Oral Morphology of the Suspension-feeding American Shad, *Alosa sapidissima*

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Oral Morphology of the Suspension-feeding American shad, *Alosa sapidissima*

A thesis submitted in partial fulfillment of the requirement for the degree of Bachelors of Science in Biology from The College of William and Mary

by

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Accepted for Honors

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Williamsburg, VA
April 29th, 2009
Abstract:

American shad, *Alosa sapidissima*, is a species of anadromous suspension-feeding fish native to the east coast of North America. Past studies of the oral cavity of American shad have not taken into consideration both the microscopic and macroscopic morphology, and have focused on a specific region of the mouth. This study differs by providing an overview of the entire oral cavity, including all four gill arches, medial and lateral rakers, and the pre-esophageal tissue. Macroscopic examination and photography are coupled with scanning electron microscopy to develop a comprehensive understanding of oral cavity structure. Based on the structure of the filtration apparatus, hypotheses for filtration models such as crossflow filtration and dead-end sieving are evaluated. Suggestions for further study are proposed to explore questions raised regarding the functional morphology and the mechanisms of filtration in the American shad.
Introduction:

The American shad, *Alosa sapidissima*, is an anadromous suspension-feeding fish belonging to the family Clupeidae. Native to the Atlantic coast of North America extending from Newfoundland south to Central Florida, the American shad returns to freshwater streams only to breed in the spring. American shad feed primarily on the zooplankton species of mysid shrimp (*Neomysis americana*) and calanoid copepods. Northern krill (*Meganyctiphanes norvegica*), cumaceans (hooded shrimp), sevenspine bay shrimp (*Crangon septemspinosa*), and gammarid amphipods (scuds) compose the remainder of American shad diets, depending on geographical location and salinity levels (Walter & Olney, 2003).

Suspension-feeding fish remove small prey such as phytoplankton and zooplankton from a suspended state in the water (Moyle & Cech, 2004). Other well-known suspension-feeding clupeids closely related to the American shad include herring and menhaden. Suspension-feeding fish are important to maintaining ecosystem stability by controlling the levels of photosynthetic plankton in the water column that contribute to hypoxic zones (Durbin & Durbin, 1998). They also contribute to food webs by supporting a wide range of secondary consumers (Langeland & Nøst, 1995). Many suspension-feeding fish species, such as menhaden, also contribute to global economics through human consumption and use for various industries. The mechanisms allowing the high efficiency at which suspension-feeding fish filter particulate food out of the water have yet to be completely understood and replicated by humans. By understanding the mechanisms through which suspension-feeding fish feed, humans may find more
efficient ways to filter particulate matter from water in sewage treatment plants and beverage production facilities.

 Unlike some suspension feeders that use a suction method to pump water through their oral cavity and gill rakers, American shad swim with their mouths open. By doing so, American shad effectively utilize the continuous ram filtration method to separate suspended food particles from the water through which they swim (Sanderson et al., 2001). As the water and food particles enter the mouth, they are forced over the gill raker/denticle comb. Water passes through the spaces between the rakers and denticles, leaving concentrated food particles in the oral cavity for the fish to swallow (Sanderson et al., 2001).

 Exactly how water passes through these spaces and where food particles are retained are highly disputed topics. Figure 1 shows a schematic of two potential filtration models. Typically, the mixture of food and water is thought to come in contact with the gill raker/denticle comb in a mechanical filtration process described as a dead-end sieve, similar to a strainer or colander (Friedland, 1985). In such a sieve, particles are captured as the flow encounters the filter comb with an angle of approach of approximately 90°, allowing filtrate to continue out of the oral cavity while retaining food particles on the comb. However, my current study challenges the dead-end sieve theory, instead suggesting a method of crossflow filtration. In crossflow filtration, the mixture passes tangential to the comb, simultaneously retaining food particles and moving them towards the posterior of the oral cavity. This process allows for continual filtration with minimal clogging of the spaces through which water exits (Sanderson et al., 2001). Water exiting
from the oral cavity through the comb then passes over the gill lamellae where gas exchange takes place, before exiting from the fish through the opercular grooves.

My study will provide detailed and thorough descriptions and quantification of both the micro- and macro-morphology of American shad gill arches, rakers, and denticles which are involved in suspension feeding. SEM micrographs with intact gill raker epithelia will be utilized during morphometric analysis in addition to a visual aid in the form of a physical model to provide an understanding of how crossflow filtration methods may be implemented in the feeding of such fish. Theories of dead-end sieve filtration during feeding can also be considered for suspensions with a 90° approach to the raker and denticle comb. Although the crossflow filtration model has been evaluated for pump suspension-feeding fish species that use suction to draw water into their oral cavities (Sanderson et al., 2001), crossflow filtration models have not been explored for ram suspension-feeding species such as American shad.

Methods:

Seven adult American shad (Table 1) were acquired from Mike Isel1 and Catherine Lim2 at the Virginia Department of Game and Inland Fisheries. The fish were captured using gill nets at the Pamunkey River (37.57°N/77.02°W) and were placed on ice during transportation to minimize degradation of the tissue occurring between catch and dissection.

