

PARTICLE RETENTION IN SUSPENSION-FEEDING FISH

Kinematics, Oral Flow Speed, and Particle Retention during Tilapia Suspension Feeding
with Gill Rakers Intact vs. Removed

A Thesis

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Jennifer Claire Smith

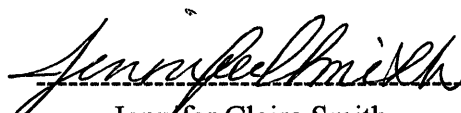
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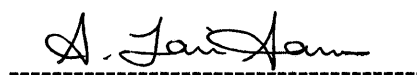
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Gregory Capelli

To Mike, for all of his love and support, and who without fail, remains my knight in shining armor.

TABLE OF CONTENTS

	Page
Acknowledgements	v
List of Tables	vi
List of Figures	vii
Abstract	viii
Chapter I. Kinematics and oral flow speed during fish suspension feeding with gill rakers removed vs. intact	2
Chapter II. Particle retention during suspension feeding in tilapia fish with gill rakers removed	32
References	61
Vita	67

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LIST OF TABLES

Table	Page
1. Mucus movement during feeding in four <i>O. aureus</i> with rakers intact	51
2. Peak flow speed and duration of pumps and post-pump reversals during feeding in <i>O. aureus</i>	52
3. Feeding pumps s ⁻¹ before and after gill raker removal as a measure of feeding enthusiasm	53
4. Particle retention efficiency	54

LIST OF FIGURES

Figure	Page
1. Classification of mucus shapes in <i>O. aureus</i> before gill raker removal	55
2. Representative flow speeds recorded during ventilation and suspension feeding in <i>O. aureus</i>	56
3. Size frequency distribution of particles retained during suspension feeding in <i>O. aureus</i> , before and after gill raker removal	57
4. Size frequency distribution of particles retained during suspension feeding in <i>O. esculentus</i> , before and after gill raker removal	58
5. Average cumulative size frequency distribution with gill rakers intact vs. gill rakers removed for <i>O. aureus</i>	59
6. Average cumulative size frequency distribution with gill rakers intact vs. gill rakers removed for <i>O. esculentus</i>	60

ABSTRACT

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 determine whether a third species of tilapia, *Oreochromis aureus*, uses crossflow filtration or hydrosol filtration with mucus to retain particles during suspension feeding with gill rakers intact as well as surgically removed. Although a large amount of mucus was present during feeding with gill rakers intact, particles were rarely retained in the mucus. The hypothesis that mucus is used for particle entrapment in this species is rejected. Mucus may function 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 mucus, probably due to the removal of mucus-secreting cells. Endoscopic analysis revealed a brief (80 msec) reversal of flow in the oropharyngeal cavity that occurred prior to every feeding pump.

Blue tilapia (*Oreochromis aureus*) and ngege tilapia (*Oreochromis esculentus*) selectively ingested microspheres larger than 50 μm during suspension feeding. Surgical removal of gill rakers and microbranchiospines did not affect feeding enthusiasm (measured in pumps s^{-1}), nor did removal affect the size selectivity of microspheres ingested by either species. The size frequency distributions of retained microspheres were similar with rakers intact and removed. These results establish that neither gill rakers nor mucus are necessary for the selective retention of particles $>50 \mu\text{m}$ during crossflow filtration in these species. Since inertial lift is proportional to the cube of the particle radius, this hydrodynamic force could play a role in particle size selectivity during crossflow filtration. Since raker removal causes a lack of mucus in the oropharyngeal cavity, There was a marked trend towards decreased particle retention efficiency after gill raker removal for both species, which could be related to a reduction in crossflow speed, a lack of mucus, and decreased vortex formation in the oropharyngeal cavity. Both *O. aureus* and *O. esculentus*, had substantial inter-individual variability in particle retention efficiency.

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CHAPTER 1
KINEMATICS AND ORAL FLOW SPEED DURING FISH SUSPENSION
FEEDING WITH GILL RAKERS REMOVED VS. INTACT

Introduction

Suspension-feeding fish are capable of filtering food particles as small as 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 (FAO, 2000). Despite the ecological and economic importance of suspension-feeding fish, food particle retention mechanisms are known for only seven species (Sanderson et al., 2001; Callan and Sanderson, 2003; Hoogenboezem et al., 1991).

Endoscopic analysis of the Nile tilapia (*Oreochromis niloticus* Linnaeus, Cichlidae) described hydrosol filtration with mucus entrapment of particles on the branchial arches as one mechanism of particle retention (Sanderson et al., 1996). A second species of tilapia, *O. esculentus* (Graham), uses crossflow filtration instead of mucus to retain particles during suspension feeding (Goodrich et al., 2000; 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 larvæ, 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*. 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.

Oreochromis aureus is found commonly throughout Africa and Israel and became established in the United States after being introduced originally in the 1960s as a biological control agent. Similar to *O. niloticus*, the diet of *O. aureus* consists of phytoplankton and organic detritus with smaller amounts of zooplankton, benthic invertebrates, and macrophytes (Spataru and Zorn, 1978; Mallin, 1985; Drenner et al., 1984). 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 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). However, surgical removal of all gill 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. (1996) suggested that Drenner et al.'s results could be explained if mucus on the gill arches functions in hydrosol filtration after the gill rakers have been removed.

The effects of gill raker removal on mucus presence and particle movement inside the oral cavity have not been studied in any suspension-feeding fish. We removed the gill rakers and microbranchiospines from all branchial arches of *O. aureus* specimens. We used a fiberoptic endoscope to compare the intra-oral movements of particles in the presence vs. the absence of gill rakers and microbranchiospines.

Quantification of fluid dynamics at the level of the gill rakers can aid in determining the type of particle encounter mechanism used as well as the efficiency and rate of suspension feeding (Shimeta and Jumars, 1991). The dynamics of flow during feeding have been described for only one pump suspension-feeding fish species, *Orthodon microlepidotus* (blackfish, Cyprinidae) (Sanderson et al., 1991). Although *O. aureus* is also a pump suspension-feeding fish, the shape of the oral cavity and the gill raker morphology are substantially different from those of *Orthodon microlepidotus*. For these reasons, we inserted a microthermistor flow probe into the oropharyngeal cavity of *O. aureus* before and after removal of the gill rakers, to measure the speed of flow during feeding.

Materials and methods

ENDOSCOPY EXPERIMENTS

O. aureus 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 in Sanderson et al. (1996). Five specimens (20.3-23.4 cm

standard length) 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) 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 x 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 hours after cannula implantation. A flexible fiberoptic endoscope (Olympus ultrathin fiberoptic type 14, 1.4 mm o.d., 1.2 m working length, 75° field of view, 0.2-0.5 cm depth of field) was threaded through the cannula. The endoscope was attached to a Kodak Intensified Imager VSG (50-500 Hz). A Kodak Ektapro Hi-Spec Motion Analyzer 1012/2 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 (Olympus Helioid ALS-6250, 250 W) provided light for the endoscope. A Sony DSR-11 DVCAM video recorder with a jog shuttle (remote control unit DSRM-20) was used for frame-by-frame analysis of the videotapes.

Data were recorded as fish were fed a slurry of finely crushed Tetramin flakes (0.1-1.0 mm diameter) mixed with water. Brine shrimp cysts (*Artemia* spp., 210-300 μ m) 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 syringe 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 fully healed.

GILL RAKER REMOVAL

Method of raker removal was modified from that of Drenner et al. (1987). *O. aureus* were anesthetized with MS-222 and all lateral and medial gill rakers and microbranchiospines were removed with microforceps from the anterior four gill arches on both sides of 5 fish. The fifth gill 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 in the surgery tray to remove a section of gill rakers and microbranchiospines, and then returned to the water in the surgery tray. The fish was then returned to its aquarium and Fungus Eliminator (Jungle Laboratories Corporation) was added to prevent infection. Fish were not adversely affected by the surgery and exhibited normal feeding behavior within 2 days. During the fifteen days following surgery, the gill arches healed and partial regeneration of rakers occurred, as described by Drenner et al. (1987) for *Sarotherodon galilaeus*. The regenerating rakers resulted in minute gill raker rudiments that were positioned randomly

on the gill arches (Drenner et al., 1987). Endoscopy and flow probe experiments were conducted on fish with rakers intact and again on the same individuals fifteen days after gill 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 gill 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 frames containing mucus were then analyzed to categorize: (1) the number of 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 branchial arches and gill rakers visibly lifted and exited from the field of view, (c) sliding along arches – mucus maintained contact with the arches and/or gill rakers while traveling posteriorly, (d) attached – mucus maintained contact with the arches and/or gill rakers and did not change location; and (3) the action of the fish as mucus that had been attached to the arches and gill rakers lifted and exited from the field of view: (a) pumps, (b) reversals, or (c) ventilation.

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. 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 gill raker before continuing posteriorly, (3) disappeared – particle traveled towards the branchial arches and disappeared either between two gill rakers or between two of the branchial arches; (4) stuck – particle stayed immobile on the arches or gill 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 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.

ORAL FLOW SPEED

Between one to 24 hours following the endoscopy experiments, a flow probe was used to measure oral flow speed in three *O. aureus*. The procedure was similar to that used for paddlefish in Sanderson et al. (1994). The flow probe was constructed from insulated wire (75 μm diameter, California Fine Wire Co., COA-101, H-ML), soldered to

the leads from a glass bead thermistor (1.09 mm diameter, Fenwal part no. 112-101BAJ-B01). The probe was temperature-compensated from 19.5 to 29.7° C and a calibrated speed controller was used to calibrate the probe from 0 to 185 cm s⁻¹ in a flume. The circuit, modified from LaBarbera and Vogel (1976), was connected to an A/D converter (Sonometrics TRX-4) with a sampling rate of 200 Hz.

For the experiments, we threaded the flow probe through the cannula so that the glass bead was fully projecting into the oropharyngeal cavity (a distance of about 1.5 mm). A sudden increase in flow speed marked the correct insertion point. This was observed through use of Sonometrics software on a Pentium computer to monitor the flow speed in real time. At the conclusion of the experiments, the cannula was removed under anesthesia and the implantation site subsequently healed fully.

Flow probe signals were recorded during ventilation and suspension feeding on a slurry of Tetramin flakes and water. External videotapes were synchronized with the data from the flow probe using a TTL-compatible trigger signal connected to a Kodak Ektapro Hi-Spec Motion Analyzer 1012/2 and the Sonometrics A/D converter. From these videotapes we were able to identify periods of time during which the fish was feeding. These segments of flow probe data were analyzed for two actions: feeding pumps and post-pump reversals.

