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Effects of currents, waves, and biofilms on motion and surface contacts by tubeworm larvae swimming above or below surfaces

M. A. R. Koehl[1,*], Elizabeth Perotti[2,3], David Sischo[2,4], Tom Hata[1], Michael G. Hadfield[2]

[1]Department of Integrative Biology, University of California, Berkeley, CA 94720-3140, USA
[2]Kewalo Marine Laboratory, University of Hawaii, Honolulu, HI 96813, USA
[3]Oregon Department of Fish and Wildlife, Newport, OR 97365, USA
[4]Department of Land and Natural Resources, Honolulu, HI 96813, USA

ABSTRACT: Larvae of many benthic marine invertebrates swim more slowly than the ambient water flow that carries them past surfaces where they might settle. Competent larvae of the tubeworm *Hydroides elegans*, abundant early colonists in warm-water fouling communities, were used to determine if active behavior by microscopic larvae carried in flowing water can affect their contacts with surfaces representing early stages of fouling community succession (clean flat surface, flat biofilm, biofilm on worm tubes). We studied larvae in waves and in the unidirectional flow used in earlier studies of larval settlement, and near surfaces above them (like those where fouling organisms recruit) or below them. We videotaped larval motion near surfaces in a small flume in which we mimicked fine-scale flow measured near surfaces in harbors. Swimming larvae within millimeters of surfaces moved up and down while being carried by horizontal flow, enhancing their contact rates with surfaces above and below them compared with dead larvae passively carried by the current. After contact, live larvae 'bounced' along the surface, sampling it many times per distance they were carried by the current, whereas dead larvae did not. Thus, active behaviors of larvae of *H. elegans*, which must touch a biofilmed surface to be stimulated to attach and metamorphose, enhance their contact with and exploration of surfaces in flowing water. These behaviors are effective in unidirectional flow and waves, for surfaces above or below larvae, and for smooth or rough surfaces, all of which are conditions that the larvae of fouling-community animals encounter in harbors.

KEY WORDS: Larval settlement · Biofilm · Fouling community · *Hydroides elegans* · Water flow · Boundary layer · Waves · Swimming

1. INTRODUCTION

Many benthic marine animals produce microscopic planktonic larvae that are transported by ocean currents (reviewed by Metaxas 2001, Levin 2006). Where these dispersed larvae recruit into benthic habitats is important ecologically because it affects the distribution and genetics of metapopulations of bottom-dwelling marine species (reviewed by Levin 2006) and is a factor in structuring benthic communities (reviewed by Ólafsson et al. 1994, Schiel 2004, Edwards & Stachowicz 2011). 'Competent' larvae have developed enough to be able to metamorphose into bottom-dwelling juveniles. To recruit into benthic habitats, competent larvae must settle from the water column onto surfaces (reviewed by McEdward 1995). A critical step in the process of larval settlement into suitable habitats is initial contact with and

*Corresponding author: cnidaria@berkeley.edu

testing of a surface (e.g. Hadfield et al. 2014), a process that occurs in flowing water in complex natural habitats (reviewed by Nowell & Jumars 1984, Butman 1987, Abelson & Denny 1997, Koehl 2007, Koehl & Cooper 2015). In this study, we examined whether the behavior of larvae that swim more slowly than the ambient water flow can affect their contacts with surfaces above or below them, and we assessed the effects of waves and substratum type on those contacts.

1.1. Effects of substratum type and water flow on larval settlement

Organisms on the substratum can affect where marine larvae settle via chemical cues dissolved in the water that affect swimming behavior, or by cues on surfaces that affect swimming, crawling, and adhesion by larvae (reviewed by Hadfield & Paul 2001). Associative settlement has been investigated for most phyla of marine invertebrates for more than 150 yr (see Crisp 1974). Most experiments on larval settlement have been carried out in dishes of still water with larvae exposed to a single substratum per dish or choices among substrata in a single container (e.g. Wilson 1952, Butman & Grassle 1992, Hadfield et al. 1994). Such experiments served to demonstrate that larvae can select particular substrata for settlement and can distinguish between available substrata, such as algal species (e.g. Ritson-Williams et al. 2014), conspecific organisms and individuals of related congeners (many examples in Crisp 1974), clean vs. biofilmed surfaces (Hadfield et al. 1994), and mono-specific biofilms of different bacterial species (Unabia & Hadfield 1999). Although some invertebrate species respond to dissolved settlement cues (e.g. Hadfield & Pennington 1990, Tamburri et al. 1992, Hadfield & Koehl 2004, Swanson et al. 2012, Maciejewski et al. 2019), most invertebrate larvae settle only after contact with specific biological surfaces (Hadfield & Paul 2001). While still-dish experiments have revealed a great amount of information about the nature of substrata upon which specific larvae will settle (i.e. induction of settlement by organisms on the benthos) and the chemical basis of the stimulatory factors, they tell little or nothing about the mechanisms by which larvae locate specific substrata in nature or remain on them long enough for induction to occur.

Larval settlement in natural marine habitats occurs in flowing water, which is turbulent and can be characterized by waves in shallow coastal habitats. Settling larvae must travel through the boundary layer (the layer of water in which a gradient of velocity develops between a surface and free-stream flow) to land on a substratum. Water flow in turbulent boundary layers affects the rates and success of larval settlement as well as the spatial patterns of where larvae land on surfaces (reviewed by Butman 1987, Abelson & Denny 1997, Koehl 2007). Measurements of larval behavior (Abelson 1997), trajectories (Jonsson et al. 1991, Tamburri et al. 1996, Finelli & Wethey 2003), and settlement onto surfaces (Butman et al. 1988, Grassle & Butman 1989, Mullineaux & Butman 1991, Pawlik et al. 1991, Turner et al. 1994) have shown that larval settlement in flowing water can be affected by flow speed and direction, and by substratum type or chemistry. However, these studies were conducted in unidirectional currents in laboratory flumes, not in the boundary layers of wave-driven flow like that measured in the field in habitats where larvae settle (e.g. Koehl & Hadfield 2004, Koehl & Reidenbach 2007, Koehl et al. 2013). Flume studies in realistic turbulent currents with superimposed waves have shown that waves can enhance vertical transport of water-borne materials (and thus of chemical cues and larvae) through the boundary layer (e.g. Reidenbach et al. 2007), and that structures on the floor improve deposition onto the substratum (Hata et al. 2017). In addition, on fine spatial scales of hundreds of microns, waves can increase pulses of acceleration and shear in the water near substrata (Pepper et al. 2015), and bursts of high hydrodynamic stress on surfaces (Reidenbach et al. 2009, Koehl et al. 2013), the frequency of which can affect larval settlement (Crimaldi et al. 2002).

Larvae of some species of benthic animals sink in response to dissolved chemical cues from benthic organisms (e.g. Tamburri et al. 1996, Zimmer-Faust et al. 1996, Hadfield & Paul 2001, Hadfield & Koehl 2004) or to bumping into an object (Pepper et al. 2015), and sink or swim downwards in response to mechanical stimuli due to turbulence (e.g. Fuchs et al. 2004, 2015, 2018, Wheeler et al. 2015). Such sinking and diving responses of larvae can enhance the probability that they will settle onto appropriate benthic sites (e.g. Tamburri et al. 1996, Finelli & Wethey 2003, Koehl et al. 2007). However, continuous passive sinking, or sinking in response to mechanical or chemical signals that indicate that a surface is nearby, are not effective strategies for landing on surfaces that are above rather than below a larva (Koehl & Cooper 2015). Communities of sessile invertebrates are found on the undersides of man-made structures such as ships and floating docks (e.g. Cal-

low & Callow 2011, Schultz et al. 2011, Bixler & Bhushan 2012, Koehl & Cooper 2015), and of natural structures such as rocks (e.g. McGuinness 1987), drifting debris (e.g. Jokiel 1990), mangrove roots (e.g. Ellison & Farnsworth 1992), and coral reefs (e.g. Martindale 1992). Behaviors that might enhance settlement onto such surfaces above larvae are not well understood.

1.2. Research system

We used competent larvae of tubeworm *Hydroides elegans* (Haswell, 1883) (Fig. 1A), an abundant early colonist in the 'fouling communities' of organisms growing on ships and docks in warm waters around the world, to study how initial contacts of settling larvae with surfaces are affected by the water flow they encounter within a few centimeters of a surface. Our study expanded beyond earlier work on how different types of substrata affect this important phase of larval settlement because we considered surfaces above as well as below the larvae, and because we investigated the effects of waves superimposed on unidirectional currents in addition to studying larvae in still water and unidirectional flow.

1.2.1. Fouling communities

The fouling communities of organisms growing on surfaces in harbors are important ecologically and economically. Fouling communities have long been used as model systems to study ecological succession (e.g. Sutherland & Karlson 1977, Bram et al. 2005, Greene & Grizzle 2007). Fouling communities have received much attention because they increase the hydrodynamic forces on ships, thereby lowering speed and raising fuel costs (e.g. Callow & Callow 2011, Schultz et al. 2011). Foulers on marine structures such as docks and drilling platforms, and in pipes delivering seawater to cool factories and electrical plants, contribute to their failure and to cleaning costs (e.g. Callow & Callow 2011, Bixler & Bhushan 2012). Thus, knowledge of the processes by which larvae of fouling organisms settle onto surfaces should enhance our understanding of ecological succession and may provide insights about ways to interfere with recruitment.

Benthic communities in bays and harbors, including those on man-made structures, experience slow currents. Near the top of the water column, communities are also exposed to small waves due to wind chop and the wakes of boats, and sometimes to large waves due to the wakes of ships (Koehl & Reidenbach 2007, Koehl et al. 2013).