Specimens I through III were frozen within four hours of collection with heads completely submerged in water until thawed for SEM preparation. Sexes of specimens

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1 Mike.Isel@dgif.virginia.gov
2 Catherine.Lim@dgif.virginia.gov
that were determined are indicated in Table 1. Specimens IV, VI, and VII were prepared for SEM within two hours of collection without freezing. Specimen V was stored in ethanol rather than being frozen in an effort to preserve epithelial tissue on the rakers. This study focuses primarily on specimens IV through VII, as the epithelia were intact on the gill rakers and gill arches of these specimens.

<table>
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<tr>
<th>ID #</th>
<th>Total Length</th>
<th>Fork Length</th>
<th>Standard Length</th>
<th>Preparation technique</th>
<th>Epithelia Intact?</th>
<th>Sex</th>
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<td>Night-of-catch</td>
<td>YES</td>
<td>Female</td>
</tr>
</tbody>
</table>

Table 1 – Specimen IDs, measurements, and preparation method

Specimen preparation

Dissection of the oral cavity was performed in such a way as to maintain the natural form of the gill arches and rakers, minimizing damage to the tissue. The operculum was removed with scissors, followed by dissection and sequential removal of the gill arches. A vigorous rinse with tap water from a squirt bottle removed as much loose mucus from the rakers as possible. Between gill arch removals, the specimen was placed in a refrigerator at 4°C and covered with a damp cloth to prevent dehydration.

Figure 2 shows the right side of an American shad via a sagittal section down the midline of the fish with the left gill arches removed. The four gill arches with filaments (Figure 3) were removed from the left side of the fish’s oral cavity and divided into

³ Epithelia, though more intact than specimens I-III, were less regular than specimens IV, VI and VII
sections depending on their size (Figures 4-7) using dissecting scissors or razor blades on
dental wax to avoid damage to the rakers. The gill filaments were then cut away from
each section. To fit the specimens on SEM stubs and to orient the specimens under the
SEM, the dorsal filaments of each arch section were cut to be noticeably shorter than the
ventral filaments. Two additional posterior sections that were observed to contain raker-
like structures were taken from pre-esophageal tissue posterior to the fourth gill arch.
The pre-esophageal tissue was mounted flat, as found in the oral cavity. Figures 4E, 5E,
6E, and 7E indicate ventral (lower) versus dorsal (upper) ends of the pre-esophageal
tissue.

The sections were fixed following the procedure used by Nguyen (1999). Each
section was placed in a labeled glass vial and fixed in 2.5% gluteraldehyde in 0.1M
phosphate buffer (pH 7.2-7.4) for thirty minutes at room temperature. The vials were
placed in a refrigerator at 4°C for two hours. They were then emptied using a transfer
pipette, and the sections were rinsed three times in 0.1M phosphate buffer. As
recommended by Dobbs (1972), this step was accompanied by the following mucus-
stripping procedure. First, each section was submerged in 16% glycerol for 24 hours at
4°C. Next, the arch sections were bathed in 20% ethanol for 24 hours at 4°C. Finally,
the specimens were dehydrated at room temperature through an ethanol series for fifteen
minutes at each of the following concentrations: 50%, 70%, 80%, 95%, 100%, 100%,
100%.

Specimens remained in 100% ethanol for approximately 24 hours (as opposed to
the three days of specimen 1 of Nguyen, 1999) and then were placed directly into a
chemical drying agent (HMDS - Hexamethyldisilazane). The sections were soaked with
HMDS for five minutes. The specimens were fixed medial side up while still damp with HMDS to metal stubs using standard SEM adhesive and carbon paint. The specimens were sputter coated with a layer of gold-palladium at seven nm/min for eight minutes (Hummer Vii sputtering system) and stored in a desiccation chamber when not in use under the microscope.

**Specimen observation**

Observation of the specimens was performed using an Amray 1810 Scanning Electron Microscope. Photography was performed with a Nikon N6006 35mm camera with Kodak Professional T-MAX 100 film. Negatives were developed and scanned into a computer using a Hewlett-Packard Scanjet 8200c Scanner. Microsoft Office Picture Manager was used to optimize image analysis by altering color saturation, color amount, and contrast.

The software package ImageJ (from the National Institutes of Health, V.1.37, updated 13 October 2006) was used to take measurements of structures in the images. By opening the selected image and using the “Straight Line Selections” tool, measurements were taken of both the scale bar on the SEM image and the object to be measured, allowing for calculation of the real distance by completing a ratio during analysis in Microsoft Excel. Figure 8 illustrates the locations of the six different measurements taken during morphometric data collection: (A) the distance from the denticle to the medial edge of a lateral raker, taken at the tenth denticle\(^4\), (B) the distance from the

\( ^4 \) The tenth denticle from the base of the raker was used as a standard from which to make a measurement. Since fewer than ten denticles were present on the pre-esophageal rakers, measurements were taken from a denticle within the third of the raker most proximal to the arch. In cases where the tenth denticle was within the region where the downstream edge of the raker curved medially, the position of the denticle that
denticle to the lateral edge of a lateral raker, taken at the tenth denticle from the base of the raker, (C) the total downstream width of a lateral raker, taken at the tenth denticle, (D) the shortest distance between midlines of adjacent rakers parallel to the arch at the base of adjacent rakers, (E) the shortest distance between tips of denticles on adjacent rakers near the base of the raker, and (F) the height of the denticle near the base of a raker from the base of the denticle flush with the raker to the tip of the denticle. Once collected in Excel, data were then summarized graphically and tested for significance using a two-way analysis of variance with replication test.

