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Role of vision in the aggregative behavior of the planktonic mysid *Mysidium columbiae*

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Abstract Aggregations of the planktonic mysid *Mysidium columbiae* use vision to help maintain position within mangrove prop-root habitats and to maintain spacing within schools. Laboratory studies show that mysids videotaped in darkness using infrared illumination did not form schools and could not hold position in currents. In daylight, mysids more effectively held position in a flow-through chamber with a high-contrast visual reference than in its absence. The compound eyes of mysids are thought to be good motion detectors, but little is known about their visual acuity or sensitivity. An optokinetic drum was used to test the visual acuity and photosensitivity of mysids using the behavioral response of mysids to vertical black and white stripes that move past their field of view. When the drum rotates, the mysids swim at the same speed and in the same direction as the moving stripes. Swimming speed of the mysids was measured to compare their speed to the turning rate of the drum using video-computer motion-analysis techniques. Detection of the moving stripes was also inferred from the proportion of mysids that followed the stripes and that reversed direction when the rotation of the drum was reversed. By varying the width of these stripes, the visual acuity of the mysids was determined. The ability of *M. columbiae* to follow stripes of 1 mm in width from a distance of 15 to 30 mm indicates that mysids can visually resolve nearby prop roots and other mysids within schools. The photosensitivity threshold for the optokinetic response was found to be 0.001 μm photons m^{-2} s^{-1}, similar to light levels during moonlight. These mysids are potential prey to a wide range of planktivorous fish, and their survival may depend upon their ability to maintain their position within schools and within the safety of the prop-root habitat during daylight hours in spite of currents and turbulence that would tend to disperse them.

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Introduction

The heterogeneity or "patchiness" of spatial distribution of planktonic organisms has important implications for food-web trophic dynamics in the sea (Haury et al. 1978). Zooplankton patchiness can be affected by both physical forces (water movements) and biological factors (behavior, growth, reproduction, predation) (Alldredge and Hamner 1980; Hamner 1988; Haury et al. 1990). Patchiness resulting from aggregative behavior has been widely reported for mysids (e.g. Steven 1961; Clutter 1969; Mauchline 1971; Wittmann 1977; O'Brien 1988; Modlin 1990; Ritz 1994). It is clear that these aggregations are formed and maintained through the behavior and social interactions of individuals, rather than being formed simply through hydrodynamic processes (reviewed in Ritz 1994). These small "planktonic" crustaceans do not passively drift with the currents, but exhibit sophisticated behavioral capabilities. Individuals within these aggregations show distinct responses to predators and other disturbances (O'Brien and Ritz 1988; Modlin 1990) and aggregations will actively remain in preferred habitats in spite of currents and turbulence that would tend to disperse them (Buskey 1998b; Roast et al. 1998). In addition to maintaining position in preferred habitats and protecting individuals from predators (O'Brien and Ritz 1988), aggregation in mysids may also lead to more efficient feeding in a patchy food environment (Ritz 1994).

Vision appears to play a very important role in aggregative behavior of mysids. Visual cues can be transmitted between aggregated groups of mysids separated

by a transparent barrier (Steven 1961; Clutter 1969). Several studies have also found that light intensity affects the inter-individual distances between mysids within aggregations (O'Brien 1989; Modlin 1990). Mysids have refracting, superposition, compound eyes (Land 1984). This design improves photosensitivity, and although visual acuity may be limited, these eyes are thought to be excellent perceptors of movement (Waterman 1961). However, it remains unknown if the visual acuity of mysid eyes is sufficient to detect movement of individual mysids within a swarm.

While many species of mysids are associated with the benthos during the day, *Mysidium* spp. are predominantly holoplanktonic (Emery 1968; Mauchline 1980). Holoplanktonic mysids are thought to use visual cues in the maintenance of shoals and schools during daylight hours (Clutter 1969; O'Brien 1988). The holoplanktonic mysid *Mysidium columbiae* is usually found in the shaded areas near mangroves (Steven 1961; Modlin 1990), but it can also be found in the vicinity of coral reefs (Emery 1968). In situ video-recordings reveal that *M. columbiae* appear to use visual cues to maintain their position within the protection of the mangrove habitat in spite of currents and wave surge that would easily disperse a truly planktonic organism (Buskey 1998b). These mysids are potential prey to a wide range of planktivorous fish, and their survival may depend upon their ability to maintain position within the prop-root habitat and to remain within schools and perform complex predator-avoidance behaviors in response to visually detected predators (O'Brien and Ritz 1988).

The mysid *Mysidium columbiae* has been shown to have a strong optomotor response to high-contrast images (Buskey 1998b). Optomotor responses, in which movements of the body compensate for the displacement of images over the eye, are well known in higher crustaceans and fishes (e.g. Fraenkel and Gunn 1940 for mysids, Pankhurst et al. 1993 for fishes). These responses can be investigated using an optokinetic drum, constructed of vertically oriented drums with vertical black and white stripes that are rotated around the outside and inside walls of a transparent, ring-shaped trough containing the experimental animals. In this study, the visual acuity of the mysid *M. columbiae* was investigated by varying the stripe width, finding the minimum stripe width that elicits a response, and determining the angle subtended by these stripes (Waterman 1961). This information allows calculation of the maximum distance at which objects of various size, including other mysids within a school, can be visually detected in clear water. The photosensitivity of these mysids was also investigated by lowering the light intensity under conditions that elicit a strong optokinetic response, providing information on the range of light intensities over which mysids are capable of vision. The role of vision in the ability of *M. columbiae* to maintain position within the mangrove prop-root environment was also examined in the laboratory by comparing the ability of mysids to hold position in currents in the presence and absence of high-contrast visual reference cues.