Zobell & Allen (1935) described community development on surfaces newly submerged in the sea: organic molecules accumulate, followed by bacterial recruitment within minutes to hours. After microbial films of bacteria, diatoms, microalgae, and fungi develop, larvae of marine invertebrates recruit and grow on the surfaces, many (e.g. sponges, hydrozans, ascidians) forming spreading colonies (e.g. Edmondson & Ingram 1939, Osman 1977). Complex biofouling communities including dozens of species develop within weeks on surfaces submerged in warm-water

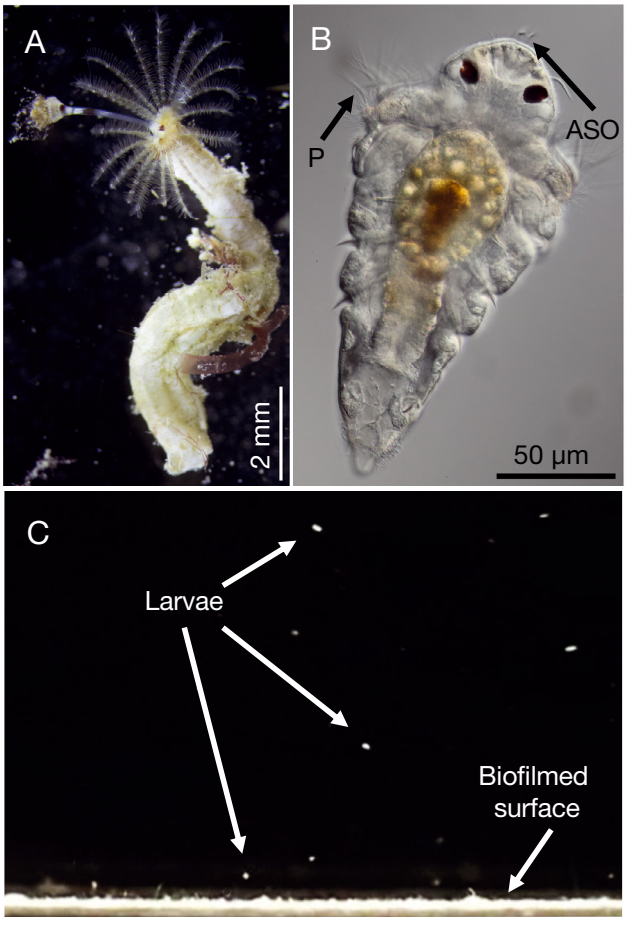

Fig. 1. (A) Calcareous tube of a *Hydroides elegans* on a ' biofilm+tubes' substratum used in the miniflume. (B) Competent nectochaete larva of *H. elegans*, which swims by beating cilia in a ring (the prototroch, P) encircling the cone-shaped body at its widest point. The anterior tuft of cilia is part of the apical sensory organ (ASO). (C) Frame of a video of larvae of *H. elegans* carried in water flowing over a biofilmed surface in the miniflume. The only larvae visible as white dots are those in the plane of light along the midline of the flume. The field of view of this video frame is 1.4 cm tall

bays and harbors (reviewed by Flemming et al. 2009). Newly submerged clean panels at our field site in Pearl Harbor, HI (USA), follow this pattern, with the serpulid worm *H. elegans* appearing within a few days (Walters et al. 1997, Holm et al. 2000, Swain et al. 2000, Shikuma & Hadfield 2005, Nedved & Hadfield 2009).

1.2.2. *Hydroides elegans*

The serpulid tubeworm *H. elegans* (Fig. 1A) is a model organism for studying polychaete development (e.g. Carpizo-Ituarte & Hadfield 1998, 2003, Holm et al. 1998), settlement patterns in response to flow (Walters et al. 1997), and settlement and metamorphosis in response to biofilms (Hadfield 2011). Competent larvae of *H. elegans* swim by beating cilia in a ring (the prototroch) encircling the body (Fig. 1B). They settle only in response to contact with a bacterially biofilmed surface (Hadfield et al. 2014), and this response is due to interactions with specific bacteria (Unabia & Hadfield 1999, Huang & Hadfield 2003, Shikuma & Hadfield 2005) and bacterial products (Huang et al. 2012, Shikuma et al. 2014, Freckelton et al. 2017). The larvae of *H. elegans* settle onto surfaces below them (e.g. Carpizo-Ituarte & Hadfield 1998, Shikuma & Hadfield 2005) and onto the undersides of horizontal surfaces above them (Hurlbut 1991, Walters 1992). After about 5 min on a biofilmed surface, the larvae of *H. elegans* attach to one spot and start making a primary organic tube (Harder et al. 2002), complete the primary tube after ~15 min, and start secreting the calcareous secondary tube (Fig. 1A) after ~1.5 h (Carpizo-Ituarte & Hadfield 1998, Hadfield et al. 2021, Huggett et al. 2021). *H. elegans* is dioecious and spawns readily in the lab, and its planktotrophic larvae achieve metamorphic competence in 4–6 d at 25°C (Hadfield et al. 1994, Nedved & Hadfield 2009).

1.3. Objectives

The overall goal of this study was to determine whether the active behavior of competent larvae of *H. elegans* that are carried past surfaces by realistic ambient water flow can affect their trajectories in the water and their contacts with those surfaces. To address this issue, the specific questions asked were:

(1) Do the different types of surfaces that characterize early stages in the development of a fouling community (clean flat surfaces, flat biofilm, biofilm on tubes of *H. elegans*) affect the trajectories and contact behaviors (percent of larvae contacting a surface, contact duration, number of contacts per streamwise distance traveled) of larvae in still and in flowing water?

(2) Which aspects of larval motions near surfaces are due to active behavior versus passive transport by ambient water flow?

(3) Are the effects of surface type on larval contact behavior the same if the larvae are carried in ambient flow under surfaces above them versus over surfaces below them?

(4) Does the superposition of waves onto an ambient unidirectional current alter the effects of surface type on larval contact behavior?

2. MATERIALS AND METHODS

We videotaped the behavior of living and dead competent larvae of *Hydroides elegans* near different surfaces on the floor and on the ceiling of a small laboratory wave-flume in which we simulated water flow measured near surfaces on which early-stage fouling communities were developing. Experiments were conducted at the Kewalo Marine Laboratory, University of Hawaii.

2.1. Water flow conditions in the wave-flume

To determine realistic flow conditions near surfaces of early-stage fouling communities so that we could mimic them in a small wave-flume (Fig. 2), we measured water velocity profiles near fouled surfaces on docks at several sites in Pearl Harbor, HI, using an electromagnetic flow meter (Koehl 2007, Koehl et al. 2013) and an acoustic Doppler velocimeter (Koehl & Hadfield 2010, Pepper et al. 2015). At our sites in Pearl Harbor, slow currents (peak free-stream velocities of 3–24 cm s^{-1}) with small waves due to wind chop flowed across these surfaces, as has been reported along dock surfaces in other harbors (Okamura 1984, Hunter 1988, Schabes 1992). Although fouling communities in harbors are occasionally briefly exposed to larger waves (e.g. ship wakes, Koehl 2007), we focused on the calm conditions that prevail in harbors most of the time.

When a water current flows past a surface, a boundary layer of slowed flow develops along the surface, so we determined the fine-scale water velocity profiles near fouled surfaces in a large wave-flume in which we replicated the water flow we

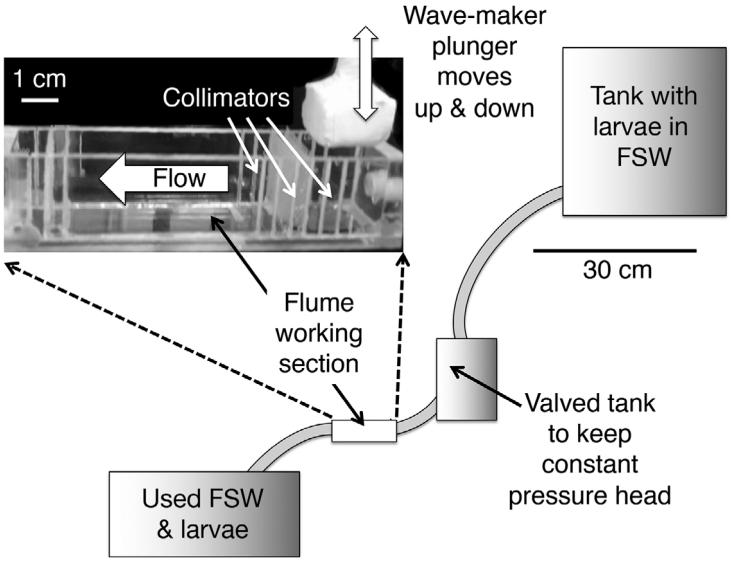

Fig. 2. Small wave-flume used in the experiments. *Hydroides elegans* larvae or particles in filtered seawater (FSW) were delivered by gravity from the supply tank to another tank that had a valve that kept the water depth constant, and thus maintained a constant pressure head to drive water through the working section of the flume. Velocity was controlled by adjusting the height of the valved tank using a lab jack. A series of collimators adjusted the velocity profile in the working section (inset photo) to mimic that measured in the field. A plunger upstream of the collimators was used to superimpose waves on the unidirectional flow to mimic the flow measured in the field (see Fig. 3). A test surface was mounted on either the floor or the ceiling of the working section. Test surfaces were the width of the working section and were held in a slot such that the test surface was flush with upstream and downstream surfaces of the floor or ceiling. After passing through the working section, the water and larvae or particles were collected in a waste tank. All larvae or particles passed through the working section only once

measured in Pearl Harbor (Koehl et al. 2013). Laser Doppler velocimetry was used to measure the very fine-scale (0.5 mm resolution) velocity gradients along surfaces of early-stage fouling communities with and without superimposed waves. In both the field and flume, orbital water motion of the waves was compressed into back-and-forth flow near the substratum and added to or reduced the instantaneous velocity of the current (Fig. 3). We used the fine-scale velocity data measured in the flume to design the velocity profiles (Fig. 4) and waves (Fig. 3) along surfaces in a small laboratory wave-flume in which videos of larval behavior could be made.

