The Journal of Experimental Biology 210, 2706-2713
Published by The Company of Biologists 2007
doi:10.1242/jeb.000703

Mucus function and crossflow filtration in a fish with gill rakers removed *versus* intact

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Accepted 20 March 2007

Summary

Filtration mechanisms are known for only two species of suspension-feeding tilapia, each of which relies on a different method of particle retention. We used high-speed video endoscopy to assess whether a third species of tilapia, *Oreochromis aureus*, with gill rakers intact as well as surgically removed, uses mucus in the oropharyngeal cavity for hydrosol filtration or uses crossflow filtration to retain particles during suspension feeding. Although a large amount of mucus was visible during feeding with rakers intact, particles were rarely retained in the mucus. The hypothesis that the presence of mucus results in particle entrapment by hydrosol filtration is rejected for *O. aureus*. Rather than functioning as a sticky filter, mucus is proposed to function in this species to regulate the loss of water between the rakers and between the anterior branchial arches, increasing crossflow speed and thereby increasing the inertial lift force that transports particles radially away from the arches. Gill raker removal resulted in an almost complete lack of observable mucus in the oropharyngeal cavity, probably due to the removal of mucus-secreting cells attached to the gill rakers. However, endoscopic videotapes showed that crossflow filtration continued to operate in the absence of gill rakers and mucus, indicating that the surfaces of the branchial arches play an important role in crossflow filtration.

Supplementary material available online at
http://jeb.biologists.org/cgi/content/full/210/15/2706/DC1

Key words: suspension feeding, filter feeding, hydrosol filtration, tilapia, *Oreochromis aureus*.

Introduction

Suspension-feeding fish are capable of filtering food particles over a range of 5–3000 μm from the water that enters the mouth and exits over the gills *via* the opercula (Sanderson and Wassersug, 1993). These fish belong to 21 families in 12 orders (Cheer et al., 2001), and comprise a quarter of the world fish catch (Food and Agriculture Organization of the United Nations, 2000). Despite the ecological and economic importance of suspension-feeding fish, food particle retention mechanisms are known for only seven species, including two species of tilapia (Callan and Sanderson, 2003; Hoogenboezem et al., 1991; Sanderson et al., 2001).

Endoscopic analysis of the oropharyngeal cavity during suspension feeding in the Nile tilapia (*Oreochromis niloticus* Linnaeus, Cichlidae) described hydrosol filtration with entrapment of particles in mucus on the gill rakers and branchial arches as one mechanism of particle encounter and retention (Sanderson et al., 1996). During hydrosol filtration, particles that are suspended in water contact filter elements as a result of physical processes [i.e. direct interception, inertial impaction, gravitational deposition, diffusional deposition, and electrostatic attraction (LaBarbera, 1984; Rubenstein and Koehl, 1977; Shimeta and Jumars, 1991)]. If the filter elements are sticky due to the presence of mucus, a particle that encounters a filter element during hydrosol filtration can be retained by adhesion, even if the particle is small enough to pass between the filter elements. In *O. niloticus*, mucus containing the trapped particles is then transported to the esophagus for swallowing (Sanderson et al., 1996).

In contrast, a second species of tilapia, *O. esculentus* (Graham), lacks observable mucus in the oropharyngeal cavity and uses crossflow filtration instead of mucus to retain particles during suspension feeding (Goodrich et al., 2000; Sanderson et al., 2001). During crossflow filtration, hydrodynamic forces such as inertial lift cause particles to remain suspended but become concentrated in the fluid traveling parallel to the filter surface, as filtrate exits between the filter elements (Brainerd, 2001; Sanderson et al., 2001). Thus, the fluid in the oropharyngeal cavity becomes increasingly more concentrated with food particles as the suspension is reduced in volume while traveling towards the esophagus. The particles are then swallowed with very little accompanying water (Sanderson et al., 2001).

Oreochromis esculentus is typically described as a specialist, feeding mostly on phytoplankton or colonial blue-green algae (Onyari, 1983). The dietary breadth of *O. niloticus* is much wider, consisting of phytoplankton, filamentous algae and

diatom-rich sediments as well as insect larvae, benthos and crustaceans (Onyari, 1983). To investigate whether there is a correlation between diet and particle retention mechanism in suspension-feeding tilapia, we used a fiberoptic endoscope to study intra-oral movements of particles during feeding in *O. aureus* (Steindachner), a species with a similar ecological niche to *O. niloticus* (Drenner et al., 1984; Mallin, 1985; Spataru and Zorn, 1978). As so few data are available on particle retention mechanisms in suspension-feeding fish, such a correlation could be a powerful predictive tool for gaining insight into the ecological implications and evolution of suspension-feeding mechanisms. Based on the dietary similarities between *O. aureus* and *O. niloticus*, we predicted that *O. aureus* uses mucus to retain particles on the branchial arches.

