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Frequencies of Suspension-Feeding Actions Vary with Prey Type in *Oreochromis niloticus* (Pisces: Cichlidae)

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FREQUENCIES OF SUSPENSION-FEEDING ACTIONS VARY WITH PREY
TYPE IN OREOCHROMIS NILOTICUS (PISCES: CICHLIDAE)

A Thesis

Presented to

The Faculty of the Department of Biology
The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of
Master of Arts

by

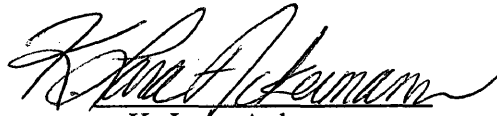
K. Lara Ackermann

1999

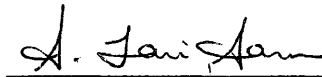
APPROVAL SHEET


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


Master of Arts


K. Lafa Ackermann

Approved, January 1999


S. Laurie Sanderson


Stewart A. Ware


Gregory M. Capelli

DEDICATION

For my parents, Mary Ellen Rose and John McGee Ackermann, who
encouraged me to try in the first place.

For my husband, John J. R. Templin, who gave me the strength to finish what
I had started.

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ABSTRACT

Few studies to date have been done on the behavioral actions of suspension feeding fish, despite the fact that a great many environmentally and economically important fish are suspension feeders.

Observations of feeding behavior in Oreochromis niloticus revealed differences in the frequencies of actions used for suspension feeding on four prey types (whole flake food, crushed flake slurry, brine shrimp, and bacteria). Five feeding actions (long pumps, short pumps, vacuums, spits, and reversals) were quantified and their functions were analyzed.

Examination of water samples by Acridine Orange Direct Counts (AODC) showed that these fish responded to the presence of naturally-occurring bacteria in the water and fed spontaneously on that bacteria when it reached a certain density.

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INTRODUCTION

Vertebrate suspension feeding involves the movement of particle-laden water into and through the buccal cavity where surfaces or structures separate the minute particles from the water, allowing the ingestion of the captured particles (Sanderson and Wassersug, 1993). Particulate feeding involves the selective ingestion of individual food particles, whereas suspension feeding is comparatively non-selective, entrapping food particles in groups rather than individually. Suspension feeders, also known as filter feeders, have been defined as "aquatic animals that have evolved special structures to process the surrounding water and to retain small suspended particles, including food particles such as phytoplankton" (Jørgensen, 1983). I would add to that definition that they may also have evolved special physical actions to aid in the processing of water for food particles, and in re-suspending particles from benthic surfaces back into the water for consumption via filter feeding.

Filter-feeding fishes have long been studied due to their importance to freshwater ecosystems (Northcote, 1988) and to mankind, since they form approximately one-third of the world fish catch (FAO, 1993). Suspension-feeding tilapia, for example, are valued in commercial fishing industries, even though they have caused disruption to native fish populations when introduced to new environments (Getachew, 1993; Kaufman, 1992; Kolding, 1993; Ogutu-Ohwayo, 1990). The ability of suspension-feeding fishes to filter huge quantities of water for tiny prey items is a source of continuing research, including studies of functional morphology and sites and mechanisms of filtration (Gerking, 1994; Sanderson and Wassersug, 1993). However, little work to date has concentrated on the behaviors

involved in collecting and processing the food particles. Behavioral actions during feeding can affect fluid flow (both direction and velocity) within the buccal cavity, which in turn affects the particle encounter and retention mechanisms involved (Rubenstein and Koehl, 1977; Shimeta and Jumars, 1991). Encounter mechanisms concern the contact between feeding structures and particles while retention mechanisms are concerned with the capture of those contacting particles (Shimeta and Jumars, 1991).

Comparison between suspension feeding and particulate feeding is hampered by the limited data on the actions involved in suspension feeding. Therefore, I have quantified the actions used during suspension feeding in O. niloticus, with the goal of determining how and why feeding actions differ with prey type. Oreochromis niloticus was chosen for this study since it is one of only three fish species whose particle retention mechanisms are known (Abramis brama, Cyprinidae, Hoogenboezem et al. 1991; Orthodon microlepidotus, Cyprinidae, Sanderson et al. 1991; Oreochromis niloticus, Cichlidae, Sanderson et al. 1996).

Field and laboratory studies have shown that O. niloticus eat a considerable variety of food types, including phytoplankton, bacteria, diatoms, zooplankton, and insect larvae (Bowen, 1982; Fryer and Iles, 1972; Moriarty and Moriarty, 1973; Onyari, 1983). Tilapia have been shown to vary suspension feeding behavior with size of prey (Drenner et al. 1984; 1987), so feeding on prey items of different sizes was investigated using crushed flake slurry versus whole flake food. The food items investigated in this study covered the range of sizes of prey items that may be consumed via suspension feeding (~1 μ m - 10 mm), and included natural prey types, bacteria and zooplankton.

MATERIALS & METHODS

Three specimens of Oreochromis niloticus were obtained from a United States aquaculture company. The fish (13.5-25.0 cm standard length) were kept in 29 gallon aquaria at 26-28 °C, in constant light, and were fed TetraMin flake food daily with occasional supplements of frozen adult brine shrimp (Artemia). The aquaria had gravel substrates (3-9 mm diameter). The fish were kept separated and without visual contact to prevent aggressive territorial behavior. All experiments were conducted during natural daytime, when these fish feed most actively (Moriarty and Moriarty, 1973).

