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SYMPOSIUM

Swimming in an Unsteady World

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Synopsis When animals swim in aquatic habitats, the water through which they move is usually flowing. Therefore, an important part of understanding the physics of how animals swim in nature is determining how they interact with the fluctuating turbulent water currents in their environment. We addressed this issue using microscopic larvae of invertebrates in "fouling communities" growing on docks and ships to ask how swimming affects the transport of larvae between moving water and surfaces from which they disperse and onto which they recruit. Field measurements of the motion of water over fouling communities were used to design realistic turbulent wavy flow in a laboratory wave-flume over early-stage fouling communities. Fine-scale measurements of rapidly-varying water-velocity fields were made using particle-image velocimetry, and of dye-concentration fields (analog for chemical cues from the substratum) were made using planar laser-induced fluorescence. We used individual-based models of larvae that were swimming, passively sinking, passively rising, or were passive and neutrally buoyant to determine how their trajectories were affected by their motion through the water, rotation by local shear, and transport by ambient flow. Swimmers moved up and down in the turbulent flow more than did neutrally buoyant larvae. Although more of the passive sinkers landed on substrata below them, and more passive risers on surfaces above, swimming was the best strategy for landing on surfaces if their location was not predictable (as is true for fouling communities). When larvae moved within 5 mm of surfaces below them, passive sinkers and neutrally-buoyant larvae landed on the substratum, whereas many of the swimmers were carried away, suggesting that settling larvae should stop swimming as they near a surface. Swimming and passively-rising larvae were best at escaping from a surface below them, as precompetent larvae must do to disperse away. Velocities, vorticities, and odor-concentrations encountered by larvae fluctuated rapidly, with peaks much higher than mean values. Encounters with concentrations of odor or with vorticities above threshold increased as larvae neared the substratum. Although microscopic organisms swim slowly, their locomotory behavior can affect where they are transported by the movement of ambient water as well as the signals they encounter when they move within a few centimeters of surfaces.

Introduction

When an organism swims in the real world, the water through which it moves is usually flowing. How does the interaction of a swimming organism with the turbulent water moving around it affect how that organism travels through the environment? We explore this question by focusing on small, weak swimmers for which the interaction with the motion of ambient water is important: microscopic marine larvae swimming in turbulent, wavy flow over surfaces from which they must escape to disperse to new sites, and onto which they must land to recruit to benthic habitats.

Larvae from fouling communities

Many benthic marine invertebrates disperse to new habitats via microscopic larvae that are transported by oceanic currents (reviewed by Metaxas 2001; Levin 2006). Once such larvae become competent to undergo metamorphosis into bottom-dwelling juveniles, they can settle onto surfaces and recruit into benthic habitats (reviewed by McEdward 1995). Escape by pre-competent larvae from their parent communities into the water column, and recruitment by competent larvae onto surfaces after dispersal are important factors affecting the structure of benthic communities (reviewed by Ólafsson et al. 1994;

Schiel 2004; Edwards and Stachowicz 2011), and the genetics of metapopulations of bottom-dwelling marine animals (reviewed by Levin 2006; Lillis et al. 2014).

We focus on the "fouling community" of organisms growing on surfaces in harbors. They contribute to failure of marine structures, increase fuel costs for ships (Callow and Callow 2002), and are model systems for studying how communities develop over time (e.g., Sutherland and Karlson 1977; Bram et al. 2005; Greene and Grizzle 2007). Fouling communities in harbors experience slow currents, small waves due to wind chop and the wakes of boats, and large waves due to the wakes of ships (Koehl et al. 2013).

Swimming and transport of microscopic organisms

Microscopic swimmers operate at low Reynolds number (Re), at which flow is laminar and viscosity is more important than inertia. Most organismal-level laboratory studies and mathematical models of the hydrodynamics or behavior of microscopic swimmers have been conducted in still water (reviewed by Vogel 1994; Lauga and Powers 2009; Guasto et al. 2012; Hadfield et al. 2014), although a few measurements and models have been made for microswimmers in steady shear or under idealized conditions of flow (e.g., turbulence without currents or waves, rectilinear acceleration) (reviewed by Guasto et al. 2012; Fuchs et al. 2013, 2015; Pepper at al. 2015). Microscopic organisms are rotated by fluid shear (e.g., Grunbaum and Strathmann 2003) and can change their behavior in response to turbulence (e.g., Fuchs et al. 2013, 2015; Wheeler et al. 2013, 2015) or chemical cues (e.g., Hadfield and Koehl 2004).

Although individual larvae operate at low Re, larval transport in oceanic currents is a high-Re process, so flow is turbulent and inertia is more important than viscosity. Field studies and mathematical models of large-scale larval transport often treat larvae as passive tracers carried by ambient flow (e.g., Stobutzki 2001; Levin 2006; Thompson et al. 2014). However, some individual-based models have been developed that follow planktonic organisms as they are transported by currents, and incorporate growth, mortality, or age-dependent behavior (e.g., Szmant and Meadows 2006; Dorman et al. 2011). On the scale of estuaries or surf zones, individual-based models and field studies showed that the transport of larvae is affected by their vertical position in the water column (Epifanio 1988; Shanks 1995; McDonald 2012; Morgan et al. 2012; Fujimura et al. 2014), mode of swimming (Daigle et al. 2014), and locomotory responses to local cues (Fuchs and Reidenbach 2013). These large-scale studies use statistical descriptions of ambient flow (e.g., mean velocities of currents, dissipation rates of turbulent energy) and do not provide information on temporal and spatial patterns of the motion of water encountered by individual larvae.

Our goal is to couple approaches used at the organismal and habitat scales to ask how interactions of microscopic organisms with realistic ambient flow of water affect their movements in natural environments. We focus on motion of larvae in the turbulent boundary layer that forms as water flows across fouling communities in harbors, so the important spatial scales are millimeters to centimeters, and the relevant temporal scales are fractions of a second to minutes.

Larval transport in turbulent boundary layers

Larvae must traverse a turbulent benthic boundary layer (the layer of water in which a gradient of velocity develops between a substratum and free-stream flow) to escape from, or settle into, the community of organisms on the substratum. Boundary-layer flow affects the spatial patterns and rates of larval settlement (reviewed by Butman 1987; Abelson and Denny 1997; Koehl 2007). Measurements of larval behavior (Abelson 1997), trajectories (Jonsson et al. 1991; Tamburri et al. 1996; Finelli and Wethey 2003), and settlement onto surfaces (Butman et al. 1988; Pawlik et al. 1991; Turner et al. 1994) have been made in unidirectional currents in laboratory flumes, but not in the wave-driven flow that is characteristic of many shallow coastal sites.

Measurements of the temporal patterns of encounters by larvae with mechanical or chemical cues as they travel in realistic ambient flow of water are needed to design ecologically-relevant laboratory experiments in which instantaneous responses of larvae can be quantified. To determine those temporal patterns, we can travel with individual larvae by using a Lagrangian approach (moving with the local velocity of the fluid) rather than a Eulerian perspective (staying at one point in space). The Lagrangian approach has been used in a few recent studies conducted in turbulence tanks in which individual larvae were tracked to measure local water velocities around them over time, and these data were used to estimate statistical descriptions of the flow near larvae (Fuchs et al. 2013, 2015; Wheeler et al. 2013, 2015). In these studies mid-water turbulence was simulated using vibrating

grids, and in one case the accelerations due to waves were approximated using seiche flow or rectilinear accelerations (Fuchs et al. 2015), so the effects of waves and turbulence along a substratum were not explored. Although a Lagrangian approach was used to determine temporal patterns of accelerations and shears encountered by model larvae in turbulent wavy flow over a rough substratum (Pepper et al. 2015), the effects of behavior on the performance of larvae at settling on, or escaping from, a surface exposed to such flow has not been explored, nor have been the patterns of the chemical cues encountered by the larvae.