Gill rakers attached to the branchial arches have been hypothesized to be a component of all filtration mechanisms in fish (Hoogenboezem et al., 1991; Sanderson et al., 1991; Sanderson et al., 1996; Sanderson et al., 2001). In tilapia, toothed projections (~150 μm high) on the external faces of the arches, termed microbranchiospines, have also been proposed as filtering structures (Beveridge et al., 1988a; Beveridge et al., 1988b). However, surgical removal of all rakers and microbranchiospines from the suspension-feeding tilapia *Sarotherodon galilaeus* did not significantly affect the size distribution of ingested particles or the efficiency of particle retention (Drenner et al., 1987). Sanderson et al. (Sanderson et al., 1996) suggested that Drenner et al.'s results could be explained if mucus on the arches functions to retain particles during hydrosol filtration after the rakers have been removed.

We used a fiberoptic endoscope to quantify and compare the intra-oral movements of particles in the presence *versus* the absence of rakers and microbranchiospines in *O. aureus*. The effects of raker removal on mucus presence and particle movement inside the oral cavity have not been studied in any fish species. We removed the rakers and microbranchiospines from all branchial arches. Our objective was to test the hypothesis that mucus, if present on the branchial arches, functions to retain particles during hydrosol filtration before and after removal of the gill rakers in *O. aureus*.

Materials and methods

Endoscopy experiments

Oreochromis aureus (Steindachner) were obtained from pure stock raised at the University of Arizona. Tilapia were held individually or in pairs in 110-liter aquaria with a gravel substrate (0.3–1.0 cm diameter). They were maintained on a diet of Tetramin™ flakes and kept at a constant temperature of 25–28°C. The methods used for the endoscopy experiments were similar to those described (Sanderson et al., 1996). Five specimens (20.3–23.4 cm standard length, *SL*) were used for the endoscopy experiments. Fish were anesthetized with MS-222 and a polyethylene cannula (45 cm long, 2.15 mm i.d., 3.25 mm o.d., Intramedic PE 280, Sparks, MD, USA) was implanted into the oropharyngeal cavity through a hole drilled in the left preopercular bone. To prevent the cannula from being pulled through the hole, a flange (approximately 1 mm wide) around the circumference of one end of the cannula lay flush with the tissue of the oropharyngeal cavity. The cannula fitted snugly, eliminating any water flow through the hole in the preopercular bone. The external section of the cannula was then threaded through a second flanged polyethylene cannula (2.5 cm long, 3.76 mm i.d., 4.82 mm o.d., Intramedic PE 360), preventing any slippage back into the oropharyngeal cavity. To reduce irritation, a small piece of neoprene rubber (0.8 cm×0.8 cm) was placed between the second flanged cannula and the skin. After this the fish was returned to the aquarium.

The experiments were conducted 4 h after cannula implantation. A flexible fiberoptic endoscope (ultrathin fiberoptic type 14, 1.4 mm o.d., 1.2 m working length, 75° field of view, 0.2–5.0 cm depth of field, Olympus, New York, NY, USA) was threaded through the cannula. The endoscope was attached to an Intensified Imager VSG (50–500 Hz, Kodak, San Diego, CA, USA). An Ektapro Hi-Spec Motion Analyzer 1012/2 (Kodak, San Diego, CA, USA) with split-screen imaging was used to record external views of the oral jaws simultaneously with the endoscopic views, to correlate external feeding behaviors with the movements of intra-oral structures and particles in the internal endoscopy video. A high-intensity light source (Helioid ALS-6250, 250 W, Olympus) provided light for the endoscope. A Sony DSR-11 DVCAM video recorder with a jog shuttle (remote control unit DSRM-20, Sony, Tokyo, Japan) was used for frame-by-frame analysis of the videotapes. The digitized video images used for publication were processed by convolving them with a mean kernel (4×4 pixels) using NIH Image 1.62, which smoothed the fine honeycomb pattern caused by individual fibers in the fiberoptic bundle.

Data were recorded as fish were fed a slurry of finely crushed Tetramin™ flakes (0.1–1.0 mm diameter) mixed with water. Pre-hydrated brine shrimp cysts (*Artemia* sp., 210–300 μm) were added to the slurry to serve as additional tracer particles when viewed through the endoscope. The slurry was administered into the water directly above the fish through a short tube attached to a 30 ml syringe. Tilapia engulfed particles directly from the tip of the tube or as the particles descended through the water column. Fish were anesthetized for cannula removal at the conclusion of each experiment, following which the insertion site healed fully.

