators to the Keck Environmental Field Laboratory at the College of William and Mary. Fish were maintained in two 284 L glass aquaria at 19-21 °C with external bio-ball filtration in 49 L sumps coupled with 1325 Lh<sup>-1</sup> hour 9 L canister filtration (Cascade 1500). Nitrofurazone (Jungle Fungus Eliminator®) and a copper-based medication (Mardel CopperSafe®) were added to the aquaria upon arrival at the laboratory and were continued until fish had recovered from the stress of capture and transport. Nitrofurazone was added to holding aquaria only and was never present in experimental aquaria. A stabilized chlorine oxide solution (Mardel Maroxy®) was also used in holding aquaria to treat fungal or bacterial infection. Gizzard shad were fed Tetramin® flake food daily. Fish were given a minimum of five days to acclimate to laboratory conditions before use in experimental trials.

**Mucus Collection:** To determine the nutrient value (carbon and nitrogen) of gizzard shad mucus, eleven adult fish (245-280 mm SL) were euthanized by severing the postcranial vertebral column followed by pithing. External mucus was collected by sliding a rubber-tipped probe along the flanks of the fish. Internal mucus was collected from surfaces within the

oropharyngeal and opercular cavities such as gill rakers, gill arches, gills, and internal suspensorium by sliding a rubber-tipped probe over them. Collected mucus was placed onto tared Perkin Elmer Disk-2000 tin disks. All samples were placed in a drying oven (Fisher Scientific Isotemp Oven) at 60 °C for at least 24 hours before being analyzed.

Feeding Experiment 1: For the first six trials, two gizzard shad (for social purposes) were transferred from the holding aquaria to a 110L aquarium containing 70L of water (31 cm from bottom of aquarium to surface) 24 hours prior to the start of a trial, allowing fish to acclimate and to empty the foregut of all contents. In previous experiments, 24 hours proved to be sufficient for gastric emptying in gizzard shad (Lammons 2009). Big Strike® brand food 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 by adding 10.00 g sieved dry food particles to the experimental aquarium. Four Little Giant® model PE-A submersible water pumps (150 Lh<sup>-1</sup>) were placed in the four corners of the aquarium, each paired and attached to perforated tygon tubing. Air stones (15 cm in length) were placed along the bottom of the aquarium. This design was used to maintain a homogeneous mixture of food within the water column during trials. The pumps and air stones created currents in the experimental aquarium that prevented food particles from settling or sorting by different physical characteristics within the water column. During each trial, fish were allowed to feed for one hour. Three water column samples were taken at 2, 30, and 60 minutes after food particles had been added to the experimental aquarium. These samples served as a measure of the food available to the gizzard shad. To take the water column samples, a plastic tube (2.5 cm in diameter) was moved vertically through the

water column onto a randomly placed rubber stopper lying on the bottom of the aquarium. This allowed for the retention of a 125 ml water sample of food particles present in the water column along the height of the water above the rubber stopper in the experimental aquarium.

At the end of the trial (one hour), one fish chosen at random was sacrificed by severing the vertebrae just behind the head, followed by pithing. One fish was chosen to avoid pseudoreplication in the form of multiple non-independent samples. The fish was dissected within 3-5 minutes of capture. The entire foregut (esophagus and gizzard) contents were extracted using blunt, flat forceps to lift the contents without scraping the foregut. Foregut contents were placed in a vial containing deionized water. The entire contents of both epibranchial organs, if any, were also collected and placed in a separate vial.

For the last trial in this experiment, a modified protocol was used. Food particles were sieved using VWR™ U.S.A. Standard Testing sieves with mesh no. 120 (125  $\mu\text{m}$ ) and no. 60 (250  $\mu\text{m}$ ) to a range of 125-250  $\mu\text{m}$ . These sieves were much larger and allowed for more efficient sieving to collect particles at the specific size range. Only one fish was used during this trial. This allowed for more space for the fish to swim. Fish proved to eat just as extensively if not better when in experimental aquaria by themselves compared to being with a companion. The solitary fish was not fed for 48 hours prior to the trial rather than 24 hours, to increase the likelihood that the fish would feed during the trial. To ensure a homogeneous mixture of food particles, the submersible pumps attached to tygon tubing were not used. Instead, three Fisher Scientific Isotemp stir plates were placed under the experimental aquarium which was elevated on wooden blocks 7.5 cm above the table surface. Two air stones (15 cm in length) were hung on the sides of the aquarium rather than placed on the bottom, which allowed for aeration and

some mixing. The associated stir bars (6.5 cm length, 1 cm diameter) were then placed in the experimental aquarium. The spinning of the three stir bars (approximately 300 rpm) in conjunction with the two air stones caused a more efficient homogenization of food particles and allowed more room within the aquarium for gizzard shad to swim without having to maneuver around cables and tubing. All other protocols were performed as in the first six trials.

Feeding experiment 2: Methods were conducted in the same manner as during the last trial in experiment 1 with the exception of the size range of food particles. Big Strike® food pellets were ground and sieved to a 75-125  $\mu\text{m}$  range using VWR™ U.S.A. Standard Testing sieves with mesh no. 120 (125  $\mu\text{m}$ ) and no. 200 (75  $\mu\text{m}$ ), to investigate whether there were differences in feeding based on food particle size. This was necessary because preliminary experiments with sediment (see feeding experiment 3) indicated that sediment particles in the size range of 125-250  $\mu\text{m}$  could not be maintained in suspension in experimental aquaria.

Feeding Experiment 3: In experiments 1 and 2, 10.00 g of a high-quality food source (high N and C content) was fed to gizzard shad in the experimental aquarium in the form of Big Strike® brand food pellets. In experiment 3, 10.00 g of a low-quality food source (low N and C content) was fed to gizzard shad in the form of sediment. Benthic sediment composed of detritus and inorganic particles was collected on three occasions from the main channel of Lake Matoaka near the western bank in 3-4 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 that was sampled from the bottom was scraped off and retained since this layer is most likely consumed by gizzard shad in nature. Collected sediment was dried in an oven at 60 °C for 3-4 days. Sediment was

then ground using a Black and Decker® electric coffee grinder (model CBM205) and sieved to a size range of 75-125 µm using VWR™ U.S.A. Standard Testing sieves with mesh no. 120 (125 µm) and no. 200 (75 µm). Elemental analyses were conducted for the three sediment collections to be sure the sediment that was collected was similar in nutrient value (Table 1).

Samples	% Total C	% Organic C	%N
Sediment 1	7.68±0.15(3)	5.97±0.11(3)	0.96±0.06(3)
Sediment 2	8.30±0.02(3)	5.68±0.10(3)	0.95±0.01(3)
Sediment 3	9.29±0.11(3)	5.25±0.12(3)	0.91±0.04(3)

Table 1: Nutrient analysis of sediment used in laboratory experiments. Mean±SE(n).

The three Fisher Scientific Isotemp stir plates proved insufficient to prevent 75-125 µm sediment particles from settling on the bottom of the experimental aquarium. Therefore, four smaller Hannah Speedsafe™ stir plates were placed under the corners of the experimental aquarium with their associated stir bars (3.75 cm length, 1 cm diameter) and two Fisher Scientific Isotemp stir/hot plates were placed under the middle. Accompanied by the two air stones hung on the sides of the aquarium, the six stir bars created sufficient water movement to suspend and homogenize sediment particles in the 75-125 µm size range without disrupting the swimming movements of the fish. All other methods were conducted in the same manner as they were in experiment 2 and the last trial of experiment 1.

Feeding Experiment 4: Methods for experiment 4 were conducted in the same manner as during experiment 3 with the exception of the food source added to the experimental aquarium. 5.00 g of the high-quality food source (Big Strike® food pellets) ground and sieved to 75-125 µm was combined with 5.00 g of the low-quality food source (benthic sediment) ground and sieved to 75-125 µm, and then added to the experimental aquarium. This 50/50 percent mixture of high-quality and low-quality food sources was used to investigate gizzard shad feeding when

equal masses of two different food sources were mixed homogeneously and maintained in suspension rather than being allowed to settle.

Feeding Experiment 5: Methods for experiment 5 were conducted in the same manner as in experiment 4 with the exception of the ratio of food sources added to the experimental aquarium. 7.50 g of benthic sediment was combined with 2.50 g of Big Strike® food pellets to form a 25/75 percent mixture to be administered. This mixture was used to investigate differences in gizzard shad feeding when different proportions of two different food sources were presented.

Feeding Experiment 6: To investigate how gizzard shad feeding reacted to no homogenization of food sources, all mixing equipment was removed from the experimental aquarium (no stir units were used) with the exception of the two hanging air stones used for aeration. 10.00g of both benthic sediment and Big Strike® food pellets (75-150 µm) (20.00 g total) was added to the experimental aquarium at the start of a trial. This was to ensure enough food would be available in the water column for feeding over the one hour time period since particles tended to settle quickly in the absence of mixing units. Thirty minutes after the food sources had been introduced, a sample was taken of the water using the methods described previously.

Immediately after the water column sampling, the particles that had settled on the bottom of the experimental aquarium were sampled using a pipette to remove all particles from the glass surface (approximately 2 ml of water and particles) in an area selected randomly and approximately equidistant from the two airstones.

The foregut contents of each gizzard shad were split into two samples by taking half from the esophagus and half from the gizzard to make each sample. The first sample of each was used to

measure total nutrient content and the second sample of each was used for inorganic analysis. The analysis of the second sample was conducted only if there was more than 2.50 mg dry mass of each sample after splitting foregut content into two separate samples. If a sufficient amount was not available in each sample, only total nutrient analyses were conducted by combining the two samples. All other methods were conducted in the same manner as in experiment 5.

**Stratification Experiment:** To investigate the settling of particles, the methods of experiment 6 were used with the exception that no fish were added. The experimental aquarium was labeled externally with three horizontal sections, each 10.3 cm in height. The 20.00 g 50/50 mixture of high-quality and low-quality food sources was added to the aquarium. After the food had settled for 30 minutes, two samples (each approximately 125 ml) were siphoned from the center of the top, middle, and lower sections using plastic tubing (3.0 mm internal diameter). Two vertical water column samples and two samples from the bottom surface of the aquarium were then collected as described in experiment 6. In addition, two water samples were siphoned within 1-2 cm of the substrate using the plastic tubing while simulating fish movement by moving one hand slightly to generate water currents that lifted some particles off the bottom of the aquarium (“weak fish movement”). Two water samples from directly above the substrate were also collected while simulating fish movement by moving one hand more vigorously to generate water currents of higher speeds (“strong fish movement”). One sample from each location was used for total nutrient analysis and the other was used for inorganic analysis.

