Sensory-Motor Systems of Copepods involved in their Escape from Suction Feeding

Jeannette Yen,1,* David W. Murphy,† Lin Fan‡ and Donald R. Webster§

*School of Biology, Georgia Institute of Technology, Atlanta, GA 30332, USA; †Department of Mechanical Engineering, The Johns Hopkins University, Baltimore, MD 21218, USA; ‡Department of Mechanical Engineering, Stanford University, Stanford, CA 94305, USA; §School of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta, GA 30332, USA

From the symposium “New Insights into Suction Feeding Biomechanics and Evolution” presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2015 at West Palm Beach, Florida.

1E-mail: jeannette.yen@biology.gatech.edu

Synopsis  Copepods escape well by detecting minute gradients in the flow field; they react quickly, and swim away strongly. As a key link in the aquatic food web, these small planktonic organisms often encounter suction-feeding fish. Studies have identified certain hydrodynamic features that are created by the approach of this visual predator and the generation of its suction flow for capturing food. Similarly, studies have identified certain hydrodynamic features that evoke the evasive response of copepods. This is a review of the copepod sensory motor system as pertains to understanding their response to suction-feeding fish. Analyses of the reaction time, threshold sensitivity, structure of sensors, and evasive behavior by this key prey of fish can be useful for evaluating the effectiveness of feeding tactics in response to suction flow. To illustrate, we present results comparing a copepod from a fishless lake (Hesperodiaptomus shoshone) to a copepod from a rich fishing ground (Calanus finmarchicus). We designed a flow mimic that produces a realistic mushroom-cap-shaped flow field and realistic accelerations of flow; the copepods treated the mimic as a threat and performed jumps directed up and away from the siphon. Calanus finmarchicus responded at an average threshold strain rate of 18.7/s, escaped at 0.46 m/s, and traveled 5.99 mm, most frequently as a single jump. Hesperodiaptomus shoshone responded at a strain rate of 15.1/s that is not significantly different, escaped more slowly at 0.22 m/s and traveled a shorter distance of 3.01 mm using a series of hops. The high variability noted in the initial angle of the body and the maximum change in body angle suggests that unpredictability in the escape maneuver is another aspect of the tactic of copepods. The speed of the escape by small copepods 2–3 mm long is overwhelmed by the speed of the attack by the much larger, faster fish; if the copepod reacts when it is within the fish’s arena of capture (<1.5 mm from mouth), it will be eaten. The copepod, however, has an acutely sensitive array of mechanosensors that perceive the flow field of the fish at distances of 3–6 mm, or outside the fish’s range of capture. The copepod also has a rapid and strong locomotory response, thereby increasing the odds that the copepod will survive—but speed is unlikely to be the best tactic for staying alive. Instead, the copepod accelerates from 61.3 to 96.5 m/s² or more than 20 times stronger than the lunge of a fish. This collection of capabilities of copepods enables them to remain one of the most abundant multicellular organisms on our planet.

Copepods have incredibly diverse sensory structures (Yen and Nicoll 1990; Boxshall et al. 1997; Boxshall and Huys 1998) and frequently exhibit responses both to chemical and to hydrodynamic signals in foraging, mating, and escaping (Fields and Yen 1996; Yen et al. 1998; Doall et al. 2002), which have allowed them to survive as one of the most abundant multicellular organisms on earth (Humes 1994). The sensing of flow by the mechanoreceptive cuticular arrays of sensors on copepods has been shaped by over 500 million years of evolution (Strausfeld and Andrew 2011) and plays an important role in avoiding predators, foraging, mating, and rheotaxis (Casas and Dangles 2010). Copepods perform sophisticated sensory-motor responses such as accurate 3 Dimension (3 D) spatial localization of deformations of flow (Doall et al. 2002), rapid milli second signal conduction, and reactions (Lenz et al. 2000; Fields and Weissburg 2004), and locomotory acceleration at several times that of gravity (Yen 2000).

Externally, the characteristic T-shape of copepods (Fig. 1) highlights their key sensors: the paired anterior antennules. Like our two ears, stereoscopy in
localizing the source of the signal is possible using paired sensors. Spatial resolution is increased by the additional setal sensors that line the linear array of the antennules (Yen and Nicoll 1990; Boxshall et al. 1997). These sensors can be mechanoreceptive (Yen et al. 1992; Fields and Weissburg 2004) and chemoreceptive (Boxshall and Huys 1998; Yen et al. 1998). Chemosensitive navigation by copepods along laminar chemical trails has been one of the most dramatic responses performed at these intermediate Re range (1–1000) (Naganuma 1996; Yen et al. 1998). Organisms that are adapted to a viscosity-dominated versus inertia-dominated fluid system exhibit abilities to balance these two forces of viscosity and inertia with novel strategies for propulsion and sensing. Furthermore, this interface between laminar and turbulent regimes is more dynamic than either regime in terms of hydrodynamic structure because small changes in size, speed, and viscosity produce significant variations wake signatures.