Gill raker removal

The method of raker removal was modified from that of Drenner et al. (Drenner et al., 1987). *O. aureus* were anesthetized with MS-222 and the tissue supporting all lateral and medial rakers and microbranchiospines was removed with microforceps from the anterior four branchial arches on both sides of five fish. The fifth arches form the lower pharyngeal jaw, which was left unaltered. The procedure lasted an average of 90 min, during which the fish was lifted periodically from the water containing MS-222 to remove a section of rakers and microbranchiospines, and then returned to the water in the surgery tray. The fish was then returned to its aquarium and Fungus Eliminator (5 g 20 l^{-1}; Jungle Laboratories Corporation, Cibolo, TX, USA) was added once to prevent infection. Fish were not adversely affected by the surgery and exhibited normal feeding behavior within 2 days. During the 15 days following surgery, the arches healed as described by Drenner et al. for *Sarotherodon galilaeus* (Drenner et al., 1987). Endoscopy experiments were conducted on fish with rakers intact and again on the same individuals 15 days after raker removal.

Mucus presence and classification

For each of five specimens, endoscopic video footage of slurry feeding and ventilation were analyzed frame-by-frame for the presence of mucus before and after removal of rakers and microbranchiospines. First, the sequences with the clearest, most focused views were identified. From these, 2–4 sequences per fish were chosen at random for analysis. All video frames containing mucus were then analyzed and categorized as follows. (1) The number of sequences and the number of video frames in which each of the following types of mucus was observed: (a) aggregate (an irregularly shaped opaque clump), (b) strand (a single opaque string of mucus), (c) sheet, stretching across the entire field of view while covering the rakers or passing through the field of view. (2) The movement of mucus: (a) pass (mucus moved through the field of view without contacting any oropharyngeal surface), (b) lift and pass (mucus that had been attached to the arches and rakers visibly lifted and exited from the field of view), (c) sliding along arches (mucus maintained contact with the arches and/or rakers while traveling posteriorly), (d) attached (mucus maintained contact with the arches and/or rakers and did not change location). (3) The action of the fish as mucus that had been attached to the arches and rakers lifted and exited from the field of view: (a) pumps, (b) reversals or (c) ventilation. Data are reported as means ± s.d. unless stated otherwise.

Particle analysis

Frame-by-frame video analysis of 100 slurry particles or brine shrimp cysts passing the endoscopic field of view during feeding was conducted for each of three specimens with rakers intact, as well as after raker removal. For this analysis, 25–50 particles (33±12, *N*=18 sequences) were selected randomly within each of 2–4 feeding sequences per specimen. The movement of each particle was described as one of four actions: (1) straight, passed the field of view in a posterior direction without contacting any oropharyngeal surface; (2) bounced, particle was seen to graze or bounce off either the oral roof, the branchial arches, or a raker before continuing posteriorly; (3) disappeared, particle traveled towards the arches and disappeared either between two rakers or between two of the arches; (4) stuck, particle stayed immobile on the arches or rakers before traveling posteriorly.

To determine the extent to which mucus was involved in particle capture, the longest feeding sequence with the best lighting in which mucus was present was analyzed for two fish with rakers intact. All slurry particles and brine shrimp cysts passing through the field of view (volume of approximately 1 ml) during this feeding sequence were counted. The number of particles caught in mucus during the course of the feeding sequence was then tallied and compared to the total number of particles passing through the field of view during the sequence.

Results

Endoscopic view

The endoscope entered the oropharyngeal cavity directly lateral to the left tissue pad located on the roof of the pharynx. This position was approximately 65% of the distance from the front of the oral jaws to the esophagus. The left ventral sections (ceratobranchials) of arches II–IV could be seen most frequently, and the left ceratobranchial of arch I entered the field of view periodically. Prior to raker removal, the rakers were visible as projections from the arches. When the endoscopy experiments were conducted 15 days after raker removal, the arches were smooth with no visible rakers or microbranchiospines.

Pumps and reversals

O. aureus suspension-fed on the Tetramin™ slurry using a series of pumping actions. During a feeding pump, water entered the mouth and continued to flow posteriorly through the oropharyngeal cavity (see Movie 1 in supplementary material) until exiting *via* the operculum. Pumps were frequently interrupted by a reversal, during which all of the suspended particles were seen through the endoscope to travel with the water from posterior to anterior inside the oropharyngeal cavity. This reversal of flow to a posterior to anterior direction has been termed stage 1 of a reversal (Sanderson et al., 1996). During stage 2 of a reversal, the particles were viewed resuming an anterior to posterior flow inside the oropharyngeal cavity.

Mucus presence and classification with gill rakers intact

A frame-by-frame video analysis of five *O. aureus* during suspension feeding on slurry and during ventilation was conducted on a total of 29 641 and 28 749 frames, respectively, (125 Hz) before raker removal. During feeding, mucus was present in 53±37% of the frames analyzed, compared to mucus present in 61±26% of the video frames analyzed during ventilation.