**Elemental Analysis:** All samples from the feeding experiments and stratification experiments were filtered onto tared 25mm glass Whatman® GF/C microfiber filters for total C and N analyses. Once filtered, samples were stored in a drying oven at 60 °C for at least 24 hours



before they were weighed to the nearest hundredth of a mg on a Perkin Elmer AD 6 microbalance to determine dry mass. A Perkin-Elmer 2400 Analyzer was used to determine the percent total C and N by dry mass of each of the samples.

In cases where gizzard shad foregut content was too abundant from one fish to be analyzed as a single sample, the foregut content was subdivided by filtering the sample onto 2-3 separate tared filters. This was necessary because samples that were over 80 mg dry mass typically contained more carbon than the analyzer could register, and would give a reading of 100% carbon. Therefore, each of the filters from the subdivided samples was analyzed and a weighted value for C and N percent dry mass of the total sample was calculated using the separate values for C and N percent dry mass and the dry mass of each subdivided sample (see Appendix I). In experiments where organic and inorganic C are reported (experiment 6 and 7), total N is reported because inorganic N accounted for less than 5% of total N.

In the event that a foregut or epibranchial sample was too small for analyses (dry mass of  $\leq 2.50$  mg), the sample was discarded. This minimum value was identified using a scatter plot (see Appendix II) of values of C and N from all samples taken. Samples with a dry mass of 2.50 mg or less had very erratic values of %C and %N associated with them. This could possibly be due to the limitations of the analyzer. These samples may not have enough material for the analyzer to accurately measure.

Mucus samples, once dried, were weighed on a Perkin Elmer AD 6 micro balance to determine dry mass and were analyzed in the same fashion for total C and N. All internal mucus samples had a dry mass less than 2.50 mg. Therefore, 2-3 internal mucus samples were analyzed together with a combined mass so that a sufficient mass would be available.

To investigate the relationship between total C and inorganic C found in the samples taken and because fish do not assimilate inorganic C, organic C was quantified separately in some trials. For this purpose, samples were filtered using the method described earlier and placed in a muffle furnace (Fisher Scientific Isotemp Muffle Furnace) at 450 °C for 3 hours to burn off organic matter. Using the elemental analyzer as mentioned before, the inorganic carbon that remained was measured and subtracted from the total C value to find the organic C value for each sample.

**Selectivity Indices:** For experiments 1-5, a selectivity index was calculated for each gizzard shad in each trial for both C and N. This was calculated by taking the percent nutrient per gram dry mass value of the foregut content and dividing it by the percent nutrient per gram dry mass value of the water column sample. For each trial, the water column value used in the selectivity index was calculated by averaging the percent nutrient per gram dry mass value of the three samples taken during the trial (2, 30, and 60 minutes). For experiment 6, a selectivity index was calculated for the foregut vs. water column content and for the foregut vs. bottom of the experimental aquarium content for comparison. The total C, inorganic C, and organic C selectivity indices were calculated when applicable. Within each experiment, average percent nutrient per gram dry mass for water column, foregut content, and epibranchial organ content were calculated and graphed for direct comparison.

**Statistical Analysis:** Repeated measures ANOVAs were performed within each experiment to test for statistical differences between the 2, 30, and 60 minute water column samples taken from the aquaria for both C and N. If there was no statistical difference, the three values were averaged within each trial and used as one water column value. One-way ANOVAs were

performed to test for statistical differences between water column, foregut, and epibranchial organ values for experiments 1 through 5 and to test for statistical differences between water column, foregut, epibranchial organ, and bottom values for experiment 6. If statistical differences were found using one-way ANOVA, a Tukey-Kramer HSD test was used to test each of the pairs. Sequential Bonferroni corrections were used to account for the number of statistical tests performed (Rice, 1989).

## **Results**

**Gizzard Shad Behavior:** Gizzard shad swimming behavior was characterized by constant swimming in full loops around the experimental aquarium. Fish typically swam in the lower half of the aquarium. Swimming behavior did not vary with experimental treatments. Gizzard shad feeding behavior during the experimental treatments was characterized by buccal movements while swimming in the same manner as described above. Fish did not feed on the bottom surface of the aquarium, with an exception observed during one trial of experiment 6 where the fish did feed directly off the bottom surface of the aquarium in addition to feeding in the water column.

**Feeding Experiment 1:** Seven trials were performed using high-quality food only (Big Strike © brand) in the 125-250  $\mu\text{m}$  size range; fish ate in five of the trials. Repeated measures ANOVAs performed on the three time values (2, 30, and 60 minutes) for both %C and %N were found to be non-significant ( $p = 0.30$  for C,  $n=5$ ;  $p = 0.18$  for N,  $n=5$ ). Thus, the three values were averaged within each trial to give one water column value for C and N (WC avg). These water column samples were comparable to those of the foregut and epibranchial organs (Table 2, Figs 1 and 2). One-way ANOVA was performed to compare the water column, foregut, and

epibranchial organs. For both C and N there was no statistical difference ( $p = 0.62$  for C, WC  $n=5$ , Foregut  $n=4$ , Epibranchial  $n=4$ ;  $p = 0.37$  for N, WC  $n=5$ , Foregut  $n=5$ , Epibranchial  $n=4$ ). Selectivity indices (SI) were approximately 1.0.

Location	%C	%N
WC avg	45.90±1.19(5)	6.30±0.16(5)
Foregut	43.50±2.50(4)	6.68±0.28(5)
Epibranchial	45.78±1.00(4)	6.69±0.16(4)

Table 2: High-quality food only, 125-250  $\mu\text{m}$ . Mean  $\pm$ SE(n) for %C and %N in the water column, foregut, and epibranchial organs for experiment 1.

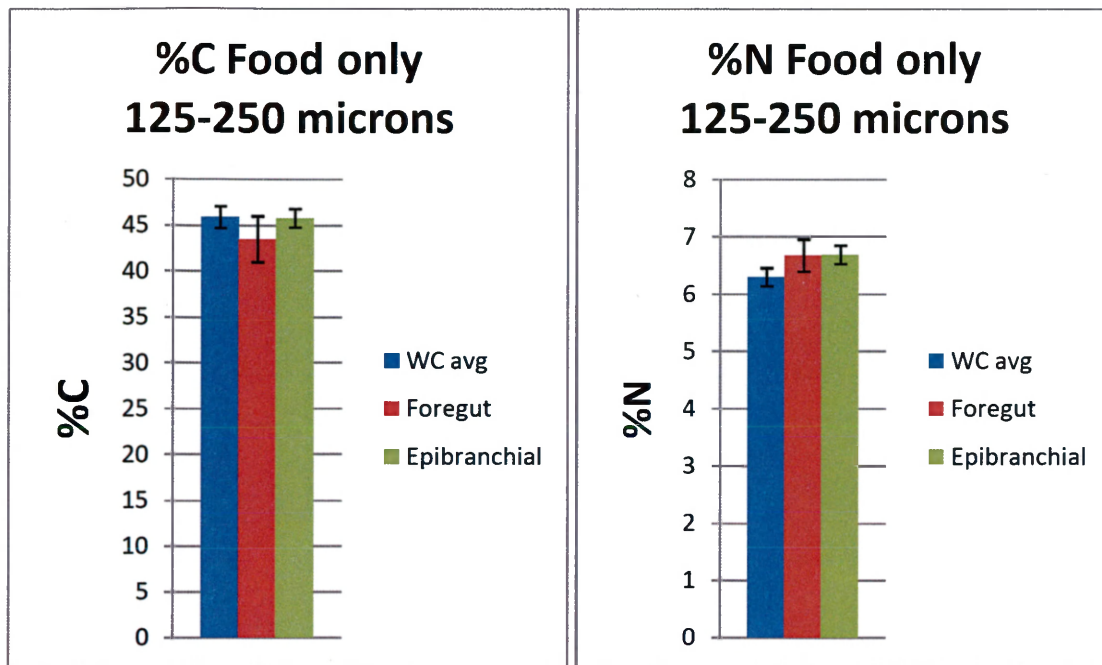


Fig 1: %C found in the water column, foregut and epibranchial organs for experiment 1, high-quality food only. SI (foregut) =  $0.95 \pm 0.05(4)$ , SI (epibranchial) =  $1.02 \pm 0.02(4)$ . Mean $\pm$ SE(n).

Fig 2: %N found in the water column, foregut and epibranchial organs for experiment 1, high-quality food only. SI (foregut) =  $1.05 \pm 0.04(5)$ , SI (epibranchial) =  $1.08 \pm 0.01(4)$ . Mean $\pm$ SE(n).

Feeding Experiment 2: Four trials were performed using high-quality food only (Big Strike © brand) in the 75-125  $\mu\text{m}$  size range. Repeated measures ANOVAs performed on the three time values for both %C and %N were non-significant ( $p = 0.42$  for C,  $n=4$ ;  $p = 0.38$  for N,  $n=4$ ). Thus, the three values were averaged within each trial to give one water column value for C and N (WC avg). These water column samples were comparable to those of the foregut and epibranchial organs (Table 3, Figs 3 and 4). One-way ANOVA was performed to compare the water column and foregut. For both C and N there was no statistical difference ( $p = 0.18$  for C,  $p = 0.21$  for N; WC  $n=4$ , Foregut  $n=4$ ). Selectivity indices (SI) were approximately 1.0.

Location	%C	%N
WC avg	43.58±0.55(4)	5.97±0.08(4)
Foregut	39.99±1.60(4)	6.45±0.24(4)
Epibranchial	42.41(1)	6.49(1)

Table 3: High-quality food only, 75-125  $\mu\text{m}$ . Mean  $\pm$ SE(n) for %C and %N in the water column, foregut, and epibranchial organs for experiment 2.