Materials and methods

Field studies were carried out at the Smithsonian Institution's field station on Carrie Bow Cay in Belize. Mysids for laboratory study were collected in Twin Bays of Twin Cays, a pair of mangrove covered islands ca. 2 km to the NW of the laboratory. Fresh mysids were captured each day between 8:00 and 10:00 hrs, while snorkeling near the mangrove prop-root environment, using a hand-held aquarium net (25 × 18 cm opening). Mysids were immediately transferred to a large insulated jug filled with seawater and taken back to the laboratory on Carrie Bow Cay. At the laboratory on Carrie Bow Cay, the mysids were held in large rectangular plastic trays (60 × 40 × 20 cm) filled with fresh seawater. All experiments were performed on mysids the same day that they were collected from the field.

To assess the possible role of vision in the aggregative behavior of *Mysidium columbiae*, several groups of ca. 100 mysids were videotaped for 10 min each in a clear plastic aquarium (30 × 10 × 20 cm), first during the day in diffuse sunlight (ca. 20 μM photons m^{-2} s^{-1}, measured with a LICOR LI-250 photometer). The same mysids were videotaped again at night for 10 min using dark-field illumination from a 20 cm-diam. ring of infrared light-emitting diodes (Optek OP-293 A, emitting 825 ± 25 nm) to observe their behavior in the absence of visible light. Mysid swimming was videotaped in the vertical plane with a field of view of ca. 15 × 15 cm. These observations were repeated on three days with different groups of mysids. The density of mysids used in these experiments was lower (ca. 17 mysids l^{-1}) than that used in the experiments described below (ca. 60 mysids l^{-1}). This provided the mysids with more free space to interact so that the tendency to form aggregations would not be affected by confinement.

The ability of mysids to hold position in a current in the presence and absence of visual cues was measured using a variable speed, flow-through chamber (Buskey 1998a). The chamber was constructed of clear acrylic plastic, with overall outside dimensions of 17 × 8 × 8 cm; the observation chamber had inside dimensions of 7 × 7 × 7 cm. Seawater collected at the end of the flow-through chamber was pumped to the forward section of the chamber by a Rule 360 bilge pump. To make the flow more uniform, water passed through a plastic grate (3 mm-square openings) and a bed of small pebbles (ca. 1 cm diam.) before entering the center of the observation chamber. A wall of 153 μm mesh screening on each end retained the mysids within the center portion of the flow-through chamber. Water was pumped between sections through a stainless steel return pipe, which served as a heat exchanger to keep the water temperature constant. The chamber was placed in a flow-through water bath at 28 °C. Pump speed was controlled by varying the electrical current to the pump. Temperature of the water within the flow-through chamber and in the water bath were monitored with bead-type thermistors using a two-channel Omega Model 747 digital thermistor thermometer.

In the first set of flow experiments the swimming behavior of a group of ca. 20 mysids swimming in the light (ca. 10 μmol photons m^{-2} s^{-1}) was videotaped from the side in the vertical plane (field of view ca. 6 × 6 cm) at a flow speed of ca. 10 mm s^{-1} for 5 min. This same group of mysids was then allowed to adapt to darkness for 1 h in the absence of a current. The mysids were then videotaped again for 5 min in darkness at a flow speed of 10 mm s^{-1}, using infrared dark-field illumination to record their behavior in the dark. This was repeated with eight different groups of mysids on different days. In the second set of flow experiments, mysids were filmed in the flow-through chamber in the light, first with no added visual cues other than those existing in the room and visible through the walls of the clear acrylic chamber, and then with 11 mm black and white vertical stripes placed on the sides of the containers. This was repeated with eight different groups of mysids.

For these experiments the mysids were videotaped in the horizontal plane from above. In both experiments the objective was to judge the ability of the mysids to hold position in a current, so their movement relative to a fixed position was determined, not their true swimming speed in the moving water.

An optokinetic drum was used to test the visual acuity and photosensitivity of *Mysidium columbiae* (Fig. 1). An optokinetic drum is used to move black and white vertical stripes past the fields of view of the mysids. Previous experiments with a simple optokinetic drum design (stripes only visible on the outside edge of the cylindrical raceway) indicated a strong optokinetic response of mysids to 11 mm-width stripes (Buskey 1998b). Motion analysis of mysids swimming within the raceway of the optokinetic drum indicated that the mysids were swimming in the same direction as the stripe rotation and at approximately the same speed over a range of drum speeds from 10 to 25 mm s^{-1} (Buskey 1998b). At speeds of up to 25 mm s^{-1}, when the direction of drum rotation was reversed, the mysids would reverse their direction of swimming within a few seconds. At higher drum speeds, mysids would tend to press their eyes against the inner surface of the raceway and their swimming speeds were generally slower than that of the rotating drum. The optokinetic drum used in these experiments was built with a second smaller drum to rotate stripes in the hole of the ring-shaped raceway so that moving stripes were visible from both sides of the raceway and mysids could not avoid viewing the moving stripes.