Mucus was identified as belonging to one of six categories when viewed through the endoscope: strand, aggregate, sheet, both strand and sheet viewed simultaneously, both aggregate and sheet viewed simultaneously, or both strand and aggregate viewed simultaneously (Table 1). Only one category of mucus

Table 1. *Frequency of occurrence of mucus shape categories in sequences and in video frames during suspension feeding and ventilation in five specimens of O. aureus with intact gill rakers*

	Observed occurrence of category		
Mucus category	Sequences (number)	Sequences (%)	Video frames (%)
Feeding			
Strand	5	38.5	19.0
Aggregate	4	30.8	32.6
Sheet	7	53.8	39.9
Strand + sheet	2	15.4	3.7
Aggregate + sheet	1	7.7	4.2
Strand + aggregate	1	7.7	0.7
Ventilation			
Strand	3	23.1	20.3
Aggregate	3	23.1	21.0
Sheet	3	23.1	37.2
Strand + sheet	0	0.0	0.0
Aggregate + sheet	2	15.4	11.8
Strand + aggregate	2	15.4	9.7

Feeding: *N*=13 sequences, 12 774 video frames; ventilation: *N*=13 sequences, 16 186 video frames. See text for details.

Table 2. *Number of pumps and reversals during which ten mucus strands and aggregates were either attached to arches or lifted and moved posteriorly out of the field of view during feeding in four O. aureus with intact rakers*

	Number of occurrences			
	Mucus attached to arches		Mucus lifted, moved posteriorly	
	O. aureus	*O. niloticus**	*O. aureus*	*O. niloticus**
Pumps	41	4	1	0
Reversals	22	1	5	5
Total	63	5	6	5

All pumps and reversals that occurred while the mucus was in the field of view are included in these counts.

*For comparison, mean numbers of pumps and reversals per ten mucus strands and aggregates are included for *O. niloticus* with intact rakers (Sanderson et al., 1996).

was present within each video frame. However, since more than one category of mucus was observed consecutively within some feeding or breathing sequences, the percentage of sequences during which each category was observed totals to more than 100% in Table 1. Whether quantifying frequency of occurrence for each mucus category using percentage of sequences or percentage of video frames during which mucus in that category was observed, mucus appeared as opaque sheets most frequently. These mucus sheets could often be seen to extend across the entire endoscopic field of view.

In general, mucus remained attached to the arches and swayed (57±28% of frames with mucus during feeding, 98±3% of frames with mucus during ventilation). Less frequently, the mucus lifted from the arches and traveled posteriorly (28±26% of frames analyzed during feeding, 0% of frames analyzed during ventilation; see Movie 2 in supplementary material). Mucus sometimes passed through the endoscopic field of view during feeding (15±20%) and ventilation (2±3%) without contacting any oropharyngeal surface. Mucus was never observed sliding across the arches.

Mucus that was attached to the arches often remained on the arches for a long period of time before exiting from the field of view. To quantify the duration of mucus presence in four fish, a total of ten sequences with a mucus strand or aggregate were observed until the mucus exited from the field of view or until the endoscopic sequence ended. Mucus remained attached for a large number of pumps and reversals before the mucus lifted from the arches or the endoscopy sequence ended (Table 2). The data on duration of attached mucus for *O. aureus* in Table 2 are conservative, since the mucus was still attached when some sequences ended.

Stage 2 of a reversal following a pump was the most common action during which mucus that had been attached to the arches subsequently left the field of view in a posterior direction after being lifted off the arches during stage 1 of a reversal (65% of 23 total occurrences of mucus during feeding for five fish) (Fig. 1; see Movie 3 in supplementary material). The exit of previously attached mucus from the field of view in association with a pump was less common (35% of total occurrences for five fish). Attached mucus was never dislodged and carried posteriorly during ventilation.

Particle analysis with gill rakers intact

For each of three *O. aureus* prior to raker removal, 100 brine shrimp cysts or slurry particles were analyzed as they entered the field of view, passed posteriorly through the oropharyngeal