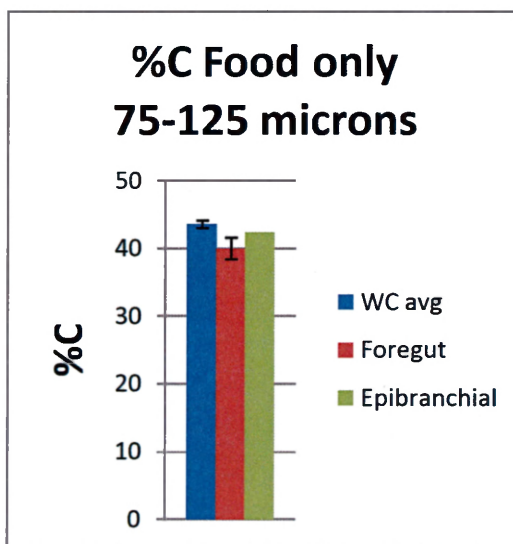


Fig 3: %C found in the water column and foregut for experiment 2, high-quality food only. SI (foregut) =  $0.92 \pm 0.04(4)$ . Mean $\pm$ SE(n).

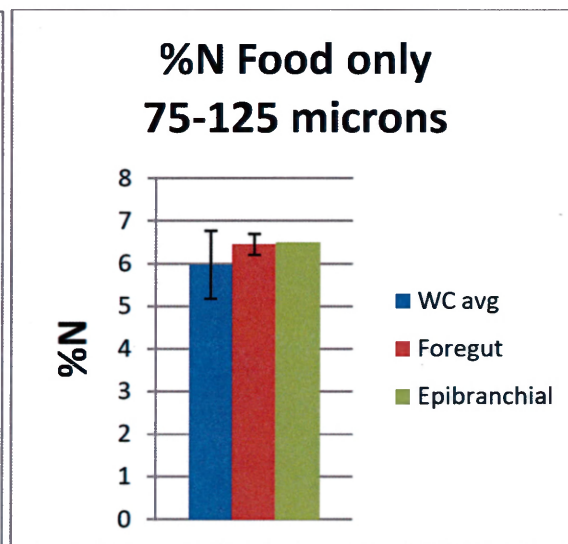


Fig 4: %N found in the water column and foregut for experiment 2, high-quality food only. SI (foregut) =  $1.08 \pm 0.04(4)$ . Mean $\pm$ SE(n).

Feeding Experiment 3: Six trials were performed using sediment only (low-quality food source) in the 75-125  $\mu\text{m}$  size range; fish ate during two of the trials. Repeated measures ANOVAs performed on the three time values for both %C and %N were found to be non-significant ( $p = 0.93$  for C,  $p = 0.83$  for N,  $n=2$ ). Thus, the three values were averaged within each trial to give

one water column value for C and N (WC avg). These water column samples were comparable to that of the foregut (Table 4, Figs 5 and 6). One-way ANOVA was performed to compare the water column and foregut. For both C and N there was no statistical difference ( $p = 0.56$  for C,  $p = 0.59$  for N; WC  $n=2$ , Foregut  $n=2$ ). Selectivity indices (SI) were slightly above 1.0, but sample sizes were small and variability between the trials was high.

Location	%C	%N
WC avg	11.06±2.36(2)	1.48±0.58(2)
Foregut	12.61±2.08(2)	1.77±0.25(2)

Table 4: Low-quality food only (sediment), 75-125  $\mu\text{m}$ . Mean  $\pm$ SE(n) for %C and %N in the water column and foregut for experiment 3.

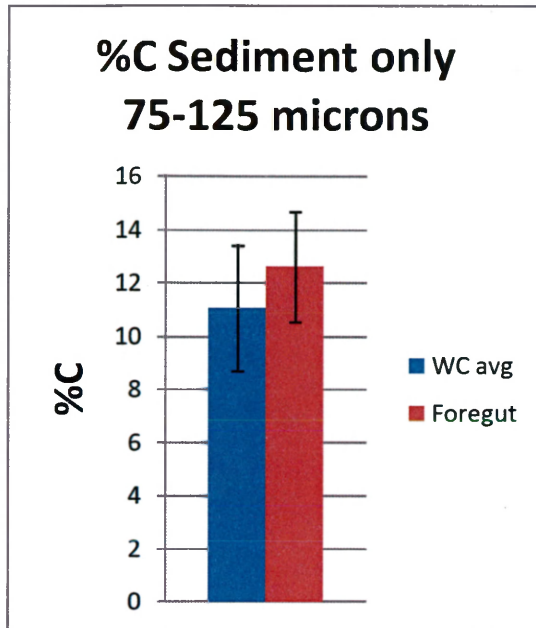


Fig 5: %C found in the water column and foregut for experiment 3, low-quality food only. SI (foregut) =  $1.15 \pm 0.04(2)$ . Mean $\pm$ SE(n).

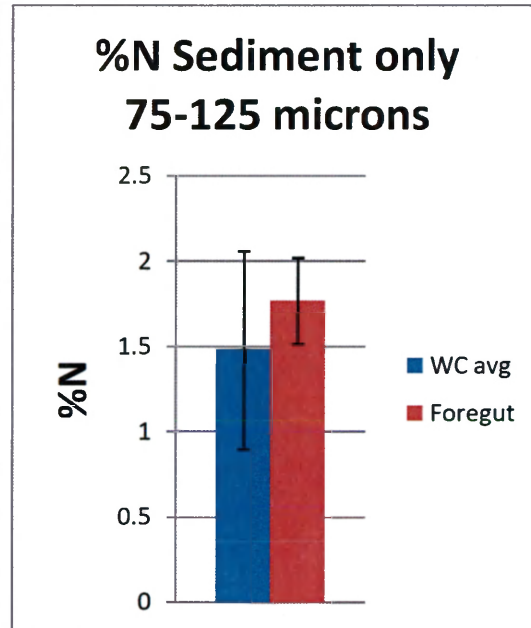


Fig 6: %N found in the water column and foregut for experiment 3, low-quality food only. SI (foregut) =  $1.27 \pm 0.03(2)$ . Mean $\pm$ SE(n).

Feeding Experiment 4: Eight trials were performed using a homogeneous 50/50 mixture of high-quality food and low-quality sediment in the 75-125  $\mu$ m size range; fish ate during six of the trials. Averages were taken of the %C and %N values for food available in the water column at 2, 30, and 60 minutes after food particles had been added to the aquarium. Repeated measures ANOVAs were performed on the three time values for both %C and %N. Results were non-significant for C but were significant for N ( $p = 0.28$  for C,  $p = 0.05$  for N,  $n=6$ ). The three values were averaged within each trial to give one water column value for C (WC avg). The three values were still averaged for N due to the low level of significance. These water column samples were comparable to those of the foregut and epibranchial organs (Table 5, Figs 7 and 8). One-way ANOVA was performed to compare the water column, foregut, and epibranchial



organs. For both C and N there was no statistical difference ( $p = 0.73$  for C,  $p = 0.73$  for N; WC  $n=6$ , Foregut  $n=6$ , Epibranchial  $n=6$ ). Selectivity indices (SI) were approximately 1.0.

Location	%C	%N
WC avg	28.44±0.26(6)	3.81±0.07(6)
Foregut	26.95±1.13(6)	3.99±0.24(6)
Epibranchial	28.19±2.16(6)	3.78±0.24(6)

Table 5: 50/50 mix of high-quality food and low-quality sediment by mass, 75-125  $\mu\text{m}$ . Mean±SE(n) for %C and %N in the water column, foregut, and epibranchial organs for experiment 4.

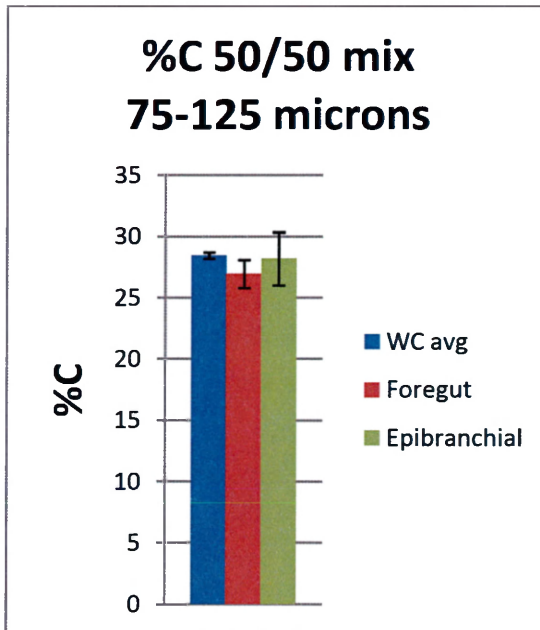


Fig 7: %C found in the water column, foregut and epibranchial organs for experiment 4. SI (foregut) =  $0.95 \pm 0.04(6)$ , SI (epibranchial) =  $0.99 \pm 0.07(6)$ . Mean±SE(n).

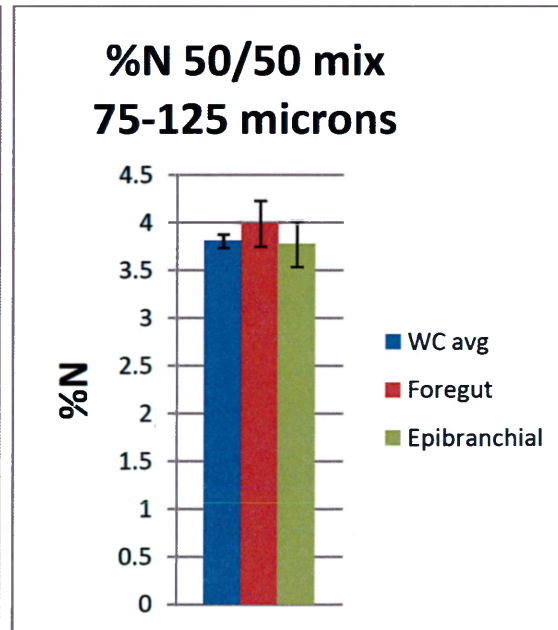


Fig 8: %N found in the water column, foregut and epibranchial organs for experiment 4. SI (foregut) =  $1.05 \pm 0.05(6)$ , SI (epibranchial) =  $0.99 \pm 0.06(6)$ . Mean±SE(n).