Other sensor-like features include pores and hairs that adorn the body (Mauchline 1998). There is a photoreceptive organ that detects shadows and flashes (Novales et al. 2000; Buskey and Hartline 2003). A comparison of the escape behavior in response to photic versus fluid-mechanical stimuli show that the latency of response of *Acartia tonsa* to a photic stimulus is 30 to over 150 ms, which is much longer than the latency of the mechanoreceptive response of ~4 ms. The photoically stimulated escape response of adult female *A. tonsa* is similar in maximum speeds of over 800 mm/s and accelerations of over 200/m² or 20 g! The distance and duration of jumps in response to the photic response are 8–13 mm and 45–75 ms, twice as far and long, respectively, as the escape evoked by mechanical stimuli. The photic response is strong and effective.

This review focuses on the structure and function of the antennules as mechanosensors and how their perception of fluid mechanical signals drive the motor response of escape from suction-feeding fish. Some of the information that mechanoreceptive aquatic organisms acquire from these wakes includes size, speed, location, and identity of the predator. Copepods' sensory system and copepod-generated hydrodynamic signals are spatially and temporally matched: the inter-setal sensor spacing is on the order of 10 s of micrometers and the variation in velocities of biologically generated flow also occur over micrometers; the millisecond latencies match the millisecond temporal dynamics of the fluid wakes left by copepods (Yen and Strickler 1996; Boxshall et al. 1997; Yen 2000; Fields and Weissburg 2004). These sensor arrays are capable of perceiving minute differences in the structure of wakes. The amplitude, frequency content, and temporal characteristics of the hydrodynamic wake..
enable recognition and localization of mates, prey, and predators.

Perception of fluid deformation depends on bending of the seta, which transduces the mechanical signal into an electrical one that then is conducted along myelin-sheathed neurons to central ganglia (Strausfeld and Andrews 2011) or directly from the afferent to motor neurons for rapid reflexive responses (<2 ms, Fields and Weissburg 2004). The sensory-motor neuronal pathways have been traced recently (Strausfeld and Andrews 2011, Wilson and Hartline 2011a, 2011b; Andrews et al. 2012,). The brain of a copepod is about 100 μm × 200 μm in size. Instead of millions of axons, only hundreds enter the brain of the copepod (Wilson and Hartline 2011a; Andrew et al. 2012). Receptor axons from, and motor axons to, the antennules are carried in the dorsal giant fiber, the largest nerve in the copepodid nervous system (Andrew et al. 2012). Many mechanoreceptors (on the order of 100) are located on the antennules (A1). According to Wilson and Hartline (2011a), the copepod escape behavior is an A1-receptor/pereiopod (swimming leg) system to which the dorsal giant fiber likely conveys signals from the A1 nerve to the thoracic motor neurons. This connection provides an antennular trigger in escape circuitry where the stereo-gradient detection of the paired antennules enables localization of stimuli (Wilson and Hartline 2011a).

Past research (Yen et al. 1992; Fields et al. 2002; Kiorboe 2008) have defined threshold sensitivities to displacements as small as 10 nm, flow speeds as weak as 20 μm/s, and shear as little as 0.05/s. Extracellular recordings taken at the base of the antennule (Yen et al. 1992; Fields et al. 2002) demonstrated that mechanical bending of the seta evoked neuronal responses, with characteristic dynamics in terms of frequency and amplitude of the action potentials, that could be associated with specific setae. Each seta also has a specific threshold sensitivity. Analyses of various setae along the antennule when bent distally versus proximally show that these sensors, as a collective array, can detect flows ranging from 40 to 160 mm/s where each seta is responsive to a smaller subrange, thus achieving range fractionation. These graded responses evoke a series of behaviors. For example, in Euchaeta rimana, (1) weak flows trigger antennal flick, perhaps reorienting the animal to the signal like a dog cocking its ear; (2) intermediate, narrow flows that bend proximal setae located in the current, evoke the capture response (Lenz and Yen 1993; Yen and Strickler 1996; Fields and Yen 2002); (3) intense, broad flows trigger escape, with the copepod using single to multiple metachronal thrusts of the 4–5 pairs of swimming legs (Fields and Yen 2002; van Duren and Videler 2003; Lenz et al. 2004; Yen 2013). Size-dependent tactics that facilitate escape from predation by fish include multiple small jumps by smaller copepods and longer, single ones by larger copepods (Wilson 2001; Yen 2013). This multi-oared locomotory system equips the copepods with an incredible ability to accelerate that is strong enough to launch them out of the water and through the air/sea interface when escaping from fish (Gemmell et al. 2012). The multi-oared metachronal gaits (van Duren and Videler 2003) rapidly and multiplied deployed during power strokes generate thrust for these supervalve behaviors.

