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## Fluid Dynamics in Suspension-Feeding Blackfish

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Measurements of flow patterns and water velocities inside the oral cavity of blackfish (Orthodon microlepidotus), made with a fiberoptic endoscope and thermistor flow probe, revealed that gill-arch structures act in blackfish as barriers that direct particle-laden water to the mucus-covered roof of the oral cavity, where particles are retained. Gill-arch structures have previously been assumed to be the site of particle retention in suspension-feeding fishes. Water does not pass between these structures in blackfish, and they do not serve as filters that separate particles from the water. These results emphasize the importance of directly assessing flow velocity and direction inside the oral cavity of vertebrate suspension feeders, particularly at the level of the filtering elements.

USPENSION-FEEDING FISHES FILTER large volumes of water past their feeding apparatus to capture minute planktonic prey. They belong to at least 12 families in nine orders (1) and make up approximately one-third of the world fish catch (2). Despite the ecological and economic importance of these fishes, hypotheses about mechanisms of particle entrapment inside their mouths have rarely been tested, and there have been no direct observations of particle retention. Most workers have assumed that rows of cartilaginous protuberances on the gill arches (for example, gill rakers, branchiospinules) act as the filter in suspension-feeding fishes (3). These structures may operate as a sieve, retaining particles larger than the pore size of the mesh (4). If the gill-arch structures have a sticky mucus coating, particles small enough to pass between the mesh may be trapped by direct interception, inertial impaction, gravitational deposition, or electrostatic entrapment (5). By developing a quantitative experimental approach, we have documented an unexpected mechanism of fish suspension feeding. The techniques we used for flow

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ed to study the function of internal feeding structures in a diversity of animals, including suspension-feeding birds and bivalves. Like other suspension-feeding species in

the family Cyprinidae, blackfish (Orthodon microlepidotus) use a series of suctions to engulf water in a pulsatile fashion. It is thought that particles captured on gill rakers are then transported by water currents to the

visualization and measurement can be adapt-

esophagus during a processing phase (6). We obtained images of particle movements and measured water velocities inside the mouths of three adult blackfish [32 to 33 cm standard length (7)] using a fiberoptic endoscope and thermistor flow probe (8) threaded through a cannula implanted in the hyomandibula or neurocranium. The unrestrained fish fed freely on a mixture of dead brine shrimp adults (4 mm long) or brine shrimp nauplii (400 to 500 µm long), hydrated brine shrimp cysts (250 µm across), and polystyrene microspheres (40 to 80 µm across).

By inserting the tips of the endoscope and thermistor less than 1.5 mm into the oral cavity through the hyomandibula cannula, we positioned the instruments above the channel between the lateral and medial rows of rakers on the ventral region (ceratobranchial) of gill arch I (Fig. 1, location B). Insertion through the neurocranium provided access to the ceratobranchial of gill arch II (Fig. 1, location C). Videotapes of the endoscopic images confirmed that particles were not entrained by any eddies around the instrument tips. Videotaped sequences were selected at random for detailed analysis.

While the fish were feeding, only 3 of 228 particles that entered the field of view (3 to 4 mm in diameter) were actually retained by the gill rakers (Fig. 2, left); the remainder of the particles traveled across the field of view in the channel between the rows of rakers. In all, 84% of the particles (n = 168) were carried dorsally toward the roof of the oral cavity, whereas 16% were transported ventrally toward the floor. Almost all particles



instruments were inserted (A, B, C, and D). (Left) Sagittal section. At location B, instruments were inserted at a right angle to the plane of the photograph. The width of the photograph is 11 cm. (Right) Head-on view of open blackfish mouth with lower jaw removed. After water and

particles entered the mouth (open arrows), particles (dots) were observed to travel (solid arrows) along gill arches and palatal organ. Arrows at left of figure indicate that water exiting between the ventral regions (ceratobranchials) of the gill arches is not filtered. Filtered water exits between the dorsal regions (epibranchials) of the gill arches (not visible). The maximum width of the head is 5 cm. [Left photograph by L. Matteson; right photograph by L. Matteson and M. Massingill]

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Fig. 2. (Left) Unusual endoscopic image taken through the hyomandibular cannula (Fig. 1, location B) showing a particle ( $\sim 80 \ \mu m$  in diameter) retained by blackfish gill rakers (jagged shapes in right background). Most particles were observed to travel rapidly along the channel between the two rows of gill rakers without contacting the rakers. In order to provide an unobstructed view of the rakers, endoscopic videotapes were also taken without the thermistor flow probe inserted. (**Right**) Typical endoscopic image of the anterior



pharynx (Fig. 1, location D) showing particles ( $\sim$ 40 to 250 µm in diameter) aggregated in mucus along the palatal organ and postlingual organ. The endoscope was retracted slightly in the cannula; the curved edge of the cannula can be seen passing through the center of the image.