Summary of previous work:

Research in the spring of 2007 was primarily for prepared specimen observation and learning of proper SEM techniques, including determining the limits of views under the SEM to help properly orient later specimens onto the observation stubs. Concepts such as depth of field and working distance were mastered, as well as the technique of taking micrographs and making measurements on ImageJ. In addition, time was spent becoming comfortable with orientation inside the American shad oral cavity, both microscopic and macroscopic. By describing the structure of the arches, rakers, and denticles, a better understanding of the anatomical orientations was achieved.

Research during the fall of 2007 was dedicated primarily to dissection and preparation of two frozen specimens. This enabled an understanding of how arches and rakers are arranged in specimens, rather than relying on artists’ depictions and compilations of micrographs. Comprehensive photography of oral structures included was just distal to the point at which the raker no longer was curved was chosen for measuring. This was done to achieve measurements that were more representative of the rakers of the section as a whole.
not only micrographs of prepared specimens, but also digital images of dissected oral cavities. With specimens II and III, cellular tissue layers that were not previously visible on specimen I were seen clearly. Patches of this tissue remaining after SEM preparation appeared to be more organized than other mucous-like material found on rakers, warranting further investigation to confirm the tissue as epithelia.

Studies during the spring of 2008 were devoted to the dissection and preparation of specimens IV through VII. Rather than freezing specimens for later preparation, three specimens were kept cool and prepared on the night-of-catch, and the head of one specimen was removed and stored in 70% ethanol until prepared a week later. A continuation of comprehensive SEM photography of the oral cavity throughout the fall semester of 2008 confirmed the presence of epithelial tissue layers over the rakers and denticles. Further investigations of raker structure and arrangement were also conducted. The model of crossflow filtration can now be applied to all of the segments of the gill arch both individually and as a full working model of suspension feeding in American shad.

**Results**

The oral cavity of the American shad extends from the oral jaws to the esophagus, which lies just posterior and medial to the posterior ridge of the operculum. The four gill arches serve both a filtration and respiration function, and extend from the ventral and dorsal midlines of the oral cavity (Figures 9 and 10). Figure 11 shows a removed arch and rakers, with the rakers directed towards the viewer and the gill filaments directed away from the viewer. Each gill arch is separated into ceratobranchial (lower) and
epibranchial (upper) sections by a definitive angle in the arch (Figure 12). While the posterior margin of the arch supports the gill filaments for gas and molecular exchange, the anterior margin of the arch supports the lateral gill rakers which form a comb through which water exits the oral cavity. The lateral rakers did not seem to be static structures, as they were easily bent by touch.

The first gill arch is the most lateral, lying just medial to the operculum (Figure 13). The second arch lies medial to the first, positioned slightly posterior to the base of the first arch along the midline. The third and fourth gill arches follow the same pattern, positioned more medial and posterior than each preceding arch (Figures 2, 14, and 15). In addition, the second and third gill arches, as well as the third and fourth gill arches, are joined ventrally by connective tissue that extends part way up the ceratobranchial section of the arches (Figure 16).

The third arch has ridges situated medially to the lateral rakers, located only on the ceratobranchial section close to the angle separating it from the epibranchial (Figure 17). The fourth arch also has additional components, called medial rakers, which appear to be similar in construct to the ridges found on the third arch (Figure 18). However, the medial rakers of the fourth arch extend from the ventral region of the arch posterior and dorsal for most of the length of the ceratobranchial section before branching away from the lateral epibranchial section to form a medial epibranchial section. From this split, the medial row of rakers proceeds towards the upper margin of the esophagus parallel to the pre-esophageal rakers, whereas the lateral row of rakers continues along the lateral epibranchial section of the fourth arch.
The most posterior region of the oral cavity is referred to in this study as the pre-esophageal tissue, termed a “keel” by Schmitz & Baker, 1969. The pre-esophageal tissue lies along the midline of the oral cavity, ventral to the opening of the esophagus, and has a single row of rakers that are situated adjacent to the medial rakers of the fourth arch (Figure 19). Two noticeably different tissue types are found on the pre-esophageal sections: a smooth tissue located lateral to the base of the rakers and a rough and irregular tissue located medial to the base of the rakers (Figure 20).

On each lateral raker of all four arches and the medial rakers of the fourth arch are found many “denticles,” also termed “branchiospinules” in menhaden (Friedland, 1985, 2006). These bony structures (Friedland, 1985) show consistency in their size, shape, orientation, and relative location along the length of the raker. Measuring close to 100 microns in length (Figure 21), the denticles are in a position to serve a role in creating the comb through which filtrate exits the oral cavity. The denticles are positioned such that their distal points are directed medially and into the flow as water passes from the oral cavity into the opercular cavity (Figure 22). Varying densities and patterns of denticles can be found on some arches (Figure 23). In addition, circular depressions were observed medially to many of the denticles on specimens that had been prepared after being frozen and therefore lacked epithelial tissue. Rarely, lateral rakers were observed to curl at the base in specific regions of the first and second arches.