These traits, some of which are quite unusual, for example, myelin insulation to reduce neuronal conduction time over millimeter distances suggests the importance of millisecond differences in reaction time or 20 g escapes by mm-sized aquatic crustaceans, have evolved in response to evolutionary pressures such as the search for a mate or a host, and the avoidance of a predator. As surmised from these surprisingly fast and powerful responses, such speeds may be required to escape from suction-feeding fish. Here we compare the escape responses of copepods from a fish mimic. The copepods are Hesperos from fishless lakes and Calanus from the rich fishing grounds of the Gulf of Maine. The hypothesis is that the copepods that evolved in fishless lakes (Sarnelle and Knapp 2004; Kramer et al. 2011) would have a limited escape response when compared to a copepod that is a dominant component in the diet of cod (Beaugrand et al. 2003). Our analyses addressed questions such as: how does the extent of the fish’s flow field relate to the escape distance of the copepod? What is the importance of the orientation of the sensor orientation and the structure of the signal? How do copepods accurately detect a predator and appropriately respond by a directed escape?

Materials and methods

Collection and maintenance of animals

Calanus finmarchicus were collected by a net towed off the coast of Oregon and shipped overnight in insulated coolers to Atlanta. Hesperodiaptomus shoshone were collected with a plankton net from Rock Pond, a shallow alpine lake above 10,000 ft in the Beartooth Mountains of Wyoming. This lake is normally frozen from September to July, and animals were collected in mid-August (Yen et al. 2012).
The animals were sorted into containers containing pond water and shipped overnight to Atlanta in coolers containing ice. At Georgia Tech, these copepods were maintained at their environmental temperature of 12°C in a temperature-controlled environmental chamber. The marine copepods (*C. finmarchicus*) were fed concentrated *Tetraselmis* spp. and *Rhodomonas lens* from the phytoplankton. *Hesperodiaptomus shoshone* were fed *Artemia salina* nauplii sieved out of their saline (10 ppt) artificial seawater. In addition, *H. shoshone* were fed the cladoceran *Daphnia middendorfiana* collected from Rock Pond. Mean prosomal lengths of *C. finmarchicus* and *H. shoshone* were 2.66 ± 0.56 mm and 2.36 ± 0.15 mm, respectively. Only male *Hesperodiaptomus* were used in the experiments; sex was not determined in the *Calanus* copepods. Twenty-three experiments were conducted for both *C. finmarchicus* and *H. shoshone*. Kinematic analysis was only performed for copepods nearest to the siphon (12 mm from the siphon at the time of escape) to more realistically mimic a fish predator, thus reducing the number of replicates (*n* = 11 for *C. finmarchicus* and *n* = 9 for *H. shoshone*). All experiments were completed within two weeks of collection of the animals.

**Development of a mimic of a predatory fish**

To expose copepods to the impulsive flow generated by a suction-feeding fish, a mimic of the flow field of a predatory fish was developed. Suction for the mimic, shown in Fig. 2, was provided by an eductor jet pump inserted into a constantly running flow-loop driven by a centrifugal pump. An eductor jet pump, also known as an ejector, is a simple device with no moving parts that uses a Venturi nozzle to create a region of low pressure that sucks fluid in from a second port. A flow line with a needle valve and normally-closed solenoid valve led from this port to the siphon inserted into the aquarium holding the copepods. By pushing a trigger, a user could simultaneously open the valve of the solenoid and begin the collection of data when a copepod appeared in a suitable location near the siphon’s tip, as monitored on a display. By providing resistance to flow, the needle valve was used to adjust the strength of the suction. The glass siphon, with inner and outer diameters of 2.4 mm and 4 mm, respectively, was bent so that the tip was horizontal.

**Fluid-structure interactions**

The tomographic Particle Image Velocimetry (PIV) system for visualizing flow in 3D, as described by Murphy et al. (2012, 2013), was used to measure both the flow induced by the siphon and the 3D kinematics of the escaping copepods. This system is composed of four high-speed cameras viewing an interrogation volume illuminated by two counter-propagating, continuous 7-W lasers firing with a near-infrared (IR) wavelength (808 nm). The flow was seeded with titanium dioxide particles with a mean diameter of <10 μm. The siphon was positioned inside the aquarium at one end of the 1–2 cm³ interrogation volume such that its tip was illuminated. Upon triggering, the synchronized cameras recorded at 500 Hz. Using identical flows through the siphon, 9 and 11 escape sequences were recorded for the *H. shoshone* and *C. finmarchicus* copepods, respectively. To characterize the siphon-induced flow and compare it to suction-feeding flow by fish, a sequence with no copepods present also was recorded. This representative sequence was processed, reconstructed, and cross-correlated to produce a time-resolved 3D velocity field surrounding the siphon, which was represented in the flow field by its visual hull (Adhikari and Longmire 2012).