Objectives

The goal of this study is to explore how behavior of microscopic larvae in realistic flow of water across a fouling community affects their motion in the habitat. We compared the performance of larvae that were actively swimming, passively sinking, passively rising, or were passive and neutrally-buoyant, focusing on: (1) the ability of larvae to escape from, or land on, the surface of the fouling community and (2) the chemical and mechanical signals they encounter.

Methods

We mimicked realistic flow measured in the field in a laboratory wave-flume where we could use particle-image velocimetry (PIV) and planar laser-induced fluorescence (PLIF) to measure instantaneous velocities of water and concentrations of chemical cues on the fine scales experienced by larvae (millimeters and fractions of seconds). We then employed an individual-based computer model to follow the trajectories of simulated larvae with different behaviors seeded randomly into those flow fields. The models were used to calculate the motions of larvae through the habitat relative to a surface on which they might land, and to determine patterns of chemical and mechanical signals encountered by the larvae along their trajectories.

Wave-flume experiments

We produced a flow regime in a wave-flume (described by Pepper et al. 2015) that fell within the range of gentle waves (wind chop, wakes of small boats) that we recorded near fouling communities on docks in Pearl Harbor, HI (Koehl 2007; Koehl et al. 2013). The flow in our flume mimicked field measurements of velocity as a function of distance from the substratum, and of temporal variations in velocities due to waves, turbulence, and eddies shed by the fouling community. We chose the temporal patterns of velocities to use in the flume by calculating the power spectral density (PSD) of each record of velocity made in the field (a Fourier transform of velocity as a function of time was made and the variation in velocity that was due to fluctuations at different frequencies was determined, as described by Welch 1967; Rabiner and Gold 1975). Comparisons of spectra of flow measured both in the field and in the flume are given by Pepper et al. (2015). In the section of the flume we imaged, the mean Kolmogorov length (size of the smallest eddies) was 0.2 cm (mean of the time-averaged values at all gird points; range of time-averaged values: 0.1–0.3 cm), and the mean rate of dissipation of turbulent energy was 0.2 $cm^2\ s^{-3}$ (mean of the time-averaged values at all gird points; range of time-averaged values: 0.002–0.1 $cm^2\ s^{-3}$) (Pepper et al. 2015).

In the field the orbital motion of water in waves was compressed into back-and-forth flow near surfaces, which we mimicked in our flume. The velocities of water in the flume varied with time in the wave cycle (wave frequency = 0.1 Hz) and height above the substratum (Fig. 1). At the midpoint of the image (6 cm above the substratum), the mean of the peak horizontal velocities in the waves was 4.67 cm s^{-1} (SD = 0.39, $n = 12$ peaks), and the mean of the peak vertical velocities was 0.08 cm s^{-1} (SD = 0.07, $n = 49$ peaks).

The substrata used in our experiments in the flume were early-stage fouling communities. Submerged surfaces in Pearl Harbor are rapidly overgrown by a biofilm and then by a succession of larger multicellular organisms (Holm et al. 2000; Shikuma and Hadfield 2006). Our early-stage fouling communities, which were dominated by tube worms and encrusting bryozoans (Koehl et al. 2013), developed on PVC panels that were hung vertically from a dock in Pearl Harbor for 1 month. The communities that develop on panels hung vertically or horizontally in Pearl Harbor are similar in composition (M. Hadfield, personal communication). We freeze-dried the communities on the panels in a Virtis Freezemobile 12ES Lyophilizer to retain their fine-scale topography, and then spray-painted them black to avoid reflections of the laser light used for measurements (Koehl and Hadfield 2010). For each experiment, a fouled panel was placed in a depression in the floor of the flume so that the top surface of the PVC panel was at the same height as the floor of the flume. Each panel covered the entire floor of the working section of the flume, extending from the upstream to the downstream

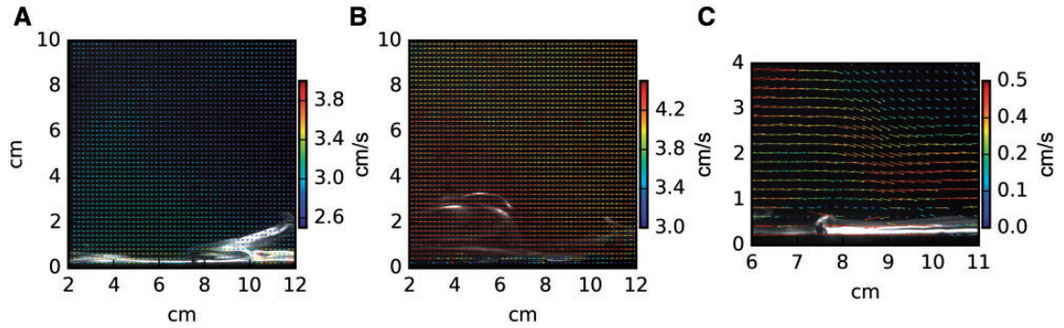

Fig. 1 Water velocity vectors measured in the wave-flume using PIV superimposed on simultaneous images of dye that oozed through the substratum and was mixed up into the flowing water. **A**, **B** and **C** represent different times in the wave cycle, and only a magnified portion of the flow field is shown in C. The color scale to the right of each image indicates water speed. Lighter pixels show higher dye concentrations measured using planar laser-induced fluorescence (PLIF). The profile of an early-stage fouling community, which was approximately 1 mm thick, is shown by the black region along the bottom of each image. The two images were taken at different times during a wave cycle. (This figure is available in black and white in print and in color at *Integrative and Comparative Biology* online.)

collomators in the direction parallel to the flow, and from wall to wall in the direction normal to the flow.

We employed PIV to measure instantaneous water velocities at many points in the flow field. Neutrally-buoyant particles (silver-coated, air-filled glass spheres 11 µm in diameter; Potter Industries) that were carried in the water were illuminated by a plane (2 mm thick) of laser light (Melles Griot DPSS 546 nm green 3W laser). Motion of the illuminated beads was video-recorded at 63 frames per second (AOS High Speed Digital Imaging System, AOS Imaging Studio V2 software, 50 mm AF Nikkor lens; images 980×850 pixels). We masked pixels in which the substratum was imaged and used MatPIV 1.6.1 software to calculate velocities and vorticities of the water for each pair of frames. Vorticity (ξ) is given by

$$\xi = \frac{\partial \vec{w}}{\partial x} - \frac{\partial \vec{u}}{\partial z} \approx \frac{(\vec{w}_{n+1} - \vec{w}_{n-1})}{2\Delta x} - \frac{(\vec{u}_{n+1} - \vec{u}_{n-1})}{2\Delta y}, \quad (1)$$

where x is the horizontal distance parallel to the current, z is the vertical distance, $\vec{w}$ and $\vec{u}$ are velocities of fluid in the z and x directions, respectively, and n is a grid step. PIV sampling windows were 32×32 pixels with windows 50% overlapped, so that the distance between adjacent velocity vectors was 2.01 mm. The mean of 12 particles per window was above the density required for accurate PIV (Raffel et al. 1998). Spurious vectors that differed by >2.0 standard deviations from neighboring vectors were removed. A Butterworth filter (2 Hz cutoff frequency) was applied at each position on the PIV grid to remove temporal noise (Biewener and Full 1992). The spectra of velocities measured by PIV at fixed points in the flume were used to determine the cutoff frequency: at frequencies above 2 Hz, the slope of the PSD as a function of frequency switched from −5/3 (indicative of fluctuations in velocity due to turbulence; e.g., Beresnyak 2011) to a slope of zero (typical of noise).