In freshly-prepared specimens, a layer of cellular tissue was found covering the medial and lateral faces of all arches as well as the rakers and denticles (Figures 24, 25, and 26). The cells on the rakers resembled simple squamous epithelial tissue and have been noted in previous studies (Friedland, 1985; Sibbing & Uribe, 1985). The surface
structures of the cells have micro-ridges and microvilli distinctive of squamous epithelia (Figure 27). Some of the cells were observed to have become ruptured, as though contents had been expelled from the cell (Figure 28). These erupting cell types have been identified in carp, *Cyprinus carpio* (Sibbing & Uribe, 1985) as mucous cells, which release their contents into the oral cavity along the rakers and denticles (Figures 29 and 30).

Other structural observations include the presence of micro-ridges along the rakers of the pre-esophageal tissue (Figure 31) and rib-like structures extending from the bases of the lateral rakers across the downstream and posterior side of all arches towards the gill filaments (Figure 32).

Figure 33 shows the graphical representations of the mean values collected during the morphometric analysis of specimen V (ethanol preparation). Figure 34 shows the graphical representations of the mean of means collected during morphometric analysis of specimens IV, VI, and VII (fresh preparation). The sample size in Figure 33 is 2-6 replicate measurements taken per specimen, and the sample size in Figure 34 is 3, the number of specimens measured. Smaller values for specimen V compared to the averages of specimens IV, VI, and VII may be the result of shrinkage during preparation. Such shrinkage was observed on the distance from denticles to the lateral edge of rakers, total downstream width of rakers, and the distance between rakers on the third arch. Ethanol preparation of specimen V may also have resulted in epithelia pulling away from rakers and denticles, resulting in larger-than-normal measurements. This observation was made on the third and fourth arches with respect to the distance from denticles to the medial edge of the rakers and the heights of denticles.
On freshly-prepared specimens (IV, VI, and VII), the mean distance from denticles to the medial edge of rakers ranged from 160 to 390 microns. Values decreased from the first arch to the third arch, then increased to a maximum along the rakers of the pre-esophageal tissue. The distance from denticles to the lateral edge of rakers ranged from 625 to 1580 microns. Values decreased from the first arch to the third arch, then increased to a maximum along the rakers of the pre-esophageal tissue. The total downstream width of rakers ranged from 800 to 1840 microns. Values decreased from the first arch to the third arch, then increased to a maximum along the rakers of the pre-esophageal tissue. The distance between denticles near the base of the rakers ranged from 200 to 425 microns. Values decreased from the first to the third arch, where they reached a minimum. The distance between denticles was greatest along the fourth arch, and second greatest along the pre-esophageal tissue. The distance between the midlines of the rakers at the base ranged from 570 to 730 microns. The minimum distance was measured on the second arch, and the maximum distance was found on the fourth arch. The length of denticles ranged from 85 to 125 microns. The minimum length was found on the first arch, and the maximum lengths were found on the third arch and pre-esophageal tissue.

The primary focus of this study was not to provide statistical analysis on data collected, but I explored the significance of my measurements as a basis for future studies. Trends are described here to stimulate further research with greater sample sizes. For the statistical studies, measurements were taken from three specimens. Statistical significance was determined using a two-factor analysis of variance with replication on each of the six different variables in Microsoft Excel. The results of the ANOVAs show
statistically significant differences between the arches in (1) the distance from the denticle to the lateral edge of lateral rakers (p=0.002), (2) the height of denticles (p=0.01), (3) the distance between denticles on adjacent rakers (p=0.02), and (4) the distance between midlines of rakers at the base (p<<0.001). Statistically significant differences were found between specimens in the distance between midlines of rakers at the base (p=0.001). Significant interaction effects were found in the height of denticles (p=0.004) and the distance between midlines of rakers (p<0.001). In these two cases, sections from all but one of the specimens were found to have similar dimensions. All other measurements showed no statistically significant differences from one arch to another or from one specimen to another.

**Discussion**

Four gill arches extend from the ventral to the dorsal midlines of the oral cavity. Moving from anterior to posterior, the first gill arch is the largest, with the second, third, and fourth arches becoming progressively smaller. Several earlier studies have focused on the morphology of these oral structures in clupeids. Schmitz and Barker (1969) provided a description of the anatomical organization of the gizzard and threadfin shad digestive systems, laying a foundation for the study of gill arches and gill rakers in clupeids. Hammann (1985) measured the lengths of and gaps between rakers in American shad, noting trends that helped create a more detailed description of the oral structures.

Friedland (1985, 2006) studied the oral morphology of the Atlantic menhaden, another ram suspension-feeding fish. He hypothesized a mechanism by which filtration
may occur during feeding in menhaden, based on a widely-accepted model of a dead-end sieve. In this model, rakers and “branchiospinules,” to which Hammann and I refer as denticles, act as sites of food particle retention as water approaches the sieve at an angle of approximately 90°. However, further investigations by Sanderson et al. (2001) using high-speed endoscopic videotapes of filtration within the oral cavity of live gizzard shad (*Dorosoma cepedianum*, Clupeidae), goldfish (*Carassius auratus*, Cyprinidae), and ngege tilapia (*Oreochromis esculentus*, Cichlidae) demonstrated that these species of pump suspension-feeding fish feed using crossflow filtration. Cheer et al. (2003) used computational models to quantify fluid dynamics in simulated oral cavities of ram suspension-feeding fish. Callan & Sanderson (2003) proposed a functional mechanism of crossflow filtration by which a wide range of suspension-feeding fish filter food particles from the water using their gill rakers. Taken together, the findings of their work provide insight into the potential for a ram suspension-feeding fish to utilize the same model of crossflow filtration during feeding.