The locations of features on the copepods’ bodies could be determined in 3D space using the multiple perspectives of the four synchronized high-speed cameras. Using the DLTdv5 software (Hedrick et al. 2008), the tips of the left and right antennules (Figs. 1 and 3) were manually tracked until the time when the copepod initiated its escape maneuver. The antennule tips could not be tracked after the escape was initiated because the antennules are drawn close to the body during high-speed jumps that minimize drag. Because the tips of the antennules bear highly sensitive mechanosensory hairs that respond to flow, the flow at the position of tip of the antennule nearer...
the siphon was characterized at the time that escape was initiated. Specifically, the maximum principal strain rate $\varepsilon$ of the flow was calculated from the representative flow field at that point in time and space in an attempt to determine a threshold signal to which the copepods consistently responded. The maximum principal strain rate, found by an eigenvalue decomposition of the measured strain-rate-tensor, represents the maximum strain rate at a certain point, regardless of the orientation of the coordinate system. Using the maximum principal strain rate thus simplifies the nine component strain rate tensors (which have directional complexity) into a single representative value that acts irrespective of the orientation of the copepod relative to the siphon.

To characterize the copepods’ responses to the siphon, their 3D escape kinematics were measured by manually tracking points on the head and tail (see white dots on anterior and posterior points on the prosome in Fig. 3) throughout each recorded video sequence. The speed and acceleration at both points were calculated at each time-point and were averaged to provide a representative speed $v$ and acceleration $a$ for the copepod. The distance traveled by each copepod during its first jump, $d_1$, also was measured.

The orientation of the copepod’s body has been shown to influence copepods’ sensitivity to stimuli from a predator (Fields 2010; Fields and Yen 1997a) and also was measured. The body’s angle of orientation $\varphi$ was calculated as the angle between a vertical line and the line formed between the points on the head and tail of the copepod just before escape. A small value of $\varphi$ thus indicates a more vertical orientation. The angle of rotation of the jump $\chi$, the change in the orientation of the body between the beginning and the end of the jump, also was measured. This parameter represents the angle over which the copepod turned its body when executing its jump.

The copepod’s directional sensitivity to the siphon’s hydrodynamic signal was investigated in the following manner: the vector reaching from the center of the siphon’s tip to the copepod’s head at the time that escape was initiated ($\vec{A}$ in the last panel of Fig. 5) was measured. A second vector ($\vec{B}$) reaching from the copepod’s initial position to its final, post-escape position also was measured. The angle between these two vectors (when placed tail to tail) is the directional sensitivity angle $\psi$. If $\psi$ is small, these vectors are well aligned (i.e., point in the same direction) and the copepod has jumped away from the siphon. For example, a directional sensitivity angle of $\psi = 0^\circ$ indicates the copepod jumped directly away from the siphon because these two vectors are perfectly aligned. Conversely, a directional sensitivity angle of $\psi = 180^\circ$ indicates the copepod jumped directly toward the siphon because these two vectors point in opposite directions.

Fig. 3 A sequence of PIV images, from one of the four cameras, showing a Calanus finmarchicus escaping from the mimic of a predator fish. Time begins when the flow starts into the siphon. The scale bar in the 10 ms panel is 2.5 mm. The white dots on the head and tail indicate the points tracked throughout the experiment. The gray dots outlined in white indicate the points on the left and right antennules tracked up to the time of the copepod escape. In the last panel, the initial position of the copepod is overlaid. The angle between vectors $\vec{A}$ and $\vec{B}$ (when laid tail to tail) is the directional sensitivity angle $\psi$. 
For all comparisons between *C. finmarchicus* and *H. shoshone*, student's *t*-tests were conducted. Because multiple (seven) comparisons were conducted, a Benjamini-Hochberg procedure was carried out to reduce the rate of false discovery (Benjamini and Hochberg 1995). Significance was, therefore, determined at the $P < 0.021$ level. Normalizing values of $d_1$, $v$, and $a$ by the individual copepod's prosomal length did not change whether those comparisons were significantly different or the $P$ level at which significance was determined.

**Results**

**Flow characterization**

Selected time points from the representative flow field are shown in Fig. 4. The visual hull of the siphon is on the right side of each image, with the vector plane bisecting it. At 0 time, no flow is yet present. At 40 ms, flow has begun. Similar to the flow induced by many suction-feeding fish, a high-speed, mushroom-cap-shaped core surrounds the tip of the siphon, with peak speeds surpassing 0.075 m/s. The volume with high velocity of flow continues to expand radially at the 80 ms and 120 ms time points, with peak speeds reaching at least 0.15 m/s at 120 ms. A steady rate of flow with no further acceleration is achieved by approximately 150 ms. At the two later time points presented in Fig. 4, a region of erroneously low flow is found directly in front of the nozzle. The high speeds in this region (and the long times of exposure required for illumination of the particle) caused streaking, thereby preventing representative PIV measurements. This type of error of measurement is characteristic of flows generated by siphons, in which it is difficult to simultaneously measure both low speeds of flow far from the siphon and high speeds of flow near the nozzle. The exposure time of the cameras was thus tuned to measure the low-speed flows to which the copepods reacted.

