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Detection and quantification of marine larvae orientation in the pelagic environment

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Abstract

The pelagic larval phase represents a major opportunity for dispersal in benthic organisms, yet behaviors controlling and potentially limiting dispersal are still largely unknown for most larvae. Here, we present a new means of observing the orientation of larvae of all developmental stages in the pelagic environment. A cylindrical frame holding a semi-open arena in which larvae are filmed is set to drift at a controlled depth within the natural environment. Larval trajectories are extracted from video data and used to quantify orientation behavior. Field tests with late-stage coral reef fish larvae show that orientation can be detected from individual larval positions in the arena and can be significantly differentiated from random movement or artifactual behavior.

Introduction

Many coastal marine organisms are benthos associated as adults but produce eggs and larvae which may disperse in the open ocean. This pelagic larval phase is often the only opportunity for dispersal in an organism's life history. As a consequence, the supply of larvae to adult populations governs the population dynamics, gene flow, and biogeography of coastal species (Doherty 2002). Until recently, marine larvae were thought to be passively carried over great distances by ocean currents. There is now strong evidence that at least coral reef fish and decapod larvae acquire swimming capabilities that develop early and increase rapidly throughout ontogeny (Kingsford et al. 2002; Leis 2006). If larvae swim randomly, however, their movement would only add noise to passive drifting trajectories and not fundamentally affect the outcome of dispersal. In contrast, modeling studies have shown that oriented horizontal swimming greatly influences dispersal outcomes (Wolanski et al. 1997). Modeling experiments also suggested that the nature of the orientation cue influences larval trajectories and that the sensory threshold is the key factor determining the supply rate of larvae onto a reef (Armsworth 2000; Codling et al. 2004; Paris et al. 2005). Therefore, to understand the dynamics of the pelagic phase, it is critical to be able to measure the orientation of larvae throughout ontogeny and gain further insight into the cues involved in this behavior.

Orientation behavior and related cues have been studied using three methods: in situ visual observations by scuba divers (starting with Leis et al. 1996), in situ fixed experiments using light traps or patch reefs where cues are manipulated (Tolimieri et al. 2000; Simpson et al. 2005), and laboratory observations in choice chambers (Stobutzki and Bellwood 1998; Tolimieri et al. 2004; Atema et al. 2002). These methods have shown that fish larvae orient and that cues such as sound and chemical plumes originating from reefs can be detected and might be used for navigation. These findings were consistent among the studies and were the subject of recent reviews (e.g., Montgomery et al. 2001, 2006; Kingsford et al. 2002; Leis 2006). However, the scope of these results is limited due to methodological constraints, as detailed below.

Following larvae on scuba allows for observation of their natural swimming behaviors, both horizontally and vertically, in an open environment with apparently insignificant influence by the presence of divers. However, scuba diving restricts the duration and depth of the observations as well as the size of the study specimen, particularly when visibility is reduced.

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Therefore, this method has been used only for daytime observations of mostly presettlement stages of coral reef fishes in clear coastal waters. In addition, this method is impractical for manipulating and inferring the cues potentially used for orientation.

Alternatively, experimental methods have provided direct evidence that sound (Tolimieri et al. 2004) or chemical (Atema et al. 2002) cues influence the orientation of reef fishes. Because these studies rely on the use of some kind of fixed device toward which larvae are attracted, they operate in shallow water habitats and/or on late-stage larvae. They are designed to identify the cues involved during settlement and not for investigating large-scale navigation during the pelagic phase of reef fishes.

In summary, the existing methods provide valuable information on the orientation of late-stage larvae relative to a limited set of coastal water cues. However, fish are known to develop swimming capacities early (Leis 2006); hence, orientation of young individuals is potentially influential to the connectivity between adult populations. The behavior of younger larvae in the pelagic environment is still completely unknown and may involve other cues, such as magnetic or electric fields, sun position, swell, and waves (Montgomery et al. 2001). Current methods are not appropriate to tackle these questions.

Here, we present a device aimed at assessing the orientation of all larval stages directly in the pelagic environment, while conserving some control over environmental cues (Paris et al. 2008). Larvae embedded in oceanic waters have no apparent frame of reference for detecting the direction of the current (Montgomery et al. 2001). Therefore the device is designed to drift with the current, and contains a circular behavioral arena in which a larva is filmed. The larva used for the experiment is thus exposed to sensory cues as a free larva would. Its trajectory is extracted from the movie recording and analyzed through circular statistics to detect orientation behavior. Simplicity, extendibility, and ease of use were major foci during the design of this instrument, while avoiding limitations in detecting and measuring orientation behavior and manipulating proximal cues. We describe the observation methodology, data acquisition, and processing and present a proof of concept using data collected with late-stage reef larvae.