Results

ENDOSCOPIC VIEW

From the insertion site in the preoperculum, the endoscope entered the oropharyngeal cavity directly lateral to the left tissue pad located on the oral roof of the

pharynx. This position was approximately 65% of the distance from the front of the oral jaws to the esophagus. The left 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 gill raker removal, the gill rakers were visible as projections from the branchial arches.

FEEDING PUMPS

O. aureus suspension-fed on the Tetramin slurry using a series of pumps (pump suspension feeding, see Lazzaro, 1987). During a pump, water entered the mouth and continued to flow posteriorly through the oropharyngeal cavity until exiting via the operculum. The duration of anterior to posterior flow during a pump was 31 ± 17 frames (mean \pm S.D., $N=3$ individuals, 10 pumps per individual, at a recording rate of 125 Hz).

External video corresponded to the internal oral movements. The mandible abducted, the premaxillae protruded and the hyoid abducted during a pump. Viewed simultaneously through the endoscope, the distance between the ceratobranchials and the oral roof increased, signifying abduction of the branchial arches. Following opercular abduction, the mandible, premaxillae, hyoid and finally the opercula were adducted. Completion of this adduction was concurrent with the return of the branchial arches to their original position.

PRE-PUMP REVERSALS

During all feeding pumps before and after gill raker removal, flow was anterior to posterior except for a brief posterior to anterior flow that occurred at the beginning of

every pump. This we termed a “pre-pump” reversal. From endoscopy footage of each of three fish prior to gill raker removal, 10 pre-pump reversals were analyzed during feeding. The duration of the pre-pump reversal, measured from the time a particle began to travel in a posterior to anterior direction until the same particle was seen to resume anterior to posterior flow, was 10 ± 3 frames (mean \pm S.D., $N=3$ individuals, at a recording rate of 125 Hz).

Pre-pump reversals began most frequently ($43.3\% \pm 0.1\%$, mean \pm S.D., $N=3$ individuals) at the same time the mouth began to open (signaled by mandibular abduction) during a pump. However, $33.3\% \pm 0.3\%$ of the pre-pump reversals began a mean of 4 ± 2 frames before the mouth began to open, and $20.0\% \pm 0.2\%$ of the pre-pump reversals began 4 ± 5 frames after the mouth began to open. Rarely, the external view of the mouth was obscured due to slurry particles in the water column ($3.3\% \pm 0.1\%$), making the correlation of the endoscopic view and the external view impossible.

POST-PUMP REVERSALS

During feeding before and after gill raker removal, 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). We refer to this as a post-pump reversal because this reversal occurred immediately after a pump, or immediately after another reversal, and is therefore distinct from the pre-pump reversals described above. Stage 1 was characterized in the external videotapes by closed oral jaws, protruded premaxillae, hyoid

abduction, and opercular adduction. Through the endoscope, this flow reversal was accompanied by a marked abduction of the branchial arches. After this the premaxillae retracted, the hyoid adducted, and the opercula abducted, signifying stage 2 of a reversal (Sanderson et al., 1996). During stage 2, the particles were viewed resuming an anterior to posterior flow inside the oropharyngeal cavity.

A typical bout of suspension feeding involved 2 to 5 sequential pumps at a rate of 1-2 pumps per second, followed by a single post-pump reversal. At the onset of feeding or when food concentration was increased, the rate of suspension feeding increased, with a pump being directly followed by a post-pump reversal and then another pump. This pattern repeated until the fish was satiated or until food concentration decreased again.

Using synchronous internal endoscopy and external video, analysis of 7 post-pump reversals was completed for each of three individuals prior to gill raker removal. Slurry particles or brine shrimp cysts were followed through the endoscopic field of view for the duration of each post-pump reversal, and the numbers of frames the particles traveled from posterior to anterior (stage 1), and from anterior to posterior (stage 2), were calculated. The mean duration of stage 1 of post-pump reversals was shorter than the mean duration of stage 2 (15 ± 3 frames vs. 51 ± 28 frames respectively, mean \pm S.D., 125 Hz).

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 gill raker removal. During feeding, mucus was present in

53%±37% (mean±S.D., $N=5$ individuals) of the video frames analyzed, compared to 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. In *O. aureus*, mucus appeared as opaque sheets most frequently, and could often be seen to extend across the entire endoscopic field of view. Overall during feeding as well as ventilation, the most common mucus occurrence was that of a single sheet or an aggregate (Fig 1).

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 attached mucus lifted from the arches and passed posteriorly during the recorded sequence (28%±26% of frames analyzed during feeding, 0% of frames analyzed during ventilation). 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 immobile for a long period of time before exiting from the field of view. To quantify the duration of mucus presence, ten mucus strands and aggregates were observed until they exited from the field of view or until the endoscopic sequence ended. Mucus remained attached for a large number of pumps and post-pump reversals before the mucus lifted from the arches or the endoscopy sequence ended (Table 1).

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 5 fish). The exit of previously attached mucus from the field of view in association with a pump was less common (35% of total occurrences for 5 fish). This occurred when the brief pre-pump reversal dislodged and lifted the mucus, and the subsequent pump carried the mucus posteriorly. Attached mucus was never dislodged and carried posteriorly during ventilation.

PARTICLE ANALYSIS

For each of three *O. aureus* prior to raker removal, 100 brine shrimp cysts or slurry particles were analyzed as they passed posteriorly through the oropharyngeal cavity. Most frequently the particles traveled posteriorly in a straight path without contacting any oropharyngeal surface ($84\% \pm 2\%$, mean \pm S.D., $N=3$ individuals). Some particles disappeared into the spaces between rakers or passed between two arches ($8\% \pm 6\%$). A small percentage of particles bounced off the rakers or arches before continuing posteriorly ($5\% \pm 2\%$), and very few particles adhered to mucus on the rakers or arches ($3\% \pm 6\%$).

For five *O. aureus* combined, Tetramin flake particles or brine shrimp cysts were seen trapped in mucus during 15% of the 12744 frames (125 Hz) with mucus present that were analyzed during feeding. To ascertain the effectiveness of mucus in particle retention, a typical feeding bout 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 bouts, 98% traveled independently without contacting 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 pumps or the frequency of reversals during suspension feeding in the absence of rakers. Just as when the gill rakers were intact, no food particles were visible exiting via the operculum after the gill rakers had been removed.

Frame-by-frame analysis of post-raker removal endoscopy video from three specimens included all unobstructed, clearly focused views (52063 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 ($2\% \pm 2\%$) was greatly reduced compared to endoscopy with intact gill rakers. During the limited number of suspension feeding frames with mucus after removal of gill 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 post-pump 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 gill raker removal. The majority of the particles ($84\pm 21\%$) traveled posteriorly in a straight path without touching any oropharyngeal surface. Many particles were visible through the endoscope while traveling straight towards the brightly lit arches, and then disappeared into the dark void between two arches ($15\pm 21\%$). Only 1% of the particles bounced off the arches before traveling posteriorly towards the esophagus. No particles adhered to mucus on the rakers or arches.

ORAL FLOW SPEED

During the experiments, the fish maintained a steady position in the water column during ventilation and pump suspension feeding. The fish exhibited a general pattern of feeding pumps and post-pump reversals consistent with typical feeding behavior. Recordings of flow speed began during ventilation. At the onset of feeding, a repeating pattern of a single pump followed by a post-pump reversal began. Each of these three actions had a distinctive flow pattern (Fig 2). Pre-pump reversals were not identifiable in the flow traces.

For each of three fish, the peak values of 15 pumps and 15 post-pump reversals were analyzed for all sequences recorded before and after gill raker removal to determine mean peak flow speed. A typical sequence began with ventilation and continued for approximately 100 sec of suspension feeding. The duration of a feeding pump and the

duration of a post-pump reversal were similar before vs. after raker removal (Table 2). The mean peak speed of the reversals was almost twice as high as that of the pumps, regardless of whether the rakers had been removed. However, the mean peak flow speeds for pumps and post-pump reversals recorded with rakers removed were significantly less (paired t-test, $t=6.24$, $p=0.02$) than those recorded with rakers intact (Table 2).

Discussion

CORRELATION BETWEEN DIET AND PARTICLE RETENTION MECHANISM

Sanderson et al. (1996) hypothesized that cichlid suspension feeders such as *O. niloticus* that retain bacteria and phytoplankton use mucus entrapment for hydrosol filtration, rather than sieving. A hydrosol filter can extract a wide range of particle sizes, including particles smaller than the pore size of the filter, and can incur lower drag than a dead-end sieve. Because particles can be retained as water passes over instead of through the filter, a hydrosol filter is less prone to clogging than a sieve. Perhaps the most notable advantage of using mucus entrapment for hydrosol filtration is that the particles are bound in mucus ready for transport to the esophagus (Sanderson et al., 1991; Sanderson et al., 1996).

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 entrapped 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 reduced from 54% in *O. niloticus* to only 2% in *O. aureus*. Overall, brine shrimp cysts (210-300 μ m 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 hydrosol filtration by mucus entrapment.

The infrequent occurrence of mucus entrapment in *O. aureus* compared to *O. niloticus* does not support the hypothesized importance (Sanderson et al., 1996) of mucus entrapment in cichlid suspension feeders that retain phytoplankton and cyanobacteria. 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. *O. niloticus* are known to retain 2-celled colonial algae, which has been hypothesized to be due to their dependence on mucus to feed (Batjakas et al., 1997; Sanderson et al., 1996). *O. aureus* can easily filter algae as large as *Ceratium* (180x50 μ m) or *Uroglenopsis* (500 μ m, Drenner et al., 1984). Cyanobacteria such as *Anabaena* and *Microcystis* (cell dimensions as small as 2x3 μ m) are common elements in the diet of both species (Moriarty and Moriarty, 1973; Spataru and Zorn, 1978; Northcott et al., 1991). 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 weight when

presented with the smaller *Chlamydomonas* (6-15 μm), which suggests an inability to filter smaller particles efficiently (McDonald, 1987). 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).

All mucus strands attached to the branchial arches were 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 post-pump reversals before lifting off the branchial 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 only 21 pumps and 6 post-pump reversals before lifting off or sliding along the arches (Sanderson et al., 1996). However, during feeding in four *O. aureus*, ten mucus strands and aggregates remained attached to the arches during 41 pumps and 22 post-pump reversals without lifting off or sliding along the arches (Table 1).

Thus, *O. aureus* has a higher abundance of mucus during feeding than *O. niloticus*, and the mucus remains attached to the branchial arches longer in *O. aureus* before being lifted and transported to the esophagus, but particles are being trapped in mucus less frequently in *O. aureus*. 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 (Shepherd, 1994). Because of the similar composition of fish and mammalian mucus, Northcott and Beveridge (1988) hypothesized that the viscosity of fish mucus may increase as acidic

glycoprotein content increases, as documented in mammals (Jones et al., 1973; Iravani and Melville, 1974; Solanki and Benjamin, 1982).