were moving individually; only one aggregated clump, containing seven particles, was observed. Because most of the particles moved up the channel as the fish were pumping water into their mouths, only a few passed the endoscope during the processing phase. Videotapes of the anterior pharynx [Fig. 1, location D, along the mucus-covered palatal organ and postlingual organ described in (9)] showed that many particles were in the form of aggregated clumps, apparently bound together with mucus, as they moved toward the esophagus during both the pumping and processing phases (Fig. 2, right).

As the signal from the thermistor flow probe was displayed on an oscilloscope in real time, we determined that the flow along the channel between the lateral and medial rows of rakers cyclically increased when the hyomandibula and gill arches abducted after the mouth opened, then decreased when they adducted [Fig. 3 (10)]. If the gill rakers acted like a sieve, flow velocity would be expected to increase during gill arch adduction as the sieve pore area decreased (11). Flow velocity along the channel was approximately half of the velocity measured at the entrance to the mouth (Fig. 1, location A), but was comparable to that along the palatal



**Fig. 3.** Flow velocity in the channel between the two rows of gill rakers on gill arch I (Fig. 1, location B) in a suspension-feeding blackfish.

organ (Table 1).

On the basis of these data, we propose a model in which particle-laden water travels rapidly along the gill arches to the lateral margins of the palatal organ (Fig. 1); particles are trapped on the palatal organ as water exits the oral cavity between the dorsal regions of the gill arches (epibranchials). Capture of suspended particles involves their transfer from a through current passing between the epibranchials to a surface current along the palatal organ. This situation is analogous to that hypothesized in suspension-feeding bivalves, in which complex flow patterns and steep velocity gradients

**Table 1.** Flow velocities (cm/s) at four locations in the oral cavity of three suspension feeding blackfish. The mean peak velocity in each feeding bout was calculated, then the mean of these values (given below) was calculated for each specimen. Locations A, B, C, and D are illustrated in Fig. 1. Numbers in parentheses are the total number of feeding bouts and the total number of peaks.

		-	-
Location	Fish	Mean peak velocity $\pm 1$ SD	Range
A	X	$118.3 \pm 11.3$ (7, 328)	101.4 to 133.9
A	r Z	$141.2 \pm 13.9 (12, 41)$ 136.3 + 11.8 (7 66)	104.8 to 147.0
	2		111.0 to 110.1
В	Х	$61.8 \pm 6.8 (9, 118)$	53.2 to 73.6
В	Y	$54.3 \pm 13.4$ (8, 73)	35.4 to 72.4
В	Z	$56.0 \pm 9.1(5, 108)$	48.7 to 70.2
С	X	$40.0 \pm 7.4 (9, 110)$	28.9 to 52.9
D	Х	$64.7 \pm 13.1 (8, 90)$	39.8 to 80.9
D	Y	$44.2 \pm 8.2 (6, 70)$	32.6 to 57.4
D	Z	$64.2 \pm 25.6 (8, 90)$	25.0 to 103.4

are generated where two feeding current systems intersect (12). If this model is correct, water that escapes between the ceratobranchials of the gill arches will not be filtered.

Our results are not consistent with evidence from x-ray films of suspension-feeding bream (Cyprinidae) (13, 14). The films indicated that 25% of individual Daphnia with a 1-mm-diameter iron sphere glued to their carapace were retained in the spaces between the gill rakers. Hoogenboezem et al. proposed a model (14) in which lateral rakers of bream move actively into the spaces between medial rakers on the adjacent arch, forming a sieve with an adjustable mesh. At no time did we observe blackfish gill rakers moving independently of the gill arches, and the gill rakers did not act as a sieve. Blackfish, unlike bream, are able to retain particles as small as 10 to 25 µm (15). Direct interception of minute particles on a nonporous mucus layer in blackfish circumvents the problems of forcing water through finely spaced filtering elements or of losing large numbers of small particles through more widely spaced elements. Endoscopic images and flow velocities obtained from the oral cavity of bream would establish whether different filtration mechanisms have evolved in confamilial species that possess the same basic filtering structures. Alternatively, many suspension-feeding cyprinids may use the mechanism described for blackfish, and the interspecific differences in size of particle retained may result from differences in the rugose morphology of the palatal organ and the viscosity of the mucus produced (16).