Feeding Experiment 5: Seven trials were performed using a homogeneous 25/75 mixture of high-quality food and low-quality food (sediment) in the 75-125  $\mu\text{m}$  size range; fish ate during three of the trials. Averages were taken of the %C and %N values for food (mixture of sediment and commercial fish food) available in the water column at 2, 30, and 60 minutes after food particles had been added to the aquarium. Repeated measures ANOVAs performed on the three time values for both %C and %N were found to be non-significant ( $p = 0.63$  for C,  $p = 0.19$  for N,  $n=3$ ). Thus, the three values were averaged within each trial to give one water column value for C and N (WC avg). These water column samples were comparable to those of the foregut and epibranchial organs (Table 6, Figs 9 and 10). One-way ANOVA was performed to compare the water column, foregut, and epibranchial organs. For both C and N there was no statistical difference ( $p = 0.27$  for C,  $p = 0.36$  for N; WC  $n=3$ , Foregut  $n=3$ , Epibranchial  $n=2$ ). Selectivity indices (SI) were slightly above 1.0.

Location	%C	%N
WC avg	18.64 $\pm$ 0.27(3)	2.48 $\pm$ 0.07(3)
Foregut	19.64 $\pm$ 2.88(3)	2.99 $\pm$ 0.64(3)
Epibranchial	19.98 $\pm$ 2.89(2)	2.66 $\pm$ 0.46(2)

Table 6: 25/75 mix of high-quality food and low-quality sediment by mass, 75-125  $\mu\text{m}$ . Mean $\pm$ SE(n) for %C and %N in the water column, foregut, and epibranchial organs for experiment 5.

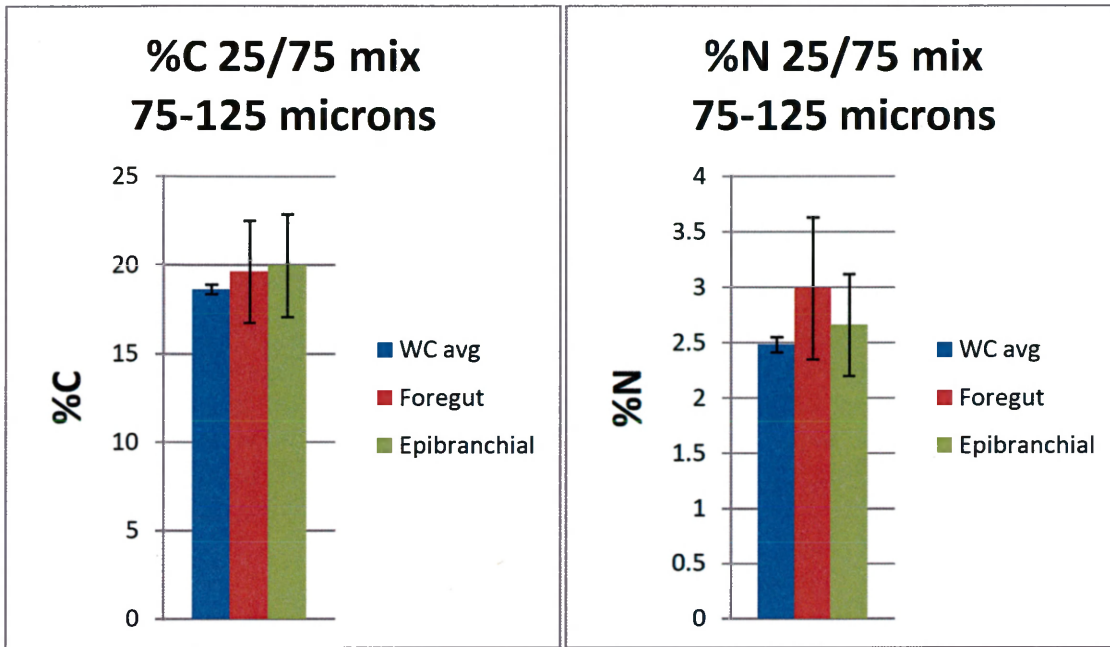


Fig 9: %C found in the water column, foregut and epibranchial organs for experiment 5. SI (foregut) =  $1.06 \pm 0.16(3)$ , SI (epibranchial) =  $1.20 \pm 0.12(2)$ . Mean  $\pm$  SE(n).

Fig 10: %N found in the water column, foregut and epibranchial organs for experiment 5. SI (foregut) =  $1.23 \pm 0.27(3)$ , SI (epibranchial) =  $1.32 \pm 0.13(2)$ . Mean  $\pm$  SE(n).

Feeding Experiment 6: Twenty-three trials were performed using a 50/50 mixture of high-quality food and low-quality sediment in the 75-125  $\mu\text{m}$  size range with no stirring of the aquaria; fish ate during eleven of the trials. In contrast to previous results, the %C and %N values for food available in the water column (WC) and the bottom surface of the aquarium were not comparable to those of the foregut and epibranchial organs (Table 7, Figs 11 and 12). One-way ANOVA was used to compare the water column, foregut, epibranchial, and bottom %C (total) and %N values. The result was significant ( $p \leq 0.0001$  for C,  $p \leq 0.0001$  for N; WC n=11, Foregut n=10, Epibranchial n=7, Bottom n=11). A Tukey-Kramer HSD test was used for post hoc pairwise analysis ( $\alpha = 0.05$ ). With respect to both %C and %N, the only pairwise comparisons for which there were no significant differences were between foregut and epibranchial organs.

Selectivity indices (SI) were approximately 1.5 with respect to the water column and approximately 2.5 with respect to the base samples from the bottom of the aquarium.

One-way ANOVA was also used to compare the water column, foregut, and bottom organic %C. The result was significant ( $p \leq 0.0001$ , WC n=8, Foregut n=8, Bottom n=8). A Tukey-Kramer HSD test was used for post hoc pairwise analysis ( $\alpha = 0.05$ ). Epibranchial values were not included in this analysis and all pairs were significantly different. Selectivity indices (SI) for organic C were similar to those for total C.

Location	%C (Total)	%C (Organic)	%N
WC	29.16±0.67(11)	28.12±0.48(8)	3.88±0.09(11)
Foregut	43.64±3.25(10)	37.55±2.74(8)	6.29±0.28(10)
Epibranchial	47.45±5.54(7)	39.37(1)	6.25±0.36(7)
Bottom	18.30±0.75(11)	15.22±0.74(8)	2.49±0.08(11)

Table 7: 50/50 mix by mass of high-quality food and low-quality sediment, 75-125  $\mu\text{m}$ , with no stirring of the aquarium. Mean±SE(n) for %C and %N in the water column, foregut, epibranchial organs, and bottom of the aquarium for experiment 6.

**%Total C 50/50 mix  
not stirred  
75-125 microns**

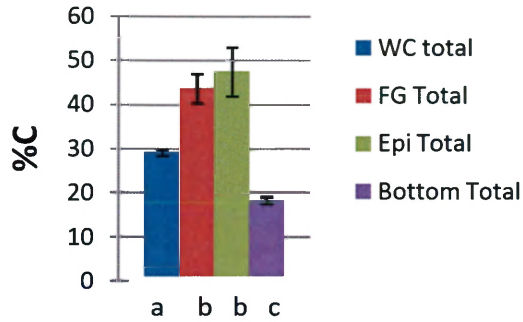


Fig 11: %C found in the water column, foregut, epibranchial organs and bottom for experiment 6. Means that are not significantly different from each other have common letters at the base of the bars. SI (foregut/water column) =  $1.52 \pm 0.12(11)$ , SI (epibranchial/water column) =  $1.58 \pm 0.17(7)$ , SI (foregut/bottom) =  $2.41 \pm 0.23(11)$ , SI (epibranchial/bottom) =  $2.40 \pm 0.11(7)$ . Mean $\pm$ SE(n).

**% N 50/50 mix  
not stirred  
75-125 microns**

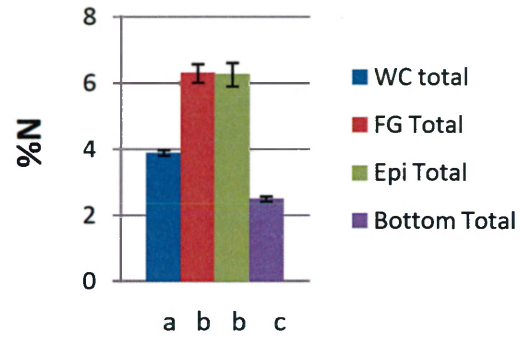


Fig 12: %N found in the water column, foregut, epibranchial organs and bottom for experiment 6. Means that are not significantly different from each other have common letters at the base of the bars. SI (foregut/water column) =  $1.65 \pm 0.10(11)$ , SI (epibranchial/water column) =  $1.57 \pm 0.08(7)$ , SI (foregut/bottom) =  $2.50 \pm 0.11(11)$ , SI (epibranchial/bottom) =  $2.41 \pm 0.11(7)$ . Mean $\pm$ SE(n).

**% Organic C 50/50 mix  
not stirred  
75-125 microns**

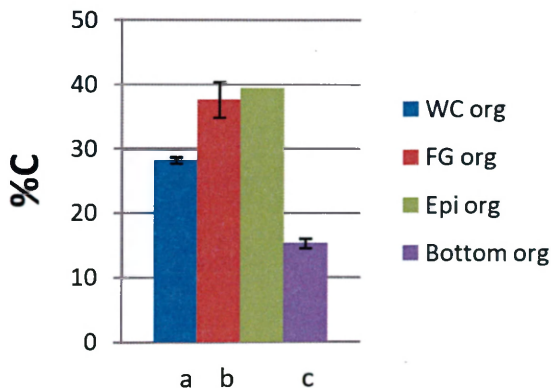


Fig 13: % organic C found in the water column, foregut, epibranchial organs and bottom for experiment 6. All pairwise comparisons of means were significantly different. SI (foregut/water column) =  $1.34 \pm 0.10(8)$ , SI (foregut/bottom) =  $2.47 \pm 0.17(8)$ . Mean $\pm$ SE(n).