The design of our small flume was described by Hadfield & Koehl (2004) and Koehl & Reidenbach (2007) and is illustrated in Fig. 2. The plexiglass working section (3 cm wide, 3 cm deep, 14.5 cm long), which had a ceiling, was small enough to permit close-up videos to be made of larval behavior. All experiments were conducted in filtered seawater (FSW) that had been passed through a 0.22 µm Millipore filter. A steady flow rate of FSW through the flume was maintained by a constant-head tank. Velocity was adjusted by raising or lowering that tank with a lab jack and fine-tuned by adjusting a valve downstream of the working section. Arrays of collimators upstream from the working section were used to create velocity profiles along the floor and ceiling (Fig. 4) that mimicked those measured in the field by Koehl et al. (2013). We could produce unidirectional flow in the flume or use a plunger upstream of the collimators to superimpose velocity oscillations on the net downstream water motion to simulate wind chop (Fig. 3).

Suspensions of larvae or particles in FSW were placed in the upstream reservoir of the flume and were carried by the moving water through the working section of the flume. The flume was designed to be a flow-through system so that each larva passed through the working section only once, and so that background levels of possible chemical cues would not build up over the course of an experiment. The head tank and flume were emptied and washed with fresh water after each replicate of an experiment.

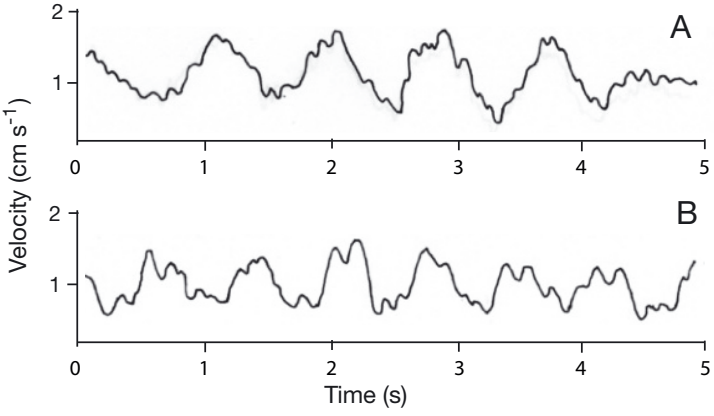

Fig. 3. Water velocity parallel to the surface measured at a distance of 2 cm (A) from a fouled panel on a dock in Pearl Harbor, HI, USA (using acoustic Doppler velocimetry; Koehl & Hadfield 2010), and (B) from the floor of the small wave-flume (using particle-tracking velocimetry) when the wave-producing plunger was operated

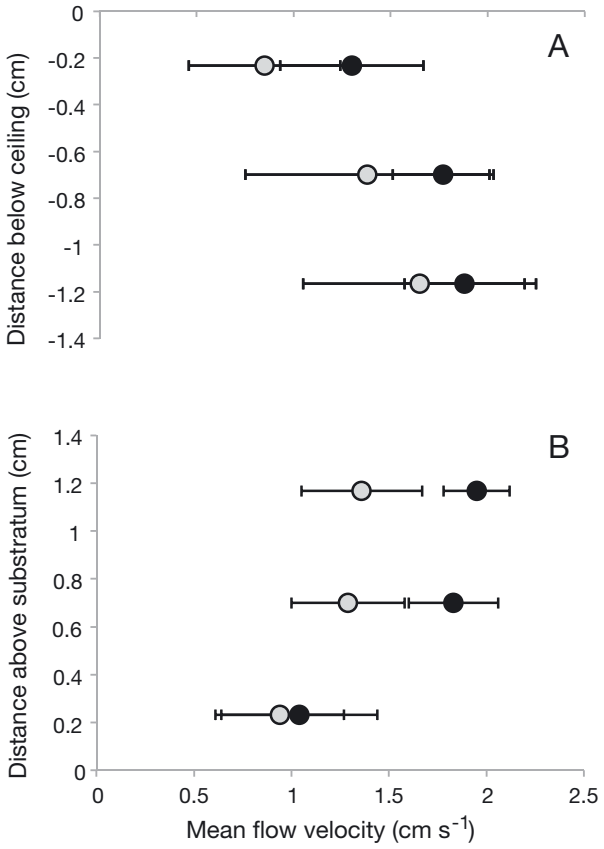

Fig. 4. Water velocity profiles along the (A) ceiling and (B) floor of the wave-flume in unidirectional flow (black) and in unidirectional flow with waves superimposed (grey; see Fig. 3). Because the field of view of the video images was 1.4 cm tall, velocities along the ceiling were measured separately from those along the floor. Mean velocities at each height were calculated for each experiment, and the grand mean of 5 replicate experiments were plotted; error bars = ±1 SD

2.2. Video analysis

Water flow and larval trajectories were measured only along the midline of the flume, which was illuminated by a sheet of light ~1 mm thick. The floor of the flume was opaque, except for a transparent slit along the midline of the working section. Light from a lamp (Portable Luminaire Model G-2134-7280) was reflected off a mirror at 45° to the horizontal plane below the flume and passed upward through the slit. Larvae illuminated in this plane of light appeared as bright white dots against a dark background (Fig. 1C).

Video recordings (60 s duration, 30 fps) were made with a Sony Handicam HDR-HC3. The camera magnification was chosen to achieve the largest field of view in which the larvae were still big enough to be clearly visible. This allowed us to image the layer of water that was ≤1.4 cm above the floor or below the ceiling of the flume. The taped video was captured using Window Movie Maker 2012, converted to uncompressed .avi files using VirtualDub 1.9, and imported into ImageJ 1.37a. The video frames were 720 × 480 pixels. Video records of a size-scale grid at the midline of the flume were made before each experiment to calibrate the vertical and horizontal distances and to determine the spatial resolution of the video images, which ranged from 34 to 56 µm pixel^{-1}, depending on the placement of the camera. Neutrally buoyant hydrated cysts of *Artemia salina* used as flow markers (~200 µm in diameter, Wheeler et al. 1979) were thus 4–6 pixels in diameter, and larvae of *H. elegans* (~150 µm in length, Hadfield 2011) were 3–4 pixels long in our videos. Only the particles or larvae that were in sharp focus in the light sheet and that were found within a defined region (2.8 cm wide × 1.4 cm tall) directly above the floor or below the ceiling of the flume were analyzed. Particle and larval tracking were done with a custom plug-in Particle Tracking Manager that we wrote.

Water velocities were measured as a function of time using particle-tracking velocimetry (PTV) of the paths of neutrally buoyant marker particles carried in the water. Cysts of *A. salina* were stirred into a beaker of FSW and left for 24 h. Cysts floating at the top of the beaker were removed and those suspended mid-water in the beaker were decanted and used as the neutrally buoyant particles. Particles were tracked only in the 2.8 × 1.4 cm region above the floor or below the ceiling of the wave-flume. This region was divided into 3 horizontal strips, and the mean velocities of the cysts in each strip were used to calculate the flow velocity in that strip (Fig. 4). These PTV flow measurements to calibrate the flow tank were made over or under glass surfaces and were done on the same days as, but separately from, the experiments with larvae.

The trajectories of larvae in still and in moving water in the flume were analyzed. The instantaneous velocities of each larva were measured and a mean was calculated for each individual. The straightness index (SI; ratio of the distance between the start and end points of a larval path during the video to the actual distance the larva moved through the water, also called 'net-to-gross-displacement ratio') was determined for larvae in still water (Hadfield & Koehl 2004). The contacts of larvae onto the floor or ceiling could be seen clearly in the videos, so the percent of larvae in a video that contacted the floor or ceiling, and the durations of the first contacts with the sur-

face ('touchdowns') were measured. In experiments in which the water was flowing, larvae or particles made their first contact with the substratum at different distances from the upstream end of the working section, so the number of touches per streamwise distance traveled was calculated using the distance between the point of first contact and the horizontal position of the particle or larva at the end of the video clip if it was still in the field of view, or the distance between the point of first contact and the downstream edge of the image if it traveled out of the field of view before the video ended.

2.3. Larvae of *Hydroides elegans*

Larvae of *H. elegans* were reared to metamorphic competence using methods described by Nedved & Hadfield (2009). A different batch of larvae was used for each replicate in this study. A subset of each batch was killed by freezing and then checked for anatomical integrity when thawed. These dead larvae were used in separate experiments as a control for the passive effects of gravity and body shape on larval trajectories and contact with the substratum. Approximately 50–100 larvae from each batch were also reserved for a settlement assay to confirm metamorphic competence.

2.4. Substrata

Substrata used in the flume represented early stages in the succession of a fouling community (Holm et al. 2000, Shikuma & Hadfield 2005): 'glass' (newly submerged flat surface); 'biofilm' (natural biofilm on a flat glass surface, Fig. 1C); and 'biofilm+tubes' (natural biofilm on a glass surface bearing some tubes of adult *H. elegans* that were about 0.5–1.0 mm in diameter and about 10–20 mm long; Fig. 1A). Our clean surfaces were acid-washed Fisherbrand glass microscope slides. Other slides were submerged in a flow-through sea table at the Kewalo Marine Laboratory for 14 d to accumulate a biofilm, or for >30 d to allow early colonizers of the fouling community to recruit (mainly *H. elegans* and a few encrusting bryozoans and small sea anemones). These slides were mounted in slots so that they were flush with the surrounding floor or ceiling of the wave-flume. We measured larval trajectories near surfaces on the ceiling as well as on the floor of the flume to determine whether gravity or the direction of the light source affected larval trajectories, and because fouling communities can develop on the undersides of objects.