While we recorded the motions of particles for PIV, we simultaneously used a second synchronized AOS camera system to record fine-scale, instantaneous distributions of concentrations of dye released from the substratum for PLIF analysis. To mimic release of dissolved substances from a fouling community, we filled a reservoir below the panel on the floor of the flume with fluorescent dye dissolved in seawater (0.2 g L^{-1} Rhodamine WT, excitation peak 558 nm, emission peak 582 nm). Dye oozed from the substratum via a row of holes (2 mm in diameter) drilled through the fouling community along the midline of the panel parallel to the direction of flow. The plane of light used for PIV also illuminated a slice of the plume of dye, causing the dye to fluoresce. The PLIF camera was fitted with a high-pass filter (552 nm) (Oriel Corporation), therefore only emitted light from the dye was imaged, and the brightness of pixels (Fig. 1) was proportional to the dye concentration (Crimaldi and Koseff 2001). Using dye to measure dispersal of other dissolved substances (e.g., odors) in moving water is justified because the Schmidt number for water is high (~1000) (Koehl and Reidenbach 2007). Schmidt number ($Sc = \nu/D$, where ν is the fluid's kinematic viscosity, and D is the molecular diffusivity of the dissolved substance in the fluid) represents the ratio of the rate at which momentum is spread through a fluid by

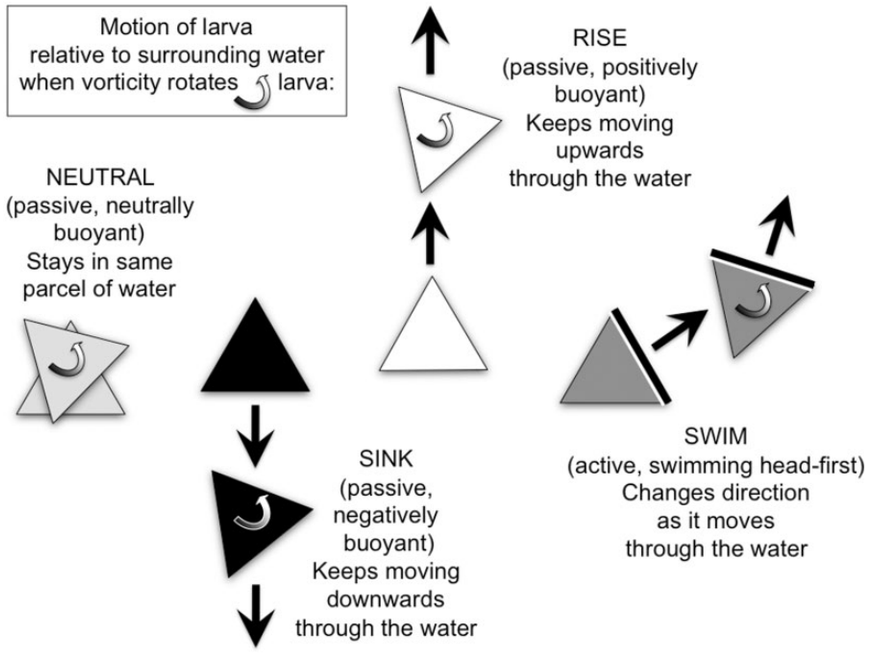

Fig. 2 Diagrams of the motion relative to the surrounding water of the types of simulated larvae used in our individual-based model.

viscosity to the rate at which chemicals (odors) are dispersed through the fluid by molecular diffusion. At high Sc, the millimeter-scale patterns of the concentration of dye or odor swept from the substratum are very similar to each other because molecular diffusivity is low relative to the water's kinematic viscosity.

Individual-based model of larvae in measured flow and concentration fields

We used an individual-based computer model to follow trajectories of simulated larvae with different behaviors as they were carried in the changing velocity fields measured by PIV and concentration fields measured by PLIF (Fig. 1). The simulations were run using Scipy (ver 0.15) binding to a LSODA integrator (http://www.netlib.org/odepack). We simulated larvae with three different behaviors (passive sink, passive rise, swim) and compared them with neutrally-buoyant passive larvae (neutrals), which had a velocity of zero relative to the water around them. The negatively-buoyant passively-sinking larvae (sinkers) had a downward velocity relative to the water of 1.5 mm s^{-1}, similar to the sinking velocities of larvae of polychaetes (Butman et al. 1988), crustaceans (Chia et al. 1984), and mollusks (Chia et al. 1984; Hadfield and Koehl 2004; Chan 2012). The positively-buoyant passively-rising larvae (risers) had an upward velocity relative to the water around them of 1.5 mm s^{-1}, within the range of rising velocities of spawned eggs from echinoderms (Thomas 1994) and corals (Szmant and Meadows 2006). Larvae that swam (swimmers) moved in an anterior direction (i.e., head first) through the water around them at 1.7 mm s^{-1}, like some molluscan veliger larvae (Hadfield and Koehl 2004).

When adjacent layers of water move at different speeds, the local vorticity (ξ, Equation (1)) can cause bodies to rotate. The consequences of rotation on how simulated larvae moved relative to the water in their vicinity are diagrammed in Fig. 2. Neutrally-buoyant, passive larvae were rotated by local vorticity, but did not move out of the parcel of water around them. Passive risers and sinkers were rotated by local vorticity, but continued to move up or down relative to the water around them regardless of their orientation. In contrast, swimmers always moved relative to the water around them with their anterior end leading. Therefore, the direction the larvae swam relative to the frame of reference of the environment (e.g., up, down, right, left) was changed as it was rotated by local instantaneous vorticity while it continued to swim in a "head-first" direction. For simplicity, we assumed that swimmers did not actively execute turns to try to swim in a particular environmental direction, and that they did not show passive righting behavior that would counteract their rotation by local vorticity. In our model, the initial orientation of swimming larvae was randomly assigned, based on the lack of a preferred

direction of swimming by competent larvae of some mollusks (Hadfield and Koehl 2004). The direction of swimming of a larva relative to the substratum was changed at each time-step by the instantaneous vorticity (ξ, Equation (1)) of the water at the position of the larva in the previous time-step. A one-dimensional cubic-spline interpolation in each dimension (Mathews and Fink 1999) was used to calculate the vorticity at the position of the larva at each time-step. The instantaneous rate of angular rotation ($\omega = \xi/2$) was used to calculate the orientation (θ_t) of the larva at time t:

$$\theta_t = \theta_{t-1} + \omega_{t-1}\Delta t, \tag{2}$$

where $t-1$ and t refer to sequential times, ω_{t-1} is the rate of angular rotation at time $t-1$, Δt is the time interval between $t-1$ and t, and θ_{t-1} is the angle of the larva at time $t-1$.