The optokinetic drum device used in these experiments consisted of a motorized rotating drum and a clear plastic raceway for holding the mysids (Fig. 1). The circular, clear acrylic plastic raceway was constructed of two concentric cylinders of plastic (0.5 cm thick) glued to a clear plastic bottom. The inside diameter of the larger cylinder was 12.7 cm, the outside diameter of the smaller cylinder was 6.3 cm, and the height of the cylinders was 5 cm. The area between these clear walls was filled with filtered seawater and formed the circular raceway in which the mysids were free to swim. Black and white stripes of various widths moved past the inner and outer walls of this container at constant speeds. The drum consisted of two concentric plastic cylinders, the outer one was 16 cm in diameter and the inner one was 4 cm in diameter; this allowed for a space of ca. 1 cm between the raceway and the drums at both the inner and outer surfaces. The two cylinders of the optokinetic drum had white inner and outer surfaces connected at the top by a clear circle of acrylic plastic. Computer-generated black stripes of various widths were printed on white paper with a laser printer and photocopied onto clear plastic acetate sheets. These sheets were taped to the inside surface of the larger drum and the outer surface of the smaller drum. The drum was rotated by a low rpm, variable-speed, reversible electric motor. The motor had a large gear on its shaft which rotated the drum using a timing belt glued to the outside of the drum. The drum rotated on three bearings mounted on an acrylic plastic base. Drum speed was regulated by varying the voltage to the motor with a potentiometer. Drum direction was reversed by reversing the polarity of the current to the motor. Since the speed of revolution increases with increased distance from the center of the drum, the stripes on the outer drum are moving faster than those on the inner drum. At a drum speed of 2 rpm, the stripes on the outer drum move at a speed of 16.7 mm s^{-1} but move past the inner surface of the outer wall of the raceway at a speed of ca. 13.3 mm s^{-1}, and the stripes of the inner drum move at a speed of 4.2 mm s^{-1} and move past the inner surface of the inner wall at a speed of 6.6 mm s^{-1}. To keep pace with the stripes, mysids near the outer wall of the raceway must swim faster than mysids near the inner wall, and mysids keeping pace with the stripes would have a speed between 6.6 and 13.3 mm s^{-1}.

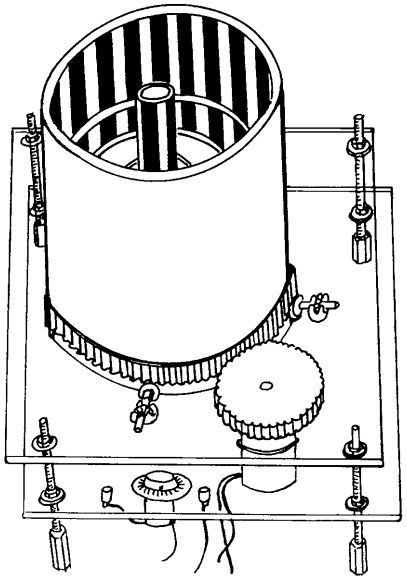

Fig. 1 Optokinetic drum. Drum assembly consisted of connected outer and inner cylinders to which black and white vertical stripes were attached. Transparent, ring-shaped raceway filled with seawater was placed between the cylinders and provided area in which mysids could swim and respond to visual stimuli. Drum was supported on bearings to allow it to turn freely, and its rotation was powered by a variable-speed electric motor fitted with a large-diameter gear

In all the optokinetic drum experiments described below, groups of ≃30 to 40 mysids were collected from holding tanks by concentrating them in a small volume with an aquarium net and then transferring them to the raceway using a large plastic pipette (21 cm in length with an 0.6 cm opening). The raceway was filled with filtered seawater and was placed within the optokinetic drum for 10 min before experiments began, allowing the mysids to adapt to their new surroundings.

Since behavioral parameters such as swimming speed of the mysids were used to test their visual acuity and photosensitivity, control experiments were run to quantify the swimming behavior of mysids within the experimental chamber and to assess the effects of drum rotation and the presence of vertical stripes on their swimming behavior. The first control experiment was designed to test if there was any change in mysid behavior due to vibrations associated with the motion of the optokinetic drum. A group of mysids was videotaped for 2 min while swimming in the raceway within the optokinetic drum with no stripes on the drum and no motion of the drum. The drum was then rotated at a speed of 2 rpm and then the same group of mysids was again videotaped for 2 min. This experiment was repeated with eight groups of mysids. The second control experiment was designed to test the effects of stationary stripes on mysid behavior within the optokinetic drum. In this experiment, groups of mysids were videotaped for two min with 11 mm black stripes taped to the white inner and outer surfaces of the optokinetic drum with no drum rotation. These stripes were then gently removed and the behavior of the same group of mysids taped again for 2 min with no stripes and no drum rotation.