2.5. Experimental design and data analysis

The trajectories of living larvae, dead larvae, or neutrally buoyant particles within 1.4 cm of each type of surface (glass, biofilm, biofilm+tubes) on the floor or on the ceiling of the wave-flume were measured in still water, in unidirectional flow, and in unidirectional flow with superimposed waves. Five independent replicates of each set of conditions were conducted, where a set of conditions was 1 type of body (live larvae, dead larvae, or neutrally buoyant spherical particles) near 1 type of surface (clean glass, biofilm, or biofilm+tubes) at 1 location (floor or ceiling) in 1 flow condition (still water, unidirectional flow, or unidirectional flow plus waves). In still water, dead larvae and particles were only videotaped for glass floors and ceilings, and in flowing water, particles were only videotaped for glass floors and ceilings, so there was a total of 38 sets of conditions. The independent replicates of each set of conditions were conducted on 5 separate days using new batches of living competent larvae, of dead larvae, or of particles, and using new surfaces. A few of the videos had technical proclems and were thus not analyzed; in those cases, the number of replicates for a given set of conditions was less than 5 (see degrees of freedom and residuals in Tables 1–4). The number of larvae or particles that were captured in a video depended on the number that happened to move through the field of view during the video, and thus could not be controlled (videos of still water, mean ± SD = 108 ± 101 larvae or particles per video, n = 47 videos; videos of flowing water, mean = 217 ± 281 larvae or particles per video, n = 183 videos).

The 1-way ANOVA with post hoc Tukey HSD analyses and the *t*-tests described below were done using the astasa Online Statistical Calculator (https://astatsa.com/OneWay_Anova_with_TukeyHSD/). Our data met the assumption of normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test); these tests were done using Statistics Kingdom Statistics Online (https://www.statskingdom.com/index.html). All data that were percentages were arcsine transformed (Sokal & Rohlf 1995) to meet the assumptions of normality and homogeneity of variance before being used in parametric statistical calculations.

In still water, we measured several aspects of the behavior of living larvae: instantaneous swimming

speed (cm s^{-1}), SI, percent of larvae that touched the surface, and duration (s) of the first touch. Living larvae changed direction as they swam, so speed was the best measure of how rapidly they were moving relative to the still water around them. Therefore, for each replicate of a set of conditions, we determined the mean of the instantaneous speeds of each living larva. We then calculated the mean of those mean swimming speeds of all larvae in a replicate, and used that population mean for the replicate as an independent sample in statistical analyses. Similarly, the mean of the SI and the mean of the first-touch duration for all the living or dead larvae or particles in a replicate were calculated. We then used the mean SI and mean touch duration from each replicate as an independent sample in statistical analyses. Each aspect of larval behavior in still water was compared between the 3 types of surfaces using 1-way ANOVA followed by Tukey HSD post hoc tests, with $\alpha = 0.05$. These ANOVA and Tukey HSD tests were done separately for the floor and ceiling.

In still water, we also measured the instantaneous velocities (cm s^{-1}) of dead larvae and of neutrally buoyant particles when they were over or under glass surfaces. The dead larvae and the particles had no horizontal components to their instantaneous velocities, so for each replicate of a set of conditions, we determined the mean vertical velocity of each dead larva or particle. A positive value signified upward motion and a negative value signified downward motion, so the mean of those values for all larvae or particles in a replicate indicated whether the population of larvae or particles in that replicate showed net upward or downward motion. We used the population mean velocity from each replicate of a set of conditions as an independent sample to calculate the grand mean and standard deviation of the vertical velocities of dead larvae and of particles.

In flowing water, we analyzed the behaviors of living larvae, dead larvae, and neutrally buoyant particles contacting the floor or ceiling. For each replicate of a set of conditions, we determined the percent of larvae or particles that touched the surface. We also calculated the mean for all larvae or particles in a replicate of the duration (s) of the first touch, and of the number of touches per distance (cm) of streamwise travel. Then we used those percentages or means from each replicate as independent samples in statistical analyses, and the grand mean and SD for each set of conditions was calculated using the means from each replicate.

We used 1-way ANOVA to compare the defined aspects of the behavior of live larvae, dead larvae, and neutrally buoyant spherical particles exposed to different types of surfaces in flowing water. There were so many sets of conditions in our study that using standard ANOVA post-hoc techniques for pairwise comparisons to control Type I error would have led to a loss of statistical power, and thus increased the likelihood of Type II error (Waite & Campbell 2006, Ruxton & Beauchamp 2008). In situations like ours for which there are so many useless comparisons (i.e. comparisons that do not test a hypothesis, such as comparing particles over a clean glass floor in unidirectional flow with live larvae under a biofilmed ceiling in waves), a better approach is to use the 'planned comparisons' technique described by Ruxton & Beauchamp (2008) to balance control for Type I and Type II errors. The planned comparisons approach does a 1-way ANOVA on all of the data, but then only calculates the likelihood of a Type I error for the comparisons that test pre-determined hypotheses. We used a *t*-test ($\alpha = 0.05$) for each pairwise comparison that tested a hypothesis, but used the residual means square from the ANOVA to calculate the likelihood of a Type I error (details described by Ruxton & Beauchamp 2008).

To further reduce the likelihood of Type II errors, we conducted separate analyses for the floor in unidirectional flow, the floor in waves, the ceiling in unidirectional flow, and the ceiling in waves. For each of these 1-way ANOVAs, there were 7 treatments: (1) living larvae, glass surface, (2) living larvae, biofilmed surface, (3) living larvae, surface with biofilm+tubes, (4) dead larvae, glass surface, (5) dead larvae, biofilmed surface, (6) dead larvae, surface with biofilm+tubes, and (7) particles, glass surface. Eight pairwise 'planned comparisons' were done following each ANOVA to test specific null hypotheses:

(1) Living and dead larvae behave the same near a clean glass surface.

(2) Living and dead larvae behave the same near a biofilmed surface.

(3) Living and dead larvae behave the same near a surface with biofilm plus *H. elegans* tubes.

(4) Living larvae behave the same near a clean glass surface as they do near a biofilmed surface.

(5) Living larvae behave the same near a clean glass surface as they do near a surface with biofilm plus *H. elegans* tubes.

(6) Living larvae behave the same near a biofilmed surface as they do near a surface with biofilm plus *H. elegans* tubes.

(7) Dead larvae and neutrally buoyant spherical particles behave the same near a clean glass surface.

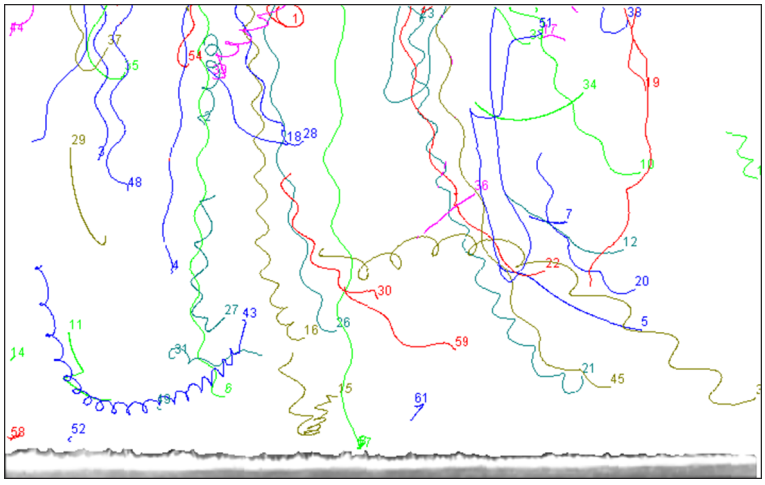

Fig. 5. Trajectories of competent larvae of *Hydroides elegans* in still water in the working section of the small wave-flume over a 'biofilm' substratum. The picture along the bottom of the image is the side view of the biofilmed surface above which these larvae were swimming, taken from a frame of this video. Each individual larva is identified by a number, which is placed at the start of its trajectory. The image is 1.4 cm tall

(8) Living larvae and neutrally buoyant spherical particles behave the same near a clean glass surface.

Comparisons that did not test any of these hypotheses were not done.

Table 1. Swimming by living *Hydroides elegans* larvae near different surfaces in still water. Comparisons were done using 1-way ANOVA followed by Tukey HSD post-hoc tests, with $\alpha = 0.05$. Pairwise comparisons for which $p \geq 0.05$ are indicated by NS (not significant)

	Swimming speed (cm s^{-1})	Straightness index
Floor: $F_{df,residual}$	$0.20_{2,12}$	$0.17_{2,12}$
Glass vs. biofilm	NS	NS
Glass vs. biofilm+tubes	NS	NS
Biofilm vs. biofilm+tubes	NS	NS
Ceiling: $F_{df,residual}$	$0.21_{2,21}$	$0.49_{2,21}$
Glass vs. biofilm	NS	NS
Glass vs. biofilm+tubes	NS	NS
Biofilm vs. biofilm+tubes	NS	NS

3. RESULTS

3.1. Behavior in still water

Competent larvae of *Hydroides elegans* swam along helical or curved paths in still water, and the direction of travel differed between individuals (Fig. 5). The swimming speeds of living competent larvae in still water were not significantly different when they swam over or under glass, biofilm, or biofilm+tubes (Table 1, Fig. 6A). The mean ± SD swimming speeds were 0.22 ± 0.10 cm s^{-1} for glass, 0.20 ± 0.09 cm s^{-1} for biofilm, and 0.20 ± 0.10 cm s^{-1} for biofilm+tubes (n = 13 replicate treatments for each type of surface, where 5 floor and 8 ceiling replicates were used). There was also no significant effect of surface type on the SI of larval trajectories (Table 1, Fig. 6B).