Feeding Action Observations and Analysis

Videotapes of feeding behavior were recorded using a hand-held Hi-8 camcorder (Sony CCD-TR81, 30 frames s⁻¹). Feeding behavior by each of the three individuals was observed on four prey types: whole flake TetraMin (3-10 mm diameter), crushed TetraMin flakes (0.1-1.0 mm diameter) in a slurry with water, frozen adult brine shrimp (Artemia, 4 mm length), and microscopic food particles suspended in the water column (bacteria and algae; see water analysis below). The first three prey types were introduced into the aquaria by the investigator, with fish beginning to feed at the moment of prey introduction. Only one prey type was introduced per day and the food types were introduced in a random order. The whole TetraMin flakes (2.2 g) were added to the tanks by hand, once at the beginning of observation periods, as per daily feedings. The brine shrimp (2.5 g) and the slurry (2.2 g crushed TetraMin in water) were added via a plastic tube attached to a 30 cc syringe in three to five portions during the first three minutes of observation. The fish

usually began to filter feed spontaneously on microscopic particles two to four hours after the last introduction of food. All feeding actions were clearly distinguished from respiratory actions, and respiratory actions were not included in analysis.

Prior to all observations, the individual being viewed fasted for 24-36 hours. Videotaped data were collected from the feeding fish for continuous time periods of 10-15 minutes after the introduction of food, or during spontaneous filter feeding on microscopic particles in the water column. These observations were repeated on separate days until 30 minutes of data had been collected, in two or three bouts of feeding, for each of the three fish feeding on each of the four prey types. In each case, the fish fed actively throughout the videotaped time, with a noticeable reduction in feeding activity after 10 - 15 minutes used as the criterion for the cessation of videotaping. In general, feeding activity continued for at least 20 minutes after feeding was initiated.

The videotapes were analyzed on a frame by frame basis, using a Hi-8 video player/recorder (Sony EVO-9700) with an editing controller jog shuttle (Sony RM-E9700). Each oral movement that took place during feeding was noted. The movements fell into five action categories: long pumps, short pumps, vacuums, spits, and reversals. There were less than ten movements in all the collected data, primarily "yawns" and "coughs", that did not fit into one of the above categories.

Water Sample Analysis

To identify and enumerate the microscopic particles in the water column, water samples were collected both within one half-hour after the beginning of spontaneous filter feeding and within one half-hour after the cessation of such filter feeding. For each aquarium, feeding and post-feeding samples were taken on the same day, for the same bout of feeding. All water samples during spontaneous filter feeding were taken a minimum of two hours and a maximum of six hours past the last introduction of external food. The fish suspension fed intermittently before

completely ceasing to feed, but post-feeding samples were taken within thirty minutes after continuous filter-feeding activity had completely ceased. Acridine Orange Direct Counts (AODC) of the contents of the water samples were done immediately following sample collection. Acridine Orange is one of the main stains used for making direct counts of aquatic bacteria by epifluorescence (Fry, 1988; Hobbie, et al. 1977) and provides better estimates of bacterial abundance than does 4'-6-diamidino-2-phenylidole (DAPI) (Suzuki, et al. 1993).

Samples were collected with a sterile 5 cc syringe. One milliliter of each sample was filtered immediately onto a black "Nucleopore" polycarbonate (0.2 μm) filter. The filters with samples were stained for five minutes with 0.3 ml of 0.01% Acridine Orange dye solution. They were then examined under an epifluorescent microscope (Olympus BH2-RFL) at 100X, which permitted identification of three general types of cells (rod bacteria, cocci bacteria, and suspended algal cells). The category of "algal cells" may include cyanobacteria since I was unable to clearly distinguish different kinds of filamentous cells. However, all cells categorized as "algal" were defined as such due to the presence of visible internal structures (none were visible in the rod or cocci bacteria).

Twenty fields were chosen for each 1 ml sample using a random number table, and all cells in each field were counted. Counts of the number of cells of each type were averaged for the 20 random fields and the numbers of cells per milliliter of water were calculated using the following equation:

$$\begin{aligned} \text{Area of filter/Area of microscope field} &= 254.46 \text{ mm}^2/0.01 \text{ mm}^2 = \\ &25446.0 = \text{number of microscope fields/filter} \\ (\text{Average number of cells/microscope field}) (25446.0 \text{ fields/filter}) &= \\ &\text{Average number of cells/filter} \\ (\text{Average number of cells/filter})/ 1\text{ml volume of sample filtered} &= \\ &\text{Average number of cells/milliliter} \end{aligned}$$

Feeding Action/Prey Type Statistical Analysis

Using JMP version 2, SAS Institute Inc. (1989), statistical analyses were performed on the total number of times each of five feeding actions (long pumps, short pumps, vacuums, spits, and reversals) occurred during 30 minute observations of feeding by each of three individual fish on each of four prey types (whole flakes, crushed flakes, brine shrimp, and microscopic cells). The variances were not homogeneous, so all data were transformed by taking the square root (Sokal and Rohlf, 1981). Once transformed, the data were normally distributed (Shapiro-Wilk W test, $P > 0.05$) and the variances were homogeneous (Bartlett's test, $P > 0.05$).

A one-way repeated measures ANOVA was performed for each of the five feeding actions. Prey type was a fixed factor and individual was a random factor. The sequential Bonferroni test was used to give an experiment-wise α of 0.05 (Rice, 1989).

Unplanned contrasts among means were conducted for each one-way ANOVA by building contrasts in terms of the least squares means of the effect. An F test was used to test jointly for all of the contrasts that were tested for each one-way ANOVA.

Water Sample Statistics

The average numbers of cells present per milliliter of aquarium water during feeding versus post-feeding were calculated for each of three aquaria. Averages were obtained for each of three cell types: two forms of bacteria (rod and cocci) and suspended algal cells. The cell counts for the water samples were not normally distributed, so a square root transformation was performed (Sokal and Rohlf, 1981). The transformed data were normally distributed (Shapiro-Wilk W test, $P > 0.05$), and the variances were homogeneous (Bartlett's test, $P > 0.05$).