In the crossflow filtration model, food particles flow downstream towards the esophagus, increasing in concentration while moving posteriorly. Although previous studies on menhaden (Friedland, 1985) proposed a mechanism by which particles are essentially passed posteriorly from the epi-ceratobranchial joint of a preceding arch to the next, no such mechanism seems to be possible in American shad. Because the arches are joined at the ventral and dorsal midlines instead of at the epi-ceratobranchial joint (Figure 15), rakers are seen to be essentially continuous from the most-anterior reaches of the first gill arch to the pre-esophageal tissue. By having a gap between the rakers of one
arch and the rakers of the next arch, flow would be able to pass with more velocity through that region, carrying with it food particles.

Water exits from the oral cavity during respiration and feeding, but little is known as to exactly where and how. It has become common knowledge that the filtrate flows between the gill arches and over the gill filaments for the purpose of gas exchange during respiration. In ram suspension-feeding fish, feeding and respiration occur simultaneously, allowing for connections between the two processes.

Denticles are microscopic projections from the dorsal and ventral faces of each raker, lying along the inter-raker spaces. Denticles point toward the medial border of the rakers of all arches and pre-esophageal tissue into the flow of the water, forming what appears to be an incompletely closed mesh, or comb. The first and second arches have only one row of long rakers, known as lateral rakers. The third gill arch has ridges located along the upper region of the ceratobranchial section, positioned medially to the lateral rakers, which appear to be similar to but much smaller than the medial rakers of the fourth arch. The medial rakers of the fourth arch are much shorter than the lateral rakers, but do have numerous denticles that could function as a comb through which filtrate exits from the oral cavity. The medial rakers, much like the lateral rakers, have denticles of similar upstream orientation and size that could also function as a filtration comb. Potential influences of the denticles of medially-directed rakers include particle capture via the dead-end sieve model and fluid dynamic manipulations upstream and downstream which may cause particles to become retained. Rakers and denticles are also found along the anterior margin of the entrance to the esophagus, on what we term the
pre-esophageal tissue. This could potentially be another site for filtration, with filtrate exiting between the posterior wall of the oral cavity and the fourth gill arch.

Macro-observation during dissection indicates that lateral gill rakers project anteriorly and slightly medially from gill arches towards the opening of the mouth during ram suspension feeding. Medial rakers on the third and fourth gill arches and pre-esophageal rakers appear to be in a position to project medially and slightly posteriorly during feeding. Therefore, with the gill arches abducted during feeding, water flowing from anterior to posterior through the oral cavity flows tangential to the lateral rakers of the arches, but comes into contact with the medial rakers at an angle of approximately 90°.

When the specimen was positioned with a closed mouth, the lateral rakers of the ceratobranchial region of each arch overlapped the lateral rakers on the epibranchial region. However, when the mouth was opened, the rakers became evenly distributed instead of overlapping along the arch, forming the anatomical position expected during ram suspension feeding. Based on gill arch position during dissection and manipulations to simulate the feeding position with gill arches abducted during ram suspension feeding, we propose that the lateral rakers lie nearly parallel to the flow of water as the water moves through the oral cavity, between the gill arches, over the gill filaments, and exits out of the opercular opening. Lateral and medial rakers were not observed to become more laterally-oriented when the arches were abducted. Therefore, the angle of approach of the water as it flows towards the lateral raker and denticle comb is closer to 180° than 90°.
Although filtrate is believed to exit through the filtration comb, the direction in which the water comes in contact with a majority of the comb does not support a direct dead-end sieve model for much of the raker/denticle comb in the oral cavity. If the water were to exit through any gap other than between rakers as described above, it might do so through any space found between the lateral rakers of two consecutive arches, such as a space between the locations where the tips of the lateral rakers of the second arch encounter the bases of the lateral rakers of the first arch. However, this seems highly unlikely, as there is no evidence of such a process and the abduction of gill arches during ram suspension feeding may essentially close this sort of gap. The medial ridges of the third arch, medial rakers found along the fourth arch, and rakers on the pre-esophageal tissue are the few regions of the raker/denticle comb that are positioned in such a way that they may come into perpendicular contact with the water flow moving anterior to posterior through the oral cavity when the mouth is opened, which may thereby allow for a functional dead-end sieve. As both perpendicular and tangential flows are thought to occur in the oral cavity during suspension feeding, this study differs by applying the concepts of crossflow filtration to the anatomical dimensions of the oral cavity. In the crossflow filtration model, the flow of material is parallel to the filtering surface of the rakers and therefore there is little or no blockage of filtrate flow by retained food particles as matter is moved posteriorly towards the esophagus in increasing concentrations.