The behavior of living larvae contacting different surfaces was compared in still water. There was no significant difference between the percentage of larvae that touched the surface for glass, biofilm, or biofilm+tubes, on both the floor and the ceiling

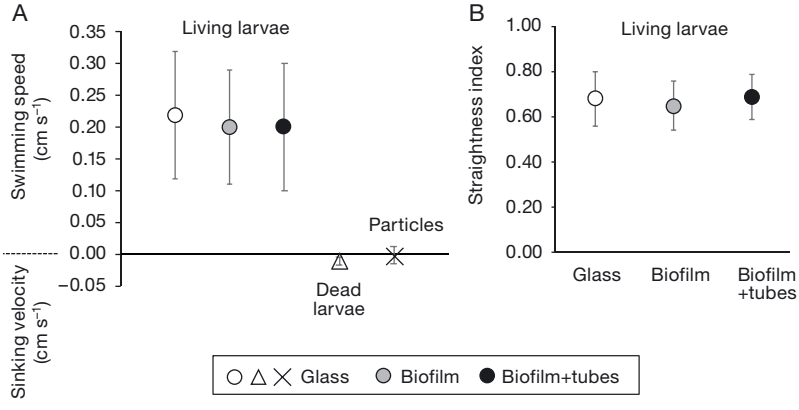

Fig. 6. Motion of *Hydroides elegans* larvae and particles in still water near different surfaces in the working section of the flume. (A) Mean swimming speeds of living competent larvae over or under glass surfaces, biofilmed surfaces, or surfaces with biofilm and tubes, and vertical velocities of dead larvae and neutrally buoyant spherical particles over or under glass. (B) Straightness indices for swimming larvae over or under glass surfaces, biofilmed surfaces, or surfaces with biofilm and tubes. Means of replicate experiments are plotted, error bars represent ±1 SD, and statistics are reported in Table 1

Table 2. Responses to surfaces in still water by living *Hydroides elegans* larvae. Comparisons were done using 1-way ANOVA followed by Tukey HSD post-hoc tests, with $\alpha = 0.05$. Pairwise comparisons for which $p \geq 0.05$ are indicated by NS (not significant)

	Arcsine transform of % of larvae that touch	Duration of first touch (s)
Floor: $F_{df,\ residual}$	$1.58_{2,11}$	$0.39_{2,12}$
Glass vs. biofilm	NS	NS
Glass vs. biofilm+tubes	NS	NS
Biofilm vs. biofilm+tubes	NS	NS
Ceiling $F_{df,residual}$	$0.65_{2,7}$	$1.38_{2,9}$
Glass vs biofilm	NS	NS
Glass vs. biofilm+tubes	NS	NS
Biofilm vs. biofilm+tubes	NS	NS

(Table 2, Fig. 7). In addition, the duration of the first touch on a surface (time a larva was in contact with a surface before resuming swimming) varied greatly between individuals (ranging on the order of 1 s to 1 min), and there was no significant difference between touch duration on glass, biofilm, or biofilm+tubes on the floor or on the ceiling (Table 2). We saw no lateral sampling of surfaces by larvae in still water.

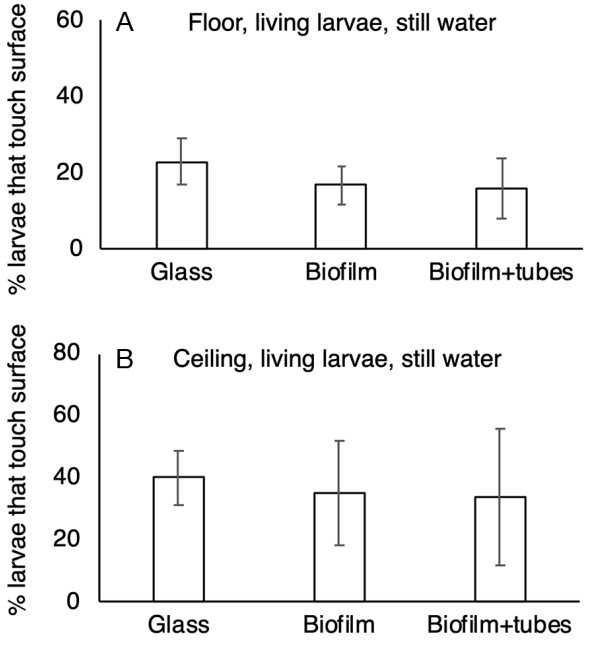

Fig. 7. Percent in each video of living *Hydroides elegans* larvae that touched (A) the floor or (B) the ceiling of the wave-flume in still water. Means of replicate experiments are plotted, error bars represent ±1 SD, and statistics are reported in Table 2

We examined the effects of larval density (weight per volume) and shape by comparing the trajectories of living larvae with those of dead larvae (same shape and density as living larvae, but no swimming behavior), and neutrally buoyant spherical particles. If a larva that is not swimming has a greater density than seawater, the rate at which it sinks towards a substratum below it (and away from a surface above it) is a function both of its density relative to seawater and of the hydrodynamic drag (which depends on its shape) resisting its sinking. Dead larvae sank, thus they were denser than the surrounding seawater and were negatively buoyant. In contrast to living larvae, dead larvae moved downwards along fairly straight paths, and their velocities through the water were an order of magnitude slower than larval swimming speeds (mean ± SD velocity of passive sinking = -0.01 ± 0.007 cm s^{-1}, n = 5 replicates for glass). Neutrally buoyant particles in still water barely moved. The mean velocity of the neutrally buoyant particles was -0.001 ± 0.01 cm s^{-1} (n = 5 replicates for glass). Both dead larvae and neutrally buoyant particles that contacted surfaces in still water remained at the point of contact thereafter.

3.2. Behavior in flowing water

The velocities of realistic ambient flow in the wave-flume (Figs. 3 & 4) were about 10 times faster than the swimming speeds of living larvae and 100 times faster than the sinking speeds of dead larvae (Fig. 6), so larvae in the water column were carried downstream at the speed of the water around them. Therefore, living and dead larvae, as well as neutrally buoyant particles, moved downstream more slowly when within a few mm of the floor and ceiling than they did at mid-depth in the wave-flume. As illustrated in Fig. 4, the velocity gradient ([Δ velocity]/[Δ distance from surface]) in the slowly moving water right next to the floor or ceiling was quite steep, so local shear in the water was much higher within a few mm of these surfaces than in the flow farther away. Therefore, larvae or particles traveling in water flowing within a few mm of a surface would be rotated by the shear in the water. The mean downstream transport rate of water and larvae was slower when the back-and-forth motion due to waves was superimposed on the unidirectional current (Fig. 4).

Examples of the trajectories of larvae over biofilmed surfaces are shown in Fig. 8. The trajectories of living and dead larvae were similar, except in the water within a few mm of a surface (Fig. 9), where

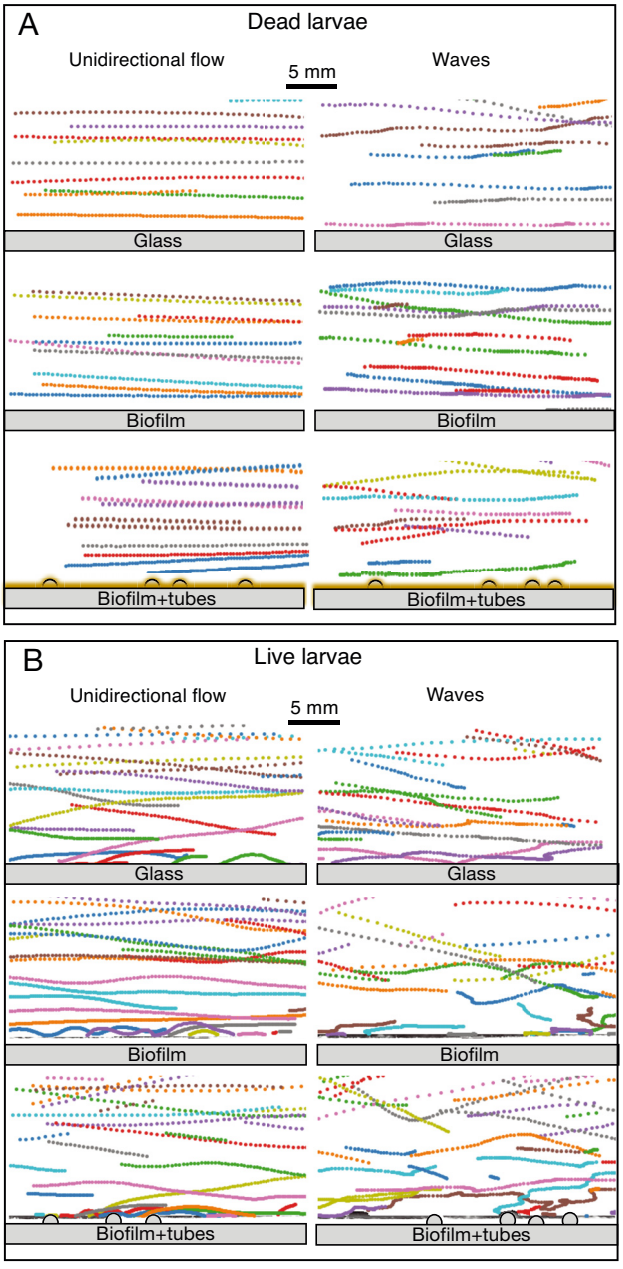

Fig. 8. Examples of trajectories of (A) dead and (B) live *Hydroides elegans* larvae in a unidirectional water current flowing from right to left over different surfaces in the wave-flume. The duration between dots in each trajectory was 0.033 s. The grey diagram along the bottom of each image illustrates a side view of the surface above which these larvae were being transported by the flowing water