Piscine suction-feeding flows are often temporally characterized by the flow at a point that is at a distance of one half of the peak of the fish’s gape away from the fish’s mouth. Similarly, Fig. 5 shows the flow speed at a distance of one half the siphon’s inner diameter from the tip of the siphon. The slope of the line represents the acceleration the fish is able to create. The acceleration found for the piscine mimic ($\sim 3.4 \text{ m/s}^2$) falls between that found by
and maximum acceleration of 127.3 m/s². Some from the siphon at a maximum speed of 0.6 m/s C. finmarchicus sequence of images of a away from the siphon. For example, Fig. 3 shows a mimic as a threat and performed jumps directed species. Most copepods of both species treated the siphon flow in the 23 replicates gathered for each There was a variety of behaviors in response to the flow field and realistic accelerations of flow. thus produces both a realistic mushroom-cap-shaped flow field and realistic accelerations of flow.

Copepods’ sensitivity

There was a variety of behaviors in response to the siphon flow in the 23 replicates gathered for each species. Most copepods of both species treated the mimic as a threat and performed jumps directed away from the siphon. For example, Fig. 3 shows a sequence of images of a C. finmarchicus escaping from the siphon at a maximum speed of 0.6 m/s and maximum acceleration of 127.3 m/s². Some copepods initially located far from the siphon swim away slowly without jumping. Some copepods performed several small hops that did not effectively remove them from the mimic. Some of these eventually performed a stronger escape jump whereas others were sucked into the siphon. Of the copepods selected for further kinematic analysis, most individuals of both species performed a single escape jump, whereas two individuals of each species performed two consecutive jumps, and two C. finmarchicus performed three consecutive ones. Generally speaking, C. finmarchicus performed more high-speed escape jumps whereas H. shoshone performed smaller, repositioning hops. The overall escape rate for C. finmarchicus was 96% whereas that for H. shoshone was 78%. In addition, two H. shoshone jumped toward the siphon and were captured.

Figure 6 shows the distance from the siphon’s center at which each copepod escaped as a function of time. This chart reveals that the copepods initiated escape at distances ranging from 3.4 mm to 11.2 mm and at times ranging from 20 ms to 670 ms after the flow started. The gray line can be thought of as the threshold of the signal eliciting escape from all copepods, and its slope represents the propagation speed of this signal away from the siphon (~12 mm/s). From Fig. 6, no particular differences in the timing of escape can be seen between the two species. The maximum principal strain rate $\varepsilon$ at the tip of the antennule when escape was initiated was measured in the representative flow field for seven individuals of each species. As shown in Table 1, the mean values of $\varepsilon$ for H. shoshone and C. finmarchicus are $15.1 \pm 10.6$/s (mean $\pm$ SD) and $18.7 \pm 7.9$/s, respectively, and do not differ significantly ($P>0.021$).

The ability of the copepod to localize the source of the mimic and respond to it by an escape maneuver was measured by the directional sensitivity angle $\psi$: $\psi = 0^\circ$ indicates the copepod jumped directly away from the siphon and $\psi = 180^\circ$ indicates the copepod jumped directly toward the siphon. As shown in Table 1, the mean values of $\psi$ for H. shoshone and C. finmarchicus were $67.4^\circ \pm 41.2^\circ$ (mean $\pm$ SD) and $44.3^\circ \pm 38.8^\circ$, respectively, and are not significantly different ($P>0.021$). The initial body orientation angles $\varphi$ of the two species, $29.3^\circ \pm 28.0^\circ$ for C. finmarchicus versus $63.6^\circ \pm 24.2^\circ$ for H. shoshone, however, were significantly different ($P<0.01$), with C. finmarchicus maintaining a more vertical stance. The angles of rotation $\chi$ of the jump of the two species, $65.6^\circ \pm 28.5^\circ$ for C. finmarchicus versus $93.8^\circ \pm 53.1^\circ$ H. shoshone, were not significantly different ($P>0.021$).

Ability of a copepod to escape

As shown in Table 1, the mean values of the maximum escape speed exhibited by the H. shoshone and C. finmarchicus were $0.22 \pm 0.1$/m/s and $0.46 \pm 0.2$/m/s, respectively, and were significantly different ($P<0.005$). Normalizing by the copepods’ individual prosomal lengths (body lengths or BL) also yields significantly different maximum escape speeds of $91.9 \pm 41.7$ BL/s and $171.2 \pm 68.3$ BL/s for H. shoshone and C. finmarchicus, respectively ($P<0.01$). The maximum acceleration of C. finmarchicus ($96.5 \pm 37.7$ m/s²) was greater than that of H. shoshone ($61.3 \pm 51.2$ m/s²), but this difference did not reach statistical significance ($P=0.093$). As might be expected, $d_i$, the distance...
traversed during the first escape jump by *C. finmarchicus* (5.99 ± 2.0 m/s) was significantly greater than that of *H. shoshone* (3.01 ± 1.0 m/s) (*P* < 0.001). This distance of travel normalized by BL is 1.27 ± 0.38 BL for *H. shoshone* and 2.3 ± 0.79 BL for *C. finmarchicus*; these values also are significantly different (*P* < 0.005).