To test the visual acuity of the mysids, they were exposed to alternating vertical black and white stripes of 11, 8, 6, 4, 3, 2, 1.5, 1, 0.75 and 0.5 mm width. Experiments were run in diffuse daylight supplemented with light from a fiber-optic illuminator diffused through a ground-glass filter, to provide a light intensity of 20 μmol photons m^{-2} s^{-1}. Approximately 30 to 40 adult mysids were placed in the drum and allowed to adapt for a period of 5 min in the absence of the stripes. The behavior of these mysids was then videotaped for 2 min in the absence of drum motion and stripes. Then the drum was fitted with a particular width of stripe, and was gently put in place and rotated at a speed of 2 rpm. The drum was allowed to rotate for 5 min before video-recording; following the adaptation period, the swimming behavior of the mysids was recorded for 2 min. The direction of the drum was then reversed for 5 min and, following this adaptation period, the swimming behavior of the mysids was videotaped again for 1 min. A new set of mysids was used in each experiment. Each stripe width was tested with 4 to 10 sets of mysids. Mysids were videotaped in the

horizontal plane from above, with a field of view of ca. 3 × 3 cm. The swimming speed of the mysids was calculated for a 60 s section of videotape before the drum began to rotate and for a 60 s period when the drum was rotating. Since there is no current within the optokinetic drum, these experiments measure the actual swimming speed of the mysids rather than movement relative to a fixed position as in the flow-through experiments. From videotapes, the percent of mysids swimming in the same direction as the drum was rotating was calculated during a 60 s period of videotape for the drum rotating in each direction (clockwise or counter-clockwise) by drawing a vertical line through the center of the video monitor and tallying the number of mysids that crossed the line in each direction.

The photosensitivity of *Mysidium columbiae* was tested by recording their optokinetic behavioral response to stripes of 11 mm width rotating at 2 rpm at five different light intensities; 1, 0.1, 0.01, 0.001 and 0.0001 μmol photons m^{-2} s^{-1}. Diffuse overhead light was provided by a Fiber-Lite 3100 fiber-optic lamp which passed through a ground-glass filter and was adjusted to an intensity of 10 μmol photons m^{-2} s^{-1} (measured with a LICOR LI-250 light meter). Both the inner and outer stripes appeared to be evenly and equally illuminated, although it was impossible to measure this directly because of size of the LICOR quantum sensor. Lower intensities were produced using combinations of 3.0, 2.0 and 1.0 neutral-density filters (Oriel Corp, 5 × 5 cm square). The optokinetic drum and video camera assembly was surrounded by an opaque black cloth in order to eliminate stray light from other instruments in the otherwise darkened room. Contrast of the mysid images at low light intensities was enhanced for video-recording using a dark-field array of infrared light-emitting diodes positioned below the clear acrylic base of the optokinetic drum.

Mysid behavior within the clear plastic raceway or within the flow-through chamber was recorded on videotape using a Cohu 3315 monochrome CCD video camera equipped with a macro lens (Micro-Nikkor 55 mm f 2.8). Images were recorded on a Sony FX-710 camcorder. Hydrated brine shrimp cysts were added to the flow-through chamber with a fine pipette and used as tracers to estimate current speed in the chamber. Calibration videotapes and tapes of mysid swimming behavior were quantified with an Expertvision Cell-Trak motion-analysis system. Videotapes were digitized with a Motion Analysis VP-110 processor, and digital outlines of mysids were sent to a personal computer at a rate of 15 frames s^{-1} (i.e. every other frame was digitized; mysid speeds were consistant enough that digitization of each frame was not required for accurate calculation of average motion). These digitized images were processed to calculate the swimming speeds (mm s^{-1}) of the mysids for the optokinetic drum experiments and movement relative to a fixed location for the flow-through experiments.

Results

Review of videotapes of mysids swimming in a clear plastic aquarium revealed that during the day *Mysidium columbiae* spends virtually all its time in aggregations. When there was little directional motion of the aggregation, they tended to be unpolarized, but when a group of mysids was swimming from one side of the aquarium to the other, they tended to have similar orientations and to form a school. Individuals that became temporarily separated from aggregations would rejoin very quickly. Physical contact between mysids within aggregations was extremely rare. These aggregations were most frequently in the upper half of the aquarium, near the surface of the water. In contrast, mysids observed at night in darkness (using video and IR illumination) did not tend to form similar aggregations. Most individuals were in contact with or near the bottom of the aquarium. They were never observed to school. Contact between individuals was observed more frequently in the dark, and resulted in rapid escape responses.