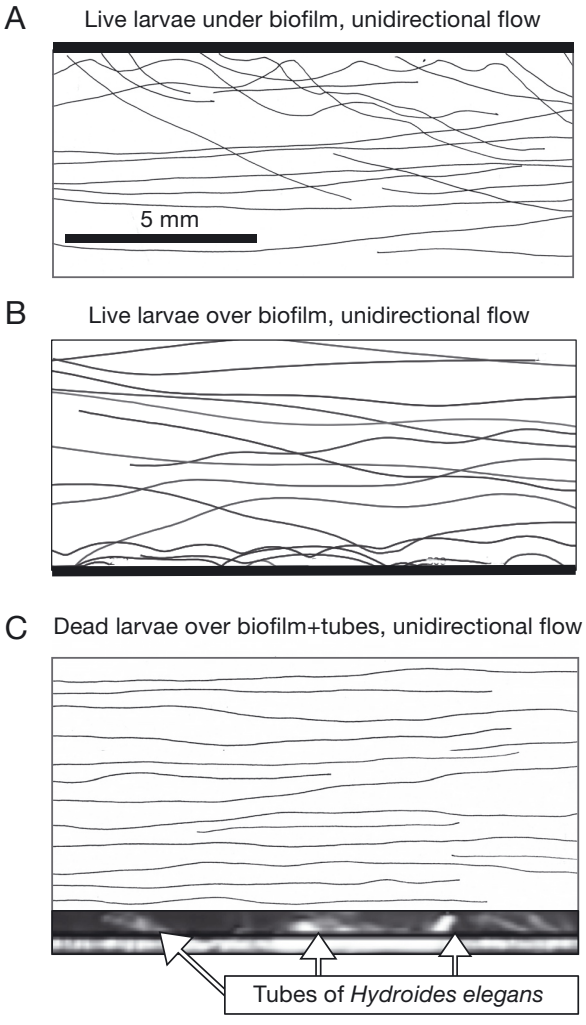

Fig. 9. Examples of magnified views of the trajectories of *Hydroides elegans* larvae in the miniflume. Flow is moving from right to left. (A) Living larvae 'bouncing' along a biofilmed surface on the ceiling in unidirectional flow. (B) Living larvae 'bouncing' along a biofilmed surface on the floor in unidirectional water flow. (C) Dead larvae in unidirectional flow along a biofilmed surface with tubes of *H. elegans* on the floor. The picture along the bottom in panel (C) is the side view of the surface above which these larvae were being transported, taken from a frame of the video in which the trajectories were digitized. In the gentle flow typical of harbors, these very small worm tubes did not cause eddies that affected the vertical motion of the water flowing above them

the flow was slow and the velocity gradient was steep (Fig. 4). Close to a surface, living larvae moved up and down more than did the slowly sinking dead larvae (Figs. 8 & 9). When living larvae contacted the surface, they appeared to 'bounce' up and down, repeatedly contacting and leaving the surface (Figs. 8 & 9A,B), whereas dead larvae did not show this behavior (Figs. 8 & 9C).

The percent of larvae in a video that contacted a surface under different treatments in flowing water are shown in Fig. 10. In all cases, a greater percentage of living larvae contacted surfaces than did dead larvae, but this difference was only significant in

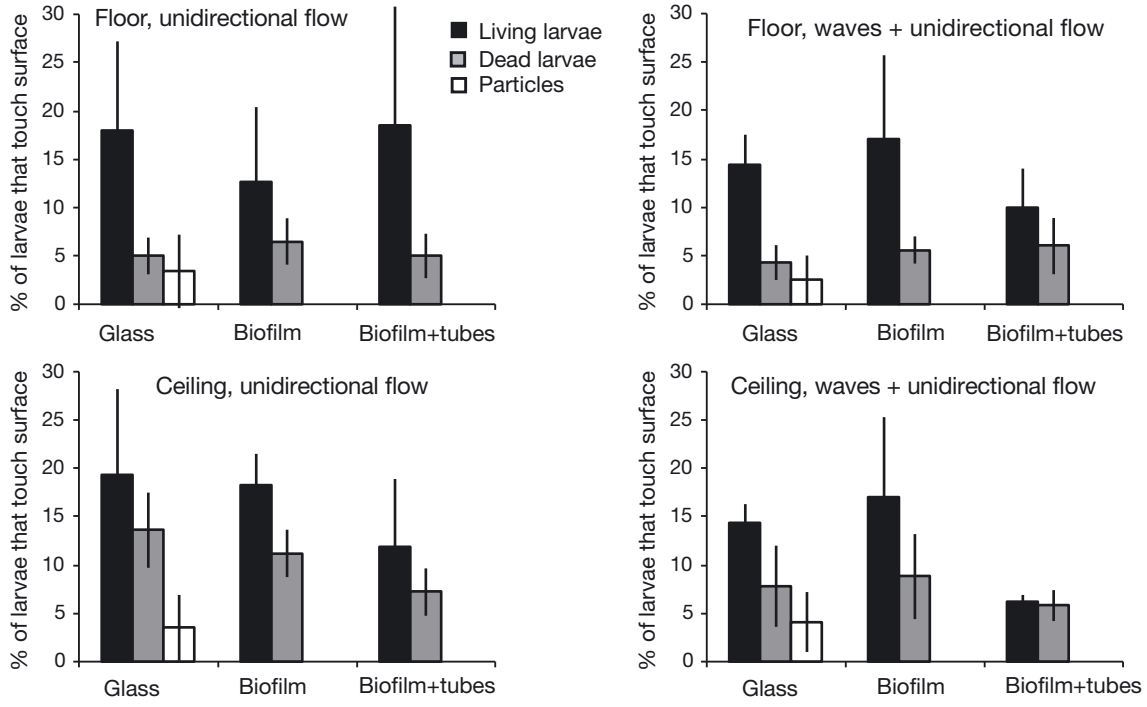

Fig. 10. Percent of the living and dead *Hydroides elegans* larvae and the neutrally buoyant particles that touched the floor or ceiling of the wave-flume. Means are plotted, error bars represent ±1 SD, and statistics are reported in Tables 3 & 4. Neutrally buoyant particles were only used over glass

some cases (Tables 3 & 4). Surface type did not affect the percent of living larvae that touched a surface. A greater percentage of both living and dead larvae contacted surfaces than did neutrally buoyant spherical particles, indicating that the density and/or shape of larvae enhanced contacts with a surface in flowing water.

In flowing water, the duration of the first touch by living larvae was not affected by surface type, and also did not differ from the touch durations of dead larvae or particles (Tables 3 & 4), all of which were highly variable. The touch durations of living larvae 'bouncing' along a surface ranged from 0.4 to 38 s. In contrast, dead larvae and particles that contacted a surface either were swept away immediately (so their touch duration was 0 s) or remained where they landed (so their touch duration lasted until the video clip ended),

Living larvae 'bounced' along a surface in flowing water, whereas dead larvae and particles did not (Figs. 8 & 9). The number of touches onto surfaces per cm of streamwise distance traveled was significantly greater for living larvae than for dead larvae or neutrally buoyant spherical particles in all cases (Fig. 11, Tables 3 & 4). Dead larvae, whose very slow sinking was overwhelmed by the much faster ambient water flow, were never significantly different from neutrally buoyant spherical particles. This indicates that the density and shape of larvae alone was not sufficient to produce 'bouncing', but rather that touching a surface multiple times while being carried along it by flowing water was due to active behavior by living larvae.

4. DISCUSSION

Our study showed that active behavior by competent larvae of *Hydroides elegans* can affect both their paths in the water as they are being carried past surfaces by realistic ambient water flow and their contacts with those surfaces. The answers to the specific questions (see Section 1.3) we posed are:

(1) The different types of surfaces that characterize early stages in the development of a fouling community (clean flat surfaces, flat biofilm, biofilm on isolated tubes of *H. elegans*) do not affect the trajectories or the contact behaviors (i.e. percent of larvae contacting a surface, contact duration, number of contacts per streamwise distance traveled) of larvae in still and in flowing water.

(2) Comparison of living larvae with dead larvae and neutrally buoyant spherical particles showed that active larval swimming behavior is responsible for the greater probability of contacting a nearby sur-

Table 3. Responses to surfaces in unidirectional flow by living *Hydroides elegans* larvae. Comparisons were done using 1-way ANOVA followed by Tukey HSD post-hoc tests, with $\alpha = 0.05$. When a pairwise comparison shows a significant difference ($p < 0.05$), we indicate which treatment was greater than the other. Pairwise comparisons for which $p \geq 0.05$ are indicated by NS (not significant)

	Arcsine transform of % of larvae that touched	Duration of first touch (s)	Touches per cm of streamwise travel
Floor: $F_{df,\ residual}$	$6.45_{6,29}$	$1.34_{6,33}$	$33.5_{6,33}$
Live vs. dead over glass	Live > dead	NS	Live > dead
Live vs. dead over biofilm	NS	NS	Live > dead
Live vs. dead over biofilm+tubes	NS	NS	Live > dead
Live vs. particles over glass	Live > particles	NS	Live > particles
Dead vs. particles over glass	Dead > particles	NS	NS
Live over glass vs. biofilm	NS	NS	NS
Live over glass vs. biofilm+tubes	NS	NS	NS
Live over biofilm vs. biofilm+tubes	NS	NS	NS
Ceiling: $F_{df,\ residual}$	$7.53_{6,16}$	$1.07_{6,26}$	$27.03_{6,25}$
Live vs. dead under glass	NS	NS	Live > dead
Live vs. dead under biofilm	NS	NS	Live > dead
Live vs. dead under biofilm+tubes	NS	NS	Live > dead
Live vs. particles under glass	Live > particles	NS	Live > particles
Dead vs. particles under glass	Dead > particles	NS	NS
Live under glass vs. biofilm	NS	NS	NS
Live under glass vs. biofilm+tubes	NS	NS	NS
Live under biofilm vs. biofilm+tubes	NS	NS	NS