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 gill rakers were large, clavate cells that produced an acidic mucosubstance. 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 gill 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. (1976) suggested that the type of mucus produced by goblet cells in the gill arches and epidermis of fish could vary depending on the habitat of each species. 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.

Similar to what was observed in *O. esculentus* which lack mucus (Goodrich et al., 2000), the majority of particles (98%) in *O. aureus* traveled posteriorly without contacting mucus or the branchial arches. These results indicate 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, a water is pumped parallel to the gill 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 (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* (combination of crossflow filtration and mucus entrapment, Sanderson et al., 1996) to *O. aureus* (crossflow filtration in the presence of mucus, but not mucus entrapment), to *O. esculentus* (crossflow filtration in the absence of mucus, Sanderson et al., 2001; Goodrich et al., 2000). Dead-end sieving by gill rakers and/or microbranchiospines is not used as a filtration method in any of these three species (Sanderson et al., 1996; Sanderson et al., 2001).

The available data suggest that these species' abilities to extract small particles also differ, with *O. niloticus* able to retain the smallest particles (Batjakas et al., 1997; Smith and Sanderson, in prep). *O. esculentus* is unable to retain 2-celled colonies of *Scenedesmus*, and does not retain 3- to 4-celled colonies (c. 30 μ m long x 18 μ m diameter, Goodrich et al. 2000) as well as *O. niloticus* (Batjakas et al., 1997). *O. esculentus* and *O. aureus* retain particles in the same size range (20-250 μ m, Smith and Sanderson, in prep).

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 this mucus in crossflow filtration is to enhance the use of the branchial arches as a surface that results in inertial lift. Also referred to as the “tubular pinch effect”, inertial lift is a hydrodynamic force that causes particles that are flowing in suspension inside tubes or channels (channel $Re > 1$) to migrate radially towards the center of the tube. Any particles near the tube walls lift away from the walls and migrate radially as they travel downstream (Brandt and Bugliarello, 1966; Segré and Silberberg, 1962). Inertial lift increases as the square of the crossflow velocity (Chellam and Weisner, 1992). Inertial lift 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 could reduce the effective sizes of the pores between the rakers and between the arches of the branchial filter. 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.

PRE-PUMP REVERSALS

The kinematic events that generate a pre-pump reversal are under investigation (Callan and Sanderson, in prep). We hypothesize that the pre-pump reversal is a result of the suction formed as the hyoid begins to abduct at the onset of a pump. This suction appears to draw water anteriorly from the posterior oropharyngeal cavity prior to the anterior to posterior flow that is established when the oral jaws open. Viewed through the endoscope, the pre-pump reversal began as the branchial arches started to abduct. This is consistent with synchronous endoscopy and external video showing a posterior to anterior flow slightly before or simultaneous with the first frame in which mandibular abduction was observed in 76% of the pumps examined. A pre-pump reversal occurred approximately 32 msec after the oral jaws began to open in 20% of the pumps.

Our report of a pre-pump reversal contradicts Callan and Sanderson (2003), who recorded from a fiberoptic endoscope at 125-500 Hz and concluded that a brief post-pump reversal occurred after 96% of the pumps during feeding in carp (*Cyprinus carpio*). However, with our synchronized internal and external video, we can state conclusively that the brief reversal in *O. aureus* always occurs pre-pump rather than post-pump. In *O. aureus*, brief pre-pump reversals were visible through the endoscope at the beginning of every pump regardless of whether the pump followed another pump or followed stage 2 of a post-pump reversal. Brief post-pump reversals were never observed in *O. aureus*, even between a pump and stage 1 of a reversal. Because carp lack stage 1 and stage 2 reversals, observations such as these could not be used by Callan and Sanderson (2003) to determine whether the brief reversal in carp occurred before or after each pump.

Pre-pump reversals were not evident in endoscopy video (30 Hz) recorded previously during suspension feeding in *O. niloticus* and *O. esculentus* (Sanderson et al., 1996; Goodrich et al., 2000). The duration of an individual frame at a recording speed of 30 Hz is approximately 30 msec. In the previous studies on *O. niloticus* and *O. esculentus*, lack of a high-speed intensified imager would have prevented detection of the brief pre-pump reversal, which had an average duration of 80 msec in *O. aureus*. Endoscopy of suspension feeding in *O. aureus* was recorded at a much higher number of frames per second (125 Hz) and was thus able to record the pre-pump reversal for the first time during feeding in tilapia.

ROLE OF FEEDING KINEMATICS

The general direction of water flow and particle movement during each feeding action can be described as follows: (1) pre-pump reversal – posterior to anterior flow, (2) feeding pump – anterior to posterior flow, (3) stage 1 of a post-pump reversal – posterior to anterior flow, and (4) stage 2 of a post-pump reversal – anterior to posterior flow. The external kinematics and endoscopic particle movements observed in *O. aureus* during stage 1 and stage 2 of reversals following a feeding pump or following another post-pump reversal were similar to those observed in *O. niloticus* and *O. esculentus* (Sanderson et al., 1996; Goodrich et al., 2000). We have termed these “post-pump” reversals to distinguish them from the pre-pump reversals that were recorded in *O. aureus*. The duration of a feeding pump was the same for both *O. niloticus* and *O. aureus* (0.37 s for both species). Stage 1 of a post-pump reversal was also similar in *O.*

niloticus (approximately 0.10 s, Sanderson et al., 1996) and *O. aureus* (0.12 s), as determined by endoscopy.

Sanderson et al. (1996) hypothesized that during feeding in *O. niloticus*, stage 1 of a post-pump reversal was responsible for lifting the mucus off the arches in preparation for transport to the esophagus during stage 2 of a post-pump reversal. Post-pump reversals were the most common action during which mucus was lifted from the arches (stage 1) and carried posteriorly (stage 2) in both *O. niloticus* and *O. aureus*. In 65% of 23 total mucus occurrences during feeding in *O. aureus*, and 56% of 59 total occurrences of mucus in *O. niloticus* (Sanderson et al., 1996), mucus that had previously been attached to the arches was lifted from the arches during stage 1 of a post-pump reversal and left the field of view during stage 2 of a post-pump reversal.

In *O. aureus*, post-pump reversals and pre-pump reversals had a similar function. A pre-pump reversal was visible at the beginning of every pump during feeding and ventilation in *O. aureus*. During feeding, the pre-pump reversal lifted attached mucus off the arches while the following pump transported the mucus posteriorly, although mucus was not lifted during a pre-pump reversal as often as during a post-pump reversal (35% vs. 65% of 23 total occurrences of mucus in *O. aureus*).

An ongoing goal of industrial crossflow filtration engineering is to minimize the concentration of particles that are near the filter surface or that have deposited onto the filter. One solution is the use of oscillating or pulsating crossflow to create a pattern of small flow reversals, or accelerating and decelerating crossflow, which reduces particle deposition on the filter and thereby increases filtration efficiency (Winzeler and Belfort, 1993; Stairmand and Bellhouse, 1985). The pre-pump and post-pump reversals that lift

mucus from the arches during feeding in *O. aureus* are comparable to the oscillatory flow that serves to lift particles from the filter surface in industry. Like flow reversals in *O. aureus*, pulsations or oscillations can increase performance by increasing the back-migration of particles from the filter surface to the bulk flow region (Winzeler and Belfort, 1993). Stairmand and Bellhouse (1985) also found that the oscillatory flow created when pulsations were applied to turbulent flow significantly increased mass transfer flux.

One notable difference in mucus transport between *O. niloticus* and *O. aureus* was the absence of mucus sliding across the arches in *O. aureus*. 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*, mucus was never observed sliding across the arches in *O. aureus*. This suggests that although feeding pumps were less important as a lifting mechanism in *O. niloticus* than in *O. aureus*, *O. niloticus* may use feeding pumps as a sliding mechanism to transport mucus out of the field of view. The lack of sliding as a transport mechanism in *O. aureus* is consistent with mucus remaining attached to the arches for a longer duration before being lifted prior to transport posteriorly.

MUCUS AND PARTICLE ANALYSES BEFORE VS. AFTER GILL RAKER REMOVAL

The large decrease in mucus presence after gill raker removal in *O. aureus* (53% of frames during feeding vs. 2% of frames during feeding) can be explained in part by the location of mucosal cells. Tilapia mucus cells are located at the base of the gill rakers,

primarily along the arch between the medial and lateral rows of gill rakers (Northcott and Beveridge, 1988). Removal of this tissue attached to the gill rakers could account for the large decrease in mucus production.

Analysis of visible mucus and particle movement after gill raker removal resulted in similar trends as were seen with gill rakers intact. The majority of mucus visible after gill raker removal was in the form of mucus attached to the arches that was then lifted and passed posteriorly (51%), somewhat higher than the percentage of lifted mucus that passed posteriorly with rakers intact (28%). Less frequently, mucus passed posteriorly through the field of view without contacting any oropharyngeal surface in both cases (49% with gill rakers removed, 15% with gill rakers intact).

Particle movement inside the oropharyngeal cavity was also similar in fish with gill rakers intact vs. removed. In both cases, 84% of particles traveled posteriorly without contacting any oropharyngeal surface. In the absence of gill rakers, more particles were observed disappearing between the arches (15%) than with gill rakers intact (8%). Particles were rarely seen bouncing off the arches before traveling posteriorly in the absence of gill rakers (1%). This was less than was observed with gill rakers intact (5%). Several factors determine whether a particle will impact a surface, including particle size, size of the filtering element, and flow speed through the filter (Rubenstein and Koehl, 1977). Upon removal of the gill rakers, there was a marked decrease in the flow speed through the oropharyngeal cavity, which could lead to less inertial impaction and greater loss of particles through the filtering elements. This could explain the decrease in the number of particles that bounced off the arches prior to traveling posteriorly. After gill raker removal, particles were never observed stuck in

mucus, compared to 3% of the particles with gill rakers intact. This result is consistent with the large decrease in mucus presence after raker removal, as well as the probable reduction in inertial impaction. After gill raker removal, particles tended to disappear more frequently between the arches rather than bounce or be retained in mucus on the arches. This is consistent with calculations indicating reduced particle retention efficiency after gill raker removal in *O. aureus* (Smith and Sanderson, in prep).