The instantaneous velocity of a larva relative to the substratum was the vector sum of the larva's velocity relative to the water and the velocity of the parcel of water around the larva relative to the substratum. Therefore, location (x_t) of the larva at time t was determined as:

$$x_t = x_{t-1} + \left(\vec{A}_{t-1}\Delta t\right) + \left(\vec{B}_{t-1}\Delta t\right), \tag{3}$$

where $t-1$ and t refer to sequential times, Δt is the time interval between $t-1$ and t, x_{t-1} is the location of the larva at time $t-1$, $\vec{A}_{t-1}$ is the $2D$ velocity of water in the image plane at $t-1$ measured using PIV, and $\vec{B}_{t-1}$ is the $2D$ velocity of the larva in the same plane relative to the parcel of water in which it was located at $t-1$. The water velocity ($\vec{A}_{t-1}$) was interpolated from the PIV grid to the location of the larva at time $t-1$ using one-dimensional cubic-spline interpolation in each dimension (Mathews and Fink 1999). Non-neutrally-buoyant bodies may be moved across streamlines into or out of eddies, but compared with larval velocities and speeds of ambient water, these effects are negligible at the low Re of larvae (Maxey and Riley 1983; Toschi and Bodenschatz 2009) so Equation (3) is a reasonable approximation of the position of a larva.

We followed the simulated trajectories of 2000 larvae for each behavior (neutral, sink, rise, swim) for each replicate panel bearing a fouling community that we studied. Our videos lasted 31 s, but we ran the model for 180 s by cycling through the PLIF/PIV data multiple times, starting with the frame in the first wave cycle of the video that matched the time in the wave cycle of the last frame of the video. A larva exiting the field on the left or right was wrapped around to the opposite side of the next frame to continue its trajectory. We stopped following the trajectory of a larva if the difference in velocity that it encountered after this wrap-around was too large: a simulation of a larva was halted if, after 500 iterations within a time-step, a solution was not reached (the mean difference in velocity for stopped trajectories was 0.73 cm s^{-1}, SD = 0.75, $n = 658$). We did not include larval trajectories that were stopped in this way (2.7% of all the trajectories started) in our calculations of the proportions of larvae that landed on the substratum or that escaped out of the top of the field of view. If a larva entered a pixel of the substratum mask, it was counted as landing on the substratum and its trajectory was stopped. If a larva moved out of the top of the field of view, it was counted as exiting out of the top and its trajectory was stopped. To assure sampling of all regions of the flow field during all phases of the wavy flow as larvae landed, or exited out the top, we seeded the flow field with larvae at random locations at a steady rate (3–4 larvae/frame for the first 650 frames). We also examined the travel of larvae nearing the substratum by categorizing the fates of larvae that entered the layer of water $\leq$5 mm above the substratum: (1) land on substratum, (2) move up and down near substratum but eventually land, or (3) leave substratum without landing.

Because fouling communities can develop on the undersides of ships, we also considered the case in which the surface was above the water in which the larvae were carried. To do this, we used the same PIV and PLIF data, but re-named rising larvae as sinkers, and sinking larvae as risers.

We followed the Lagrangian paths of each simulated larva and tallied their encounters with chemical or mechanical signals, which fluctuated with time (Fig. 3). A threshold magnitude of the signal often is required to damage or elicit a reaction from a larva (e.g., Denny et al. 2002; Hadfield and Koehl 2004; McDonald 2012; Fuchs et al. 2013). Therefore, we tallied the encounters by our simulated larvae with dye (odor) and with vorticity above threshold values. There is a linear relationship between the concentration of the dye and the brightness of a pixel (Crimaldi and Koseff 2001), so we determined the concentration of odors emanating from the substratum by calculating the average brightness (B_{mean}) of all pixels in the layer of water $\leq$2 mm above the substratum. Larvae of a sea slug stop swimming when encountering concentrations of odor from coral that are 3–17% of the concentration at the reef's surface (Hadfield and Koehl 2004), so when the brightness of a pixel in a PLIF video frame at the position of a larva was $\geq 0.1\ B_{mean}$, the odor at

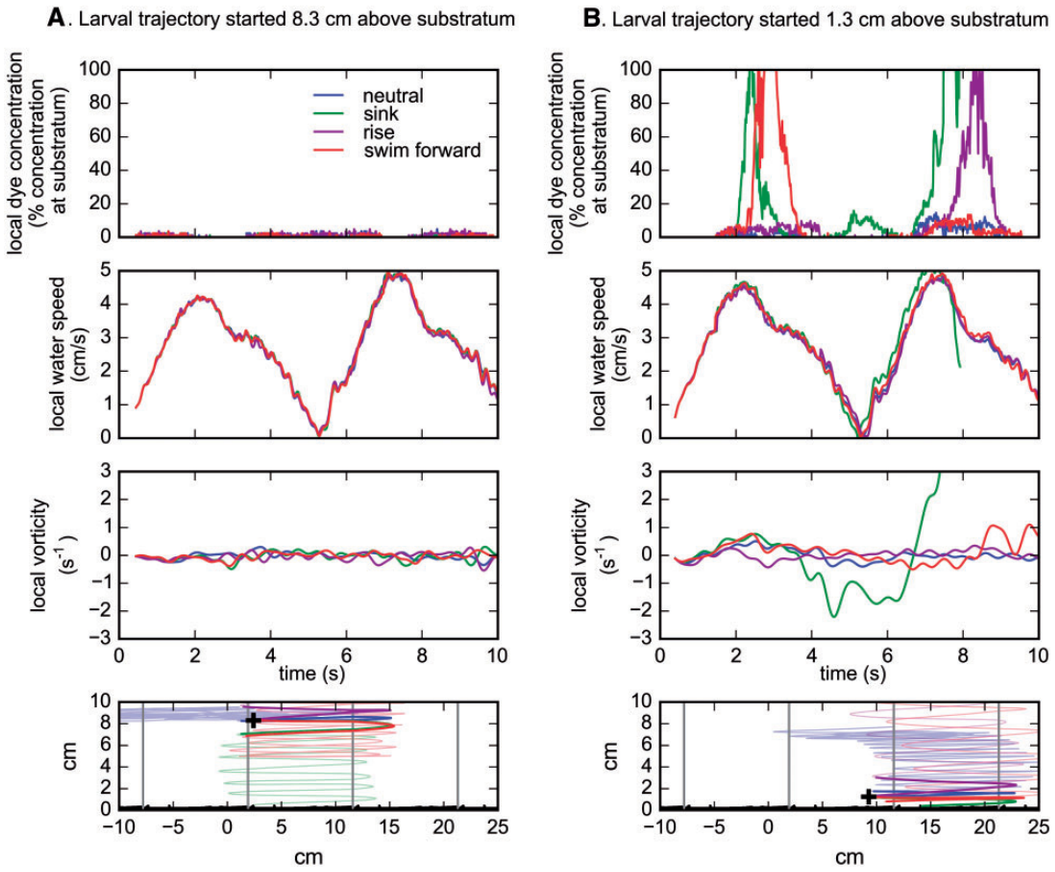

Fig. 3 Examples of local instantaneous dye concentration, water velocity, and water vorticity at the position of a larva (Equation (3)) plotted as a function of time for larvae whose trajectories started in the same video frame at the same point 8.2 cm above a substratum on the floor of the wave-flume (**A**) or at the same point 3.5 cm above the substratum (**B**). Neutral = passive, neutrally-buoyant larva; sink = passive, negatively-buoyant larva moving downwards relative to the water at 1.5 mms^{-1}; rise = passive, positively-buoyant larva moving upwards relative to the water at 1.5 mms^{-1}; swim = actively swimming larva moving head-first through the water at 1.7 mms^{-1} while changing orientation (Equation (2)) and thus swimming direction with respect to the substratum (Fig. 2) as determined by local vorticity (Equation (1)). The graph at the bottom of each column shows the trajectories of the larvae, which all started at the black +. The heavy part of each trajectory shows the path of the larva during the period when the concentrations, speeds, and vorticities are plotted above, and the pale part of each trajectory shows the subsequent path of the larva. (This figure is available in black and white in print and in color at *Integrative and Comparative Biology* online.)