### Table 1 Sensitivity characteristics and kinematic parameters of escaping copepods

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number of <em>Calanus finmarchicus</em></th>
<th>Number of <em>Hesperodiaptomis shoshone</em></th>
<th><em>Calanus finmarchicus</em></th>
<th><em>Hesperodiaptomis shoshone</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum Principal Strain Rate <em>ι</em> (per second)</td>
<td>7</td>
<td>7</td>
<td>18.7 ± 7.9</td>
<td>15.1 ± 12.0</td>
</tr>
<tr>
<td>Maximum Speed <em>v</em>&lt;sub&gt;max&lt;/sub&gt; (m/s)</td>
<td>11</td>
<td>9</td>
<td>0.46 ± 0.2*</td>
<td>0.22 ± 0.1*</td>
</tr>
<tr>
<td>Normalized Maximum Speed <em>v</em>&lt;sub&gt;max,norm&lt;/sub&gt; (BL/s)</td>
<td>11</td>
<td>9</td>
<td>171.2 ± 68.3*</td>
<td>91.9 ± 41.7*</td>
</tr>
<tr>
<td>Maximum Acceleration <em>a</em>&lt;sub&gt;max&lt;/sub&gt; (m/s&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>11</td>
<td>9</td>
<td>96.5 ± 37.7</td>
<td>61.3 ± 51.2</td>
</tr>
<tr>
<td>Directional Sensitivity Angle <em>ψ</em> (degrees)</td>
<td>10</td>
<td>9</td>
<td>44.3 ± 3.8</td>
<td>67.4 ± 41.2</td>
</tr>
<tr>
<td>Body Orientation Angle <em>φ</em> (degrees)</td>
<td>11</td>
<td>9</td>
<td>29.3 ± 28.0*</td>
<td>63.6 ± 24.2*</td>
</tr>
<tr>
<td>Jump Rotation Angle <em>χ</em> (degrees)</td>
<td>11</td>
<td>9</td>
<td>65.6 ± 28.5</td>
<td>93.8 ± 53.1</td>
</tr>
<tr>
<td>Escape Distance <em>d</em>&lt;sub&gt;1&lt;/sub&gt; (mm)</td>
<td>10</td>
<td>9</td>
<td>5.99 ± 2.0*</td>
<td>3.01 ± 1.0*</td>
</tr>
<tr>
<td>Normalized Escape Distance <em>d</em>&lt;sub&gt;1,norm&lt;/sub&gt; (BL)</td>
<td>10</td>
<td>9</td>
<td>2.3 ± 0.79*</td>
<td>1.27 ± 0.38*</td>
</tr>
</tbody>
</table>

Note: Asterisks indicate a significant difference between the two species.

### Discussion

The tomographic PIV system enables us to examine key aspects of fluid-structure interactions in the intermediate Re regime of planktonic organisms. By illuminating a volume and recording with multiple cameras, the 3D nature of the flow and swimming patterns of zooplankton can be studied nonintrusively. A high-speed camera system with high magnification quantified the zooplankton’s behavior at the appropriate time/space scale of ms/mm. Using near-IR laser illumination eliminated the evocation of phototaxis by the zooplankton. Our system thus allows for the measurement of 3D vectors of velocity in the volume around planktonic organisms at high.
spatial and temporal resolution without disturbance to the animals. Thus, tomographic PIV measured flow fields around the sensory appendages of the zooplankton so as to quantify hydrodynamic thresholds of signals for escape from the mimic.