Two-tailed paired t-tests on each of the three sets of data (rod, cocci, and algal cells) were then executed to test for significant differences between feeding and post-feeding samples.

RESULTS

Feeding Action Descriptions

Oreochromis niloticus filter feed using suction pumps that cause water to pass from the mouth through the buccal cavity and out the opercula, with edible particles filtered from this water as it passes through the buccal cavity. These pumping actions were interspersed with other feeding actions: vacuums, short pumps, spits, and reversals (described below). When food was introduced to the aquarium, the fish began to feed by using suction pumps in the open water column for several minutes. Particles slowly settled to the substrate during this feeding. When few prey items remained suspended in the water column, the fish would briefly disturb the substrate with benthic suction feeding (vacuums), and then return to water column feeding on the particles that had been re-suspended from the substrate. This pattern of alternating between benthic and water column feeding would continue until feeding activity ceases after approximately 20 minutes.

In Figure 1, a fish was traced from the videotape at intervals of 0.13 seconds (4 frames) during a representative suction pump. Most pumps occurred as part of a cycle of feeding actions and the pre-pump appearance of the fish often included adducting opercula and the adducting of the hyoid from the previous pump (Fig 1a). As a pump began (Fig 1b), the mouth opened and the oral valve retracted as the premaxillae began to protrude. The opercula and hyoid remained adducted. As the mouth opened further, abduction of the hyoid apparatus created a negative pressure within the buccal cavity, and water rushed into the mouth (Fig 1c). At the pump apex, the mouth formed a wide oval opening, the premaxillae were extended far forward, the floor of the buccal cavity was abducting, and the opercula were

abducting (Fig 1d). As the mouth closed (Fig 1e), the buccal cavity floor was fully expanded and the opercula were fully abducted. Once the mouth closed completely, the hyoid began to adduct, and water was expelled through the opercular opening (Fig 1f). Then the hyoid apparatus continued to adduct and the opercula adducted (Fig 1a), leaving the fish ready for the next action. Pumps lasted for as little as 0.4 seconds or as long as 1.25 seconds, with an average duration of 0.7 seconds ($n = 30$, $SD = 0.18$).

For *O. niloticus* two general categories of pumps were identified: long pumps and short pumps. Long duration pumps (0.6 seconds or longer, Fig 1) always occurred in the water column with a wide-open mouth, and any food particles visible near the mouth could be seen entering the buccal cavity during these pumps. Short duration pumps (less than 0.6 seconds) also occurred in the water column. However, during these short pumps, the mouth was opened only a half to a quarter as wide as during long pumps and the buccal cavity was not as expanded. Comparatively little suction was created as indicated by the fact that only particles in the immediate vicinity of the mouth entered the buccal cavity during these short pumps. In all other respects, the movements involved were similar to those that occurred in long pumps. The few yawns observed were unlike both types of pumps in that the mouth opened widely over an extremely long time period (3 or more seconds) in a stretching motion with no visible particle collection.

Vacuums were used exclusively for benthic feeding, where suction was used to take up large mouthfuls of food, gravel and detritus from the bottom of the aquarium. The physical actions of the mouth during a vacuum were very similar to those of a long pump. Vacuums were quite brief (usually 0.3 to 0.5 seconds duration), and the fish often shoved its mouth into the gravel in a rooting motion prior to the use of suction. In this paper, vacuums refer exclusively to benthic feeding, while the term long pump refers solely to water column feeding.

Short pumps occurred after a series of long pumps or interspersed with vacuuming during benthic feeding. When they occurred intermixed with vacuuming actions, gravel, food, and fecal material was clearly visible within the anterior buccal cavity on the abducted oral floor. In these cases, short pumps were followed by spitting, when unwanted material such as gravel was expelled from the mouth in a quick burst. Spits occasionally followed vacuums directly.

Lasting 0.5 sec or less, spits ejected material through a wide-open mouth. Spits were similar to reversals in all buccal movements except that the mouth was open in a spit, whereas the closed mouth during the reversal prevented any loss of material from the buccal cavity. At no time was material observed exiting via the opercula; all uneaten material was ejected through the mouth by these fish during spits.

A total of three coughs were observed in these specimens. A cough was defined as an abrupt opening of the mouth very wide accompanied by rapid abduction and adduction of the opercula, with no visible ejection of material. Water flow was assumed to reverse in the mouth during a cough, similar to a spit, but the lack of ejected material made the direction of water flow difficult to determine. More data is required to clarify the purpose of coughing in this species.

Long pumps, short pumps, vacuums, and spits were all periodically interspersed with reversals, although reversals most often occurred following a pump or another reversal. A reversal was characterized by an abrupt, coordinated motion of the opercula, premaxillae and hyoid apparatus generally lasting 0.5 seconds or less which created a suction in the opposite direction from a pump, causing water to flow in through the opercula and forward into the buccal cavity to the closed mouth.