In most sardines and herring (Clupeidae) and anchovies (Engraulidae) that ram suspension feed by swimming forward with an open mouth, a number of gill arches meet in the dorsal midline, and this geometry allows the gill slits and rakers to extend farther medially than in other fishes (17). In the absence of an expansive mucus-covered roof, the filtration mechanism described for blackfish cannot operate. However, the unexpected fluid dynamics that we observed in the blackfish oral cavity prompt an examination of flow patterns and velocities in ram suspension feeders. The entire process of particle capture and transport to the epibranchial organs located dorsal to the esophagus in such fishes (18) may be mediated by water currents directed passively by the morphology of the branchial apparatus itself as the fish swims forward. Mathematical models of fluid motion at the level of the gill rakers will allow prediction of the flow velocities and raker morphologies that are associated with the functioning of rakers as barriers versus filters (19).

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## A Molecular Genetic Classification of Zooxanthellae and the Evolution of Animal-Algal Symbioses

## **ROB ROWAN AND DENNIS A. POWERS**

Zooxanthellae are unicellular algae that occur as endosymbionts in many hundreds of marine invertebrate species. Because zooxanthellae have traditionally been difficult to classify, little is known about the natural history of these symbioses. Zooxanthellae were isolated from 131 individuals in 22 host taxa and characterized by the use of restriction fragment length polymorphisms (RFLPs) in nuclear genes that encode small ribosomal subunit RNA (ssRNA). Six algal RFLPs, distributed host species specifically, were detected. Individual hosts contained one algal RFLP. Zooxanthella phylogenetic relationships were estimated from 22 algal ssRNA sequences-one from each host species. Closely related algae were found in dissimilar hosts, suggesting that animal and algal lineages have maintained a flexible evolutionary relation with each other.

NIMAL-ALGAL SYMBIOSES ARE UBIQuitous and typically dominant features of shallow tropical seas. Of the several kinds of unicellular algal participants (1), the most abundant are coccoid, yellowbrown dinoflagellates that are known as zooxanthellae (2, 3). Photosynthetic production by these symbioses, especially the reef-building corals, is largely responsible for the life and growth of tropical reef communities (4). Zooxanthella hosts include various invertebrate taxa, but zooxanthella diversity (3, 5-7) is far less obvious and has been difficult to evaluate. Most zooxanthellae are referred to the genus Symbiodinium Freudenthal (3, 8), which presently includes four species described as in vitro cultures and many cryptic forms (8, 9). Symbiodinium taxonomy has been hindered by a paucity of informative morphology, especially in the vegetative (symbiotic) state (10), by the possibility of host-associated phenotypic plasticity (5), by difficulties in obtaining in vitro cultures (5), and by the absence of sex, which precludes formal genetic analyses (3). The poor status of Symbiodinium taxonomy has limited our understanding of symbiosis ecology and evolution. We classified 22 zooxanthella isolates using the polymerase chain reaction (PCR) (11) to amplify small ribosomal subunit RNA (ssRNA) genes from Symbiodinium (12), and ssRNA sequence data.

Symbiodinium-like zooxanthellae were isolated from hosts (Table 1) and their nuclearencoded ssRNA genes were PCR-amplified with zooxanthella-specific primers as de-

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scribed (12). The amplified DNA, which corresponds to the ssRNA molecule less 103 nucleotides at either end (12), was first analyzed with restriction enzymes. Three common and three unique Taq I digestion patterns [restriction fragment length polymorphisms (RFLPs)] were observed (Fig. 1). Tag I RFLPs A and B are predicted by the ssRNA sequences from cultured Symbiodinium isolates (13), and all six RFLPs can be related to one another by a single Taq I site gain or loss (14). This implies that individual host samples contain only one of six detectable zooxanthella genotypes (15). Zooxanthellae obtained from different individuals of the same host species (16) always exhibited the same RFLP genotype (in a total of 129 algal isolates from 20 host taxa collected from nature) (Table 1). Since these genotypes apparently represent biologically distinct algae that associate specifically with one or more host species, algal taxonomy was investigated in greater detail.

Phylogenetic relationships among zooxanthellae were estimated from ssRNA gene sequences. Four cultured (13) and 18 fresh isolates were compared. PCR-amplified DNA was digested with Xba I and cloned into the vector M13mp18 (17), and two relatively variable regions (13) were sequenced, yielding a total of 472 to 476 nucleotide positions from each isolate. These sequences are obtained from a single Xba I fragment and should represent about one-half of the variability contained in entire Symbiodinium ssRNA molecules (13). Sequences were identical to one another or differed by up to 35 nucleotides in 83

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