Copepods are prey for a wide variety of aquatic species. Predators whose interactions with copepods have been studied include other copepods (Doall et al. 2002), predatory cladocerans (Browman et al. 1989), mysids (Viitasalo and Rautio 1998; Viitasalo et al. 1998, 2001), krill (Abrahamsen et al. 2010), medusae (Suchman 2000), and fish (Clarke et al. 2005; Heuch et al. 2007; Waggett and Buskey 2007). Copepods sense their fluid environment with setae-bearing antennules (Strickler and Bal 1973; Yen et al. 1992; Yen and Strickler 1996), and dissimilar hydrodynamics signals created by these predator groups may induce different responses by copepods. For instance, copepods and mysids produce a current that entrains prey. Depending on the prey’s sensitivity to the current’s structure and its ability to escape, prey then can be individually picked out of this ‘scanning current’ by the predator (Fields and Yen 1997a; Viitasalo and Rautio 1998; Doall et al. 2002; Kiørboe 2010). The ability of copepods to accurately detect a predator and appropriately respond with a directed escape response has been investigated using various predators and mimics of predators. For example, steady siphon flows have been used to define a strain rate threshold above which a particular copepod species will escape (Fields and Yen 1996; 1997b; Kiørboe et al. 1999), to test the relative abilities of various species to escape (Viitasalo et al. 1998; Viitasalo et al. 2001; Burdick et al. 2007), to test the effect of various conditions of flow (such as turbulence) on copepods’ ability to escape (Clarke et al. 2005; Gilbert and Buskey 2005; Robinson et al. 2007), and to investigate the directional bias in copepods’ sensitivity (Fields 2010). The response is often dramatic, yielding escape jumps by calanoid copepods of speeds up to 1 m/s (or 500 BL/s) and accelerations over seven times that of gravity (Yen 2000; Murphy et al. 2012).

The disturbance created to a fluid by fish feeding in it is quite different from that of a feeding current generated by copepods or mysids. Fish predominantly use a combination of ram feeding (swimming toward the prey with the predator’s mouth open) and suction feeding (impulsively generating suction to pull the prey into the mouth) (Higham et al. 2005). Ram feeding creates a bow wave in front of the animal, whereas suction feeding generates a sudden, high-speed flow into the animal’s mouth (Day et al. 2005; Higham et al. 2005; Day et al. 2007). Measurements of flow created by suction-feeding fish have shown that planktivorous adult bluegill sunfish can open their mouth to peak gape (maximum diameter) in as little as 12 ms and achieve maximum flow speeds at the mouth shortly thereafter of almost 1 m/s (Day et al. 2005). Similarly, Holzman et al. (2008) showed that young-of-year bluegill sunfish (standard length of 57 mm) can achieve peak gape in the same time period and shortly thereafter achieve peak flow speeds of ~0.1 m/s. For suction-feeding fish, flow speeds dramatically decrease with distance from the mouth and are generally <5% of peak flow speed at one mouth diameter away (Day et al. 2005); the volume affected by the flow thus resembles the top of a mushroom centered on the fish’s mouth (Day et al. 2007).

As noted by Holzman and Wainwright (2009), previous studies of fish-copepod interactions have focused on the hydrodynamic signal generated by the fish’s bow wave, but suction may be the more important signal for the copepod (Viitasalo et al. 1998; Kiørboe and Visser 1999; Visser 2001; Heuch et al. 2007). To be sure, the approaching fish’s bow wave can warn the copepod. For instance, Heuch et al. (2007) showed that Acartia spp. copepods escaped from the bow wave created by a moving fish-head mimic whereas the same bow wave attracted a parasitic copepod to the mimic. In addition, Viitasalo et al. (1998) showed that the speed at which stickleback approach was linked to the distance at which its copepod prey, Eurytemora affinis, escaped. The fish were most successful when able to approach to <1 mm from the prey before initiating attack (Viitasalo et al. 1998). Holzman and Wainwright (2009), however, measured flow caused by feeding bluegill and showed that a hypothetical copepod prey may detect a striking fish principally by its suction-induced disturbance to flow rather than by the bow wave the predator creates.

The sensitivity and reaction of a copepod to the dynamic, high-acceleration flow created by a suction-feeding fish have been explored here. It is reasonable to expect that the reaction when the copepod experiences a sharply increasing strain rate in an impulsive suction-feeding event may be different than that to a mimic generating a steady flow. In the present study, a realistic mimic of a suction-feeding predatory fish was developed and tested with a copepod species (Calanus finmarchicus) originating from a fish-containing (marine) environment and compared to a copepod species (Hesperodiaptomus shoshone) from a fish-less alpine lake. Hesperodiaptomus shoshone is the primary predator in its environment...
(Williams 2012) and detects and feeds on smaller plankton, like cladocerans or juvenile fairy shrimp. The mimic of the flow field produced by suction-feeding fish that we created was validated against published measurements of fish that were suction feeding. Using volumetric measurements of flow, the hydrodynamic signal measured along each copepod’s antennules at the point of escape, and the success and kinematics of escape (velocity and acceleration) were compared between species. With this bio-inspired suction-feeding mimic, we wanted to demonstrate whether or not prey copepods that successfully evade capture tune their mechanosensitivity to a location where their escape thrust exceeds the suction forces to the extent that their response has a timing just shorter than that of the predator’s reaction, and an escape distance reaching just beyond the predator’s capture zone. We expected that copepods from the marine environment with abundant fish would exhibit enhanced sensitivity (i.e., a lower strain rate threshold) and lesser ability to escape in comparison to those from the fish-less environment.