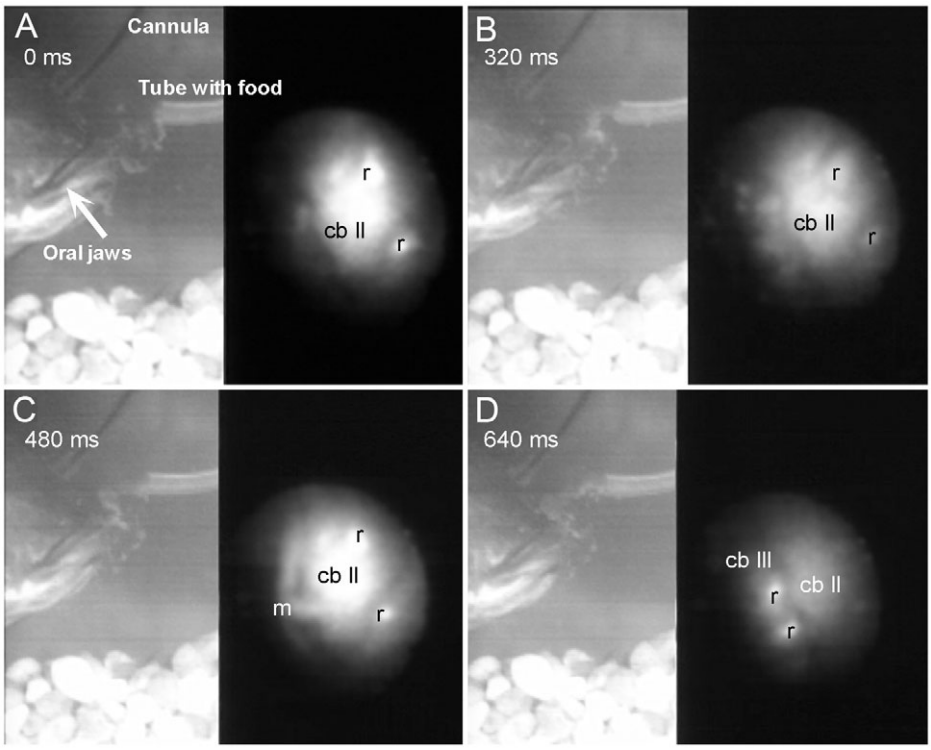

Fig. 1. Endoscopic images (right panels) and synchronized external images (left panels) digitized from DVCAM videotapes recorded at 125 Hz. The anterior of the fish is at the bottom of the endoscopic view, and at the right of the external view. The cannula through which the endoscope is inserted can be seen at the upper left of the external view. (A) Prior to the next feeding pump, food is introduced in front of the oral jaws through a tube. The second branchial arch (cb II) and the tips of two lateral rakers (r) on cb II are visible in the endoscopic view. These raker tips are approximately 0.2 mm wide. (B) The oral jaws have abducted, and food particles can be seen passing through the endoscopic field of view. (C) As stage 1 of a reversal begins, a mucus aggregate (m) is seen resting on the second and third branchial arches. (D) As stage 2 of a reversal clears mucus from the field of view, the row of lateral gill rakers (r) is visible on ceratobranchial III (cb III).

cavity, and exited from the field of view during feeding. Most frequently the particles traveled posteriorly in a straight path without contacting any oropharyngeal surface (84±2%, Table·3; see Movie 4 in supplementary material). Some particles disappeared into the spaces between rakers or passed between two arches. A small percentage of particles bounced off the rakers or arches before continuing posteriorly, and very few particles were stuck on the rakers or arches.

For five *O. aureus* combined, Tetramin™ flake particles or brine shrimp cysts were seen trapped in mucus during 15% of the 12 744 frames (125 Hz) with mucus present that were analyzed during feeding. To ascertain the effectiveness of mucus in particle retention, a typical feeding sequence was analyzed for each of two fish to determine the total number of particles that passed through the endoscopic field of view compared with the total number of particles that were retained in mucus during the feeding sequence. Of the total of 642 particles that passed posteriorly during the two feeding sequences, 98% traveled independently without being retained in the mucus while only 2% of the particles were retained in mucus on the arches or rakers.

Mucus and particle analysis with gill rakers removed

Typical feeding behavior was observed after the rakers were removed. There were no observable differences in the number of sequential pumps or the frequency of reversals during suspension feeding in the absence of rakers. Just as when the rakers were intact, no food particles were visible exiting *via* the operculum after the rakers had been removed.

Frame-by-frame analysis of post-raker removal endoscopic videotapes from three specimens included all unobstructed, clearly focused views (52 063 frames of feeding on slurry and 8020 frames of ventilation, 125 Hz). No mucus was seen during ventilation without rakers, and the total number of frames with mucus present during suspension feeding (2±2%) was greatly reduced compared to endoscopy with intact rakers. During the limited number of suspension-feeding frames with mucus after the removal of rakers, there was an equal percentage (33% of frames with mucus present) of strands, aggregates and sheets of mucus visible through the endoscope. Mucus swayed while attached to the arches until lifted from the arches (stage 1) and cleared from the field of view (stage 2) with a reversal in 51% of the frames in which mucus was present during feeding. Mucus was also frequently seen passing straight through the field of view in a posterior direction without contacting any oropharyngeal surface during feeding pumps (49% of total frames analyzed).

For each of the three fish, 100 brine shrimp cysts or slurry particles were followed through the field of view to determine particle movement while suspension feeding after raker removal. The majority of the particles (84±21%, Table 3) traveled posteriorly in a straight path without touching any oropharyngeal surface. Many particles were visible through the endoscope while traveling straight towards the arches, and then disappeared into the dark void between two arches (15±21%).

Table 3. *Frequency of particle movements inside the oropharyngeal cavity before and after raker removal in O. aureus*

	Frequency of particle movement (%)	
Category of particle movement	Gill rakers intact	Gill rakers removed
Traveled posteriorly without contacting oropharyngeal surfaces	84±2	84±21
Disappeared between rakers/arches	8±6	15±21
Bounced off rakers/arches	5±2	1±0
Stuck on rakers/arches	3±6	0

Values are means ± s.d., *N*=3 individuals.