ORAL FLOW

The mean peak flow speeds of both pumps and post-pump reversals in *O. aureus* were much lower after raker removal than with gill rakers intact. Removal of gill rakers may reduce resistance to water flow between the arches, resulting in a greater volume of water exiting from the oropharyngeal cavity between the anterior arches during pumps. We hypothesize that in the absence of gill rakers a larger volume of flow passing between the arches results in less crossflow parallel to the arches. The reduced speed of the crossflow would be expected to reduce the inertial lift force within the oropharyngeal cavity. This could explain in part the reduction of feeding efficiency observed in *O. aureus* after gill raker removal (Smith and Sanderson, in prep).

The consistent decrease in post-pump reversal flow speed after raker removal may be the result of flow reversing from the opercular cavity into the oropharyngeal cavity between the arches when negative pressure in the oropharyngeal cavity creates posterior to anterior flow within the oropharynx. This would reduce the speed of anteriorly directed flow passing the flow probe during a reversal in the absence of gill rakers.

The mean peak flow speed recorded during feeding in *O. aureus* was much lower than that recorded in other suspension-feeding fish. The pump suspension-feeding Sacramento blackfish (*Orthodon microlepidotus*) was observed to have a mean peak flow speed of 54-62 cm s⁻¹ near the ceratobranchial of gill arch I during pumps (Sanderson et al., 1991). This is eight times faster than the mean peak speed of *O. aureus* feeding pumps (rakers intact, 6.7±2.8 cm s⁻¹) and more than four times faster than the mean peak speed during a post-pump reversal in *O. aureus* (rakers intact, 11.2±3.2 cm s⁻¹). Although both of these are pump suspension-feeding fish, there are differences between the two species that may explain this discrepancy in flow speed.

Blackfish have a slit-like oropharyngeal cavity (Sanderson et al., 1998) and do not exhibit post-pump reversals during feeding. Thus, the uninterrupted repetition of pumps produces a fast flow of water that carries particles along the channel between the lateral and medial rows of rakers on each arch to the oral roof, where the particles are trapped in mucus (Sanderson et al., 1991). The close proximity of the arches to the oral roof is evident when the mouth is closed and the arches fit into grooves along the lateral palatal organ. Sanderson et al. (1998) hypothesized that the tufted, closely spaced gill rakers in blackfish direct particles along the arches to the oral roof, resulting in transfer of particles from the arches onto the lateral sides of the mucus-covered palatal organ.

O. aureus does not exhibit the grooved palatal organ characteristic of blackfish (Sanderson et al., 1998). Unlike blackfish, *O. aureus* does not force particle-laden water at high speed along grooves in the oral roof. *O. aureus* are mouthbrooders and, compared to blackfish, have an oropharyngeal cavity with a greater dorso-ventral height and a larger volume in which they hold their young. Mouthbrooders such as *O. aureus* use

vigorous oral movements (termed “churning”) distinct from normal ventilation to aerate and re-suspend the non-adhesive eggs inside the oropharyngeal cavity (Keenleyside, 1991). The external kinematics of these churning movements resemble those of the post-pump reversals that alternate with feeding pumps in *O. niloticus* (Sanderson et al., 1996) and *O. aureus*. These post-pump reversals could compensate for the low flow speed in *O. aureus* compared to blackfish by enhancing back-migration of particles into the crossflow and thereby increasing the retention of particles (Winzeler and Belfort, 1993). This could represent a functional trade-off that exchanges a slit-like oropharyngeal cavity and repetitive pumping with a high flow speed during feeding in blackfish, for an oropharyngeal cavity with a large volume and slower flow speed with reversals for the dual functions of mouthbrooding and feeding in *Oreochromis*. Further study is needed to determine whether the “churning” that occurs during mouthbrooding is identical in kinematics and function to the post-pump reversals that occur during suspension feeding in *Oreochromis* species.

Data recorded from hyomandibular and neurocranial flow probe insertion sites of a ram suspension-feeding paddlefish (*Polyodon spathula*) resulted in a mean peak oral flow speed of $19.3 \pm 1.7 \text{ s}^{-1}$ during feeding (Sanderson et al., 1994). Compared to measurements taken directly anterior to the gill rakers of pump suspension-feeding blackfish ($54\text{-}62 \text{ cm s}^{-1}$; Sanderson et al., 1991), and anterior to the gill arches in bream, *Abramis brama* ($115 \pm 63 \text{ cm s}^{-1}$; Hoogenboezem et al., 1991), this suggested that ram suspension feeders may operate at a much lower flow speed than pump suspension feeders (Sanderson et al., 1994). Our data on *O. aureus* do not disprove this hypothesis but suggest that oropharyngeal cavity morphology and the associated fluid dynamics may

be more important in determining intraoral flow speed than method of suspension feeding (ram versus pump suspension feeding).

The absence of a visible pre-pump reversal in the majority of flow speed recordings is of interest. The short duration of the pre-pump flow reversal in a posterior to anterior direction (0.08 sec) in comparison with the duration of the anterior to posterior flows that precede and follow the pre-pump reversal (stage 2 of a post-pump reversal, 0.4 ± 0.2 sec; feeding pump 0.25 ± 0.14 sec) could account for the lack of pre-pump reversals in the flow speed recordings. The turbulence created as anterior to posterior flow decelerates during a pump or during stage 2 of a post-pump reversal, and as this flow is followed immediately by posterior to anterior flow of brief duration during a pre-pump reversal, could mask the transition between the two events on the flow recording. During ventilation however, there is a visible pre-pump reversal, perhaps due to the slower flow speed and longer duration of ventilation pumps compared to feeding pumps. The lower flow speed of ventilation does not generate the turbulence that is visible through the endoscope during transitions between post-pump reversals and feeding pumps, and allows the brief pre-pump reversal to be detectable in the flow recording.

CHAPTER 2

PARTICLE RETENTION DURING SUSPENSION FEEDING IN TILAPIA FISH WITH GILL RAKERS REMOVED

Introduction

Suspension-feeding fish that filter food particles as small as 5-3000 μm can play an important role in structuring phytoplankton and zooplankton communities (Drenner et al. 1984a; Drenner et al. 1987; McDonald 1987). The dietary breadth of a suspension-feeding fish and the size range of particles ingested should be dependent on the particle retention mechanism employed by the fish (Brainerd 2001; Sanderson et al. 1996). Although the potential predictive power of this theory is high, tests of the theory have been hampered by a lack of knowledge on particle retention mechanisms. Of the more than 70 species of fish that have been reported to suspension feed, particle retention mechanisms are known for only seven (Cheer et al. 2001; Sanderson et al. 2001; Callan and Sanderson 2003; Hoogenboezem et al. 1991).

Functions of gill rakers and the contributions of gill rakers to the particle retention mechanism employed by suspension-feeding fish have rarely been quantified experimentally. If the rakers function as a dead-end sieve, then particles that are too large to pass through the pores of the sieve will be retained on the filter surface when water exits perpendicular to the sieve. In contrast, during hydrosol filtration, a number of fluid mechanical processes can result in contact between particles and a filter that has adhesive properties (Shimeta and Jumars 1991; LaBarbera 1984; Rubenstein and Kohl

1977). Particles that are small enough to pass between the filter elements may then be retained by adhesion to the sticky (e.g. mucus-covered) surface of the filter.

Crossflow filtration is another mechanism that may result in the retention of particles that are small enough to pass between the filter elements. During crossflow filtration in pump suspension-feeding fish, a high-velocity crossflow is pumped parallel to the gill rakers, transporting particles towards the esophagus. As the oropharyngeal cavity narrows posteriorly, particles remain suspended in the crossflow and become more concentrated as filtrate exits between the rakers (Sanderson et al. 2001). Unlike industrial crossflow filtration, during fish crossflow filtration there is no accumulation of particles on the filter surface (i.e., the branchial arches and the gill rakers). Inertial lift has been hypothesized as an important factor in maintaining particles in suspension so that the filter does not become clogged (Sanderson et al. 2001). Due to this hydrodynamic force, particles near the tube or channel walls lift away from the walls and migrate radially towards the center of the tube or channel (channel $Re > 1$) as they travel downstream (Brandt and Bugliarello 1966; Segré and Silberberg 1962).

While the spaces between the rakers of suspension-feeding cichlids are too large to retain the particles that are typically consumed by these fish, the microbranchiospines on the branchial arches have been evaluated as a potential dead-end sieve (Gosse 1956; Beveridge et al. 1988a; Beveridge et al. 1988b). Drenner et al. (1987) developed a novel technique to investigate the importance of gill rakers and microbranchiospines during suspension feeding. Surgical removal of gill rakers and microbranchiospines in *Sarotherodon galilaeus* (Cichlidae) by Drenner et al. (1987) resulted in no discernible change in particle retention ability. While the particle retention mechanism for *S.*

galilaeus is still unknown, the use of hydrosol filtration with mucus for particle entrapment has been predicted (Sanderson et al. 1996; Smith and Sanderson in prep). If this is the case, and mucus remains after removal of the gill rakers and microbranchiospines, the absence of these structures would not be expected to result in a substantial loss of particle retention capabilities. However, if removal of the gill rakers and microbranchiospines results in coincidental removal of mucus-secreting tissue and a consequent lack of mucus, particle retention capability would be expected to decline. If particle retention is unchanged in the absence of mucus, then the importance of mucus as a hydrosol filtration mechanism is disproven. Since it is not known whether the removal of gill rakers and microbranchiospines affected mucus secretion on the branchial arches of *S. galilaeus*, we cannot distinguish among the above alternative interpretations of the results obtained by Drenner et al.

To investigate the roles of gill rakers, microbranchiospines, and mucus during suspension feeding, we have modified the method of Drenner et al. (1987). We removed the gill rakers and microbranchiospines from two cichlid species with known particle retention mechanisms to quantify the effect that gill raker and microbranchiospine removal have on particle retention abilities such as the size frequency distribution of particles retained by each species and the efficiency of particle retention. The blue tilapia (*Oreochromis aureus* Steindachner) uses crossflow filtration in the presence of mucus (Smith and Sanderson in prep), whereas the ngege tilapia (*Oreochromis esculentus* Graham) uses crossflow filtration without mucus present (Goodrich et al. 2000). Particle retention abilities have never been quantified in a species known to use crossflow filtration. Rather than quantifying particle retention indirectly through water samples, we

used a more direct method by measuring and counting microspheres that were excreted in the feces of the fish (Sanderson and Cech 1995; Sanderson et al. 1998).

Materials and Methods

O. aureus were obtained from pure stock raised at the University of Arizona. *O. esculentus* were from pure stock bred at the Museum of Science in Boston. Methods were based on those from Sanderson and Cech (1995; Sanderson et al. 1998). Tilapia were held individually or partitioned in pairs in 110-L aquaria with a gravel substrate. They were maintained on a diet of Tetramin flakes and kept at a constant temperature of 25-28° C.