that instant was defined as being above threshold. Vorticity is one indicator of mechanical signals that larvae might sense if they perceive being rotated, or if they sense shear in the water around them. Based on measurements of vorticity that triggers a change in swimming by molluscan larvae (Fuchs et al. 2013), we used a threshold vorticity of 0.4 s^{-1} in our simulations. Because there is a lag time between the time that larvae encounter a chemical or mechanical signal and the time that they respond to it (durations reviewed by Pepper et al. 2015), we used a temporal sampling window of 0.1 s as we followed the trajectory of each larva (i.e., multiple encounters with a signal above threshold occurring within a 0.1 s window were counted as one encounter). Larvae in our model did not change their behavior in response to environmental signals encountered along their trajectories.

Statistical analyses

One-way ANOVA and Bonferroni–Dunn tests for pair-wise comparisons were conducted using Statview 5.0 ($P<0.0083$ or $P<0.0167$ for significance at the 5% level for comparisons of four or three types of behavior, respectively). Proportions were transformed by the arc-sine of the square-root of the variate before ANOVA (Sokal and Rohlf 2011).

Results

Vertical distribution of odor and vorticity

Water velocities and concentrations of dye varied on fine spatial scales (Fig. 1) and changed rapidly with

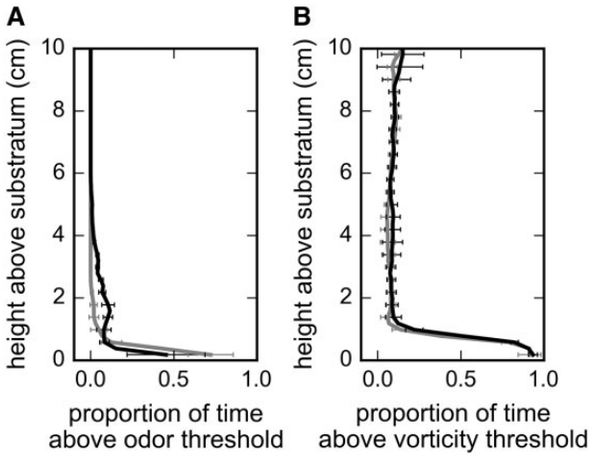

Fig. 4 Proportion of the time in a video lasting 31 s (approximately three wave cycles) that the odor concentration in the water was above $\geq 0.1\ B_{mean}$ (**A**), and that the vorticity was $\geq 0.4\ s^{-1}$ (**B**) at different heights in the water column above a fouling community. Each curve represents a different fouling community. The mean values of all the points at a given height are plotted, and error bars show one standard deviation ($n = 980$ pixels per height for odors; $n = 60$ grid points for vorticity).

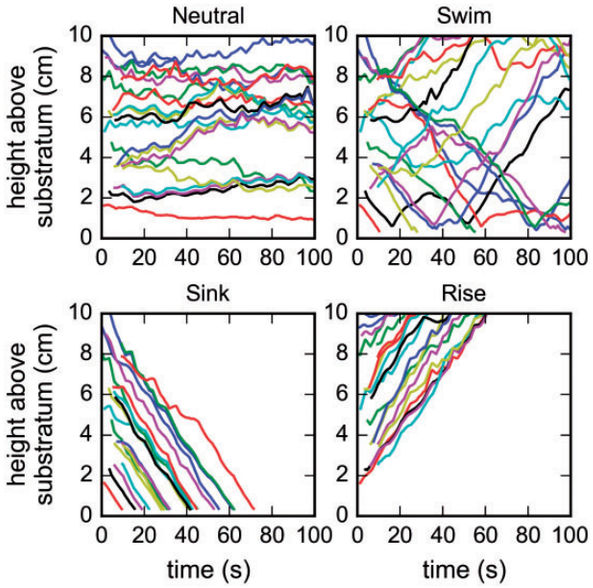

Fig. 5 Vertical positions of simulated larvae plotted as a function of time. A few larvae at the start of a video were chosen to represent different heights above the substratum, and their vertical positions at each time step are shown if they were passive and neutrally-buoyant (Neutral), actively swimming (Swim), passively sinking (Sink), or passively rising (Rise). The starting time in the wave cycle and the initial orientations and positions of the larvae in the water column were the same for all four plots. (This figure is available in black and white in print and in color at *Integrative and Comparative Biology* online.)

time. The proportion of time that vorticity of the water was above threshold was much greater in the steep gradient of velocity near the substratum than it was higher in the water column (Fig. 4A). Similarly, the proportion of time that the concentration of odor from the fouling community was above threshold was much greater near the substratum (Fig. 4B).

Larval motions through a habitat

Examples of vertical positions of simulated larvae are plotted as a function of time in Fig. 5. Small, regular up-and-down motions were due to the orbital flow of water in the waves, the vertical component of which was reduced close to the substratum. Over time in turbulent flow, swimmers moved up and down much more than did passive larvae: sinkers moved downward, risers moved upward, and neutrally-buoyant larvae barely changed their vertical position. Therefore, the numbers of the different types of larvae at each height above the substratum changed with time (Fig. 6). Sinkers accumulated near the substratum, where vorticity and concentration of odor were high (Fig. 4). Risers became more abundant high in the water, while swimmers and neutrally-buoyant larvae remained spread out vertically.

Effects of behavior on the fates of larvae carried in ambient flow near early-stage fouling communities are summarized in Fig. 7. A greater proportion of sinkers landed on a substratum below them than of swimmers, while almost no neutrally-buoyant larvae or risers landed (Fig. 7A). Exiting out of the top of the video when the substratum was below was a measure of the ability of larvae or eggs to escape from the parent community. Most risers and swimmers escaped, while almost no neutrally-buoyant larvae or sinkers got away (Fig. 7B). In contrast, when the fouling community was above the larvae (as for the bottoms of boats), more of the risers landed than did swimmers, while few neutrally-buoyant larvae and few sinkers landed (Fig. 7C). When the fouled surface was above, sinkers and swimmers escaped, while few neutrally-buoyant larvae or risers did.

The times required for larvae with different behaviors to settle or exit out the top when the substratum was below are shown in Fig. 8. Swimmers took longer to land than did sinkers, and to exit out the top than did risers or neutrally-buoyant larvae.