Stratification Experiment: Six trials were performed using a 50/50 mixture of high-quality food and low-quality sediment in the 75-125  $\mu\text{m}$  size range with no stirring of the aquaria and no fish (Table 8). Two different types of bottom samples were taken while simulating fish movement at the conclusion of five of the six trials (Table 9).

Location	%C	%N
Top total	24.82±0.83	3.25±0.33
Top inorg	1.52±0.11	NA
Top org	23.30±0.81	NA
Mid total	25.44±0.58	3.18±0.08
Mid inorg	1.36±0.04	NA
Mid org	24.08±0.60	NA
Low total	26.32±1.32	3.27±0.16
Low inorg	1.21±0.07	NA
Low org	25.12±1.38	NA
Bot total	28.82±1.15	3.60±0.16
Bot inorg	5.27±0.64	NA
Bot org	23.55±1.76	NA
WC tot	26.32±0.73	3.26±0.08
WC inorg	1.31±0.04	NA
WC org	25.01±0.75	NA

Table 8: 50/50 mix of high-quality food and low-quality sediment in the 75-125  $\mu\text{m}$  size range with no stirring and no fish. Mean±SE(n = 6). Top = top third of aquarium, Mid = middle third of aquarium, Low = lower third of water in aquarium, Bot = bottom surface of aquarium, WC = vertical sample of water column. NA = not applicable.

One-way ANOVAs were performed comparing nutrient content in the water from the top third (Top), the middle third (Mid), and the lower third (Low) of the aquarium. The results for total %C, total %N, and organic %C were not significant ( $p = 0.55$ ,  $p = 0.96$ , and  $p = 0.44$ , respectively;  $n = 6$ ).

Location	%C	%N
LowF total	29.64±2.07(6)	3.76±0.28(6)
LowF inorg	1.50±0.18(6)	NA
LowF org	28.14±0.19(6)	NA
LowF2 total	34.30±0.08(5)	4.58±0.11(5)
LowF2 inorg	2.58±0.27(5)	NA
LowF2 org	31.73±0.85(5)	NA

Table 9: 50/50 mix of high-quality food and low-quality sediment in the 75-125  $\mu\text{m}$  size range with no stirring and no fish, but with simulated fish movements. Mean±SE(n). Low F = simulation of weak fish movement above the bottom surface of the aquarium, Low F2 = simulation of strong fish movement above the bottom surface of the aquarium. NA = not applicable.

One-way ANOVAs were performed comparing the water column (WC), weak fish simulation in lower third (LowF), strong fish simulation in lower third (LowF2), and bottom surface of the aquarium (Bot) for total %C, total %N, and organic %C. The results for total %C were significant ( $p = 0.0055$ , WC  $n=6$ , LowF  $n=6$ , LowF2  $n=5$ , Bot  $n=6$ ; Fig 14). A Tukey-Kramer HSD test ( $\alpha = 0.05$ ) found that the only pairs that were significantly different for total %C were WC and LowF2, and Bottom and LowF2. The results of the one-way ANOVA for total %N were significant ( $p = 0.001$ , WC  $n=6$ , LowF  $n=6$ , LowF2  $n=5$ , Bot  $n=6$ ; Fig 15). A Tukey-Kramer HSD test ( $\alpha = 0.05$ ) found that the pairs that were significantly different for total %N were WC and LowF2, LowF and LowF2, and Bottom and LowF2. The results of the one-way ANOVA for organic %C were significant ( $p = 0.012$ , WC  $n=6$ , LowF  $n=6$ , LowF2  $n=5$ , Bot  $n=6$ ; Fig 16). A Tukey-Kramer HSD test ( $\alpha = 0.05$ ) found that WC and LowF2, and Bottom and LowF2 were significantly different for organic %C.

Selectivity indices (Table 10) were then calculated using the water column values from Table 8 (WC) and the strong fish simulation movements from Table 9 (LowF2). Vertical water column samples were used as in the selectivity indices calculated for the previous experiments.

Selectivity indices (Table 11) were also calculated using the bottom values from Table 8 (Bot) and the strong fish simulation movements from Table 9 (LowF2).

Nutrient Fraction	C	N
Total	1.34±0.05(5)	1.43±0.05(5)
Inorganic	1.96±0.21(5)	
Organic	1.31±0.05(5)	

Table 10: Selectivity indices comparing LowF2 to water column values for total, inorganic, and organic C and total N. Mean±SE(n).

Nutrient Fraction	C	N
Total	1.22±0.07(5)	1.31±0.06(5)
Inorganic	0.52±0.09(5)	
Organic	1.43±0.18(5)	

Table 11: Selectivity indices comparing LowF2 to bottom values for total, inorganic, and organic C and total N. Mean ±SE(n).

**% Total C 50/50 mix  
not stirred  
Stratification Expt  
75-125 microns**

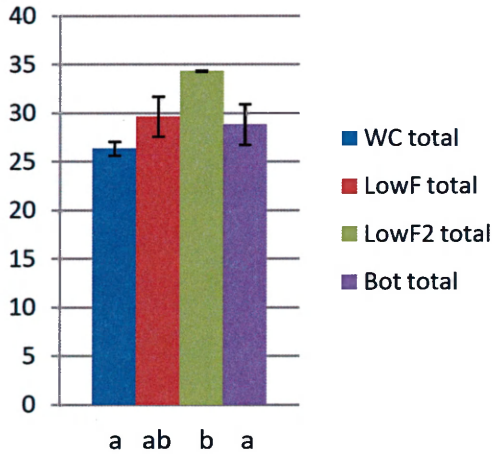


Fig 14: Total %C found in the water column, LowF, LowF2, and bottom for the stratification experiment. Means that are not significantly different from each other have common letters at the base of the bars. SI (LowF2/water column) = 1.34±0.05(5). SI (Low F2/Bottom) = 1.22±0.07(5). Mean±SE(n).

**%N 50/50 mix  
not stirred  
Stratification Expt  
75-125 microns**

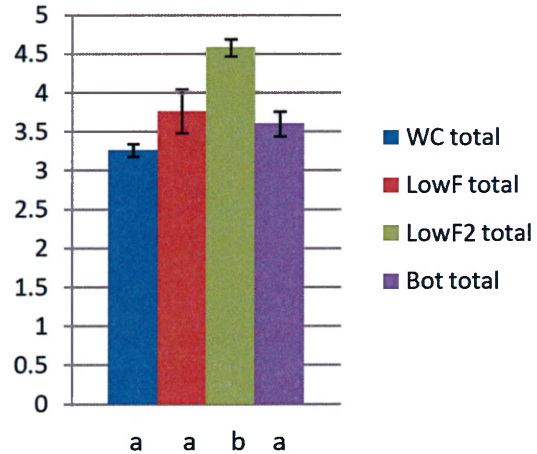


Fig 15: Total %N found in the water column, LowF, LowF2 and bottom for the stratification experiment. Means that are not significantly different from each other have common letters at the base of the bars. SI (LowF2/water column) = 1.43±0.05(5). SI (Low F2/Low F) = 1.22±0.12(5). SI (Low F2/Bottom) = 1.31±0.06(5). Mean±SE(n).



**% Organic C 50/50  
mix not stirred  
Stratification Expt  
75-125 microns**

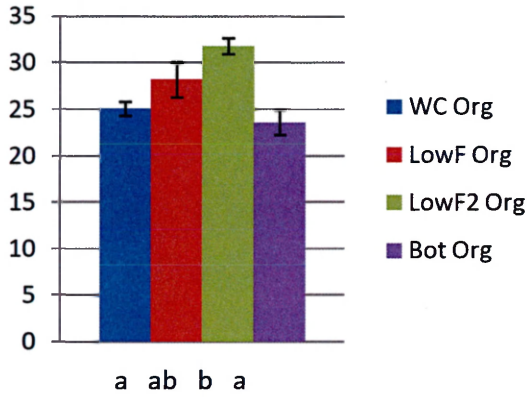


Fig 16: % organic C found in the water column, LowF, LowF2, and bottom for the stratification experiment. Means that are not significantly different from each other have common letters at the base of the bars. SI (LowF2/water column) =  $1.31 \pm 0.05(5)$ . SI (Low F2/Bottom) =  $1.43 \pm 0.18(5)$ . Mean  $\pm$  SE(n).

Mucus Collection: Mucus collection results show that internal mucus composition is comparable to external mucus composition (Tables 12 and 13). A one-ANOVA was performed to compare external mucus to internal mucus for both %C and %N. The results for %C were non-significant ( $p = 0.18$ , external mucus  $n = 10$ , internal mucus  $n=5$ ). The results for %N were non-significant ( $p = 0.32$ , external mucus  $n = 10$ , internal mucus  $n=5$ ).

External Mucus Samples	%C	%N
1	45.98	10.11
2	47.11	10.64
3	45.79	10.09
4	46.30	10.36
5	47.05	10.82
6	46.97	10.42
7	46.55	10.58
8	45.62	10.06
9	42.45	10.20
10	46.66	10.19
Avg	46.05±0.41(10)	10.35±0.08(10)

Table 12: %C and %N of external mucus samples. Mean±SE(n).

Internal Mucus Samples	%C	%N
1, 2	44.53	10.28
3, 4, 5	44.02	10.56
6, 7	44.20	10.37
8, 9	45.29	10.62
10, 11	46.59	10.57
Avg	44.93±0.46(5)	10.48±0.07(5)

Table 13: %C and %N of internal mucus samples. Mean±SE(n).

## Discussion

Our results provide no evidence for mechanical selectivity of nutrient-rich particles within the oropharyngeal cavity of gizzard shad. Foregut contents would have had to be significantly higher in nutrient quality than the water column in a homogeneous environment for there to be evidence for mechanical selectivity. However, our results do provide support for behavioral selectivity of nutrient-rich particles within the environment.