Table 4. Responses to surfaces in unidirectional flow with superimposed waves by living *Hydroides elegans* larvae. Comparisons were done using 1-way ANOVA followed by Tukey HSD post-hoc tests, with $\alpha = 0.05$. When a pairwise comparison shows a significant difference ($p < 0.05$), we indicate which treatment was greater than the other. Pairwise comparisons for which $p \geq 0.05$ are indicated by NS (not significant)

	Arcsine transform of % of larvae that touched	Duration of first touch (s)	Touches per cm of streamwise travel
Floor: $F_{df,\ redisudal}$	$9.45_{6,33}$	$3.44_{6,33}$	$13.52_{6,33}$
Live vs. dead over glass	Live > dead	NS	Live > dead
Live vs. dead over biofilm	Live > dead	NS	Live > dead
Live vs. dead over biofilm+tubes	NS	NS	Live > dead
Live vs. particles over glass	Live > particles	NS	Live > particles
Dead vs. particles over glass	Dead > particles	NS	NS
Live over glass vs. biofilm	NS	NS	NS
Live over glass vs. biofilm+tubes	NS	NS	NS
Live over biofilm vs. biofilm+tubes	NS	NS	NS
Ceiling: $F_{df,residual}$	$6.39_{6,18}$	$0.87_{6,24}$	$44.86_{6,23}$
Live vs. dead under glass	NS	NS	Live > dead
Live vs. dead under biofilm	NS	NS	Live > dead
Live vs. dead under biofilm+tubes	NS	NS	Live > dead
Live vs. particles under glass	Live > particles	NS	Live > particles
Dead vs. particles under glass	Dead > particles	NS	NS
Live under glass vs. biofilm	NS	NS	NS
Live under glass vs. biofilm+tubes	NS	NS	NS
Live under biofilm vs. biofilm+tubes	NS	NS	NS

face, and for the 'bouncing' behavior along surfaces whereby larvae make multiple contacts per distance they are carried by the ambient flow.

(3) These patterns of contact behaviors (described in 1 and 2 above) are the same for larvae swept under ceilings and over floors.

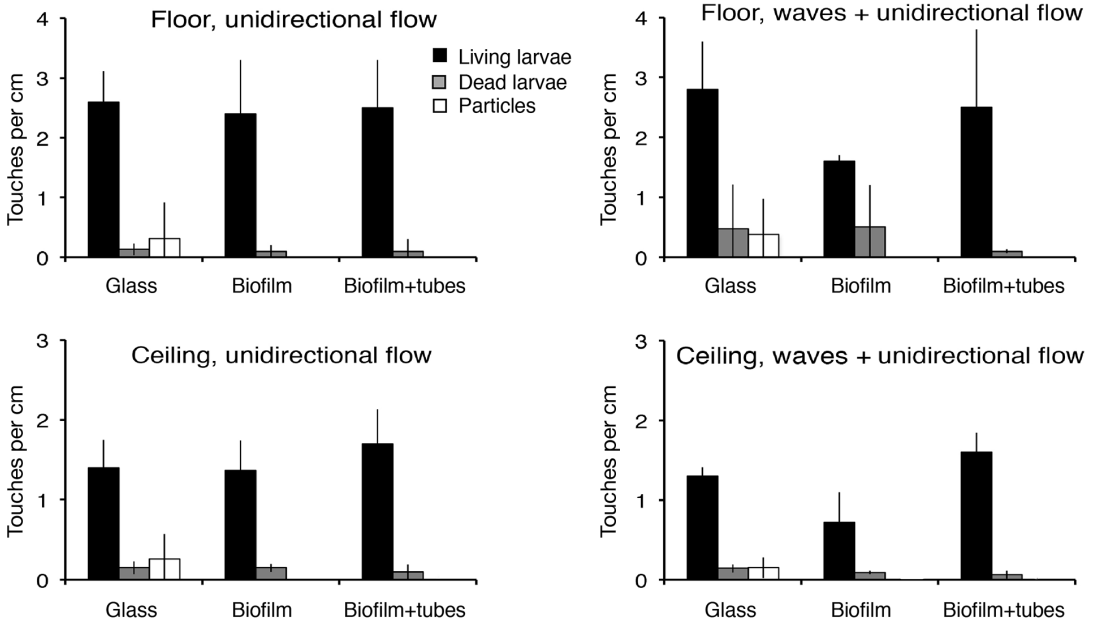

Fig. 11. Touches onto the surface per streamwise distance (cm) traveled by living and dead *Hydroides elegans* larvae and neutrally buoyant particles in the wave-flume. Means are plotted, error bars represent ±1 SD, and statistics are reported in Tables 3 & 4. Neutrally buoyant particles were only used over glass

(4) These patterns of contact behaviors are not affected by the superposition of waves onto ambient unidirectional water flow.

4.1. Comparison of swimming and sinking by *Hydroides elegans* to that of other larvae

The swimming and sinking behavior of competent larvae of *H. elegans* is similar to that reported for larvae of other polychaetes. The trajectories of *H. elegans* larvae are helical like those of other polychaete larvae (Bolton & Havenhand 1997), and have SIs of ~60 to ~80, as do other polychaete larvae (Butman et al. 1988). Larvae of *H. elegans* swim at speeds of ~0.2 cm s^{-1}, which is within the range of swimming speeds (~0.1 to ~0.4 cm s^{-1}) reported for other polychaete larvae (Chia et al.1984, Butman et al. 1988, Bolton & Havenhand 1997, Beaulieu et al. 2015). Dead larvae of *H. elegans* sink extremely slowly (sinking velocity of −0.01 cm s^{-1}), which falls within the range of sinking velocities (−0.007 to −0.1 cm s^{-1}) reported for other polychaete larvae (Butman et al. 1988, Bolton & Havenhand 1997, Beaulieu et al. 2015).

Because they are so small (Fig. 1B) and have no heavy mineralized shell or skeleton, dead larvae of *H. elegans* sink at velocities that are 1–2 orders of magnitude slower than the sinking velocities of larvae that have shells or exoskeletons. For example, when molluscan larvae with shells stop swimming, they sink at velocities ranging from −0.2 to −1.0 cm s^{-1} (Chia et al. 1984, Dekshenieks et al. 1996, Fuchs et al. 2004, Hadfield & Koehl 2004, Kim et al. 2010), and non-swimming crustacean larvae sink at velocities of −0.2 to −2.6 cm s^{-1} (Chia et al. 1984).

4.2. Swimming enhances contacts with surfaces above and below larvae in flowing water

Our data show that larvae of *H. elegans* in both still and flowing water do not change their behavior in response to water-borne chemical cues from biofilms (Tables 1–4), which is consistent with results for larvae of *H. elegans* in still water (Hadfield et al. 2014). However, our data suggest that the continuous swimming by larvae of *H. elegans* larvae can enhance their contacts with surfaces above and below them.

Even when larvae are carried in flowing water moving faster than they swim or sink, they can enhance their chances of being carried to benthic substrata below them by using various behaviors. For example, mathematical models of larval settlement in unidirectional flow (Eckman 1990, Gross et al. 1992, Eckman et al. 1994, McNair et al. 1997) and in waves (Koehl et al. 2007, Koehl & Cooper 2015), as well as flume studies in unidirectional flow (e.g. But-

man 1987, Tamburri et al. 1996, Finelli & Wethey 2003) show that in flowing water, both ceasing to swim (i.e. sinking passively) or swimming downwards rapidly can concentrate larvae close to the substratum. These analyses were done for larvae that move downwards through the water more rapidly than do dead larvae of *H. elegans*, which sink 100 times more slowly than the ambient currents that carry them across the environment. Although such slow sinking relative to ambient flow can make little difference to larval contacts with small surfaces typically colonized by fouling organisms (e.g. pilings, buoys, rafts, boats), it could enhance travel towards a substratum (e.g. the sea floor, a coral reef) that is big enough to allow time for slowly sinking larvae to reach it as they are carried across it by the ambient current. Similarly, slowly sinking larvae carried in water flowing under a very large aircraft carrier could have time to fall away from the surface above them.

In contrast to larvae of animals that live on the sea floor, the larvae of animals that are members of the fouling community recruit to natural or man-made surfaces that are above them and next to them, as well as to substrata below them. A model of the transport of larvae in water flow like that across surfaces in Pearl Harbor (Koehl & Cooper 2015) showed that passive sinking is the worst strategy for encountering the underside of a horizontal surface above larvae (e.g. the bottom of a ship or floating dock), but is an effective strategy for contacting a surface below them. Conversely, passive rising increases the probability of contacting a ceiling, but reduces the chances of contacting a floor. The model predicted that active swimmers are more likely to contact a ceiling or floor than are passive neutrally buoyant particles. The model also showed that swimming is the most effective way of contacting vertical surfaces next to larvae (e.g. pilings), and that swimming is the second-best way (after passive sinking or rising) for encountering horizontal surfaces below or above larvae. Therefore, swimming should be the best strategy for contacting surfaces whose location and orientation are unpredictable, as they are for the larvae of fouling organisms.

The larvae of *H. elegans* settle onto biofilmed surfaces below them (e.g. Carpizo-Ituarte & Hadfield 1998, Shikuma & Hadfield 2005, Hadfield et al. 2014), and in field studies, *H. elegans* also recruit onto vertical surfaces (Huggett et al. 2009) and onto the undersides of horizontal surfaces (Hurlbut 1991, Walters 1992). As described above, continuous swimming is an effective way of enhancing encounters of larvae with surfaces above, below, and next to them (Koehl & Cooper 2015). The larvae of *H. elegans* swim continuously and do not stop swimming or slow down in response to dissolved chemical cues in the water (Hadfield et al. 2014), or when near to clean or biofilmed surfaces that they have not yet touched (Fig. 6). Therefore, it is not surprising that we found that live larvae of *H. elegans* carried in ambient water flow were more likely to contact surfaces both above and below them than were dead larvae, which fall through the water 10 times more slowly than living larvae swim (Fig. 10, Tables 3 & 4).