Travel relative to the substratum by larvae entering the layer of water ≤ 5 mm above a fouling community is summarized in Fig. 9. Many of the swimmers that entered this layer exited without landing on the substratum, whereas all sinkers landed. Most of the neutrally-buoyant larvae that came

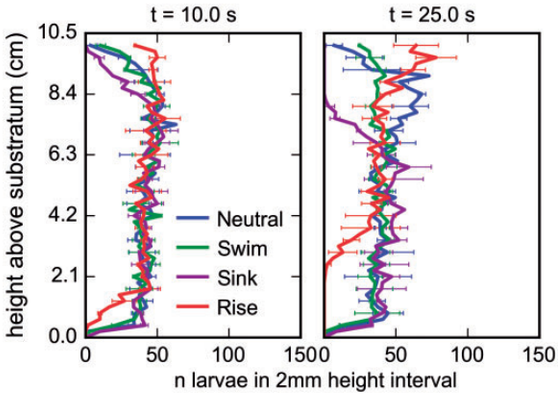

Fig. 6 Number of larvae at different heights above a fouling community on the floor of the wave-flume 10.0 s after the simulation started (left graph) and 25.0 s after the simulation started (right graph). Means for two different fouling communities are plotted. Error bars show one standard deviation. (This figure is available in black and white in print and in color at *Integrative and Comparative Biology* online.)

within 5 mm of the substratum moved up and down, but eventually landed. None of the risers came within 5 mm of the substratum, and those starting there all left.

Environmental signals encountered by larvae

Concentration of odor, speed of the water, and vorticities encountered by larvae varied rapidly, and the frequency of encounters with large peaks tended to be greater for larvae near the substratum than for those higher above it (Fig. 3).

Larval encounters with odor from the substratum at concentrations above threshold (10% B_{mean}) are summarized in Fig. 10. We followed larvae only during the time period when they were in water that was 5–10 cm above the substratum, and we followed larvae only during the period when they were <5 cm above the substratum so that we could see how encounters with odor changed as larvae neared the benthic community. Behavior did not affect the frequency of encounters with odor (Fig. 10A). The proportion of time larvae spent in concentrations of odor above threshold, and the fraction of larvae that encountered odor were low and unaffected by behavior when larvae were 5–10 cm from the substratum (Fig. 10B, C). However, when closer to the substratum where these proportions were much higher, more sinkers encountered odors and spent longer times in odor than did risers, while swimming had no effect.

Encounters with vorticity above threshold ($\geq 0.4\ s^{-1}$) are shown in Fig. 11. We followed larvae only during the time period when they were

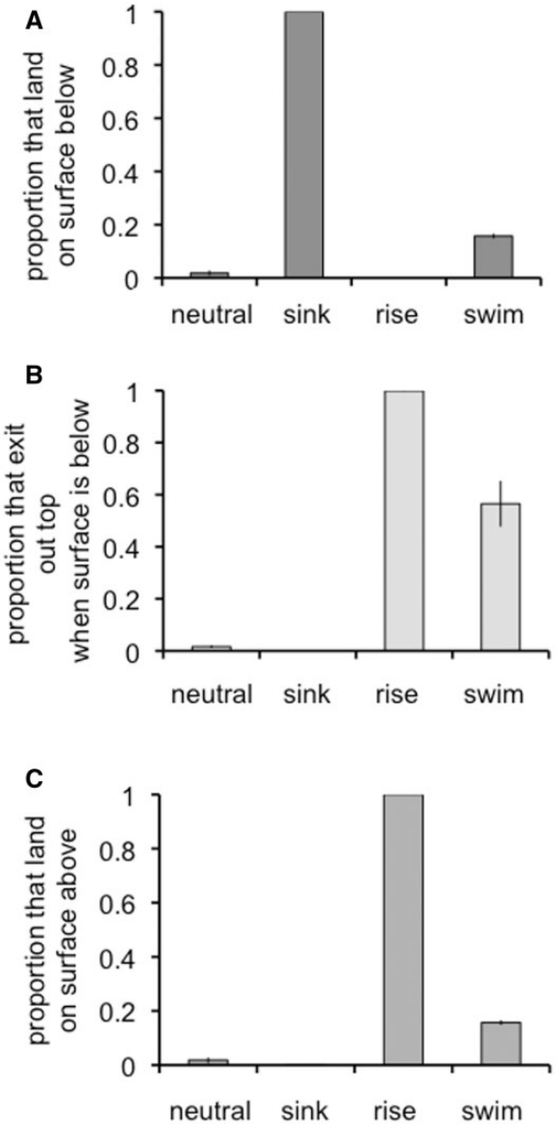

Fig. 7 Proportions of larvae using different behaviors that land on a solid surface below the water in which they are carried (**A**), that exit out of the top of the video frame when the solid surface was below them (**B**), and that land on a solid surface that was above the water in which they are carried (**C**). Means of the values for two different fouled panels are plotted, and error bars indicate one SD. A. Significant differences in proportion that landed on a surface below them: swim > neutral and rise; sink > swim, neutral and rise (ANOVA, Bonferroni/Dunn, $P < 0.0001$). B. Significant differences in proportion that exited out top: swim > neutral and sink; rise > swim, neutral and sink (ANOVA, Bonferroni/Dunn, $P < 0.0001$). C. Significant differences in proportion that landed on a surface above them: swim > neutral and sink; rise > swim, neutral and sink (ANOVA, Bonferroni/Dunn, $P < 0.0001$).

in water that was 5–10 cm above the benthos, and we followed larvae only during the period when they were <5 cm above the benthos so that we could determine how encounters with vorticity changed as

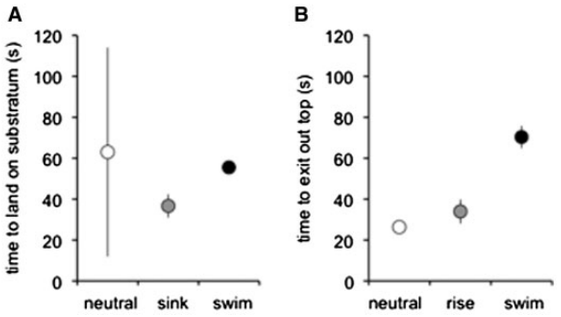

Fig. 8 Time for larvae to land on the substratum (**A**) or to exit out of the top of the video frame (**B**) when the substratum was below them. Means of the times for larvae above each fouled panel were calculated; the grand mean of the means for both panels tested is plotted here, and error bars show one SD. A. If neutrally buoyant larvae, which had enormous variation in time to land, were not considered, then it took significantly longer for swimmers to land on the substratum than sinkers (ANOVA, $P < 0.043$). No risers landed on the substratum. B. Significant differences in the time it takes for larvae to exit out top: swim > rise and neutral (ANOVA, Bonferroni/Dunn, $P < 0.0044$). No sinkers exited out the top.