Discussion

Particle retention mechanisms in tilapia

Sanderson et al. (Sanderson et al., 1996) hypothesized that cichlid suspension feeders that retain bacteria and phytoplankton use hydrosol filtration with mucus entrapment, as does *O. niloticus*. A hydrosol filter can extract a wide range of particle sizes, including particles smaller than the pore size between the filter elements, because particles can be retained on sticky surfaces of a hydrosol filter. Since particles can be retained as water passes over instead of through the filter, a hydrosol filter is less prone to clogging than a sieve on which particles larger than the pores of the mesh are retained when filtrate exits through the pores. Perhaps the most notable advantage of using hydrosol filtration with mucus entrapment is that the particles are bound in mucus ready for transport to the esophagus (Sanderson et al., 1991; Sanderson et al., 1996).

Based on the similarities in diet and ecological niche of *O. niloticus* and *O. aureus*, we hypothesized that *O. aureus* uses hydrosol filtration with mucus entrapment. Although *O. aureus* with rakers intact had mucus present twice as often during feeding as *O. niloticus* (53% of the video frames analyzed *versus* 26%, respectively), the mucus did not appear to serve as a particle entrapment mechanism in *O. aureus*. Particles were seen trapped in mucus 97.9% of the time when mucus was present during feeding in *O. niloticus* (Sanderson et al., 1996), but only 15% of the time in *O. aureus*. The percent of particles trapped in mucus during feeding on Tetramin™ slurry was higher in *O. niloticus* (54%) compared to *O. aureus* (2%). Overall, brine shrimp cysts (210–300 μm diameter) and slurry particles (0.1–1.0 mm diameter) were retained much less frequently in *O. aureus* mucus than in *O. niloticus* mucus. Our data on *O. aureus* demonstrate that the presence of mucus strands, sheets and aggregates inside the oral cavity during suspension feeding is not necessarily indicative of the use of mucus to trap particles. The infrequent occurrence of particles retained in mucus in *O. aureus* compared to *O. niloticus* does not support the prediction (Sanderson et al., 1996) that cichlid suspension feeders that retain phytoplankton and cyanobacteria will use mucus entrapment.

As observed in *O. esculentus* (Goodrich et al., 2000), the majority of particles (98%) in *O. aureus* traveled posteriorly without contacting mucus or the arches. These results demonstrate that *O. aureus*, like *O. esculentus*, uses crossflow filtration as a particle retention mechanism (Sanderson et al., 2001). During crossflow filtration in pump suspension-feeding fish, water is pumped parallel to the rakers, transporting

particles towards the esophagus. As the oral cavity narrows posteriorly, particles remain suspended in the mainstream flow above the rakers and become more concentrated as filtrate exits between the rakers (Brainerd, 2001; Sanderson et al., 2001).

The filtration mechanisms of the three tilapia species that have been studied with a fiberoptic endoscope can be placed along a continuum from *O. niloticus*, with its combination of crossflow filtration and mucus entrapment (Sanderson et al., 1996), to *O. aureus*, with crossflow filtration in the presence of mucus, but not mucus entrapment, to *O. esculentus*, with crossflow filtration in the absence of mucus (Goodrich et al., 2000; Sanderson et al., 2001). Dead-end sieving by rakers and/or microbranchiospines, during which the fluid to be filtered passes perpendicularly through the pores between the rakers and/or the microbranchiospines while particles larger than the pores are retained on the sieve, is not used as a filtration method in any of these three species (Sanderson et al., 1996; Sanderson et al., 2001). While muscular control of rakers during feeding has been hypothesized to allow reduction in the diameter of the channels between rakers in common bream (Hoogenboezem et al., 1991; van den Berg et al., 1994), changes in channel diameter as a result of raker movement have not been observed to occur in endoscopic videotapes of tilapia species (Goodrich et al., 2000; Sanderson et al., 1996; Sanderson et al., 2001).

Correlation between diet and particle retention mechanism

Diet analysis of *O. niloticus* and *O. aureus* showed similarities in the prey species ingested in the field. However, there is some evidence from the literature suggesting that *O. niloticus* has a greater ability to retain small particles than does *O. aureus*, supporting the hypothesized link (Sanderson et al., 1996) between mucus entrapment and the retention of small food particles. Cyanobacteria such as *Anabaena* and *Microcystis* (cell dimensions as small as 2 μm×3 μm) are common elements in the diet of both species (Moriarty and Moriarty, 1973; Northcott et al., 1991; Spataru and Zorn, 1978). However, ingestion rates calculated for *O. aureus* feeding on *Anabaena* appear to be less than that of *O. niloticus*, although this could be due to starvation of *O. niloticus* prior to experimentation (Northcott et al., 1991). *O. aureus* lost mass when presented with *Chlamydomonas* (6 μm–15 μm), which suggests an inability to filter smaller particles efficiently (McDonald, 1987). Sanderson et al. (Sanderson et al., 1996) showed that *O. niloticus* relies more on mucus to retain small particles (Tetramin™ slurry particles, 0.1–1.0 mm in diameter) than larger particles (whole Tetramin™ flakes, 3–10 mm diameter).