Experiment 1 used high-quality food only (Big Strike © food pellets) ground to a uniform size range of 125-250  $\mu\text{m}$ . The aquarium environment was homogeneous in that particles were maintained in suspension and were not allowed to settle. Results of statistical tests showed that there were no significant differences between the nutrients (C and N) in the water column, the foregut, and the epibranchial organs of the fish. The water column values represented the nutrients available to the fish during feeding and the foregut and epibranchial organ values represented the nutrients ingested. The nutrients that had been ingested by the fish matched that of the environment (Figs 1 and 2), indicating that the gizzard shad did not select or sort specific 125-250  $\mu\text{m}$  particles of commercial fish food. Alternatively, if nutrients ingested by the fish had been higher than those in the environment, that would have suggested that gizzard shad could select or sort particles mechanically within the oropharyngeal cavity. The selectivity indices for foregut/water column and epibranchial organs/water column were approximately 1.00. Thus, the particles that fish consumed closely matched those in the environment, further supporting the conclusion that the fish did not select or sort specific particles.

Experiment 2 used high-quality food only ground to a uniform size range of 75-125  $\mu\text{m}$  and was a homogeneous environment. A smaller particle size was used to investigate whether size has

an impact on gizzard shads' ability to feed selectively or causes the gizzard shad to sort within the oropharyngeal cavity. Drenner et al. (1984) observed that gizzard shad in a size range of 13.6 – 16.3 cm SL fed on particle sizes 60  $\mu\text{m}$  and above. Again, results of statistical tests showed that there was no significant difference between the nutrients in the water column and the foregut of the fish (Figs 3 and 4). This indicates that size (75-125  $\mu\text{m}$ ) of food particles had no effect on the gizzard shads' ability to select particles within the oropharyngeal cavity, nor did it cause sorting. These results are consistent with those of the Experiment 1, demonstrating that the nutrients that the fish consumed matched those available in the environment. The selectivity indices for foregut/water column were approximately 1.00, further supporting the conclusion that the fish did not select or sort specific particles.

Experiment 3 used low-quality food only (sediment collected from Lake Matoaka) ground to a uniform size range of 75-125  $\mu\text{m}$  and was a homogeneous environment. A low-quality (lower %C and %N) food source was used to investigate whether food quality has an impact on gizzard shads' ability to sort within the oropharyngeal cavity or whether it would cause sorting. Results of statistical tests showed that there were no significant differences between nutrients in the water column, the foregut, and the epibranchial organs of the fish (Figs 5 and 6). The selectivity indices for foregut/water column were approximately 1.00. This indicates that nutrient quality had no effect on the gizzard shads' ability to select particles, nor did it cause sorting. These results are consistent with those of the previous experiments, indicating that the gizzard shad did not sort particles.

Experiment 4 used a 50/50 mix by dry mass of high-quality and low-quality food sources ground to a uniform size range of 75-125  $\mu\text{m}$  and was a homogeneous environment. A mix of high-

quality and low-quality food was used to investigate whether gizzard shad could sort particles within the oropharyngeal cavity when there were two very different types of food available to them in a homogeneous environment. Results of statistical tests showed that there were no significant differences between nutrients in the water column, the foregut, and the epibranchial organs of the fish (Figs 7 and 8). The selectivity indices for foregut/water column and epibranchial organs/water column were approximately 1.00. This indicates that particle sorting did not occur when two distinctly different food sources were mixed homogeneously. The results are consistent with those of the previous experiments, indicating that gizzard shad did not select or sort particles in a homogeneous environment.

Experiment 5 used a 25/75 mix by dry mass of high-quality and low-quality food sources ground to a uniform size range of 75-125  $\mu\text{m}$  and was a homogeneous environment. This mix was used to investigate whether a lower concentration of high-quality food relative to low-quality food would cause oropharyngeal sorting by gizzard shad. Results of the statistical tests showed that there was no significant difference between nutrients in the water column, the foregut, and the epibranchial organs of the fish (Figs 9 and 10). The selectivity indices for foregut/water column and epibranchial organs/water column were approximately 1.00. These results indicate that a reduction in the overall nutrient quality of a homogeneous mixture of high-quality and low-quality particles had no effect on the gizzard shads' ability to select particles, nor did it cause sorting.

Experiment 6 used a 50/50 mix by dry mass of high-quality and low-quality food sources ground to a uniform size range of 75-125  $\mu\text{m}$ . There was no attempt to make the environment homogeneous, which allowed particles to sink and separate based on size and density. Twice

the dry mass of high-quality food and low-quality sediment relative to previous experiments was added to the experimental aquarium to ensure there was enough food for gizzard shad to eat in the water column as particles sank to the bottom. Significant differences in nutrient content were found between the water column and the foregut, and between the water column and the epibranchial organs (Figs 11, 12, and 13). The selectivity indices for foregut/water column and epibranchial organs/water column were approximately 1.5. Thus, the particles that the fish consumed were higher in nutrient quality than what was found in the water column. These findings show evidence of particle selectivity by gizzard shad. In a homogeneous environment (Experiments 1-5), nutrient content of the foregut and epibranchial organs matched that of the environment. However, in a heterogeneous environment (Experiment 6), the nutrient content of the foregut and epibranchial organs was higher in quality than that of the environment. In the unstirred aquarium used in Experiment 6, particles were able to separate by density and thus by quality. More dense, lower-quality particles will sink faster and tend to remain on the bottom whereas less dense, higher-quality particles will stay suspended or will tend to be re-suspended more readily (Bowen 1983, Shepherd 1994). This would create regions of higher- and lower-quality particles within the aquarium. Gizzard shad could then swim to nutrient-rich areas or avoid nutrient-poor areas when feeding (Bowen 1983).

The stratification experiment used a 50/50 mix by dry mass of high-quality and low-quality food sources ground to a uniform size range of 75-125  $\mu\text{m}$ . There was again no attempt to make a homogeneous environment, allowing particles to sink and separate by density. The same dry mass of food was used as in Experiment 6 but fish were not used. This experiment was performed to quantify particle stratification in a heterogeneous environment. The sampling method used did not detect significant differences in nutrient content of the water from the

top, middle, and lower thirds of the water column (Table 8). However, when strong fish movement was simulated above the bottom surface of the aquarium (LowF2), nutrient content of the water directly above the bottom was significantly higher than in a vertical sample of the entire water column or on the bottom surface of the aquarium (Figs 14, 15, and 16). Simulated fish movement could re-suspend the less dense more nutrient-rich particles and leave most of the denser less nutrient-rich particles on the bottom. This theory is supported by the inorganic C portion of the selectivity index tables (Tables 10 and 11). When LowF2 (~2.6% inorganic C) was compared to the water column (~1.3% inorganic C) the selectivity index was high (1.96), indicating that the simulated strong fish movement re-suspended inorganic particles. However, when LowF2 (~2.6% inorganic C) was compared to the bottom (~5.3% inorganic C) the selectivity index was low (0.52), indicating that most inorganic particles remained settled on the bottom rather than being stirred into suspension by the simulated strong fish movement. The selectivity indices for organic C and total N in Tables 10 and 11 and the data in Figures 15 and 16 demonstrate that the simulated strong fish movement created a region directly above the bottom surface of the aquarium in which nutrient content was significantly higher than in the water column or on the bottom surface of the aquarium. This suggests that less dense higher-quality particles were re-suspended more readily than lower-quality particles. The selectivity indices for total C and total N comparing the area of strong simulated fish movement (LowF2) and the water column (approximately 1.3-1.4, Figs 14 and 15) were similar to the selectivity indices comparing the foregut and the water column in Experiment 6 (approximately 1.5-1.6, Figs 11 and 12). These results suggest that gizzard shad could select particles behaviorally by swimming and feeding directly above the bottom to re-suspend and ingest less dense more nutrient-rich particles.

There was no statistical difference in the nutrient composition of the water collected at 2, 30 and 60 minute time intervals during experiments 1-5. Thus, the three values were averaged to produce one water column value for each trial in each experiment. This indicates that the environment produced by the stir bars did not stratify over time due to sinking of particles and a homogeneous suspension was maintained in the experimental aquaria. This result also suggests that the feeding rate of the gizzard shad did not have a detectable impact on the composition of the environment and that the food particles available to the fish were effectively constant over time.

Mucus samples were analyzed from the exterior of gizzard shad and the interior of the oropharyngeal cavity (Tables 12 and 13). There were no statistical differences in mucus nutrient quality between the two locations. The nutrient composition of mucus that might have been secreted into the water from the exterior of the fish and consumed was similar to that of the mucus produced within the oropharyngeal cavity. In previous studies of some external fish mucus, % total C and %N were similar to those found in gizzard shad. For example, *Thalassoma duperrey* mucus contained 35.2 %C and 8.8 %N. However, *Zebrafish flavescens* mucus contained only 14.6 %C and 3.4 %N (Gorlick 1980). This provides evidence for the variability of fish mucus content.

Mucus total C values closely matched those of the high-quality food source that was provided. Thus, ingested mucus should not have affected the total C values in the foregut and epibranchial organs during feeding on high-quality particles. However, mucus may have had a measurable impact on % total N in the foregut and epibranchial organs in the experiments using the high-quality food source, as well as the % total N and % total C in the foregut and epibranchial organs



in the experiments using the low-quality food source. This is suggested by the N selectivity indices that were slightly over 1.00 in experiments using high-quality food only (Figs 1,2,3, and 4), as well as the N and C selectivity indices greater than 1.00 seen in experiments using sediment only (Figs 5 and 6). Lammons (2009) reported that mucus constituted 30% to 69% by dry mass of gizzard shad foregut contents, which could be responsible for higher nutrients in the foregut. However, since selectivity indices were approximately 1.00 for our experiments in a homogeneous environment we have used a series of derived equations to conclude that mucus constituted  $10\% \pm 5\%$  (9) of the foregut contents and  $12\% \pm 1\%$  (5) of the epibranchial organ content by dry mass (Appendix III; Mean  $\pm$  SE(n)). Experiment 1 and 2 were used for these calculations because these experiments used the high-quality food source only and had a sufficiently large sample size. %N was used in these calculations because the %N in mucus was substantially higher than the %N in the high-quality food available to the gizzard shad. A possible explanation for the discrepancy between our results and those of Lammons (2009) is that Lammons used smaller fish (65-95 mm SL) and thus obtained smaller foregut samples. As mentioned previously, the elemental analyzer became imprecise when sample dry mass was  $\leq 2.50$  mg. This could also explain the large range of foregut nutrient content seen in Lammons' study.