Based on videotapes obtained by inserting a fiberoptic endoscope into the buccal cavity, Sanderson *et al.* (1996) identified three stages of the reversal in *O. niloticus*: the pre-reversal stage, reversal stage 1, and reversal stage 2. In Figure 2, a

fish was traced from exterior videotape at intervals of 0.067 seconds (2 frames) during a representative reversal. Before a reversal began, the mouth was closed and the floor of the buccal cavity was either fully adducted or still slightly abducted from a previous action (Fig 2a). Immediately preceding a reversal, the opercula abducted and the hyoid apparatus and buccal cavity floor continued to adduct if not yet fully adducted (Fig 2b). In stage 1 of a reversal, as the buccal cavity filled with water from the backwards influx from the opercula, the opercula abruptly adducted, the hyoid apparatus was abducting and the premaxillae began a very rapid protrusion, which moved the closed mouth forward (Fig 2c). The premaxillae continued to extend to the maximum protrusion (Fig 2d). This movement expanded the volume of the anterior buccal cavity as the floor of the buccal cavity distended further, which caused a suction that pulled the water in the opercular and buccal cavities from the posterior to the anterior. Observations of internal water flow patterns via an endoscope have confirmed this posterior to anterior flow during stage 1 of a reversal (Sanderson, *et al.* 1996). Then stage 2 began as the closed mouth and premaxillae began to retract, the hyoid apparatus adducted, and the opercula abducted again (Fig 2e). As the reversal ended (Fig 2f), the mouth returned toward its pre-reversal position with the hyoid apparatus adducting as water left through the open opercula.

Feeding Action/Prey Type

The specimens ate vigorously when all food items were introduced to the aquaria. The data collected for the five feeding actions for each of the four prey types are shown in Figure 3.

Applying the sequential Bonferroni test with an experiment-wise error rate of $\alpha = 0.05$, the one-way ANOVAs demonstrated that there were significant differences between prey types for all five actions (Table 1). There were also significant differences between individuals for short pumps and reversals (b and e, Table 1).

The unplanned contrasts were performed on selected subsets of prey types for the ANOVAs of each action (Table 2). For all five feeding actions, microscopic food was responsible for the most significant differences among prey types. Long pumps occurred more often with microscopic food than with any other prey type, while all other actions (short pumps, vacuums, spits, and reversals) occurred least frequently with microscopic prey. There were also significantly fewer spits with brine shrimp than with whole flake or crushed flake prey types. However, no other trends tested among prey types were significant.

Water Samples

All water samples showed the presence of rod and cocci bacteria and algal cells (Table 3). The algal cells included both fragments of filamentous algae, presumably broken off of the aquarium walls, and unicellular phytoplankton. Filamentous algae fragments were small, rarely having more than three or four cells in a strand. A significantly larger number of bacteria were present in the water during feeding behavior than when no feeding was taking place (rods $P = 0.002$, cocci $P < 0.001$). However, there was no significant difference between the number of algal cells present during feeding and post-feeding behavior ($P = 0.4$). There were substantially fewer algal cells present in the water than bacterial cells of either type.

DISCUSSION

Feeding Actions

Prior to this study, most research focused on the mechanisms for particle encounter and retention and the diets of suspension-feeding fishes, rather than on the actions involved in food capture. Different broad categories of feeding behavior, or feeding modes, have been noted previously for some suspension-feeding fishes (Janssen, 1976; 1978). Janssen (1976) identified three such modes in the alewife: filtering, gulping, and particulate feeding. Filter feeding was not size selective, and gulping and particulate feeding were size selective, non-suspension-feeding modes. However, I have found that prey type and size affect food collection and processing actions within the filter-feeding mode. Oreochromis niloticus used different frequencies of five action types in response to the four food types in this study.

The functions of the five feeding actions could be inferred from close observations of these actions in real time and in frame-by-frame analysis of video footage. Long pumps were observed to draw suspended food particles into the buccal cavity. Since these particles did not exit via the buccal or opercular cavities, the term "collecting pump" can be used to describe the function of long pumps. Since vacuums were observed to suction food and detritus from the substrate, they can be described as specialized collecting pumps used solely for benthic feeding. However, vacuums also re-suspend particles into the water column as a side effect of the benthic-feeding action.

Short pumps were characterized by a far less open mouth and less expanded buccal cavity than long pumps and caused relatively few particles visible in the water column to enter the buccal cavity, which suggested that they induced far less suction

than long pumps. Rather than collecting food from the water column, short pumps appeared to have the function of separating organic particles from inedible materials, such as gravel or sand which entered the mouth during vacuums. Through the partially open mouth during short pumps, gravel, feces, and food could be seen lying in the anterior buccal cavity, just ventral and posterior to the lower jaw. Small amounts of water entering the mouth during a short pump would wash across this material, potentially washing less dense particles upwards and to the posterior buccal cavity. Therefore, short pumps were most likely used for sorting edible particles from inedible materials that were collected together, suggesting that the term "sorting pumps" be used to distinguish these actions. Short pumps commonly intermingled with vacuums, reversals and spits, usually with inedible material visible in the anterior buccal cavity. Spits simply expelled water and unwanted matter through the mouth in a forceful burst. Reversals, however, appeared to have far more complex functions.

A backward movement of water flow in the buccal cavity had been previously mentioned in this species as a "coughing action" (Dempster, et al, 1995), but had not been fully examined prior to this study. Reversals caused a posterior to anterior movement of water within the buccal cavity while the mouth was closed (Sanderson, et al, 1996), shifting collected particles inside the cavity while preventing the loss of particles. On their own, or in close combination with short pumps, reversals may help to separate food particles from inorganic materials by re-suspending less dense organic particles within the buccal cavity. This re-suspension should aid in the retention of edible particles by providing additional opportunities for entrapment of small particles in mucus on the branchial arches. Sanderson, et al (1996) reported that O. niloticus used mucus entrapment during suspension feeding on whole and crushed flakes, and suggested that reversals also aid in the transport of mucus-bound particles to the esophagus during this form of aerosol filtration.

Therefore, reversals may be important for both mucus transport and re-suspension of food within the buccal cavity.