Experiments were conducted on five *O. aureus* (22.7-27.7 cm standard length) and five *O. esculentus* (15.7-19.1 cm standard length) to determine the size frequency distribution of particles retained during suspension feeding for each species. Metal rings were attached with DAP silicone aquarium sealant in two arcs to the bottom of a 110-L aquarium filled with 60 L of water that had been measured using a 1 L graduated cylinder. To ensure water circulation, a submersible water pump (Little Giant, 304 L/h; 4 pumps total) was attached to both ends of each of two tygon tubes (1.2 cm i.d., 1.5 cm o.d., 40 cm long) that were then threaded through the rings on the bottom of the aquarium. During feeding, the pumps were turned on to force water through holes (2 mm diameter) drilled along the length of the tubing. The flow did not disrupt the fish, but was sufficient to maintain particles in suspension. Three air stones (Aquamist, 1.5 x 2.5 cm) were used at the corners of the aquarium as an additional method to prevent particles from settling. A solution of microspheres composed of inert, cross-linked Dextran

polymer was added to the aquarium to achieve a concentration of approximately 10 particles/mL in the aquarium (Sigma Aldrich Sephadex G-25 beads; 20-50 μm diameter, 0.01 g; 50-150 μm diameter, 0.165 g; weighed to the nearest 0.001 g). Microspheres were hydrated in aquarium water for 24 h prior to each experiment. We established that there was no additional swelling after the initial hydration period by hydrating a sample of microspheres for up to 42 h, and measuring the diameter of the microspheres at 8 h intervals.

Each fish was placed individually in the aquarium described above and allowed to suspension feed for 3 min on a slurry of Tetramin flakes and water. The slurry was added to the aquarium via a short piece of tubing attached to a 30 mL syringe. Fish engulfed food particles as they drifted down through the water column. The aquarium water was stirred periodically with a rod as an additional method to prevent settling of the food particles and microspheres. After 3 min of suspension feeding, whole Tetramin flakes were added to the aquarium by hand, and the fish continued to consume the Tetramin slurry and flakes for 1 min. Fish were videotaped during the experiments using a handheld Sony CCD-TR81 video camera recorder (30 frames s^{-1}) to aid in calculating feeding efficiency.

At the conclusion of each experiment, the fish was removed from the aquarium and placed in an 18.9 L bucket of fresh water. Each fish was rinsed externally with water using a squirt bottle to remove any microspheres trapped in external mucus. Each fish was then placed individually in a heated and aerated holding tank (27.8 L for *O. esculentus*, 55.6 L for *O. aureus*). Grating at the bottom of the holding tank prevented the feces from being resuspended by the activity of the fish. Within several hours, the

fish was transferred temporarily to a fresh bucket of water and fed whole Tetramin flakes. All feces were then collected from the holding tank using a pipette and placed under coverslips on glass microscope slides moistened with water. The holding tank was filled with fresh water and the fish was returned. Any feces that the fish produced in the bucket of water were also collected and placed on microscope slides. This entire process was repeated four or five times at approximately 12 h intervals, until the feces were devoid of microspheres. The fish was then returned to its aquarium. All of the fish remained healthy.

An Olympus BH2 phase contrast light microscope with an ocular micrometer was used to scan the entire microscope slide at 10x and measure microspheres at 20x to the nearest 5 μm . The microspheres were encased in a thin transparent sheath that surrounded the fecal strings, and the outlines of microspheres were clearly visible in the field of view as circles. Approximately 600 microspheres were measured from microscope slides selected randomly for each fish. In addition to the 600 measured microspheres, for three fish of each species the total number of microspheres retained on all microscope slides was counted to quantify feeding efficiency. Also, samples of the microsphere solution that was added to the aquaria at the beginning of the experiments were placed on slides, and microspheres (997) were measured to determine the size frequency distribution in the solution.

Approximately 7 days after each of the above particle retention experiments, gill rakers and microbranchiospines were removed from each fish. Method of removal was modified from that of Drenner et al. (1987). *O. aureus* and *O. esculentus* were anesthetized with MS-222 and all microbranchiospines and lateral and medial gill rakers

were removed with microforceps from the anterior four branchial arches on each side of five fish for each species. We refer to this procedure as “gill raker removal.” The fifth gill arches form the lower pharyngeal jaw, which was left unaltered. The procedure lasted an average of 75 min, during which the fish was lifted periodically from the water containing MS-222 in the surgery tray to remove a section of gill rakers and microbranchiospines, and then returned to the water in the surgery tray. Following the surgery, the fish was returned to its aquarium and Fungus Eliminator (Jungle Laboratories Corporation) was added to prevent infection. Fish were not adversely affected by the procedure and exhibited normal feeding behavior within two days. During the twelve days following surgery, the branchial arches healed and partial regeneration of rakers began, as described by Drenner et al. (1987) for *Sarotherodon galilaeus*. The regenerating rakers resulted in minute gill raker and microbranchiospines rudiments that were positioned randomly on the gill arches (Drenner et al. 1987). Twelve days after gill raker removal, the particle retention experiments described above were repeated on the same individuals.

For each of three *O. aureus* and three *O. esculentus*, the total number of microspheres retained after gill raker removal divided by the total number retained with gill rakers intact, expressed as a percentage, yielded the observed particle retention efficiency in the absence of gill rakers. This calculation of observed retention efficiency assumes that the fish actually fed during an equal amount of time before vs. after gill raker removal. To account for potential differences in the duration of feeding before vs. after gill raker removal, we calculated a corrected value of retention efficiency for each experiment. First, we analyzed videotapes from each experiment before as well as after

raker removal to quantify the number of video frames during which the fish could actually be seen feeding. The number of video frames during which the mouth was not visible in the videotapes had to be taken into account. The mouth of the fish was not visible when the fish was facing towards the back of the aquarium, passed behind a Little Giant water pump, or swam into a stream of bubbles.

To calculate the corrected value of retention efficiency, we made two assumptions: (1) the fish was feeding during the entire time when the mouth was not visible before the gill rakers had been removed, and (2) the fish was ventilating but not feeding during the entire time when the mouth was not visible after the gill rakers had been removed. Thus, for each experiment prior to gill raker removal, we added the number of frames during which feeding was observed plus the number of frames during which the mouth was not visible. For each experiment after gill raker removal, the time during which the fish could be seen feeding in the videotape was assumed to be the only time that the fish spent feeding. These assumptions provide the maximum possible estimate for the time spent feeding before raker removal, and the minimum possible estimate for the time spent feeding after raker removal.

After using the above assumptions to estimate the time spent feeding, we calculated the corrected particle retention efficiency. The observed particle retention efficiency was multiplied by the estimated time spent feeding with gill rakers intact, and divided by the estimated time spent feeding with gill rakers removed. The result of this calculation, expressed as a percentage, is the corrected particle retention efficiency in the absence of rakers. This corrected particle retention efficiency represents the highest

possible estimate of the efficiency of microsphere retention after gill raker removal relative to before gill raker removal.

Using videotapes, the total number of feeding pumps during each experiment was counted for all frames in which the mouth of the fish was visible. The total number of pumps was then divided by the total time spent feeding while the mouth was visible during each experiment to determine any change in feeding enthusiasm (rate of pumps s^{-1}) that may have contributed to a change in feeding efficiency. For example, a decrease in feeding enthusiasm (fewer pumps s^{-1}) after gill raker removal could result in a decrease in feeding efficiency. This calculation was performed for each fish before and after gill raker removal.

Results

For *O. aureus* and *O. esculentus*, particle retention experiments were conducted on five fish of each species before and after gill raker removal. For each fish, 600 microspheres were measured ranging from 11-210 μm in diameter. The first null hypothesis was that fish with gill rakers intact would retain the same size frequency distribution of microspheres as was present in the aquarium water. However, both *O. aureus* and *O. esculentus* retained proportionately fewer microspheres 11-50 μm in diameter than were present in the aquarium water, but proportionately more microspheres >50 μm in diameter than were present in the water (Figs 1,2). According to the cumulative size frequency distributions (Figs 5,6), although 55% of the microspheres in the aquarium water were greater than 50 μm , an average of 70% of the microspheres

ingested by *O. aureus* (and approximately 75% ingested by *O. esculentus*) were greater than 50 μm with rakers intact.

The second null hypothesis was that fish with gill rakers removed would retain the same size frequency distribution of microspheres as fish with gill rakers intact. There was not a substantial difference in size frequency distributions before vs. after gill raker removal. Comparing Figures 1 and 2 illustrates that there was no trend towards retaining smaller or larger particles with gill rakers intact vs. after gill raker removal for either species. Also, similar to what was observed with gill rakers intact, after gill rakers were removed an average of 75% of the microspheres ingested by both *O. aureus* and *O. esculentus* were greater than 50 μm (Figs 5,6).

Three individuals of each species were analyzed for feeding efficiency. The number of pumps per second during feeding was similar before and after raker removal (Table 3) for *O. aureus* (1.0 ± 0.1 pumps s^{-1} rakers intact vs. 0.7 ± 0.2 pumps s^{-1} rakers removed) and *O. esculentus* (1.5 ± 0.2 pumps s^{-1} rakers intact vs. 1.5 ± 0.1 pumps s^{-1} rakers removed). No differences in external feeding behavior or kinematics were observed after rakers had been removed compared to before raker removal. Consequently, no correction in the calculation of feeding efficiency was made for changes in “feeding enthusiasm.” The observed particle retention efficiency varied substantially among individuals (Observed column, Table 4) and was corrected for potential differences in time spent feeding before vs. after raker removal. The resulting corrected particle retention efficiency also showed substantial variation among individuals (Corrected column, Table 4).

For all three *O. esculentus*, and two *O. aureus* (#1, #3), the observed number of microspheres retained after gill raker removal was less than the observed number of microspheres retained with gill rakers intact. *O. aureus* #2 retained substantially more microspheres after gill raker removal, and showed a marked increase in observed feeding efficiency (Table 4). Correcting for the potential difference in time spent feeding after gill raker removal led to slight changes in the number of microspheres retained and in particle retention efficiency. The corrected number of microspheres retained with gill rakers removed was less than the observed number of microspheres retained with gill rakers intact for two *O. esculentus* (#1, #2) and two *O. aureus* (#1, #3; Table 4). Along with *O. aureus* #2, one *O. esculentus* (#3) retained more microspheres with gill rakers removed after the data was corrected for feeding time.