The information gathered in this study, both from the macroscopic and microscopic perspectives, suggests that crossflow filtration may occur along lateral rakers whereas dead-end sieving may occur on medial rakers of the third and fourth arch and pre-esophageal rakers. All of the lateral rakers are seen to be oriented antero-medially,
tangential to the flow of water as it exits between the gill rakers and arches. Medial rakers, which lie medial to the lateral rakers on the third and fourth gill arches, as well as the rakers on the pre-esophageal tissue, are in a position to function as dead-end sieves. By appearing to be able to collect particles at angles closer to 90° than the tangential flows that appear to occur along the lateral rakers, based on information from microscopic (SEM) and macroscopic (during dissection) observation, these rakers have the potential to serve as dead-end sieves. With a more posterior location than the other arches, the medial rakers of the third and fourth arches and the rakers of the pre-esophageal tissue may assist in food particle concentration as water exits the oral cavity at the most posterior sites, between the third and fourth gill arches and between the fourth gill arch and the posterior wall lateral to the pre-esophageal tissue.

Directions for future study:

Many directions of further study are possible to provide additional understanding of how crossflow filtration may be used by American shad during feeding. Aside from the challenging task of observing and filming live specimens in flow tanks and aquaria with internal endoscopic video, many opportunities exist to develop a more in-depth wealth of information about the highly-efficient filtration system in the oral cavity of shad.

Additional studies to qualitatively and quantitatively determine the gut contents of American shad would help in further development of filtration hypotheses. Small detritus and phytoplankton particles may have been missed by previous studies, possibly accounting for much of the biomass of the gut contents. Also, sexual dimorphism in oral morphology of American shad may account for differing sizes of particles retained,
depending on sex. Additional studies with both male and female American shad specimens would add significantly more depth to the overall study.

Several aspects of this study could have used a more precise means, mostly in regard to taking measurements. Micrographs were taken to maximize measurements available on each image, but sometimes in doing so, the precision of taking such measurements suffered. A more efficient and more accurate method would enhance measurements and perhaps the statistical significance of the data collected. This is not to detract from the great amount of information about the anatomical arrangement of the oral cavity that was learned from the microscopy and macro-photography of the study.

Further understanding of the tissue types in the oral cavity could provide additional insight to the function of different regions throughout the oral cavity. Histological studies on different tissues on the raker, those found adjacent to rakers on arches, and those found on lateral and medial sides of the pre-esophageal rakers may have some implications for sensory detection and particle retention. Additional histology may give rise to a better understanding of raker and arch development in maturing fish. Histology may also uncover the origins of developing denticles, the pits located medially to them, and an explanation of the varying frequency of denticles in a downstream plane along the rakers.

As the range of motion of the lateral and medial rakers is not known in a ram suspension-feeding fish, obtaining data with respect to flexibility and movement of the arch and rakers would help to provide a more comprehensive understanding of the filtration process. Comparisons between flexibilities of medial and lateral rakers could
also provide insight into different filtration techniques and particle-clearing mechanisms used.

If data were collected regarding swimming speed during ram feeding and the velocity of water flowing into the mouth, physical models and computational models could accurately replicate the physical environment of the oral cavity during feeding. Analysis of movement capabilities of the fish’s gill arches, whether voluntary or involuntary, would also help to determine the structural arrangement of the oral cavity during feeding. The swimming velocity during feeding relative to body size (and therefore gill arch and raker size) and the velocity of flow inside the oral cavity during feeding would also have to be taken into consideration. A correlation between the speed at which a shad ram feeds and its size would add to the accuracy of this study by providing data regarding the velocity of water flow through the raker/denticle comb depending on size of specimens. Physical models would need to be scale replicas of gill arches and rakers, with accurate dimensions as laid out by the measurements in this study. Dentine numbers and positions in a given region of the arch would have to be determined based on general trends seen on rakers in respective regions of the arch.

Studies of physical models in a flow tank (with appropriate Reynolds numbers velocity of flow, etc.) would to help show how water flows over the raker structure. Considerations would have to be made for different velocities and directions of flow along different sections of the four arches and pre-esophageal arches, unless a full-scale model of an oral cavity was to be used. Having only one segment of an arch with rakers studied for flow would not account for the influence of the other structures on the flow through the oral cavity. Also, the presence and position of an operculum on the lateral
The methods used to observe particle movement in gizzard shad, studied by Sanderson et al. (2001), would be particularly useful for a study on American shad. Issues arise, however, as the feeding style of American shad differs from the gizzard shad. The gizzard shad is a suction suspension feeder, using expansion of the oral cavity to suck water into the mouth for filtration and respiration, a method known as buccal pumping. Buccal pumping requires no forward movement, and endoscopic video footage is thus relatively easy to obtain. However, with a ram suspension feeder, forward movement is continuous, as water must constantly be flowing through the oral cavity for feeding and over the gill filaments for respiration. Video footage of the functional oral cavity of an American shad, if obtained, would help in understanding raker and arch angles during feeding-associated and during non-feeding-associated movement. The
availability of in vivo video endoscopic footage of water movement in the posterior region of the oral cavity would help to clarify the flow of water through the medial rakers of the fourth arch and the rakers of the pre-esophageal tissue. Data are lacking on directions, turbulence, and velocities of flows in this region. Whether the pre-esophageal rakers function as an epibranchial organ by directing flow to further concentrate food particles, or function as another filter comb, could be explored using such techniques.
References