Reversals, or reversal-like movements, may serve these functions in other fish species as well. A "closed protrusion", a movement similar to a reversal, has been noted in suspension-feeding carp (*Cyprinus carpio*, Cyprinidae) by Sibbing *et al.* (1986). These authors also suggested that this action aids in the sorting of edible from inedible matter and in the positioning of particles within the buccal cavity. Both carp and *O. niloticus* maintain completely closed mouths during these actions. This helps to distinguish the reversal and the closed protrusion from oral winnowing, a behavior used by non-suspension-feeding surfperch species to separate edible and inedible items in the mouth (Drucker and Jensen, 1991). Winnowing involves a partially open mouth, allowing the escape of water, inedible materials, and tiny food particles. Winnowing fish are feeding on the larger particles which do not escape via the mouth.

Feeding Actions/Prey Type

Significant differences existed between prey types for all actions and between individuals for short pumps and reversals (Table 1). Fish 1 was anomalous, showing fewer actions per unit time than the other two individuals for short pumps and reversals (Figure 3). However, the overall pattern of actions for the prey types remained consistent so that the individual differences did not mask the differences between prey types (Figure 3).

The greatest differences within actions took place with microscopic prey. Long pumps occurred significantly more frequently during feeding on microscopic prey than during feeding on any other prey type (Table 2). There were significantly fewer occurrences of the other four actions (short pumps, vacuums, spits, and reversals) during microscopic feeding versus feeding on the other prey types (Table 2, Figure 3). Long pumps may have occurred more often with microscopic prey since

these particles were suspended in the water column, where this action was used for collection of food.

The feeding on microscopic food was spontaneous, with no bacteria or algal suspension introduced into the aquarium to induce feeding. In *O. niloticus*, bacteria and other microscopic food are probably retained in mucus on the branchial arches (Sanderson, et al., 1996), but during feeding on microscopic prey many long pumps were apparently needed before reversals became necessary for the transport of mucus to the esophagus (Figure 3). Relatively few short pumps and spits were apparently needed during feeding on microscopic prey, possibly because large inedible particles such as gravel were not engulfed. Likewise, vacuums to stir up food particles from the substrate for continued suspension feeding were least frequent during microscopic feeding (Table 2, Figure 3).

All non-microscopic prey types could be observed to fall to the substrate fairly rapidly, so that water column feeding could occur only during the first few minutes after introduction of the food. After that, feeding had to take place on the substrate, leading to a greater number of short pumps, vacuums, spits and reversals for non-microscopic prey types relative to microscopic prey. The significantly lower number of spits for shrimp versus whole flake or crushed flake slurry prey types may be related to the ease with which the prey were retrieved from the substrate. Whole flake and crushed flake slurry collected inside crevices in the gravel as well as in a thin two-dimensional layer on the substrate. During vacuums, gravel was often collected with the flake or slurry particles, which may have led to the high number of spits to clear the gravel from the buccal cavity. The adult brine shrimp, having a three-dimensional shape, did not sink down into the gravel crevices, which may have led to less gravel collection and a reduced number of spits. However, short pumps did not differ significantly between prey types, suggesting more research is needed to clarify the uses of this action.

Water Samples

The significantly higher numbers of rod and cocci bacteria in the water column during microscopic feeding compared to post-feeding periods (Table 3) are consistent with reports that O. niloticus can detect and suspension feed on bacteria (Beveridge et al. 1989). The bacteria concentrations found in the aquaria during feeding and post-feeding periods were within reported ranges for natural oligotrophic and mesotrophic lakes (Kusenzow, 1970 in Rheinheimer, 1992). Since at least some forms of bacteria form a food source for this species (Bowen, 1982; Fryer and Iles, 1972), the fish may have mechanisms for the detection of bacteria in high enough concentrations to make feeding on them energetically efficient. These specimens do appear to respond to bacterial concentration in the water since they do not feed continuously, but at specific times. The intervals of two to four hours that elapsed between the introduction of prey (flake or shrimp) and later spontaneous feeding on microscopic prey in the water column may have reflected the time required for enough bacteria to reproduce for feeding to be worthwhile for these fish. Ingestion rates increase with particle concentration (microspheres and zooplankton) in another suspension-feeding cichlid, Tilapia galilaea (Drenner et al. 1987). This assumes that the bacteria are using any uneaten food as an energy source, resulting in an increased concentration of bacteria that is detectable by the fish. Spontaneous filtering of the aquarium water was not merely a function of hunger level since the fish filtered spontaneously two to four hours after consuming external food but were never observed to filter spontaneously prior to the introduction of external food.

The small number of reversals compared to long pumps during microscopic feeding indicated that mucus was positioned and swallowed only after a considerable time spent collecting, presumably after a large number of particles had bound to the mucus (Figure 3). This suggests that there may be a threshold quantity of particles bound to the mucus before mucus transport is stimulated, but that threshold remains

unknown at this time. Oreochromis niloticus can adjust mucus production in response to particle presence and size (Sanderson et al. 1996) and now appears to likewise behaviorally regulate mucus transport and consumption through reversal frequency.

There was no relationship between concentration of algal cells and the occurrence of spontaneous suspension feeding (Table 3). Oreochromis niloticus feed on phytoplankton and diatoms in the wild (Moriarty and Moriarty, 1973; Onyari, 1983; Getachew, 1993) and in the laboratory this species has been shown to consume periphytic cyanobacteria (Dempster et al. 1993) and planktonic cyanobacteria and algae (Northcott et al. 1991; Beveridge et al. 1993; Keshavanath et al. 1994; Robinson et al. 1995). Dempster et al. (1995) showed by bioenergetic modeling that four tilapia species (including O. niloticus) cannot maintain their weight solely by filter feeding on phytoplankton. I propose that there was too little suspended algae to stimulate feeding without a corresponding increase in the concentration of bacterial cells. In other words, algal cell numbers rose and fell within too small a range and with too few cells overall to stimulate suspension feeding. However, when bacteria levels reached the point where feeding occurred, then algal cells were undoubtedly also consumed simply by being present in the water being filtered. Another possibility is that the algal particles involved were too small to stimulate feeding, since it has been shown that O. niloticus feeding on planktonic algae increase feeding rates with particle size (Northcott et al. 1991; Robinson et al. 1995).