The role of vision in position-holding behavior of mysids was tested in the laboratory by comparing the ability of mysids to hold position within the flow-through chamber in diffuse light during the late afternoon and in darkness at night. Mysid behavior was recorded from a fixed location as the mysids swam into a current with the camera perpendicular to the direction of flow of the current. The speed of the current was ca. 10 mm s^{-1}. From the perspective of the camera, a mysid swimming at exactly the same speed as the current directly into the current would appear to be stationary. The more motion a mysid displays by swimming faster than the current and moving forward, or swimming slower than the current and moving backward, the greater the motion detected from the fixed reference point. In diffuse indirect daylight (ca. 20 μmol photons m^{-2} s^{-1}), the grand mean relative motion with respect to the fixed observation point for eight groups of mysids was 3.7 ± 0.6 mm s^{-1}, indicating considerable ability to hold position within the current (Fig. 2). These mysids generally swam at or near the speed of the current and remained within the view of the video camera for extended periods of time. When the same group of mysids was allowed to adapt to the dark for 1 h and exposed to the same flow regime, their grand mean speed relative to

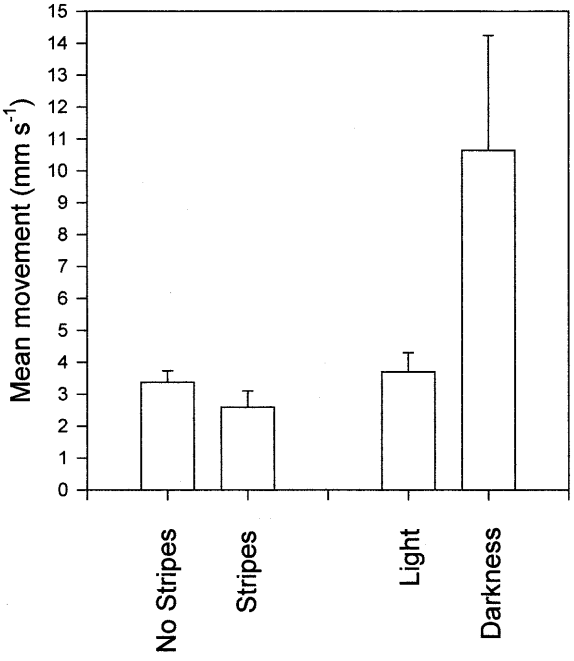

Fig. 2 *Mysidium columbiae*. Mean (+SD) rates of movement relative to fixed position for eight replicate experiments with mysids swimming in flow-through chamber in light and then in dark after 1 h adaptation period (*right-hand columns*); a second experiment compared swimming speeds for eight replicate experiments with mysids swimming in a flow in light with and without vertical black and white stripes (*left-hand columns*). Current speed was ca. 10 mm s^{-1}

the fixed observation point increased significantly to 10.6 ± 3.6 mm s^{-1} (Student's *t*-test with paired design, $\alpha = 0.05$). In the dark, mysids tended to be carried back by the flow and then surge forward into the flow, especially when they contacted the mesh at the rear of the observation chamber. In a second experiment, mysids in diffuse indirect daylight (ca. 20 μmol photons m^{-2} s^{-1}), with 11 mm-width vertical stripes as additional visual markers, displayed significantly less motion relative to the fixed observation point (2.6 ± 0.5 mm s^{-1} compared to 3.4 ± 0.4 mm s^{-1} for the same groups of mysids without stripes: Student's *t*-test with paired design, $\alpha = 0.05$: Fig. 2). Although the difference in grand means is not large, the pooled distribution of swimming speeds from all experiments shows a clear difference between treatments (Fig. 3).

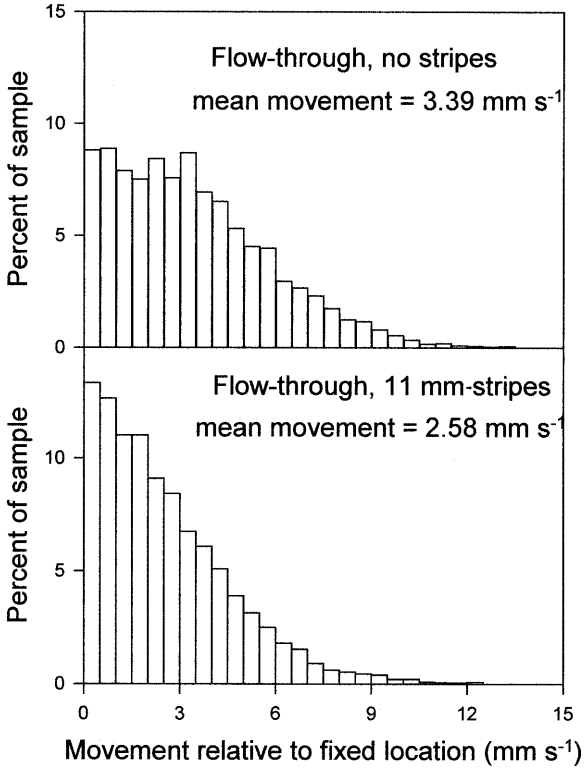

Fig. 3 *Mysidium columbiae*. Position-holding behavior experiments. Combined distributions for eight replicate experiments of motion of mysids in a flow of ca. 10 mm s^{-1} relative to a stationary camera for mysids without alternating black and white stripes as a visual cue (*top graph*) and with 11 mm black and white stripes present (*bottom graph*)

The control experiments for the optokinetic drum showed no indication of effects of the apparatus on mysid behavior. The mean swimming speed of the mysids was calculated for each replicate, and for mysids swimming in the raceway of the optokinetic drum with no stripes and no motion, the grand mean of their mean speeds was 7.29 ± 2.31 mm s^{-1}. For the same groups of mysids with a plain white drum rotating at 2 rpm, the grand mean of their mean speeds was 6.75 ± 2.11 mm s^{-1}. Any vibrations or other signals associated with the motion of the rotating drum had no quantifiable effect on the swimming behavior of *Mysidium columbiae*, and there was no significant change in swimming speed for mysids with a plain white drum rotating around them (Student's *t*-test with paired design, $\alpha = 0.05$). In a second control experiment, when mysids were initially swimming in the raceway with no stripes and no motion, the mean of their mean speeds was 6.89 ± 1.94 mm s^{-1}. In the absence of drum motion and in the presence of 11 mm stripes, these same mysids swam with a grand mean speed of 5.24 mm s^{-1}, and there was no significant difference between treatments (Student's *t*-test with paired design, $\alpha = 0.05$).