Unlike *O. niloticus*, *O. esculentus* appears to be unable to retain 2-celled colonies of *Scenedesmus* (Batjakas et al., 1997). In addition, *O. niloticus* consumed significantly more 3- to 4-celled *Scenedesmus* colonies (c. 30 μm long×18 μm diameter) (Goodrich et al., 2000) than *O. esculentus* (Batjakas et al., 1997). Thus, the abilities of *O. niloticus*, *O. aureus* and *O. esculentus* to extract small particles differ, with *O. niloticus* able to retain the smallest particles.

The dietary data discussed above and data from endoscopic videotapes support the hypothesis that the entrapment of particles in mucus during hydrosol filtration in *O. niloticus* allows for the retention of smaller particles than does crossflow filtration in *O. esculentus* and *O. aureus*. Our study demonstrated that mucus that is visible in the oropharyngeal cavity during suspension feeding in *O. aureus* is not used to retain particles during hydrosol filtration. While the available data indicate a correlation between the smallest particle size in the diet and the particle retention mechanism used by each of these three species, we did not find a correlation between range of particle size in the diet and particle retention mechanism in these tilapia species. *O. niloticus* and *O. aureus* both have a more generalized diet with a greater range of food particle sizes than *O. esculentus*, but the particle retention mechanism in *O. niloticus* differs from that of *O. aureus* and *O. esculentus*.

Role of mucus

All mucus attached to the arches was observed for *O. niloticus* (Sanderson et al., 1996) and *O. aureus*, until either the mucus was lifted off the arches or the endoscopy sequence ended. Mucus remained attached during fewer pumps and reversals before lifting off the arches in *O. niloticus* than in *O. aureus*. During feeding in three *O. niloticus*, 60 mucus strands and aggregates remained attached to the arches during a total of only 21 pumps and six reversals before lifting off or sliding along the arches (Sanderson et al., 1996). However, during feeding in four *O. aureus*, ten mucus strands, aggregates, and/or sheets remained attached to the arches during 41 pumps and 22 reversals without lifting off or sliding along the arches (Table 2). Another distinction between the two species is that opaque sheets of mucus extending across the arches were not present in *O. niloticus* (Sanderson et al., 1996), but were the most common category of mucus observed in *O. aureus* (Table 1).

Thus, mucus is present more often during feeding in *O. aureus* than in *O. niloticus*, and the mucus remains attached to the arches during more pumps and reversals in *O. aureus* before being lifted and transported to the esophagus, but particles are being trapped in mucus less frequently in *O. aureus*. There are no indications that this difference in mucus function between the two species can be accounted for by differences in oral flow patterns or flow speed (J.C.S. and S.L.S., unpublished). A possible explanation that deserves study is that the mucus may have different properties in these two species. The glycoproteins present in fish mucus can either remain neutral or, in the presence of sialic acid or sulphated monosaccharides, become acidic. The full extent to which the glycoproteins influence the properties or contribute to specific functions of mucus is still controversial (Shephard, 1994). Based on the similar composition of fish and mammalian mucus, Northcott and Beveridge (Northcott and Beveridge, 1988) hypothesized that the viscosity of fish mucus may increase as acidic glycoprotein content increases.

A histological study of the gill rakers and branchial arches in *O. niloticus* revealed two morphologically distinct types of mucus cells (Northcott and Beveridge, 1988). The mucus cells located on the trailing keel of the rakers were large, clavate cells that produced an acidic mucosubstance. Northcott and Beveridge (Northcott and Beveridge, 1988) suggested that this mucus with charged acidic groups may have increased particle retention properties. Smaller goblet cells lined the anterior face

and side of the arches and secreted neutral or neutral/acidic mucus. This mucus may be less viscous and could aid in transport of captured particles towards the esophagus (Northcott and Beveridge, 1988). Differences in types of mucus produced are evident not only in different areas of the oropharyngeal cavity, but also among different species. From a histological study of the gills and epidermis of plaice, flounder and trout, Fletcher et al. suggested that the type of mucus produced by goblet cells in the arches and epidermis of fish could vary depending on the habitat of each species (Fletcher et al., 1976). In *Oreochromis mossambicus*, the proportions of mucosubstances present in the oral mucosa even varied seasonally. During mouthbrooding, the concentrations of glycogen, sialomucins and sulfomucins increased compared to non-brooding seasons (Varute and Jirge, 1971). Thus, the oropharyngeal mucus of *O. aureus* may differ in acidity and viscosity from that of *O. niloticus*, and consequently differ in function.

Since the mucus is not serving as the primary particle entrapment mechanism in *O. aureus*, are there potential functions for the abundant mucus that is present? Mucus can form unstirred layers over surfaces that are involved in ion or water transport (Shephard, 1994). An unstirred layer is a static region of fluid immediately adjacent to a membrane that does not mix even when the bulk solution is stirred. Thermal convection or density gradients do not cause significant mixing of the region of slow laminar flow over the static layer (Barry and Diamond, 1984).