CONCLUSION

O. niloticus consumes prey items from bacteria (~1 μm) to flake food (3-10 mm diameter) via filter feeding, using five actions: long pumps, short pumps, vacuums, spits, and reversals. The frequencies of feeding actions change in response to the type and concentration of prey being captured and the amount of processing required (separation from inorganic particles, mucus transport). These findings should stimulate further research, since the frequencies of feeding actions could have effects on particle encounter and retention methods and sites within the buccal cavity.

Further studies are needed to determine the concentration of food, especially bacteria, necessary for detection by the fish and for stimulation of feeding. An analysis of the varied feeding patterns of pumps and reversals in microscopic feeding is also needed. Similar studies in more species of suspension feeders will help to clarify the functions of the above described feeding actions. Other uses for the reversal action would be especially interesting; for instance, if mouth brooding specimens use reversals to reorganize or aerate eggs in the buccal cavity. Further clarification is also needed of any interactions between mucus processing and the described feeding actions.

APPENDIX

FIGURE LEGENDS

Figure 1. A representative pump, traced from videotape at intervals of 0.13 seconds (4 frames). Arrows indicate (1) maximum premaxillary protrusion, (2) maximum hyoid abduction, and (3) maximum opercular abduction. Opercular stippling indicates opercular abduction. (a) pre-pump appearance with opercula and hyoid adducting, (b) mouth opening, oral valve retracts, and premaxillae begin to protrude, (c) mouth opens further, hyoid abducting, (d) pump apex: mouth wide, premaxillae extended, and hyoid and opercula abducting, (e) mouth closing, hyoid and opercula fully abducted, (f) mouth closed, hyoid adducting, water expelled through opercular opening.

Figure 2. A representative reversal, traced from videotape at intervals of 0.067 seconds (2 frames). Arrows indicate (1) maximum premaxillary protrusion, (2) maximum hyoid abduction, and (3) maximum opercular abduction. Opercular stippling indicates opercular abduction. (a) end of previous action: hyoid slightly abducted, (b) pre-reversal: opercula abduct, hyoid fully adducted, (c) stage 1: opercula rapidly adduct, hyoid abducts, and premaxillae begin rapid protrusion, (d) premaxillae reach maximum protrusion, (e) stage 2: premaxillae retracting, hyoid adducting, opercula abducting, (f) hyoid adducts to pre-reversal position.

Figure 3. The total number of actions per thirty minute observation of each prey type. X-axis: the number of actions. Y-axis: action types are listed by individual fish (1, 2, 3).