Figure 1
A schematic showing the fundamental characteristics of the dead-end sieve and crossflow filtration models. Medial is up, lateral is down, anterior is to the left, and posterior is to the right. The net movement of food particles is anterior to posterior, as indicated by the narrow horizontal arrow. Heavy arrows indicate the direction of water flow approaching the raker/denticle comb (gray bars). Flow in dead-end sieving approaches the filter at an angle of approximately 90°, resulting in a collection of food particles on the raker/denticle comb. However, the mainstream flow in crossflow filtration moves tangential to the filter, transporting concentrated food particles towards the esophagus at posterior of the denticle/raker comb.
Figure 2
Right side of the American shad head following a sagittal cut through the midline. Anterior is to the left. The first (1), second (2), third (3), and fourth (4) gill arches are shown, in addition to the pre-esophageal tissue (PE), esophagus (E), and oral jaws (J). The location of the eye (Eye) is also labeled for reference. Specimen preparation method: Ethanol.
Figure 3
The first (1), second (2), third (3), and fourth (4) gill arches are shown from left to right as removed from the left side of an American shad. The first, second, and third gill arches are positioned as found in the oral cavity in lateral view. The fourth gill arch has been positioned to provide a view of both the lateral rakers (l) and medial rakers (m). The lateral and medial rakers of the fourth arch are projecting upwards towards the viewer, as the fourth arch has been rotated laterally and posteriorly, providing an anterior view rather than a lateral view. Although pre-esophageal tissue is not shown, the rakers of the pre-esophageal tissue would lie adjacent to the medial rakers of the fourth arch when seen from an anterior perspective. Specimen preparation method: Frozen.
Figure 4
Diagram of the sections cut from specimen IV, prepared fresh. Sections from the four arches (plates A, B, C, and D) were mounted to provide a lateral view of the lateral rakers. The gill filaments along the dorsal ends of each section were cut shorter than the filaments along the ventral ends to allow for quick reference under the microscope. Sections from the pre-esophageal tissue (plate E) were mounted to show the anterior view, with rakers along the lateral border of the tissue (on the right) projecting upward towards the viewer.
Figure 5
Diagram of the sections cut from specimen V, preserved in ethanol prior to dissection. Sections 2A and 2B (plate B) were mounted to provide a medial view of the lateral rakers. All other arch sections (plates A, B, C, and D) were mounted to provide a lateral view of the lateral rakers. The gill filaments along the dorsal ends of each section were cut shorter than the filaments along the ventral ends to allow for quick reference under the microscope. Sections from the pre-esophageal tissue (plate E) were mounted to show the anterior view, with rakers along the lateral border of the tissue (on the right) projecting upward towards the viewer.
Figure 6
Diagram of the sections cut from specimen VI, prepared fresh. Arch sections (plates A, B, C, and D) were mounted to provide a medial view of the lateral rakers. Section 2F (plate B) was mounted with the rakers directed upward towards the viewer, allowing for better visibility of both the medial and lateral borders of the arch adjacent to the rakers. The gill filaments along the dorsal ends of each section were cut shorter than the filaments along the ventral ends to allow for quick reference under the microscope. Sections from the pre-esophageal tissue (plate E) were mounted to show the anterior view, with rakers along the lateral border of the tissue (on the right) projecting upward towards the viewer.
Figure 7
Diagram of the sections cut from specimen VII, prepared fresh. Arch sections (plates A, B, C, and D) were mounted to provide a medial view of the lateral rakers. The gill filaments along the dorsal ends of each section were cut shorter than the filaments along the ventral ends to allow for quick reference under the microscope. Sections from the pre-esophageal tissue (plate E) were mounted to show the anterior view, with rakers along the lateral border of the tissue (on the right) projecting upward towards the viewer.
Figure 8
Illustration of locations for measurements taken on micrographs. Measurements include (A) the distance from the midpoint of the base of a denticle to the medial edge of a lateral raker, (B) distance from the midpoint of the base of a denticle to the lateral edge of a lateral raker, (C) total downstream width of a raker, (D) distance between midlines of adjacent rakers parallel to the arch at base of rakers, (E) shortest distance between tips of denticles on adjacent rakers near the base of the rakers, and (F) height of denticle near the base of the rakers from base of denticle to tip of denticle. The tip-to-tip denticle measurement (E) was conducted on the denticles closest to the arch, near the base of the raker (i.e., if four measurements were taken, the four denticles closest to the base of the raker were measured). Several measurements of D, E, and F were taken from each section when feasible to increase sample size. Specimen preparation method: Fresh.
**Figure 9**
Rakers on the ventral midline of the oral cavity anterior to the origin of distinct left and right second arches. Anterior is to the lower left. Specimen preparation method: Frozen.
Micrograph of the fourth gill arch branching along the ventral midline of the oral cavity (ventral is at top). Lateral rakers of the right gill arch are in the upper left corner, and lateral rakers of the left gill arch are in the center of the figure. Specimen preparation method: Frozen.
Figure 11