Possible water- and ion-regulatory roles for mucus are based on the formation of these unstirred layers (Shephard, 1994). We propose that a potential function for mucus in crossflow filtration is to contribute to the formation of an unstirred layer and thereby enhance the use of the branchial arches as a surface that leads to the radial migration of particles. Lift is a hydrodynamic force that causes particles flowing in suspension inside tubes or channels to migrate radially towards the center of the tube at a tube Reynolds number (Re)>1. The oral cavity Re for *O. aureus*, calculated using the dorso-ventral height of the oral cavity and the mean peak flow speed during feeding pumps, was ~300. At Re>1, particles lift away from the tube walls and migrate radially as they travel downstream (Eloot et al., 2004; Matas et al., 2004). This radial migration is an important component of crossflow filtration because particles that remain suspended in the crossflow are not lost through the pores of the filter, nor do the suspended particles clog the pores. The formation of an unstirred layer directly over each arch and between the rakers could reduce the effective sizes of the pores between the rakers and between the arches of the branchial filter. Inertial lift increases as the square of the crossflow velocity (Chellam and Weisner, 1992). By helping to regulate the loss of water between the rakers and between the arches, mucus could increase the crossflow speed inside the oropharyngeal cavity and thereby increase inertial lift.

Whereas hydrosol filtration mechanisms are either independent of particle radius or dependent on particle radius to the first power (Rubenstein and Koehl, 1977; Shimeta and Jumars, 1991), inertial lift increases as the cube of the particle radius (Chellam and Weisner, 1992). Thus, crossflow filtration using inertial lift is predicted to exhibit greater dependence on particle size than is hydrosol filtration using mucus entrapment. The inability of *O. esculentus* and *O. aureus* to retain particles as small as those retained by *O. niloticus* (Batjakas et al., 1997) (J.C.S. and S.L.S., unpublished) is consistent with the reliance of these two species on inertial lift generated during crossflow filtration rather than the use of hydrosol filtration.

One notable difference in mucus transport between *O. niloticus* and *O. aureus* was the absence of mucus sliding across the arches in *O. aureus*. *O. niloticus* uses feeding pumps for sliding transport of mucus and retained particles towards the esophagus. Whereas mucus was observed sliding along the arch surfaces before being transported out of the field of view in 29% of 59 total mucus occurrences during feeding in *O. niloticus* (Sanderson et al., 1996), mucus was never observed sliding across the arches in *O. aureus*. The lack of sliding for mucus transport in *O. aureus* is consistent with mucus remaining attached to the arches for a longer duration before being lifted prior to transport posteriorly. The lack of sliding is also consistent with the use of mucus, particularly the frequent sheets and aggregates (Table 1), as a mechanism in *O. aureus* to restrict the inter-raker gap distance rather than as a hydrosol filtration mechanism.

Mucus and particle analyses before versus after gill raker removal

The large decrease in mucus presence after raker removal in *O. aureus* (53% of frames during feeding *versus* 2% of frames during feeding) can be explained in part by the location of tilapia mucus cells at the base of the rakers, primarily along the arch between the medial and lateral rows of rakers (Northcott and Beveridge, 1988). Surgical removal of gill rakers in *O. aureus* did not significantly affect the movement of particles inside the oropharyngeal cavity. Regardless of whether the rakers were intact or removed, 84% of particles traveled posteriorly without contacting any oropharyngeal surface (Table 3). In the absence of rakers, slightly more particles were observed disappearing between the arches (15%) than with rakers intact (8%), but this difference was not statistically significant (P=0.3, one-tailed t-test, d.f.=4).

Although we hypothesize that mucus on the arches serves to reduce the loss of water between the rakers and between the arches, thereby increasing crossflow speed and inertial lift, a decrease in inertial lift force in the absence of mucus would have to be dramatic to be detectable from particle movement through the endoscopic field of view. Our finding that crossflow filtration continued to operate in the absence of gill rakers and mucus indicates that the surfaces of the branchial arches themselves play an important role in crossflow filtration. Studies are in progress to determine the effects of gill raker removal on particle retention efficiency and particle size selectivity during crossflow filtration in *O. aureus*.

P. Heideman and G. Capelli and two anonymous reviewers provided valuable comments on the manuscript. We also thank K. Fitzsimmons (University of Arizona) for assistance in obtaining specimens, and S. Bottorff, R. Broughton, T. Callan, A. Herradura, T. Huber, M. Mirabella, and D. Suskin for data collection and analysis. The animal care procedures and experimental protocol were approved by the Institutional

Animal Care and Use Committee (IACUC-406). This research was supported by NSF grant IBN-0131293 and a grant from the Thomas F. Jeffress and Kate Miller Jeffress Memorial Trust to S.L.S.

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