Mundahl and Wissing (1988) compared gizzard shad foregut content to food available in the environment in Acton Lake, Ohio. Available food was measured by placing plexiglas discs on the lake bottom to collect settling sediment for 18-20 hours. They found that selectivity indices were high, 2.4-2.6 for total carbon and 3.2-6.1 for total nitrogen (Mundahl and Wissing 1988). Mundahl and Wissing concluded that gizzard shad use some means of selectivity for more nutrient-rich particles when feeding. Higgins et al. (2006) compared gizzard shad foregut

content to food available in the environment from three different reservoirs in Ohio. Available food was measured by taking sediment cores from the upstream portion of the reservoirs and analyzing the top 2cm of the core. They also found high selectivity indices, 3.5-5.88 for organic carbon and 6.00-13.33 for total nitrogen (Higgins et al. 2006). Higgins et al. also concluded that gizzard shad use some means of selectivity for more nutrient-rich particles. These studies were conducted in the field and both provided evidence for some type of selectivity in a natural setting.

Mundahl and Wissing (1988) also performed a laboratory study in which they fed gizzard shad in a living stream system either a high-quality food source (commercial fish food, 44.6-45.3 total %C and 6.5-6.8 %N) or a low-quality food source (aufwuchs, 4.3-4.4 total %C and 0.2-0.4 %N). In comparison, prior to the addition of food to our experimental aquaria, our high-quality food source contained 42.3-43.0 % total C and 5.0-5.2 %N (n=3) and our low-quality food source contained 7.7-9.3 % total C and 0.9-1.0 %N (n=3). In their experiment, Mundahl and Wissing did not homogenize the environment, allowing food to sink, and they did not directly measure the living stream system for nutrients but rather pre-analyzed the food that they were administering. When the gizzard shad were fed low-quality food in the living stream system, the selectivity indices were 4.4 for total carbon and 13.2 for nitrogen. When fed high-quality food, the selectivity indices were 1.0 for total carbon and 1.3 for nitrogen. The selectivity indices were calculated by dividing foregut nutrient content by nutrient content of food that was analyzed before it was administered to the living stream system.

Bowen (1983) suggested that high-quality particles tend to be less dense and stay in suspension longer whereas low-quality particles tend to be denser and sink more readily. In our

stratification experiment, a mixture of high-quality and low-quality food was allowed to sink. After a given amount of time, stratification could be seen clearly on the bottom of the aquarium. Darker denser particles tended to settle first and were then covered by lighter-colored less dense particles. The high-quality particles were more easily re-suspended while the low-quality particles tended to remain settled (Tables 10 and 11 for the stratification experiment; Table 7 for Experiment 6). When strong fish movement was simulated in the stratification experiment, the LowF2 water sample taken directly above the bottom surface of the aquarium contained significantly higher % organic C and % total N than was present on the bottom or in the entire water column (Figs 15 and 16). The simulated strong fish movement caused high-quality organic particles to re-suspend and become available in the water directly above the bottom surface of the aquarium. The fish in our experiments suspension fed in the lower half of the water column rather than suction feeding on particles that had settled on the bottom surface of the aquarium. This would explain why selectivity indices of approximately 1.5 were seen for foregut/water column comparisons and approximately 2.5 for foregut/bottom comparisons in Experiment 6 (Figs 11, 12, and 13). The less dense particles that were seen settling on top of the darker particles in the stratification experiment could have been re-suspended by swimming fish and subsequently ingested in Experiment 6 by fish suspension feeding in the lower half of the aquarium.

Ahlgren (1996) performed experiments that compared % total organics, amino acids (mg per 100 mg Ash Free Dry Mass AFDM), and energy (KJ per gram AFDM) of the foregut to the food source (pond detritus), which was measured before being added to experimental aquaria, in juvenile white suckers (*Catostomus commersoni*, Catostomidae) in a laboratory setting. When fed particles  $\leq 45 \mu\text{m}$  only, the selectivity indices were 2.6 for % organics, 1.9 for amino acids,

and 1.3 for energy. Ahlgren concluded that suckers can selectively consume fine particle detritus with the highest nutritional value when there are no invertebrates present (Ahlgren 1996). In contrast to gizzard shad, juvenile white suckers feed exclusively off the bottom and will entirely reject detritus when invertebrates are present (Ahlgren 1990). Ahlgren (1996) suggested that they are capable of selective consumption of fine particle detritus because the gill rakers possess rows of small spines that can be manipulated within the oropharyngeal cavity. She suggested that, while fine particles are suspended within the oropharyngeal cavity and are trapped in the passages between gill rakers due to the spines, larger particles are not trapped in the gill rakers and inorganic grains settle out due to gravity and are expelled through the operculum or by spitting (Ahlgren 1996). This was hypothesized by Ahlgren to be a mechanical filter. Unlike Ahlgren's (1996) results for juvenile white sucker, our studies' results suggest that gizzard shad gill rakers are incapable of mechanically sorting particles (75-250  $\mu\text{m}$ ) by nutrient value.

In our study, when in a homogeneous environment, fish foregut nutrient content was not significantly different from that of the environment. This result was obtained for several mixtures of high-quality food (commercial fish food) and low-quality food (sediment): 100% commercial fish food in the 125-250  $\mu\text{m}$  size range, 100% commercial fish food in the 75-125  $\mu\text{m}$  size range, 100% sediment in the 75-125  $\mu\text{m}$  size range, 50/50 mixture of commercial fish food and sediment in the 75-125  $\mu\text{m}$  size range, and a 25/75 mixture of commercial fish food and sediment in the 75-125  $\mu\text{m}$  size range. Nutrient content of the food added to the aquaria ranged from approximately 8-43 % total C and 1-5 % total N. The homogeneous environment did not allow gizzard shad to select for particles through behavior or movement. Thus, in Experiments 1-5 the only type of particle selection that could have been used was mechanical means within

the oropharyngeal cavity via physical structures. Our results indicated that gizzard shad were only able to ingest what was available to them in a homogeneous environment, and there was no evidence for a mechanical means of selection. However, when the environment was not homogeneous, as in the Higgins et al. (2006) and Mundahl and Wissing (1988) field studies, the Mundahl and Wissing (1988) laboratory studies, and our Experiment 6, selectivity indices rose which indicated selectivity for nutrient-rich particles (Table 14).

Study	C Selectivity Index	N Selectivity Index
Higgins et al. (2006) Field Study	3.5-5.88	6.00-13.33
Mundahl and Wissing (1988) Field Study	2.4-2.6	3.2-6.1
Mundahl and Wissing (1988) Laboratory Study Low-quality Diet	4.4	13.2
Mundahl and Wissing (1988) Laboratory Study High-quality Diet	1	1.32
Experiment 6 (this study; mix of high-quality and low-quality diet)	1.52-2.41	1.65

Table 14: Comparison of selectivity indices between our Experiment 6, Higgins et al. (2006) field study, Mundahl and Wissing (1988) field study, and the two Mundahl and Wissing (1988) laboratory studies.

The difference in selectivity indices between the field studies' results and ours might be explained by the fact that Mundahl and Wissing (1988) and Higgins et al. (2006) collected only the particles that had settled and did not collect particles that were suspended in the water column. This was based on the assumption that gizzard shad feed on the bottom and not in the water column. In Experiment 6, the fish chose to feed in the water column, with the exception of one fish that fed both on the bottom and in the water column.

Our results were similar to the Mundahl and Wissing (1988) laboratory results with regards to the high-quality diet but not with regards to the low-quality diet. This might be explained by the

fact that Mundahl and Wissing calculated selectivity indices using the nutrient content of food before it was administered to the living stream system. This could have caused a discrepancy between what was administered to the living stream system and the food that was actually available to the gizzard shad, depending on the location where the fish fed. Mundahl and Wissing reported that their fish fed on the bottom but did not mention whether the fish fed from the water column. In regard to the low-quality diet, the aufwuchs used may have contained an organic, nutrient-rich fraction. The organic fraction could have stayed suspended longer or been re-suspended more easily making it readily ingested. Thus, when foregut content was compared to the diet source it was much higher in nutrient quality.

Bowen (1983) discussed how suspension-feeding fish can behaviorally select food particles when feeding. He discussed moving to higher levels in the water column because higher-quality less dense food particles stay suspended longer than denser lower-quality food particles. He also mentioned moving to areas of slower-moving water that allows denser particles to settle more readily leaving less dense particles suspended longer. According to Smoot (1999), suspension-feeding fish can also move into these areas of slow-moving water where particles have settled and can disturb the sediment-water interface, causing less dense particles to be suspended and ingested while leaving denser particles settled. These theories for behavioral selectivity of nutrient-rich particles are supported by the results of Experiment 6 and the stratification experiment.

Based on the data collected in Experiments 1-5, there is no evidence that gizzard shad have the means to mechanically select more nutrient-rich particles within the oropharyngeal cavity. However, based on Experiment 6 and the stratification experiment, there is evidence that

gizzard shad could behaviorally select for nutrient-rich particles by disturbing the sediment-water interface, re-suspending nutrient-rich particles, and feeding on the re-suspended particles. The organic C and total N portions of Tables 10 and 11 indicate that simulated strong fish movement caused the water directly above the bottom surface of the aquarium to be richer in organic C and total N compared to the entire water column and the bottom. The denser nutrient-poor particles tended to remain settled on the bottom, as indicated by the inorganic C portion of Tables 10 and 11. The hypotheses that (1) when in a homogeneous environment gizzard shad will not show any sign of selection, and foregut content will closely match that of the environment and (2) when in a heterogeneous environment there will be evidence of selection, and foregut content will have higher nutrient content than that of the environment were both supported by our findings.

Gizzard shad are very important in the recycling and translocation of nutrients. They assimilate nutrients from food within the water column and at the sediment-water interface and excrete those nutrients back into the upper levels of the water column. These nutrients then become available to phytoplankton, and thus gizzard shad support the base of aquatic food webs.

Gizzard shad are also an important trophic link between detritus and piscivorous organisms higher in the food chain. Understanding how gizzard shad obtain their food is an important part of understanding any food web in which gizzard shad are involved (Vanni 2002).