Left second arch removed to show lateral rakers. The medial/upstream faces of the rakers are directed upwards and towards the viewer. Gill filaments are behind the arch, directed away from the viewer. Specimen preparation method: Frozen
Figure 12
Lateral view of the left side of the American shad head with the operculum removed to allow a view of the first gill arch (a), rakers (r), and filaments (f). The segment of the arch ventral and anterior to (a) is referred to as the ceratobranchial section, and the segment dorsal and anterior of (a) is referred to as the epibranchial section. Specimen preparation method: Frozen.
Figure 13
Ventrolateral view of the left side of the American shad head with the operculum removed to allow a view of the first gill arch on the left side removed ventrally and abducted to provide a medial view of the first arch (1) and a lateral view of the lateral rakers of the second arch (2). Specimen preparation method: Frozen.
Figure 14
The most dorsal ends of the first (1), second (2), third (3), and fourth (4) gill arches on the right side of the oral cavity and the pre-esophageal tissue (PE) on the left side of the oral cavity. The left gill arches have been removed. Anterior is on the lower left. Specimen preparation method: Frozen.
Figure 15
The oral cavity as viewed from the oral jaws, looking posteriorly. The rakers of the first (1), second (2), third (3), and fourth (4) gill arches on the right side of the fish are shown, in addition to the pre-esophageal tissue (PE). The first, second, and third arches on the left side of the fish have been removed. Specimen preparation method: Frozen.
Figure 16
Lateral view of ventral connective tissue (*) present between the medial side of the third (3) gill arch and lateral side of the fourth (4) gill arch on the left side of the fish. Analogous tissue was found between the second and third arches. Specimen preparation method: Frozen.
Figure 17
Medial view of the bases of the lateral rakers (right) and ridges (left) of the third arch. The midline of the third gill arch runs from the bottom left to the top right of the image. The ridges shown here resemble medial rakers found on the fourth arch, but have few or no denticles. Specimen preparation method: Frozen.
Figure 18
Medial view of the lateral rakers (shown on right) and medial rakers (shown on left) on the ceratobranchial section of the fourth arch. This section is ventral to the location where the row of medial rakers branches from the row of lateral rakers. Specimen preparation method: Frozen.
**Figure 19**
Lateral view of the rakers and arches on the right side of the oral cavity. The left gill arches have been removed. The lateral rakers on the ceratobranchials of the first (1r), second (2r), third (3r), and fourth (4r) arches and the rakers of pre-esophageal tissue (PEr) are labeled. Also labeled with arrows are the lateral rakers (4r(epi)) of the epibranchial section of the fourth arch and the medial rakers (4r(m)) of the fourth arch. Specimen preparation method: Ethanol
Figure 20
Medial view of different tissue layers on the medial vs. lateral sides of the rakers along the pre-esophageal tissue. Smooth and regular tissue lateral to the rakers is shown in the top of the figure, whereas irregular tissue medial to the rakers is shown at the bottom of the figure. Specimen preparation method: Frozen.
Figure 21
A micrograph of a denticle from the lateral view, with the tip pointed away from the viewer. Tissue covers most of the denticle after preparation for SEM. Specimen preparation method: Fresh.
Figure 22
Medial view of lateral rakers on the second arch, with denticles directed medially. Irregular tissue is located on the arch medial to the lateral rakers. Specimen preparation method: Frozen.
Figure 23
The variability in numbers and patterns of consecutive denticles found along the downstream width of some rakers is illustrated in this medial view of rakers from the fourth arch. Specimen preparation method: Frozen.
Figure 24
Cellular tissue layer located along the second arch lateral to the base of the lateral rakers. The cells found on the arch are less smooth and regular than those found on the rakers. Specimen preparation method: Fresh.
Figure 25
Medial view of cellular layer covering the lateral rakers and denticles of the second arch. Specimen preparation method: Fresh.
Figure 26
A row of denticles along a lateral raker of the third arch with intact epithelial tissue. Tissue is shown to cover all of the denticle after SEM preparation. The upstream/medial edge of the raker is shown at the bottom of the figure. An adjacent raker is pictured in the upper left corner. Specimen preparation method: Frozen.
Figure 27
Smooth and regular layer of intact epithelial cells along a lateral raker. Specimen preparation method: Fresh.
**Figure 28**
High magnification view of structure identified as a mucous-secreting cell on a lateral raker. Vesicles described by Sibbing & Uribe (1985) as being involved in mucous secretion are shown. Specimen preparation method: Fresh.
Figure 29
Epithelial tissue layer (E) intact along the upstream (medial) edge of a lateral raker. A ruptured mucous cell (MC) is shown. Specimen preparation method: Fresh.
Figure 30
A layer of epithelial cells along the perimeter of a denticle, shown in the lower left corner. Irregular objects along cellular layer are believed to be detritus/mucous from ethanol preparation. Specimen preparation method: Ethanol.
Figure 31
Lateral view of the micro-ridges along the rakers of the pre-esophageal tissue. Specimen preparation method: Frozen.
Figure 32
The “ribs” (b) extending from the bases of the lateral rakers (r) across the downstream/posterior side of the third arch towards the gill filaments (f). Specimen preparation method: Fresh.
Figure 33
Data collected during the morphometric analysis of specimen V (mean and standard deviation, n = 2 to 6)
Figure 34
Data collected during the morphometric analysis of specimens IV, VI, and VII (mean and standard error, n = 3)