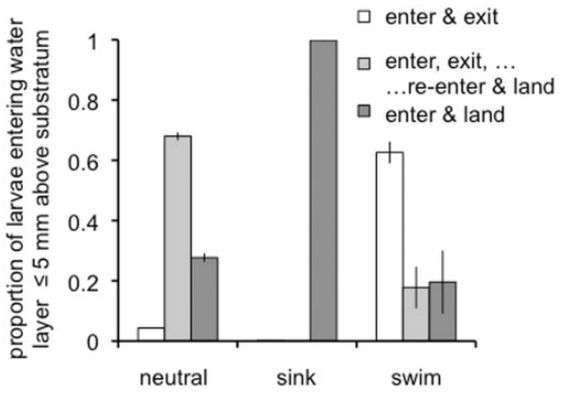

Fig. 9 Performance of larvae that entered the layer of water ≤5 mm above the surface of the fouling community on the floor of the flume: dark gray = proportion that entered the layer once and landed on the substratum; light gray = proportion that entered the layer, exited the layer, and re-entered the layer one or more times, and eventually landed; white = proportion that entered the layer and then exited after one entry or after exiting and re-entering. Means of the values for two different fouled panels are plotted, and error bars indicate one SD. No risers entered this layer. Significantly more swimmers exited the layer of water near the substratum than did sinkers or neutrally buoyant larvae (ANOVA, Bonferroni/Dunn, $P < 0.0002$). Significantly more neutrally buoyant larvae moved up and down, but landed than did swimmers or sinkers (ANOVA, Bonferroni/Dunn, $P < 0.0009$). Significantly more sinkers entered the layer once and landed than did neutrals or swimmers (ANOVA, Bonferroni/Dunn, $P < 0.0003$).

larvae moved closer to the substratum. Behavior had no effect on the frequency of encounters when larvae were 5–10 cm away from the substratum, but sinkers encountered high vorticity more often than did risers when near the substratum (Fig. 11A). When >5 cm from the substratum, fewer sinkers encountered high vorticity than did swimmers and neutrally-buoyant larvae (Fig. 11C), although behavior did not affect how much time they spent in such vorticity (Fig. 11B). In contrast, when closer to the substratum, fewer risers encountered high vorticity than did the animals engaged in other behaviors (Fig. 11C), and swimmers spent less time in high vorticity than did neutrally-buoyant larvae or sinkers, but more than did risers (Fig. 11B).

Discussion

Where do larvae go?

Although microscopic organisms swim slowly, their locomotory behavior can affect where they are transported by ambient water flow. We found that swimmers move up and down in turbulent, wave-driven flow more than do passive larvae (Fig. 5), suggesting that swimming might enhance the probability of encountering surfaces above, below, or alongside a larva. Although more passive sinkers land on substrata below them, and more passive risers on surfaces above them, swimming appears to be the best strategy for landing on surfaces whose location is unpredictable, as is the case for fouling communities on ships and docks (Fig. 7).

In still water, some larvae show directional swimming in response to light or gravity (reviewed by McEdward 1995; McDonald 2012). However, if the swimming directions of larvae are repeatedly changed as they are rotated by fluctuating ambient vorticity, then the importance of phototaxis and geotaxis in affecting larval motion in nature should decrease in high turbulence and near substrata where vorticity is high. Furthermore, experiments and models run in steady water-shear have shown that if the distribution of weight within a microswimmer produces a gravitational torque that provides passive righting, their vertical transport and aggregation can be affected (e.g., Kessler 1986; Grunbaum and Strathmann 2003; Clay and Grünbaum 2010; McDonald 2012). Similar analyses carried out in realistic rapidly-fluctuating shear typical of different locations in the ocean should reveal where such mechanisms might be important in nature.

We found that when larvae move within 5 mm of surfaces below them, sinkers land, most passive neutrally-buoyant larvae move up and down in the flow

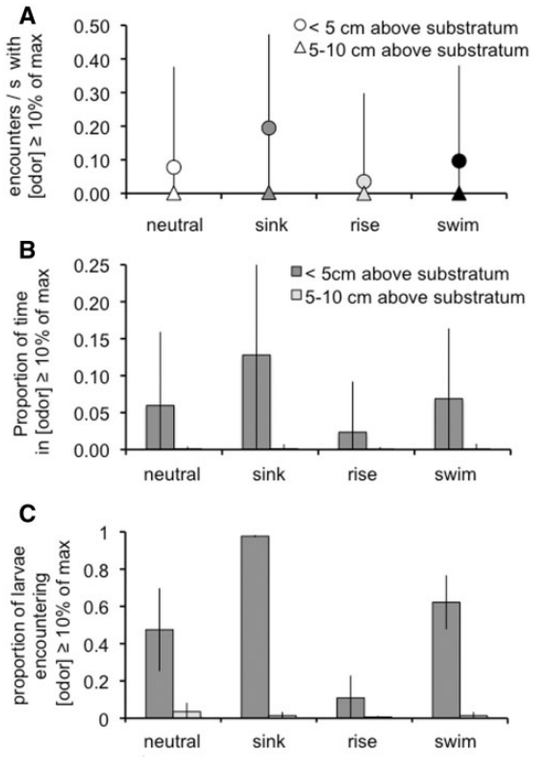

Fig. 10 Encounters by larvae using different behaviors with odor above threshold concentration (i.e., dye from the fouling community at concentrations >10% of the maximum concentration, which was the mean of the concentrations along the substratum). (**A**) Encounter rates by larvae that were between 5 and 10 cm above the substratum (fouling community) on the floor of the flume (circles) and larvae that were <5 cm above the substratum (triangles). Means of odor encounters/s for larvae above each fouled panel were calculated; the grand mean of the means for both panels tested is plotted here, and error bars show one SD. There was no significant effect of behavior on encounter rates. (**B**, **C**). Dark gray bars indicate values for larvae that were between 5 and 10 cm above the substratum (far) and light gray bars refer to larvae that were <5 cm above the substratum (close). Means of the values for two different fouled panels are plotted, and error bars indicate one SD. B. Proportion of their time that larvae spent in odor concentrations above threshold. Behavior had no significant effect when larvae were far from the substratum. When larvae were close to the substratum, risers spent a significantly lower proportion of their time in odor than did sinkers (ANOVA, Bonferroni/Dunn, $P < 0.002$), but there were no other significant differences between behaviors. C. Proportion of the larvae that had one or more encounters with odor above threshold. Behavior had no significant effect when larvae were far from the substratum. When larvae were close to the substratum, the proportion of sinkers that had encounters with odor above threshold was significantly greater than for neutrals or risers (ANOVA, Bonferroni/Dunn, $P < 0.006$), but there were no other significant differences between behaviors.

and land, while many of the swimmers move up and down but do not land (Fig. 9). Although the tumbling of swimming larvae in high shear along the substratum tends to keep those larvae near the bottom (Jonsson et al. 1991), our results suggest that settling larvae are more likely to land on the substratum if they stop swimming as they near a surface. Settling larvae of many species explore benthic habitats by touching down and then leaving surfaces, both in still water (reviewed by Hadfield et al. 2014) and in flowing water (reviewed by Koehl 2007). Our results suggest that the flow of water along a surface enhances such up-and-down motion for larvae swimming near the substratum.

If eggs, embryos, and pre-competent larvae released from parents or egg cases on the substratum are able to move upwards rapidly, their exposure to benthic predators is reduced (reviewed by McDonald 2012) and their dispersal away from their natal site is enhanced (e.g., Strathmann 1982; Coombs et al. 1985; Kelman and Emlet 1999). Our study showed that positively-buoyant, passively-rising larvae are best at escaping from a surface below them, but swimmers are also much more effective than passive neutrally- or negatively-buoyant larvae at exiting the layer of water along the substratum (Fig. 9). Furthermore, although rotation by ambient turbulent flow repeatedly changes the direction of swimmers, they escape out of the top of the field of view at a greater rate than do passive neutrally-buoyant larvae or sinkers (Fig. 4).