Mysidium columbiae showed some level of optokinetic response to black and white stripes ranging in width from 1 to 11 mm (Table 1). With stripes in these widths, >80% of the mysids swam in the same direction as the stripes were rotating, and *M. columbiae* swam significantly faster than in the presence of stationary stripes, indicating that the mysids could detect the

Table 1 *Mysidium columbiae*. Test of visual acuity using alternating black and white vertical stripes of differing widths. Grand mean swimming speed of groups of mysids in absence of stripes and drum rotation (*no rotation*) compared to grand mean swimming speeds of same groups of mysids with stripes rotating on drum at 2 rpm (*rotation*). [*significant increase in swimming speed with drum rotation (Student's *t*-test with paired comparison design, $\alpha = 0.05$); *N* number of groups of mysids tested for each stripe width]. Grand mean percent of mysids following stripes (*% following*) and of those following stripes after the direction of rotation was reversed (*% following reversed*) are also shown. Light intensity was 20 μmol photons m^{-2} s^{-1}

Stripe width (mm)	[*N*]	Speed, mm s^{-1} (SD)		% following (SD)	% following reversed (SD)
		no rotation	rotation		
11	[4]	5.43 (0.63)	9.05* (0.83)	97 (3)	92 (4)
8	[4]	4.42 (0.71)	8.48* (0.59)	99 (2)	93 (5)
6	[4]	4.73 (1.07)	9.50* (1.03)	99 (1)	97 (3)
4	[4]	5.75 (0.52)	8.94* (0.70)	95 (3)	89 (7)
3	[4]	3.48 (0.54)	9.50* (0.91)	95 (3)	76 (17)
2	[6]	4.69 (0.93)	7.95* (0.91)	89 (7)	46 (21)
1.5	[6]	5.53 (0.92)	10.04* (1.54)	79 (20)	28 (24)
1	[10]	5.42 (0.79)	7.29* (1.32)	81 (18)	19 (17)
0.75	[10]	5.90 (1.01)	6.40 (1.64)	56 (15)	47 (16)
0.5	[6]	4.63 (1.27)	4.85 (1.14)	48 (19)	41 (18)

stripes and their motion (Student's *t*-test with paired design, $\alpha = 0.05$). At stripe widths of 0.5 and 0.75 mm, the mysids exhibited slightly faster mean swimming speeds than without stripes, but the increase was not significant. Furthermore, for stripes of these widths (0.5 and 0.75 mm), $< 60\%$ of the mysids were swimming in the same direction as stripe motion. This indicates that moving stripes less than 1 mm in width can not be resolved. For stripes of widths from 4 to 11 mm, nearly 90% or more mysids would reverse their swimming direction after the direction of stripe rotation was reversed. This percentage of swimming direction reversal dropped to 76% for 3 mm stripes and to $< 50\%$ for stripes of ≤ 2 mm. When exposed to stripes of < 3 mm, mysids tended to continue swimming in the same direction as the original drum motion, even after the drum motion had reversed. The stimulus of the presence of other swimming mysids was probably stronger than that provided by the narrower stripes. Stripes (< 3 mm) travelling in the opposite direction as the mysids were swimming would have passed the eyes much more quickly, and temporal resolution of the photoreceptors may have affected spatial resolution (Srinivasan and Bernard 1975).

Using the optokinetic response of the mysids to 11 mm-width stripes at a rotational speed of 2 rpm as a behavioral test for photosensitivity, *Mysidium columbiae* exhibits an optokinetic response at light intensities greater than 0.001 μmol photons m^{-2} s^{-1} (Table 2). More than 90% of the mysids swam in the direction of motion of the stripes at light intensities above this threshold, and mysids swam significantly faster than when the stripes were not moving, indicating that they could visually perceive the motion of the stripes. At light intensities > 0.001 μmol photons m^{-2} s^{-1}, $> 80\%$ of the mysids followed the stripes after the drum had reversed direction, although $< 60\%$ reversed direction at 0.001 μmol photons m^{-2} s^{-1}. The photosensitivity threshold for the optokinetic response appears to be 0.001 μmol photons m^{-2} s^{-1}. It is interesting to note that mysids swam slightly faster than the speed of outer drum rotation (13.4 mm s^{-1}) at low light intensities (0.01 to 1 μmol photons m^{-2} s^{-1}); this did not occur at higher light intensities with the same stripe width (Table 1).