Figure 1

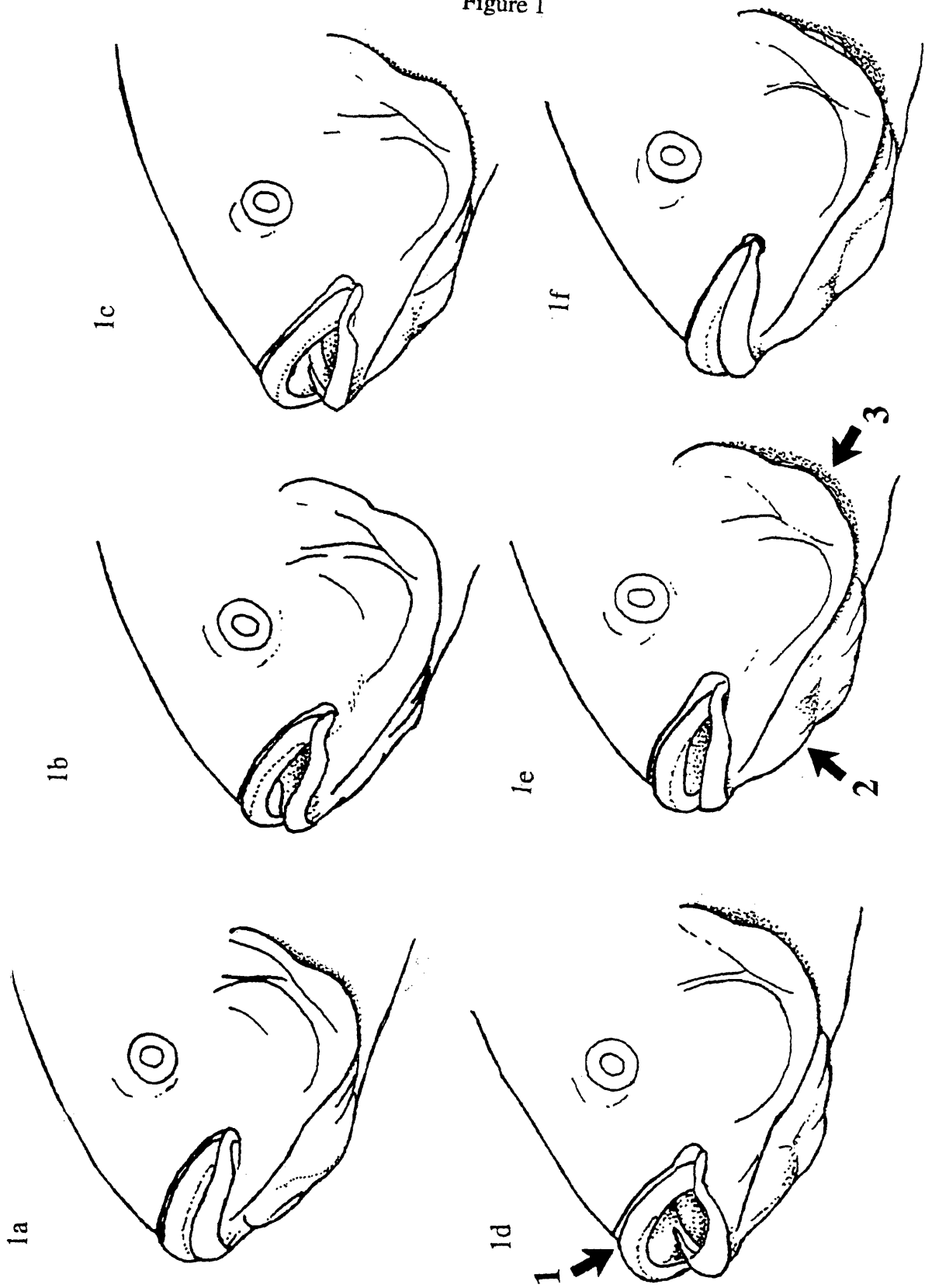


Figure 2

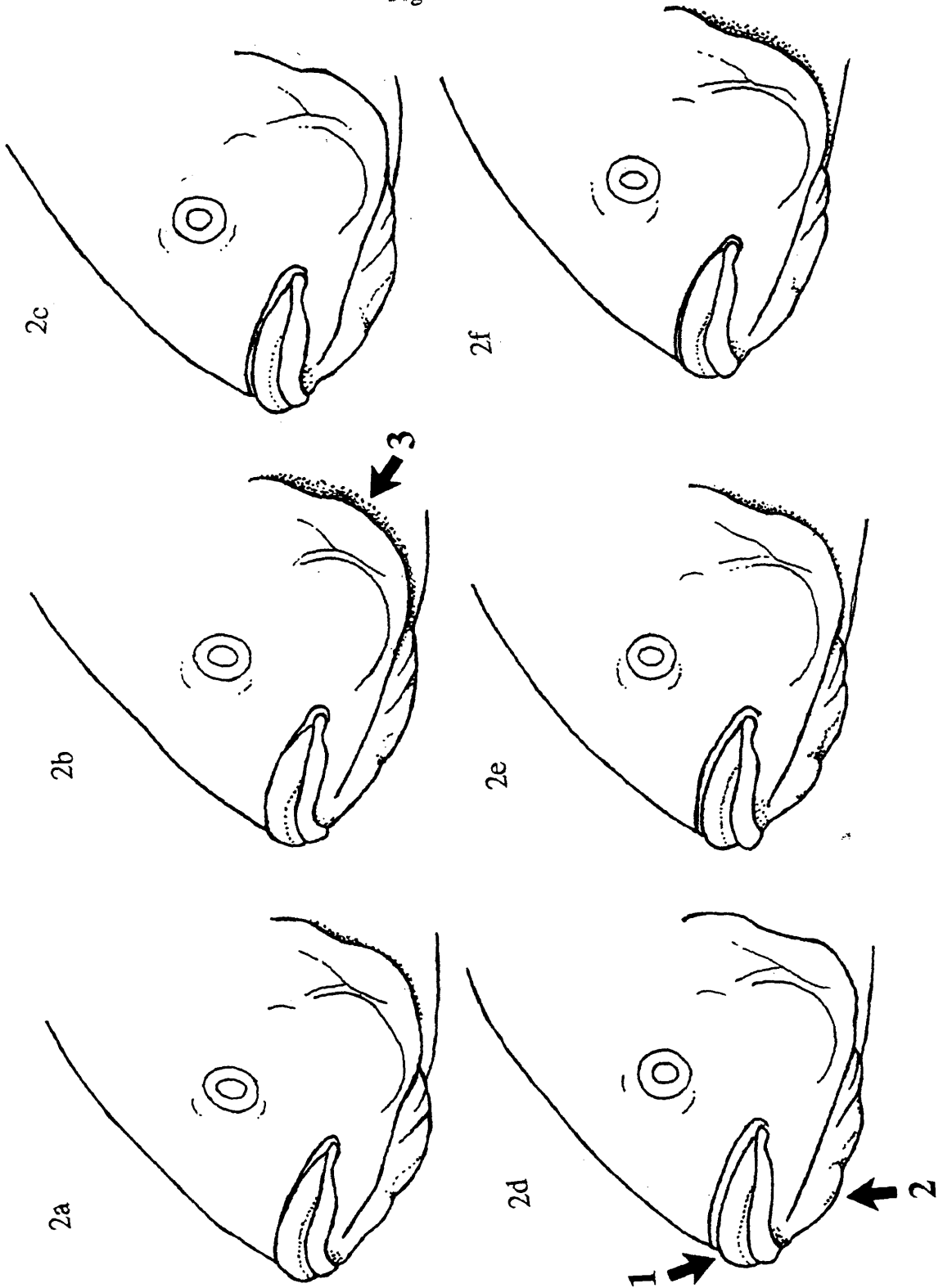


Table 1. One-way ANOVAs of prey type and individual fish during feeding actions observed over 30 minutes (a) long pumps, (b) short pumps, (c) vacuums, (d) spits, and (e) reversals.