Threshold sensitivity

Our study enabled us to document the ephemeral moment when a copepod is entrained, detects, and escapes from a suction-feeding flow field. We found that copepods escape at an average strain rate of $17/\text{s}$. For our siphon flow, the signal traveled outward from the siphon at a constant speed of $12 \text{ mm/s}$. This could potentially be useful in identifying the type of signal to be vorticity or to be strain in a certain direction. We found that the speed at which the strain-rate signal traveled from the source (Fig. 6B) has a slope that gave a ‘propagation speed’ of $13.6 \text{ mm/s}$. The close agreement with the speed of $12 \text{ mm/s}$ based on the locations where escape occurred suggests that strain rate may be a key trigger of the escape response. Data are too sparse for a similar analysis to assess whether a vorticity signal of $0.5/\text{s}$ (the likely threshold as found by Webster and Young [2015]) might also serve as a trigger. From the copepod’s perspective, it would be better if the signal traveled quickly so that the copepod would be at a greater distance from the fish when beginning to escape. From the fish’s perspective, a slowly traveling signal would be beneficial since the copepod would have less time to react. As Gemmell et al. (2014) found, fish are known to manipulate the flow field (with the mouth) so that, by the time the gradient of the flow has alerted the copepod, it is too late for it to escape (which means that getting as close as possible to the copepod is of key importance). For good success in capturing prey, the field of flow around the fish should have a structure in which the high-velocity gradients are as close to the mouth as possible and the flow field is manipulated so that the signal propagates outward slowly.

The perceptive range of 3–11 mm for Calanus and Hesperos found in this study is similar to the average perceptive distances found for Acartia (6.54 mm), Temora (2.72 mm), and Calanus (7.12 mm) by Wilson (2001 as cited by Yen 2013). Successful escapes occurred as close as 3.4 mm and as far as 11.2 mm from the siphon within 20 ms and at 670 ms, respectively, after the onset of suction. To connect the sensing ability of copepods to the signal generated by the suction emanating from the fish, we compared the copepods’ threshold sensitivity and reaction times to the strength of the signal in the flow field at similar distances from the fish and at different intervals of time (Fig. 7). Within 3–8 mm, the threshold strain rate occurs within a narrow window of 40–50 ms (Fig. 7). Since copepods escape within 20 ms when that close to the fish’s mouth, they are likely successful in their escape. Furthermore, copepods also continue to escape for 70–290 ms (Wilson 2001) while the duration of the strike by the fish is on the order of 10 ms. At 670 ms, the threshold is about 5 mm from the fish’s mouth (Fig. 7), that is, outside the range for capture by suction feeders, and again, the copepods are likely to be successful in their escape. At 20 ms, the threshold strain is within the capture range of 1.5 mm from the fish’s mouth. However, the acceleration of the fish is only $20 \text{ m/s}^2$ (R. Holzman personal communication), which is much lower than that of copepods. The maximum speed of copepods ranged from 0.22–0.46 m/s. The speed of the advancing mouth (the sum of swimming speed + the speed of jaw protrusion) was 0.13 m/s, which is relatively slow (speeds for bluegills are up to 1.5 m/s; R. Holzman personal communication). Speed is unlikely to be the best tactic for staying alive. However, the maximum acceleration of copepods averaged from 61.3–96.5 m/ sec$^2$; this is more than 20 times stronger than the acceleration of the fish’s mouth of 2.9 m/s$^2$ (R. Holzman personal communication). The uncanny capability of copepods to propel themselves so nonlinearly may be the key to their success!

The effects of orientation of the body on sensing and escaping

Most predatory fish see and attack copepods from the bottom or side (angles relative to horizontal of about 45°). Fields (2010) showed that copepods are
As expected, the speed of the escape by the 2–3 mm copepod is overwhelmed by the speed of the attack by the much larger fish; if the copepod reacts when it is within the fish’s capture arena (<1.5 mm from mouth), it will be eaten. The copepod, however, has an acutely sensitive array of mechanosensors to perceive the flow field of the fish and a rapid and strong locomotory response to execute an effective escape response while outside the fish’s capture field, thus increasing the odds that the copepod will survive. The copepod also has the ability to accelerate much faster than the lunge of a fish which points out the importance of timing: fish may be fast but copepods react faster in shorter times. The copepods have tuned their mechanosensitivity, which can be adjusted by the orientation of the body and by the sensor’s structure to a location where their escape thrust exceeds the force of the suction. Furthermore, the unpredictability and duration of the escape trajectory take the copepod outside the capture arena of the fish. Our analysis of the interplay between prey and their predators has revealed the key escape tactics used by copepods for survival in the aquatic environment.

Acknowledgments
We wish to express our great thanks to Peter Wainwright and Tim Higham for getting us together for this interdisciplinary workshop hosted by NIMBIOS. Discussions with Matt McHenry and Roi Holzman were especially insightful and important for this article.

Funding
The authors gratefully acknowledge financial support of the National Science Foundation (OCE-0928491, OPP-1245296, DUE-1022778).

References