Table 2 *Mysidium columbiae*. Test of photosensitivity using optokinetic response to 11 mm alternating black and white stripes at various light intensities. Grand mean swimming speed of groups of mysids in absence of stripes and drum rotation (*no rotation*) compared to grand mean swimming speeds of same groups with stripes rotating on drum at 2 rpm (*rotation*). [*significant increase in swimming speed with drum rotation (Student's *t*-test with paired comparison design, $\alpha = 0.05$)]. Eight groups of mysids were tested at each light intensity. Grand mean percent of mysids following drum (*% following*) and of those following drum after the direction of rotation was reversed are also shown. Light intensities are in μmol photons m^{-2} s^{-1}

Light intensity (μmol)	Speed, mm s^{-1} (SD)		% following (SD)	% following reversed (SD)
	no rotation	rotation		
1	5.83 (0.99)	14.48 (2.08)*	96.3 (3.7)	98.5 (2.7)
0.1	5.22 (0.52)	14.32 (2.32)*	98.7 (2.9)	92.0 (3.8)
0.01	6.53 (1.04)	14.13 (1.94)*	97.9 (1.8)	83.6 (5.4)
0.001	5.21 (1.21)	10.72 (0.78)*	92.6 (4.0)	57.5 (11.1)
0.0001	5.85 (1.11)	7.33 (0.79)	64.8 (16.8)	31.5 (12.9)

Discussion

Aggregative behavior has been reported for a variety of near-shore mysid species. The adaptive value of this behavior is thought to include improved foraging, facilitation of reproduction, and protection from predators (Ritz 1994). Although there have been no direct experimental studies of the adaptive value of aggregation to *Mysidium columbiae*, it seems likely that the major benefit of this behavior involves avoidance of predators. When confined within an aquarium, *M. columbiae* are rapidly consumed by a wide variety of small reef fishes including juvenile *Abudefduf saxatilus*, juvenile *Haemulon* spp., juvenile *Stegaste* spp., and *Acanthemblemaria* spp. (Buskey 1998b, and unpublished observations). However, predation of *M. columbiae* in nature is rarely observed, provided that groups stay intact (Modlin 1993; Buskey personal observations). Mysids released individually into the environment in the vicinity of small reef fishes are often attacked and consumed (Buskey unpublished observations). The one report of repeated predation of schooling *M. columbiae* in nature occurred within mixed schools of mysids and post-larval grunts, when the fish increased in size and began preying on mysids within the mixed aggregations (McFarland and Kotchian 1982). When mysid aggregations are attacked from outside the school, they benefit from coordinated group avoidance and evasive tactics. When a predator is first detected, the aggregation condenses, polarizes, and swims away from the threat. When an attack occurs, evasive tactics such as "flash expansion" occur and aggregations may temporarily split into smaller units (O'Brien and Ritz 1988). The affinity of *M. columbiae* schools for the fringe of the mangrove prop-root habitat also suggest that this environment helps them avoid predation. The complex mosaic of sunlit patches and shade as well as the structural complexity of the prop roots and associated epibiota may enhance the predator-avoidance behaviors of these aggregating mysids. In addition, attacks by small fishes on mysid schools could draw the attention of large predatory fishes such as barracuda and mangrove snappers that also reside in the shaded areas near the margins of mangroves.

Position-holding behavior has been previously described in the holoplanktonic mysid *Mysidium columbiae* (Buskey 1998b) as well as in hyperbenthic mysids (Hough and Naylor 1992; Roast et al. 1998). While hyperbenthic mysids tend to take refuge in the benthic boundary layer, and maintain contact with bottom when exposed to strong currents (Roast et al.

1998), holoplanktonic mysids such as *M. columbiae* use visual references that allow them to swim to match the speed of the current and remain in place. Underwater video footage shows that *M. columbiae* can hold position in the water column remarkably well in the presence of surging and even reversing currents and turbulence, requiring a large expenditure of energy (Buskey 1998b). In laboratory studies, *M. columbiae* was able to hold position in a laboratory flume in daylight but not in darkness (Fig. 2); in addition, position-holding behavior improved when a high-contrast visual reference was provided. These observations indicate that vision plays an important role in position-holding behavior in nature. Although some mysids that exhibit position-holding behavior appear to show affinity for particular substrates, this does not appear to be the case for *M. columbiae*, which normally swims in the mid-water column, away from the substrate (Modlin 1993). Rather, *M. columbiae* seems to show an affinity for sunlit areas within the overall shade provided by the mangroves near the shoreline.