4.3. Effects of currents and of waves on larval behavior near and on surfaces

Ambient water flow near surfaces can affect the motions of marine larvae near and on those surfaces (e.g. reviewed by Abelson & Denny 1997, Koehl 2007, Koehl & Cooper 2015), so studies of larval responses to substrata in still water may not reveal ecologically relevant behaviors for larvae that in nature must contact and explore surfaces exposed to moving water.

Although the superposition of the orbital water motion of waves onto a unidirectional current can enhance vertical transport of larvae and other materials across the benthic boundary layer on a scale of centimeters to meters (e.g. Reidenbach et al. 2007), we found that larvae that were already in the layer of water right next to a surface above or below them (≤1.4 cm from the surface) were no more likely to contact that surface when small waves were added to the current than they were in unidirectional flow (Fig. 10). These results may have been due to the compression of the orbital water motion in waves into back-and-forth flow near a surface (e.g. Bascom 1980). Superimposing small waves (to mimic wind chop) on a unidirectional current had little effect on vertical flow right next to the surfaces we studied, but rather just added to or reduced the instantaneous horizontal velocity of the current (Figs. 3 & 4). Although pulses of rapid flow within a few hundred microns of surfaces can dislodge larvae (Reidenbach et al. 2009, Koehl et al. 2013), the hydrodynamic forces on larvae of *H. elegans* hit by such pulses in the gentle wind chop we mimicked were too small to wash the larvae off surfaces. Thus, the behaviors used by *H. elegans* to contact and explore surfaces were just as effective in flow affected by wind chop as they were in unidirectional currents.

Once larvae are in flowing water near a surface, they encounter signals that trigger some species to alter their behavior. For larvae that respond to waterborne chemical cues from the benthos, encounters with cues in concentrations high enough to affect behavior are more likely close to the substratum in unidirectional flow (e.g. Turner et al. 1994, Zimmer-Faust et al. 1996) and in waves (Koehl et al. 2007). Similarly, for larvae that respond to physical stimuli, hydrodynamic cues that a surface is nearby (e.g. pulses of acceleration and shear in the water) occur more frequently as larvae approach a surface exposed to realistic water flow (Reidenbach et al. 2009, Pepper et al. 2015). In contrast to larvae that change their behavior in response to such cues that a surface is nearby, competent larvae of *H. elegans* just keep swimming steadily, which is an effective strategy for fouling organisms.

4.4. Behavior on surfaces

Larvae of many species of benthic invertebrates explore surfaces after landing on them, so the positions where they eventually settle and undergo metamorphosis can be affected by their behavior after contact (e.g. reviewed by Koehl 2007). Larvae of various species that live in diverse microhabitats use different behaviors to explore surfaces, as have been reported for larvae in still water (e.g. reviewed by Crisp 1974, Hadfield et al. 2014) and in flowing water (e.g. Walters et al. 1999; reviewed by Abelson & Denny 1997, Koehl 2007). Chemical cues can alter the behavior of larvae encountering surfaces (e.g. Krug & Zimmer 2000, Matson et al. 2010), as can the fine-scale topography of a surface (e.g. Walters 1992, Walters & Wethey 1996).

We found that after competent larvae of *H. elegans* touched a surface (clean glass, biofilm, or biofilm+tubes), they remained in contact with the surface for several seconds, then left the surface, and then repeated this behavior. Larvae of *H. elegans* use the apical tuft (Fig. 1B) to sense surfaces (Nedved et al. 2021), so perhaps they were sampling surface chemical signals during their periods of contact. Competent larvae of *H. elegans* in still water also showed this 'swimming-and-touching' behavior over both clean and biofilmed surfaces (Hadfield et al. 2014).

When larvae of *H. elegans* use their swimming-and-touching behavior in flowing water, they appear to 'bounce' along the substratum (Figs. 8 & 9). Such bouncing by larvae along a surface has long been thought to be a mechanism for sampling the substratum to select a spot to settle (e.g. Butman & Grassle 1992). Dead larvae of *H. elegans* in flowing water did not show up-and-down trajectories along a surface (Figs. 8 & 9C), thus the bouncing was due to active behavior by the larvae. One mechanism that has been suggested to produce such bouncing is that larvae that swim in helical paths move closer and farther from a surface when they swim along the surface in the slow flow near a substratum (Tamburri et al. 1996). Larvae of *H. elegans* do swim along helical paths in still water (Fig. 5), as do many other invertebrate larvae (e.g. Maciejewski et al. 2019). However, the observation that larvae of *H. elegans* stop moving when they touch a surface, both in still water and in flowing water, suggests that helical swimming along a surface is not the mechanism that produces their bouncing behavior in flow. We propose a different mechanism for bouncing by *H. elegans*. When swimming bodies are rotated by local shear in the ambient flow around them, their swimming direction changes in ways that depend on their shape (Clay & Grünbaum 2010, Pujara et al. 2018), and they move through turbulent flow differently from passive particles of the same shape (Pujara et al. 2018). We suggest that when the larvae of *H. elegans* touch a surface, they stop swimming and maintain contact with the surface for a number of seconds, and then when they resume swimming, the high shear in the flow along the surface rotates them so that they once again swim into contact with the surface. Such a rotation mechanism that returns swimming larvae to surfaces in flowing water was proposed for bivalve larvae by Jonsson et al. (1991), and larvae of various species in flumes have been observed to tumble end-over-end along the substratum (Jonsson et al. 1991, Pawlik & Butman 1993). Furthermore, mathematical models of the hydrodynamics of swimming larvae being rotated by the high shear near a surface produced bouncing trajectories like those we observed for the larvae of *H. elegans*, but not for non-swimming passive bodies (Zilman et al. 2008, Koehl & Cooper 2015) such as dead larvae or particles. Thus, we suggest that the duration of contact with the substratum is determined by the behavioral response of a larva to touching a surface, but that return to the substratum is controlled by hydrodynamics, which rotates a swimming larva.

Substratum topography affects settlement rates and locations for the larvae of many species (e.g. Prendergas et al. 2008, Scardino et al. 2009, Fingerut et al. 2011, Koehl et al. 2013, Hata et al. 2017; earlier papers reviewed by Koehl 2007). Roughness

elements (i.e. protrusions from surfaces) alter the water flow right next to surfaces, which in turn can affect both where larvae first contact surfaces and where they can remain without washing away in local refuges from rapid flow, such as crevices and the bases of bumps (e.g. Koehl & Hadfield 2004, Reidenbach et al. 2009, Whitman & Reidenbach 2012, Koehl et al. 2013, Hata et al. 2017; earlier papers reviewed by Abelson & Denny 1997, Koehl 2007). The wakes produced by larger benthic organisms can add turbulence to a boundary layer (reviewed by Koehl 2007), so dense aggregations of tubes of *H. elegans* that can sometimes develop later in the succession of fouling communities probably have a similar effect on the flow. In contrast, our study focused on an earlier successional stage characterized by isolated tubes of *H. elegans* (which were attached with their long axes parallel to the surface and had diameters of ~1–2 mm) (Fig. 1A). These tubes were too small to produce any noticeable effect on the vertical motion of the water right above them (Fig. 9C) and did not affect larval contact behavior (Tables 3 & 4). However, in realistic harbor flow, tubes of *H. elegans* cause regions of lowered Reynolds stress behind and raised Reynolds stress above them within 500 µm of their surfaces (Koehl et al. 2013). These fine-scale flow differences around a worm tube might affect where crawling larvae attach and undergo metamorphosis. Both surface texture (e.g. Prendergast et al. 2008) and local flow velocity along a surface (e.g. Crisp 1955) can affect the ability of larvae to crawl. Furthermore, local flow has been shown to be a signal used by a variety of larvae to help them choose the right spot to attach to a surface (reviewed by Abelson & Denny 1997). Walters et al. (1997) found that the larvae of *H. elegans* recruited behind *H. elegans* tubes and physical models of those tubes in flowing water, but not in still water, and suggested this was due to passive deposition of larvae in crevices beside tubes, followed by attachment if a suitable biofilm was present. In contrast, we found that the presence of worm tubes on surfaces exposed to flowing water did not affect the likelihood of larvae contacting a surface, nor did the tubes alter the behavior after contact with biofilmed surfaces by larvae of *H. elegans*. The discrepancy between our observations of first contacts with surfaces and the observations of Walters et al. (1997) of recruitment patterns in flowing water might be explained if crawling larvae use local flow signals to choose their attachment sites after exploring the substratum.

5. CONCLUSIONS

Although microscopic larvae are small and swim slowly relative to the ambient water flow carrying them past surfaces onto which they might settle, their active behavior can affect their contacts with those surfaces. To understand the significance of specific aspects of larval behavior to settlement success in their habitats in the ocean, we can study larval motions in water flow that mimics the fine-scale flow they encounter in the field. The larvae of *Hydroides elegans*, which must contact a biofilmed surface to be stimulated to attach and metamorphose, have active behaviors that can enhance their contacts with and exploration of surfaces (continuous swimming until they touch a surface, and then alternating surface contact with swimming such that they 'bounce' along the surface in flowing water). These behaviors are effective for structures that are above as well as below the larvae, for surfaces that are smooth or rough, and in unidirectional flow as well as in waves, all of which are conditions that the larvae of fouling-community animals encounter in harbors.

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