Discussion

Filtration Mechanisms and Particle Size Selectivity

Previous researchers have examined gill raker morphology to assess the correlation between inter-raker gap distances and sizes of prey retained by planktivores. Particle retention in gizzard shad (*Dorosoma cepedianum*, Clupeidae) was consistent with a model in which the gaps between rakers serve as the pores of a dead-end sieve (Drenner et al. 1984a). However, in some other fish species, the actual prey sizes consumed have been reported to be much smaller than the minimum sizes predicted to be retained by dead-end sieving on the basis of inter-raker gap distances (Langeland and Nøst 1995; Seghers 1975). Although many published texts and biological reference books assume that dead-end sieving is the primary method of particle retention in

suspension-feeding fish (e.g., Gerking 1994), it has been reported for only one species, bream (*Abramis brama*, Cyprinidae, Hoogenboezem et al. 1991). In fish that rely on hydrosol filtration using mucus entrapment or on crossflow filtration, the sizes of the gaps between rakers do not necessarily serve as prey size thresholds.

Northcott and Beveridge (1988) examined the branchial arches and gill rakers in the Nile tilapia, *Oreochromis niloticus*, proposing that mucus produced from the gill rakers may act as a particle entrapment mechanism. Use of a fiberoptic endoscope by Sanderson et al. (1996) established that *O. niloticus* uses mucus to entrap particles during hydrosol filtration. *O. esculentus* however, lacks mucus on the branchial arches (Goodrich et al. 2000). Although present, mucus does not contribute substantially to particle entrapment in *O. aureus*. Instead, as in *O. esculentus*, crossflow filtration is employed as a mechanism of particle retention (Smith and Sanderson in prep).

O. esculentus feeds primarily on diatoms or colonial algae in the water column (Onyari 1983), whereas *O. aureus* consumes a much wider range of prey, including phytoplankton, detritus, zooplankton, benthic invertebrates, and macrophytes (Spataru and Zorn 1978; Mallin 1985; Drenner et al. 1984b). The diet of *O. niloticus* is comparable to that of *O. aureus*, consisting of phytoplankton, filamentous algae, diatom-rich sediments, insect larvae, benthos, and crustaceans (Onyari 1983). Smith and Sanderson (in prep) suggest that filtration mechanisms of these tilapia species can be placed along a continuum from *O. niloticus* (combination of crossflow filtration and mucus entrapment, Sanderson et al. 1996) to *O. aureus* (crossflow filtration in the presence of mucus, but not mucus entrapment), to *O. esculentus* (crossflow filtration in the absence of mucus, Sanderson et al. 2001; Goodrich et al. 2000).

Data available in the literature indicate that *O. niloticus* is able to retain smaller particles than the other two species. Unlike *O. niloticus*, *O. esculentus* did not retain 2-celled *Scenedesmus* colonies, and was unable to retain 4-celled colonies as well as *O. niloticus* (30 μm x 18 μm , Goodrich et al. 2000; Batjakas et al. 1987). When McDonald (1987) presented *O. aureus* with small *Chlamydomonas* (6-15 μm), *O. aureus* actually lost weight. This is consistent with our findings that, although *O. aureus* can retain particles as small as 15 μm , they preferentially retained particles larger than 50 μm . We hypothesize that hydrosol filtration using mucus entrapment enables *O. niloticus* to retain smaller particles than *O. aureus* and *O. esculentus*.

Both *O. aureus* and *O. esculentus* retained proportionately fewer microspheres <50 μm in diameter than were present in the aquarium water, and retained proportionately more microspheres >50 μm in diameter (Figs 1, 2). Sanderson et al. (1996) suggested that hydrosol filtration with a mucus entrapment system could explain such size selectivity in tilapia. While this suggestion could apply to *O. niloticus*, it is not relevant for *O. aureus* or for *O. esculentus*. Smith and Sanderson (in prep), and Goodrich et al. (2000) have demonstrated that neither *O. aureus* nor *O. esculentus* relies on mucus entrapment for particle retention during feeding. Here, we have quantified particle size selectivity in *O. aureus* and *O. esculentus* in the absence of mucus entrapment as well as in the absence of gill rakers.

Raker removal results in a dramatic decrease in mucus during feeding in *O. aureus* (Smith and Sanderson in prep). Despite this decrease in mucus and the absence of gill rakers, *O. aureus* are capable of retaining a similar size frequency distribution of microspheres after raker removal (Fig. 3). *O. aureus* were able to retain microspheres as

small as 15 μm in diameter, but selectively retained microspheres greater than 50 μm with gill rakers intact as well as removed (Fig. 1). This establishes that neither mucus nor gill rakers are necessary for the selective retention of particles larger than approximately 50 μm . Size selectivity in the absence of mucus and gill rakers could be explained by the use of crossflow filtration (Sanderson et al. 2001). For example, since inertial lift is proportional to the cube of the particle radius, the size frequency distribution of retained particles could be affected by the operation of this hydrodynamic force during crossflow filtration (Chellam and Wiesner 1992).

Since neither mucus nor gill rakers appears to be necessary for retaining particles in *O. aureus*, there is a question as to the importance of hydrosol filtration as a particle retention mechanism in tilapia. Performing particle retention experiments with gill rakers intact and again with rakers removed in *O. niloticus*, a species known to use mucus for particle entrapment during hydrosol filtration (Sanderson et al. 1996), will be necessary to fully understand the importance of such a filtration mechanism. Fiberoptic endoscopy performed after raker removal on *O. niloticus*, coupled with particle retention efficiency calculations, could establish whether mucus is present and to what extent gill raker removal affects the particle retention mechanism of a species that uses hydrosol filtration. Since *O. aureus* does not rely on mucus for particle entrapment (Smith and Sanderson in prep), surgical removal of mucus-producing cells resulted in little change in the overall particle retention efficiency and in the cumulative size frequency distribution of retained particles. We hypothesize that a species such as *O. niloticus* that does rely on mucus for particle entrapment would retain substantially fewer small (<50 μm) microspheres than

O. aureus or *O. esculentus* after gill raker removal, and would exhibit a marked decrease in particle retention efficiency after raker removal.

Particle Retention Efficiency

Fiberoptic endoscopy inside the oropharyngeal cavity of *O. aureus* showed that, after gill raker removal, particles tended to disappear more frequently between the arches rather than bounce on or be retained in mucus on the arches and rakers (Smith and Sanderson in prep). This is consistent with our calculations indicating reduced particle retention efficiency after gill raker removal in *O. aureus*. We suggest three possible explanations for the reduction in particle retention efficiency after gill raker removal. One possible cause could be the loss of water between the anterior branchial arches and the resulting decrease in inertial lift. Flow speed measurements from the oropharyngeal cavity of *O. aureus* before and after gill raker removal showed a significant decrease in speed after gill raker removal (Smith and Sanderson in prep). The reduction in particle retention efficiency after the removal of gill rakers (Table 4) could be related to this decrease in flow speed through the oropharyngeal cavity. Smith and Sanderson (in prep) hypothesized that the absence of gill rakers results in a larger volume of water passing between the anterior branchial arches. The water that exits between the anterior arches has been filtered only minimally by crossflow filtration, and the abnormally large volume of water could carry many particles out of the oropharyngeal cavity. In addition, the loss of this water between the anterior arches would result in a lower volume flow rate through the oropharyngeal cavity, and therefore a slower crossflow speed parallel to the arches. Inertial lift is proportional to the square of the crossflow speed (Chellam and

Weisner 1992). The hypothesized reduction in inertial lift force within the oropharyngeal cavity after raker removal would result in less back-migration of particles from the region near the filter surface towards the midline of the oropharyngeal cavity, which could lead to lower particle retention efficiency.

If removal of the gill rakers and microbranchiospines coincidentally removed the mucus-secreting cells, and if a potential function for this mucus in crossflow filtration is to enhance the use of the branchial arches as a surface that results in inertial lift (Smith and Sanderson in prep), then the consequent lack of mucus could serve as a second explanation for reduced particle retention efficiency. Although *O. aureus* do not trap particles in mucus during suspension feeding (Smith and Sanderson in prep), the presence of mucus as a surface to increase inertial lift is still of potential importance.

A third possible cause of the reduction in particle retention efficiency in the absence of gill rakers is a potential decrease in vortex production and a consequent decrease in the back-transport of particles into the mainstream flow. Smith and Sanderson (in prep) compared the flow reversals observed during feeding in *O. aureus* to the pulsatile or oscillatory flow sometimes injected into channels or tubes during industrial crossflow filtration. In fish as well as in industry, the decelerating and accelerating flow or the flow reversal can result in back-transport of particles from the filter surface to the mainstream flow (Smith and Sanderson in prep; Winzeler and Belfort 1993). Another mechanism for introducing unsteady flows to the crossflow and increasing filtration efficiency is through protuberances on the filter surface. During crossflow filtration in fish, the arches and the gill rakers could serve as these protuberances, adding roughness elements to the filter surface. Such “furrowed

channels” can produce vortices as water interacts with each furrow (Winzeler and Belfort 1993). Flow instabilities such as these vortices have been very effective in the back-transport of particles from the filter surface to the mainstream flow, resulting in less particle accumulation on the filter and increased industrial filtration efficiency, particularly in combination with flow reversals (Winzeler and Belfort 1993). By removing the gill rakers in *O. aureus*, we removed the finest roughness elements of the filter surface. This may have led to the formation of fewer vortices and less back-transport of particles. A decrease in vortex formation after gill raker removal, and a consequent decrease in particles returning to the mainstream flow, could be related to a decrease in particle retention efficiency.

After removal of gill rakers in *S. galilaeus*, Drenner et al. (1987) concluded that the gill rakers did not function as a dead-end sieve and suggested that mucus may be involved in the ability of *S. galilaeus* to retain particles in the absence of gill rakers. Vinyard et al.’s (1988) report that *S. galilaeus* is more efficient at grazing on small- and intermediate-sized phytoplankton than *O. aureus* suggests that *S. galilaeus*, like *O. niloticus*, may use a combination of crossflow filtration and mucus entrapment. To assess the importance of mucus in retaining particles after removal of gill rakers, fiberoptic endoscopy in *S. galilaeus* will be necessary before and after raker removal.

Previous studies that have used a group of fish per aquarium for each experimental trial have allowed for declines in particle concentration to be detected in the aquarium, but have masked variability among individuals. Placing each fish in an individual aquarium for the current study allowed quantification of the substantial variability in particle retention efficiency among individuals (Table 4). Smith and

Sanderson (in prep) observed similar inter-individual variability in the oral flow speed of *O. aureus*, which could be related to the variability in particle retention efficiency. Neither *O. aureus* nor *O. esculentus* showed inter-individual differences in feeding enthusiasm that could be responsible for the inter-individual differences in particle retention efficiency. Inter- and intra-individual variability in filtration processes remains an unexplored area of research that has substantial potential for increasing our understanding of the factors influencing filtration mechanisms.