Visual acuity of small marine organisms can be assessed through both structural studies of eyes and by using behavioral assays. In a study with larval fish, behavioral estimates of visual acuity using an optokinetic response indicated poorer visual acuity than theoretical estimates based on eye structure (Pankhurst et al. 1993). The disparity was thought to be due to the initial myopia of the larval fish eyes, which was corrected during growth and development, and estimates of acuity based on the two methods converged with larval age. Based on the optical geometry of compound eyes of aquatic crustaceans, Land (1984) reports spatial resolution values ranging from 0.4 to 38°. For the euphausiid *Meganyctiphanes* with a refracting superposition eye, Land et al. (1979) determined that their minimum resolution was ca. 2.9°. While no similar structural analysis has been performed for the eyes of *Mysidium columbiae*, these results are similar to the behavioral estimate of the visual acuity based on the optokinetic response (Table 1). The ability of *M. columbiae* to follow stripes of 1 mm in width from a distance of 15 to 30 mm indicates that mysids can visually resolve objects that subtend an arc of 1.9 to 3.8°. With this resolution, *M. columbiae* should be able to detect other adult mysids within a school (ca. 0.5 to 0.7 cm in length: Brattegard 1969) from a distance of 7.5 to 15 cm, a distance much greater than the range of inter-individual distances in their schools (0.5 to 2.0 cm: Modlin 1990). Similarly, in clear waters, mysids should be able to perceive a mangrove prop-root (average diameter ca. 7 cm) at a distance of >1 m.

For behavioral assays, it is important to understand that visual acuity is dependent on both the spatial and temporal resolution of the eye, and that both properties may be affected by light intensity. In a theoretical analysis of the effect of motion on the visual acuity of compound eyes, Srinivasan and Bernard (1975) proposed that above a critical value of angular velocity, the temporal resolution of photoreceptors was more important than the structural optics of the eye in determining visual acuity. Therefore, when using an optokinetic drum to determine visual acuity of *Mysidium columbiae*, care must be taken to keep the speed of the stripes moving past the eye within the range where spatial resolution is not affected by temporal resolution. Temporal resolution of crustacean eyes is typically measured as the critical flicker-fusion frequency, the maximum flicker rate that photoreceptors can resolve at a particular light intensity. Although the critical flicker-fusion frequency for *M. columbiae* is not known, it is typically in the range of 50 to 60 Hz for shallow-water crustaceans (Crozier and Wolf 1939; Crozier et al. 1939) although it can be lower for mesopelagic crustaceans (Frank 1999). A 1 mm white stripe (alternating with black stripes) moving past the mysid eyes at <13.3 mm s^{-1} (maximum speed at outer edge of raceway) would only create a flicker rate of 6.65 Hz, so it seems unlikely that temporal resolution affected the measurement of spatial resolution with the optokinetic drum.

Mysids possess superposition compound eyes (Land 1981). In these eyes, images from several lens facets may be superimposed on the same receptors, increasing the stimulus strength and thus increasing photosensitivity. While theoretical sensitivity can be estimated based on the geometric characteristics of an eye (Land 1981), behavioral assays may be needed to determine the light intensity required for organisms to respond to a specific stimulus. In this study, *Mysidium columbiae* was found to exhibit an optokinetic response at light levels as low as 0.001 μmol photons m^{-2} s^{-1}. This should be comparable to light intensities near the surface in moonlight (Munz and McFarland 1973). This level of photosensitivity is not an absolute one for the eye of *M. columbiae*; the minimum amount of light required will vary with the requirements of a particular visual situation. Measures of photosensitivity of planktonic crustaceans are often made using phototactic behavioral responses, or using physiological methods such as electroretinograms (Cronin 1986). Phototactic responses of planktonic animals such as copepods typically have much lower thresholds than those found in this study for the optokinetic response of *M. columbiae* (Stearns and Forward 1984; Buskey et al. 1989). It is unclear why mysids tended to swim slightly faster than the movement of stripes at low light intensities (Table 2) but not at higher light intensities (20 μmol photons m^{-2} s^{-1}: Table 1). One possible explanation is that mysids are most vulnerable to predation at the low light intensities of dawn and dusk, and may respond to visually perceived movement with more rapid swimming behavior to avoid potential predators.

The role of sensory modalities other than vision in the aggregative and schooling behavior of mysids is less clear. Although aggregations are most well organized during the day, several investigators have noted that aggregations persist at night, even though inter-individual spacing may increase and aggregations become

less structured (e.g. Emery 1968; Whittmann 1977; O'Brien 1988). The persistence of aggregations in darkness suggests that other sensory modalities besides vision can be used to maintain aggregations. Other reports indicate dispersal of mysid aggregations at night, however (McFarland and Kotchian 1982). It seems likely that mysids could use hydrodynamic signals from conspecifics to judge the proximity of other individuals within aggregations, but little is known of their mechanosensory capabilities. It seems unlikely that hydrodynamic signals are sufficient alone, however, since non-schooling mysids seem to collide with one another more frequently in the dark. The response to hydrodynamic stimuli has been better studied in planktonic copepods, which are clearly capable of responding to minute hydrodynamic signals with rapid escape responses (Hartline et al. 1999; Lenz and Hartline 1999). Similarly, little is known about the potential role of chemoreception in the aggregative behavior of mysids, although Modlin (1993) notes the role of olfaction in their feeding behavior. Studies with planktonic copepods have demonstrated that calanoid copepods can form aggregations in response to a chemosensory signal of dissolved amino acids (Poulet and Ouellet 1982). The long flagellae of the antennae and antennules of *Mysidium columbiae* are held out perpendicular to the head in the pattern of the letter "x", and appear to be well designed and positioned to detect hydrodynamic and chemosensory signals from other mysids when schooling. Additional studies are needed to assess the roles of other sensory modalities in the aggregative behavior of mysids.

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