Although the study reported here focused on single larval speeds and on thresholds of signal over one type of benthic community exposed to particular flow conditions, individual-based models permit us to explore the effects on larval motion of differences in these parameters (Koehl et al. 2007).

What patterns of signals do larvae encounter?

Chemical and mechanical signals encountered by larvae carried in realistic turbulent flow fluctuate rapidly, with peaks much higher than mean values (Fig. 3) (Koehl et al. 2007; Pepper et al. 2015). Encounters with concentration of odor, or with vorticity above threshold, increase as larvae move near the substratum, and behavior does not affect encounters unless larvae are within 5 cm of the substratum (Figs. 10 and 11).

Although larvae in the simulations reported here do not change their behavior in response to encounters with mechanical or chemical signals, larval responses to instantaneous local signals can be incorporated in future calculations to explore their effects on larval motion through a habitat. For example, larvae of various species show a range of responses to mechanical stimuli in flowing water that might affect their motion through the environment.

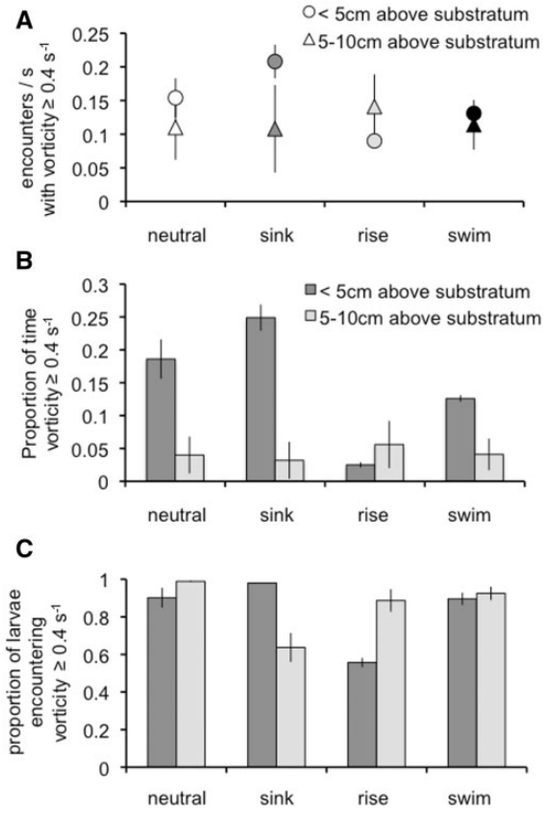

Fig. 11 Encounters by larvae using different behaviors with vorticity above threshold (i.e., vorticity $\geq 0.4\,s^{-1}$). (**A**) Encounter rates by larvae that were between 5 and 10 cm above the substratum (fouling community) on the floor of the flume (circles) and larvae that were <5 cm above the substratum (triangles). Means of vorticity encounters/s for larvae above each fouled panel were calculated; the grand mean of the means for both panels tested is plotted here, and error bars show one SD. There was no significant effect of behavior on encounter rates for larvae 5–10 cm above the substratum. When ≤5 cm above the substratum, sinkers had significantly more encounters per time with vorticity above threshold than did risers (ANOVA, Bonferroni/Dunn, $P < 0.0035$), but there were no other significant differences between behaviors. (**B**, **C**) Dark gray bars indicate values for larvae that were between 5 and 10 cm above the substratum (far) and light gray bars refer to larvae that were <5 cm above the substratum (close). Means of the values for two different fouled panels are plotted, and error bars indicate one SD. B. Proportion of their time that larvae spent in vorticity above threshold. Behavior had no significant effect when larvae were far from the substratum. When larvae were close to the substratum, there were significant differences between behaviors in the proportion of time they spent above threshold vorticity: sink > swim > rise; neutral > rise (ANOVA, Bonferroni/Dunn, $P < 0.0026$). C. Proportion of the larvae that had one or more encounters with vorticity above threshold. Behavior had significant effects when larvae were far from the substratum: swim and neutral > sink (ANOVA, Bonferroni/Dunn, $P < 0.0064$), but there were no other significant differences between behaviors. When larvae were close to the substratum, sink, swim, and neutral > rise (ANOVA, Bonferroni/Dunn, $P < 0.0022$), but there were no other significant differences in the proportion of the larvae that encountered vorticity above threshold.

In strong turbulence, some types of larvae are induced to sink (e.g., Barile et al. 1994; Young 1995; Fuchs et al. 2004; Fuchs and DiBacco 2011) or to actively dive downwards (Finelli and Wethey 2003; Fuchs et al. 2013, 2015), whereas others are induced to dive less often (Wheeler et al. 2013, 2015) or to swim upwards (Fuchs et al. 2010, 2015). In contrast, larvae of other species are stimulated to sink (Fuchs et al. 2010) or to increase their swimming speed (Fuchs and DiBacco 2011) in weak turbulence. High acceleration of water can induce upward or downward swimming by some types of larvae (Fuchs et al. 2015; Wheeler et al. 2015), and high shear can induce an increase in swimming speed in some species (McDonald 2012), but not in others (Fuchs et al. 2013). These contrasting responses to mechanical signals are thought to affect the vertical position in the water column in which larvae are carried and the types of habitats into which larvae of different species recruit. For example sinking or diving in strong turbulence have been suggested as mechanisms that move larvae closer to the substratum when they are transported into the energetic flow of shallow coastal areas (Fuchs et al. 2007), or that enhance settlement onto patches of rough substrata (e.g., oyster beds) at a site (Fuchs and DiBacco 2011; Fuchs and Reidenbach 2013). Furthermore, larvae of species that live in benthic habitats with gently-flowing water sink in weak turbulence, whereas larvae of species that live at sites exposed to rapid flow sink in strong turbulence (Fuchs et al. 2010). Our study focuses on a finer spatial scale and shows that larvae encounter more large mechanical signals within a few centimeters of a surface (Fig. 11) and suggests that sinking in response to such signals should enhance landing on surfaces below larvae, whereas swimming should enhance escape from surfaces below or above larvae (Fig. 7).

Responses to chemical cues can also affect how larvae move through an environment. For example, brief exposure to dissolved chemical cues (e.g., odors from organisms on the substratum) at concentrations above threshold can induce certain larvae to sink (Hadfield and Koehl 2004), and that sinking response during encounters with a chemical cue can enhance rates of larval settlement into benthic communities located below the water column (Koehl et al. 2007). However, since sinkers do not land on surfaces above them (Fig. 7C), such a response to odors is not a good strategy for larvae of members of the fouling community that colonize surfaces above as well as below them.

The temporal patterns of encounters with odors or mechanical signals that are revealed by following

individual larvae through the ambient flow can be used to choose ecologically-relevant frequencies, durations, and magnitudes of such signals to employ in experiments with larvae, e.g., tethered in a miniflume (Hadfield and Koehl 2004), or free in a microfluidic device, to measure their behavioral responses. Recent studies have coupled tracking of larvae with simultaneous PIV to determine responses to local, instantaneous flow (Fuchs et al. 2013, 2015; Wheeler et al. 2013, 2015). However, those studies were conducted in turbulence tanks without water currents or substrata, and thus they approximated conditions in the water column. In contrast, our study quantifies the temporal patterns both of chemical and of mechanical signals encountered by larvae in turbulent boundary layers near substrata from which pre-competent larvae need to escape, or on which competent larvae might settle.

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