The increase in particle retention efficiency to a corrected value of 155.6% for *O. esculentus* #3 could be explained by the conservative calculation of the corrected particle retention efficiency (Table 4). The observed particle retention efficiency assumed that the fish fed an equal amount of time before and after gill raker removal. However, when the mouth of the fish could not be seen in the videotape, we could not determine from the videotape whether the fish was feeding or simply ventilating. Similarly, in measurements of feeding time used to calculate filter-feeding rates of gizzard shad (*Dorosoma cepedianum*), Drenner et al. (1982) did not include periods of time during which fish swam vigorously against the side of the pool. To correct for potential differences in time spent feeding before vs. after raker removal, we assumed that the time during which the mouth of the fish was not visible on the videotape was spent feeding before raker removal but spent ventilating after raker removal. To be consistent, we corrected for each fish using the same formula, which in the case of *O. esculentus* #3 conflicted with notes from visual observations of time spent feeding that were recorded during the experiment. Observations during the experiment indicated that this particular fish was feeding rather than ventilating for the duration of the experiment, so that the

corrected particle retention efficiency is a conservative overestimate. In contrast, notes from visual observations recorded during the experiment indicated that *O. aureus* #2 was observed to be feeding the entire time with rakers intact, but was not feeding as consistently with rakers removed. However, these observations do not explain the large number of microspheres retained after gill raker removal vs. with gill rakers intact.

Our results are not consistent with those of Drenner et al. (1987), who reported that particle retention efficiency in *S. galilaeus* was not affected by gill raker removal. When fish are grouped in an experimental trial, the resulting particle retention efficiency is an average of the values for the individual fish. Using a direct method for counting particles that have been ingested by each individual fish permits declines in particle retention to be detected that might not be quantifiable in samples of aquarium water.

TABLE 1

Number of pumps and post-pump 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 rakers intact.

Action of Fish	Movement of Mucus	
	Attached to arches	Lift, move posteriorly
Pump	41	1
Reversal	22	5
Total	63	6

TABLE 2

Peak flow speed and duration of pumps and post-pump reversals during feeding in *O. aureus* (mean \pm S.D., $N=3$ individuals)

	Duration of Pump (sec)	Duration of Reversal (sec)	Pump peak speed (cm s ⁻¹)	Reversal Peak speed (cm s ⁻¹)
Rakers Intact	0.50 \pm 0.1	0.55 \pm 0.1	6.7 \pm 2.8	11.2 \pm 3.2
Rakers Removed	0.49 \pm 0.1	0.59 \pm 0.1	2.6 \pm 1.6	4.9 \pm 2.8

TABLE 3

Feeding pumps s^{-1} before and after gill raker removal as a measure of feeding enthusiasm.

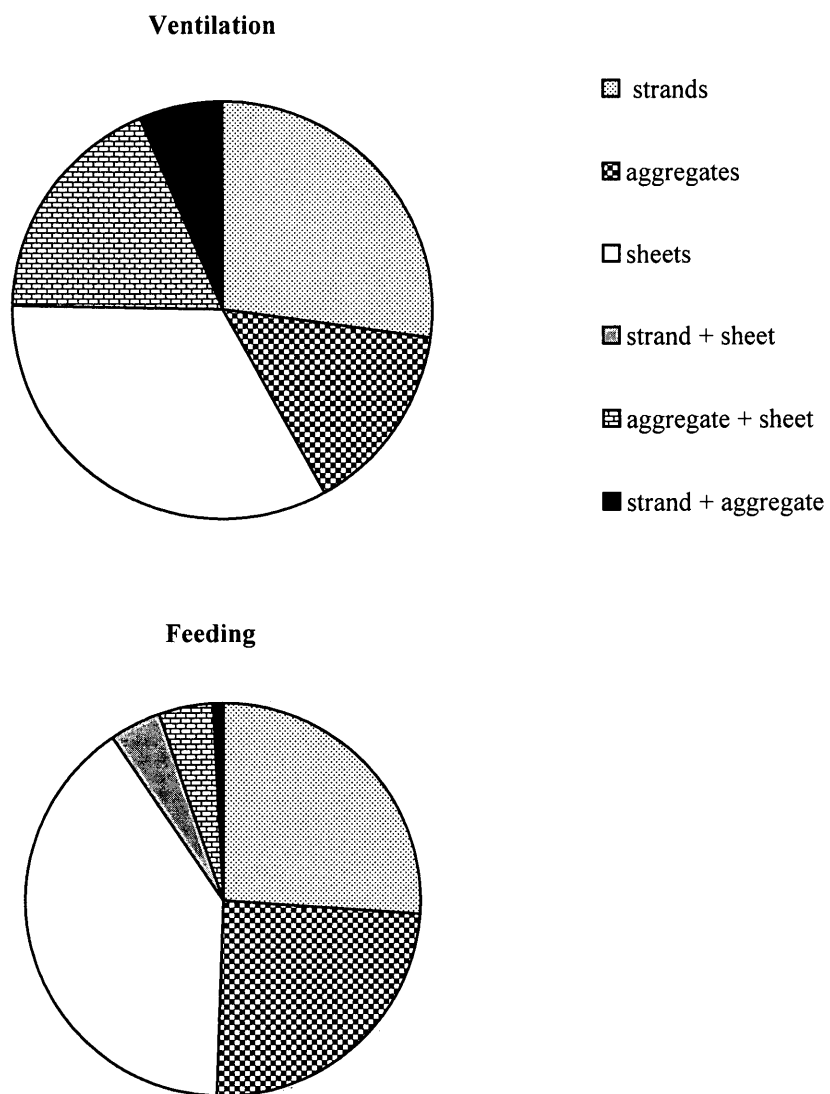
		Feeding pumps s^{-1}	
	Fish	rakers intact	rakers removed
<i>O. aureus</i>	1	0.874	0.802
	2	1.019	0.551
	3	1.029	0.873
<i>O. esculentus</i>	1	1.595	1.502
	2	1.502	1.577
	3	1.322	1.289

TABLE 4

The total number of observed microspheres retained with gill rakers intact and removed. Particle retention efficiency as the ratio of number of particles retained with gill rakers removed to number retained with gill rakers intact, expressed as a percentage (N= 3 individuals of each species).

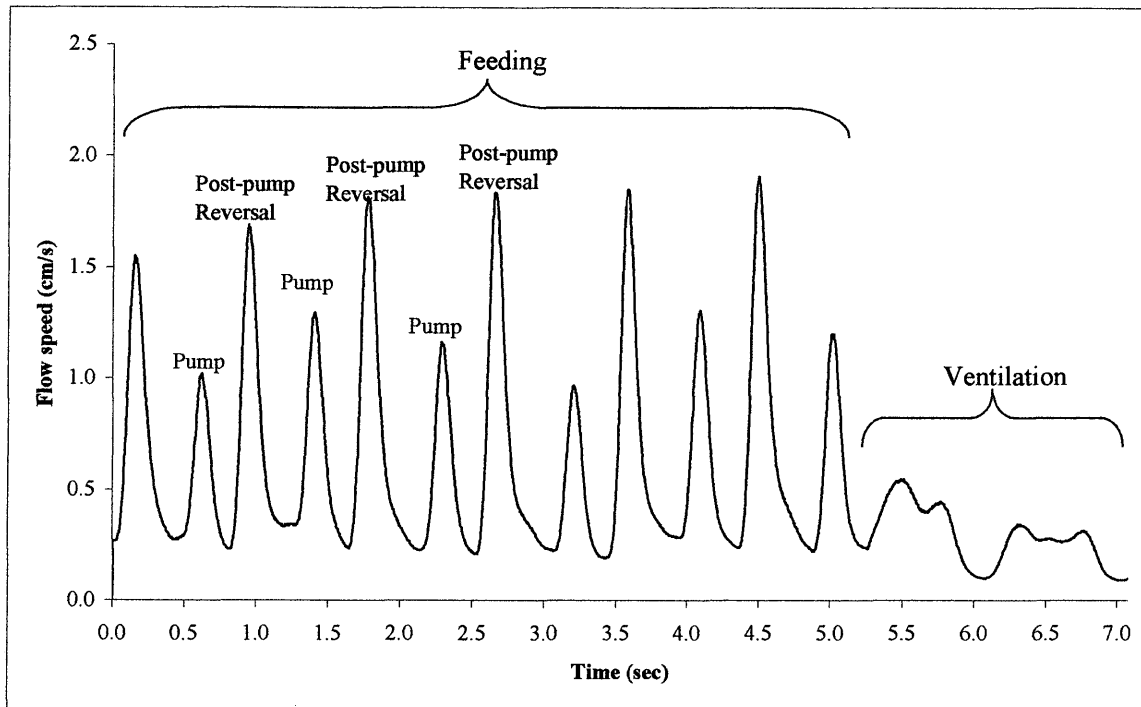
	Fish	Total # microspheres retained		Particle Retention Efficiency	
		rakers intact	rakers removed	Observed	Corrected
<i>O. aureus</i>	1	8498	209	2.5%	3.5%
	2	621	8192	1319.2%	1796.2%
	3	4065	2356	57.9%	94.5%
<i>O. esculentus</i>	1	3193	912	28.6%	55.7%
	2	619	293	47.3%	63.6%
	3	2789	1763	63.2%	155.6%

FIGURE 1



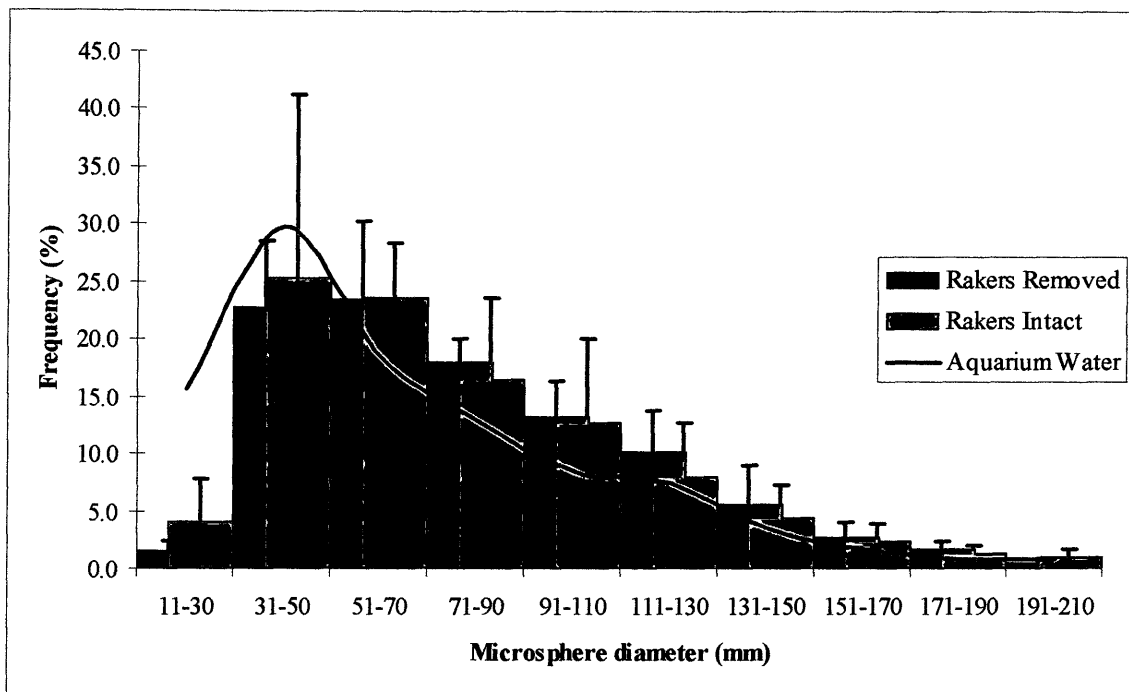
Classification of mucus shapes during ventilation and suspension feeding in *O. aureus* before gill raker removal.