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## Appendix I

**Weighted average equation for multiple foregut samples from one gizzard shad.**

$((\text{Wt gut})_1(\% \text{Nut gut})_1 + (\text{Wt gut})_2(\% \text{Nut gut})_2) / ((\text{Wt gut})_1 + (\text{Wt gut})_2) = \% \text{ nutrient per gram dry mass in total foregut}$

Wt gut = Dry mass of foregut contents in sample

%Nut gut = % nutrient per gram dry mass in foregut sample

## Appendix II

Scatter plots comparing dry mass of a sample to % nutrient.

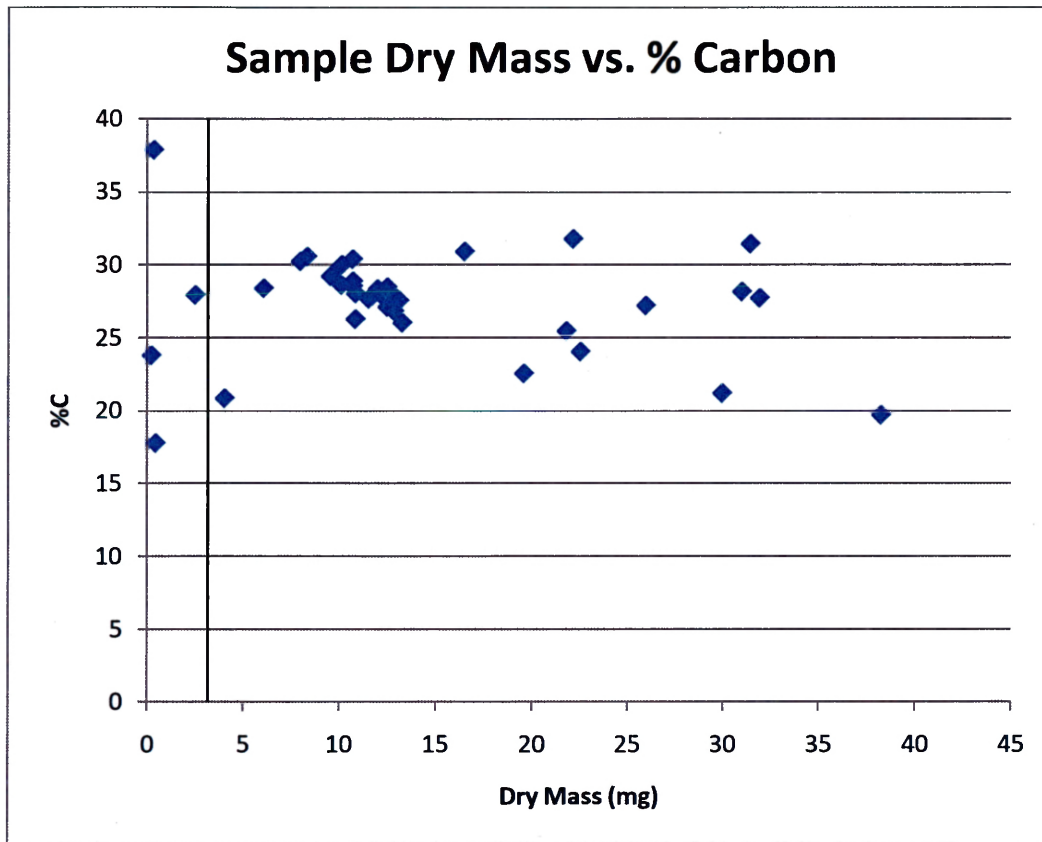


Figure 17: Scatter plot comparing sample dry mass and %C. The vertical line depicts the dry mass of the sample at which the elemental analyzer became inaccurate ( $\leq 2.50$  mg). No samples were used under this limit.

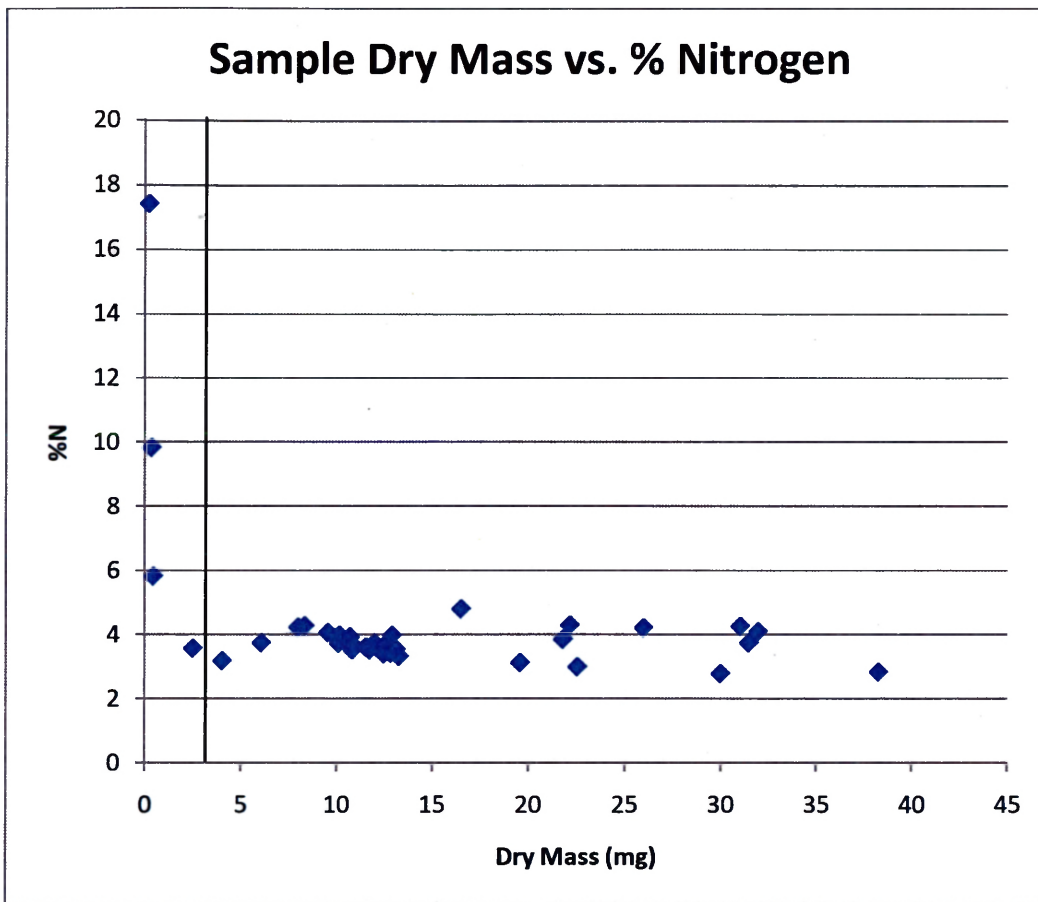


Figure 18: Scatter plot comparing sample dry mass and %N. The vertical line depicts the dry mass of the sample at which the elemental analyzer became inaccurate ( $\leq 2.50$  mg). No samples were used under this limit.

### Appendix III

#### Contribution of Mucus and High-quality food to Foregut Contents (or Epibranchial Organs), by Dry Mass

Known Variables:

(%N mucus) = % nitrogen per g dry mass in mucus

(%N food) = % nitrogen per g dry mass in food (Quantified using the average values for the vertical water samples in the experimental aquarium during each trial.)

(%N gut) = % nitrogen 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

(% food) = proportion of foregut dry mass attributable to food

(% muc) = proportion of foregut dry mass attributable to mucus

Equation 1:  $(\%N \text{ mucus})(Wt \text{ mucus}) + (\%N \text{ food})(Wt \text{ food}) = (\%N \text{ gut})(Wt \text{ gut})$

Equation 1 defines the relationship between % nutrient and dry mass of mucus, food, and the entire foregut assuming the only substances found in the gut are mucus and food (when fish are fed food only).

Equation 2:  $(Wt \text{ mucus}) = (Wt \text{ gut}) - (Wt \text{ food})$

Equation 2 defines the relationship between the dry mass of the entire gut, food, and mucus assuming the only substances found in the gut are mucus and food.

Equation 3:  $(\%N \text{ mucus})[(Wt \text{ gut}) - (Wt \text{ food})] + (\%N \text{ food})(Wt \text{ food}) = (\%N \text{ gut})(Wt \text{ gut})$

Equation 3 is derived by substituting equation 2 into equation 1, i.e., by replacing (Wt mucus) in equation 1 with [(Wt gut) – (Wt food)].

Equation 4:  $(\%N \text{ mucus})(Wt \text{ gut}) - (\%N \text{ mucus})(Wt \text{ food}) + (\%N \text{ food})(Wt \text{ food}) = (\%N \text{ gut})(Wt \text{ gut})$

Equation 5:  $[(\%N \text{ food}) - (\%N \text{ mucus})] (Wt \text{ food}) = [(\%N \text{ gut}) - (\%N \text{ mucus})] (Wt \text{ gut})$

Equation 4 is the expansion of equation 3 and equation 5 is the result of simplifying equation 4.

Equation 5.5: Set (Wt gut) = 1.0 g

By setting the (Wt gut) to 1.0 gram and solving for (Wt food) in equation 5, (Wt food) is converted into a proportion (% food) of food by dry mass found in the entire foregut, as seen in equation 6:

Equation 6: (% food) =  $[(\%N \text{ gut}) - (\%N \text{ mucus})] / [(\%N \text{ food}) - (\%N \text{ mucus})]$

Equation 7: (%mucus) = 100% - (% food)

Equation 7 can be used to find % mucus constituting the foregut using % food from equation 6. The 100% is representative of the entire foregut contents based on the assumption that the only substances found in the foregut are food and mucus.

Expt. 1 Foregut			Expt. 2 Foregut		
Trial	Food	Mucus	Trial	Food	Mucus
1	105%	-5%	12	101%	-1%
4	100%	0%	13	93%	7%
5	62%	38%	14	90%	10%
7	90%	10%	15	73%	27%
11	99%	1%			
Expt. 1 Epibranchial Organs			Expt. 2 Epibranchial Organs		
Trial	Food	Mucus	Trial	Food	Mucus
1	90%	10%	14	85%	15%
5	85%	15%			
7	89%	11%			
11	91%	9%			

Table 15: Food and mucus percentages by dry mass in the foregut and epibranchial organs calculated using % nitrogen values from experiments 1 and 2.