(a)					
Source	df	SS	MS	F	<i>P</i>
prey type	3	262.7	87.6	5.1	0.04
individual	2	21.6	10.8	0.6	0.6
(b)					
Source	df	SS	MS	F	<i>P</i>
prey type	3	180.3	60.1	21.9	0.001
individual	2	88.0	44.0	16.03	0.004
(c)					
Source	df	SS	MS	F	<i>P</i>
prey type	3	861.05	287.02	29.7	0.0005
individual	2	72.4	36.2	3.7	0.09
(d)					
Source	df	SS	MS	F	<i>P</i>
prey type	3	203.5	67.8	11.6	0.007
individual	2	28.8	14.4	2.5	0.2
(e)					
Source	df	SS	MS	F	<i>P</i>
prey type	3	487.8	162.6	47.7	0.0001
individual	2	80.4	40.2	11.8	0.008

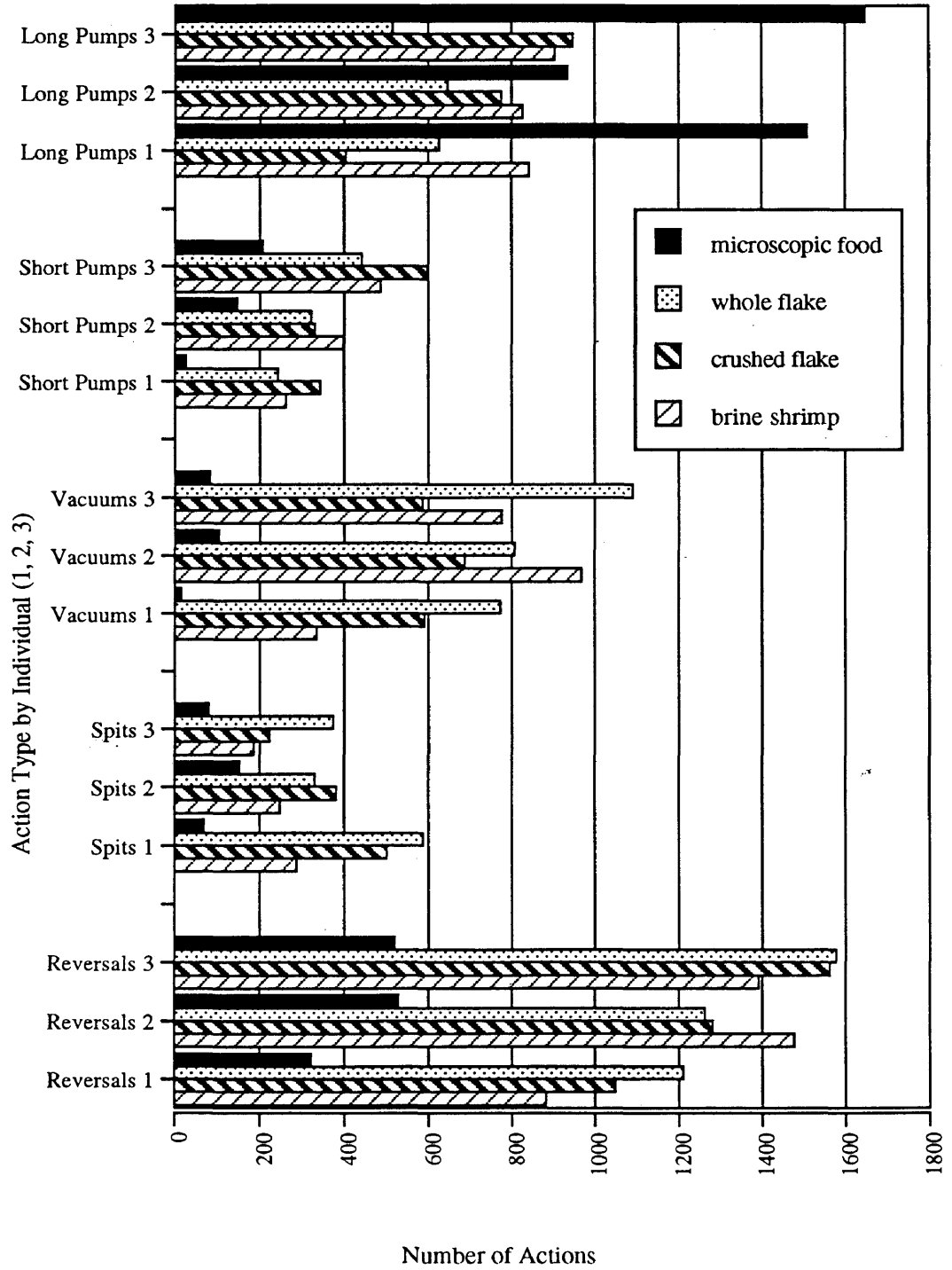
Table 2. Unplanned contrasts of prey types for each action (n. s. = > 0.05). m = microscopic food, f = whole flake, c = crushed flake, and s = brine shrimp.

Action	contrast	prob > t	prob > F
Long Pumps	m vs. f, c, s	0.01	0.04
	f vs. c, s	n. s.	
	c vs. s	n. s.	
Short Pumps	m vs. f, c, s	0.0002	0.001
	f vs. c, s	n. s.	
	c vs. s	n. s.	
Vacuums	m vs. f, c, s	< 0.0001	0.0005
	f vs. c, s	n. s.	
	c vs. s	n. s.	
Spits	m vs. f, c, s	0.002	0.007
	s vs. f, c	0.04	
	f vs. c	n. s.	
Reversals	m vs. f, c, s	< 0.0001	< 0.0001
	s vs. f, c	n. s.	
	f vs. c	n. s.	

Table 3. Results of paired t-tests comparing the number of cells counted in water samples taken during and after periods of filter feeding on microscopic prey.

Cell type feeding or post-feeding	Number of cells/milliliter (mean \pm SD, N = 3)	
Bacteria, rod		
feeding	178546 \pm 48385	<i>P</i> = 0.002
post-feeding	51528 \pm 11387	
Bacteria, cocci		
feeding	688261 \pm 234404	<i>P</i> < 0.001
post-feeding	160893 \pm 61794	
Algae		
feeding	2863 \pm 900	<i>P</i> = 0.4
post-feeding	3605 \pm 1180	

Figure 3



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