FIGURE 2



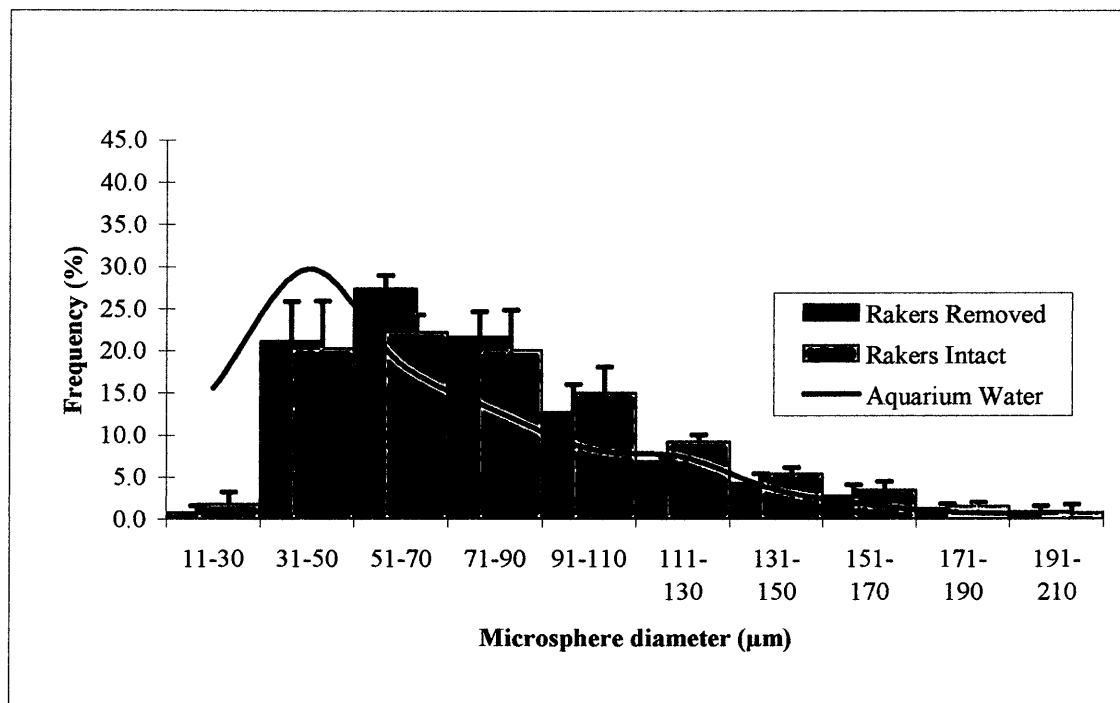
Representative flow speeds recorded during ventilation and suspension feeding in *O. aureus*.

FIGURE 3



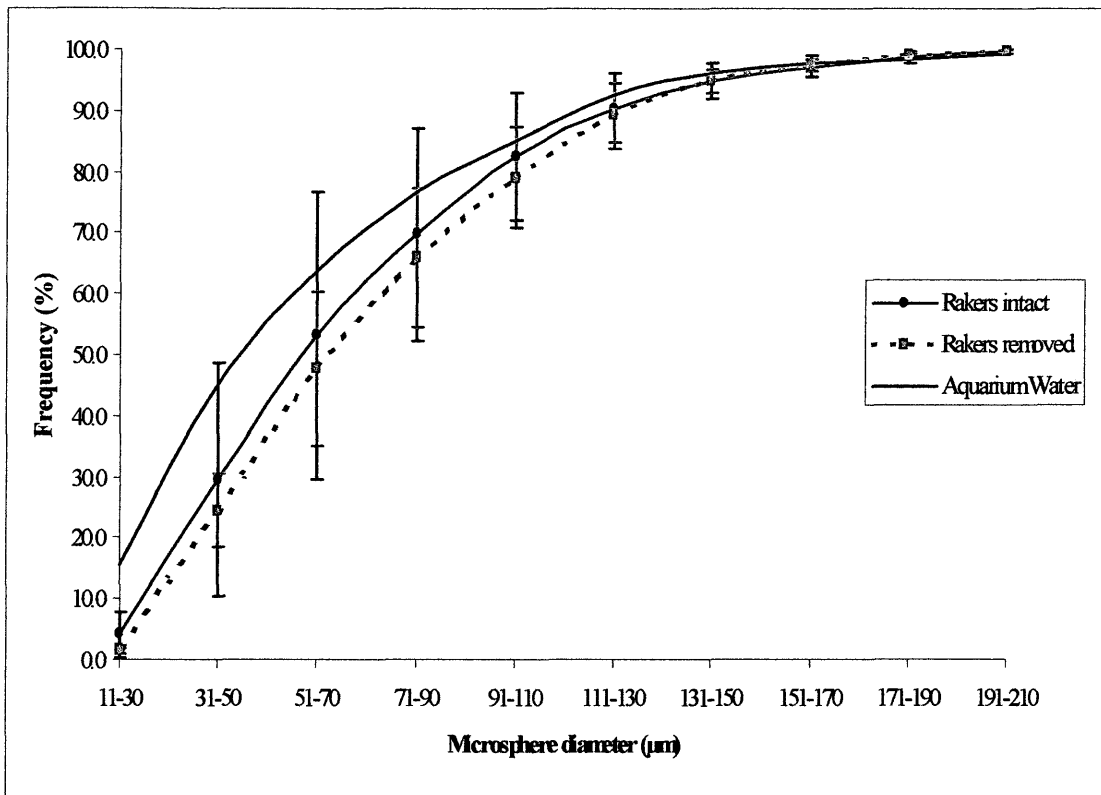
Size frequency distribution of particles retained during suspension feeding in *O. aureus*, before and after gill raker removal (mean \pm S.D., $N=5$ individuals). The curve for aquarium water illustrates the size frequency distribution of particles in the solution that was added to the aquarium at the beginning of the experiments.

FIGURE 4



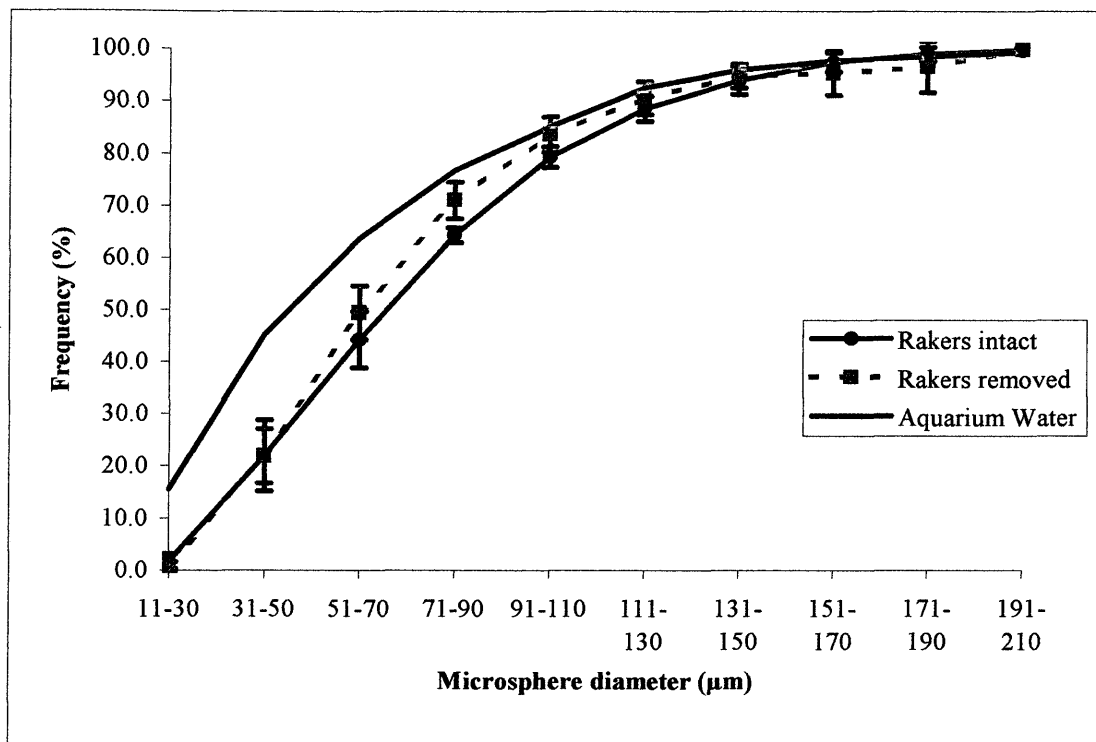
Size frequency distribution of particles retained during suspension feeding in *O. esculentus*, before and after gill raker removal (mean \pm S.D., $N=5$ individuals). The curve for aquarium water illustrates the size frequency distribution of particles in the solution that was added to the aquarium at the beginning of the experiments.

FIGURE 5



Average cumulative size frequency distribution with gill rakers intact vs. gill rakers removed for *O. aureus* (mean±S.D., $N=5$ individuals).

FIGURE 6



Average cumulative size frequency distribution with gill rakers intact vs. gill rakers removed for *O. esculentus* (mean±S.D., $N=5$ individuals).

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VITA

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