Materials and procedures

Materials—The OWNFOR (Orientation With No Frame Of Reference) apparatus is built on a hollow cylindrical frame (130.8 cm height, 45.7 cm diameter) made of four aluminum bars and three aluminum rings (1.25 cm thick) welded together (Fig. 1). Eight smaller bars, to which four strong nylon fabric sheets (130.8 cm × 29.20 cm) are secured, protrude diametrically outward from the cylinder and should lock the apparatus in the surrounding water mass. The bottom of the frame holds a cylindrical arena (12 cm height, 38 cm diameter) made of two round pieces of transparent acrylic

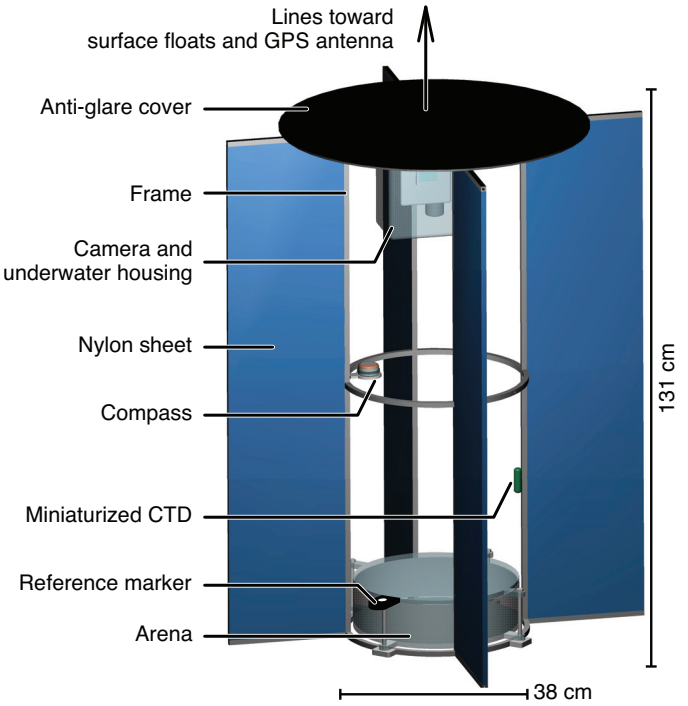

Fig. 1. 3D representation of the observation apparatus.

(1.25 cm thick), secured by transparent plastic bolts. The bolts are placed outside the arena so that the specimen cannot seek refuge behind them. The periphery of the arena is closed by 300 µm Nytex® mesh attached with Velcro® bands. The arena is entirely symmetrical to minimize visual reference for the larvae enclosed within.

At the top of the frame, an Ikelite Underwater Systems housing contains a Sony Handycam DCR-PC350® camcorder aimed down at the arena, a diving compass, and a white reference mark over a black plastic disc. This DV camera has very good low-light performance and all filming is done in available light. Frames measure 720 × 576 pixels and cover a region 45 cm wide (i.e., a 600-µm pixel resolution). The video data are recorded on 80-min Mini DV tapes in SP mode. The compass records the orientation of the arena, and the white marker provides a fixed reference point on the arena relative to the camera. Both are used for data calibration. Finally, an opaque plastic disc tops the frame to avoid glare from the sea's surface on the arena.

The submerged part of the OWNFOR device is attached to a set of three stainless steel bridles that connect to a 3-mm-diameter line leading to the surface. This line first runs through a small float, then forms a loose buckle tied with a bungee cord, and finally attaches to a larger surface float, sold as an inflatable spherical fender. The line length can be adjusted before deployment to run experiments at different depths. The use of the subsurface float and bungee cord attenuates the effect of waves on the OWNFOR apparatus below.

A custom-made spar-type float is attached to the surface float and houses a global positioning system (GPS) antenna interfaced with a GPS data logger (Geostats Inc.). The position of the device is recorded every 30 s. In addition, a mini–conductivity temperature density (CTD) logger from Starr-Oddi Inc. is attached to the frame and records environmental variables (temperature, salinity, and depth) every 30 s.

After deployment, the video data are retrieved and stored on the hard drive of a computer. Analysis of such data requires only a large enough storage space to hold the videos and 1 Gb memory to allow all the video frames to be loaded at once. The video analysis relies on software programs that are most easily installed on a Unix-like operating system. The assessment data presented here was processed on a Power Macintosh running Mac OS 10.4 and on an HP Proliant running Debian GNU/Linux 3.0 and Fedora Core Linux 7.0.

Procedures—

Deployment: The OWNFOR device's size and shape allow deployment and recovery from a small boat using only two people. The surface float is deployed downstream from the boat and the line is slowly paid out. The frame is lowered on its side alongside the vessel. While one person holds the frame half submerged, the other places one larva inside the area. Once the specimen is inside the arena the frame is slowly released. As it sinks sideways, the air escapes from the arena through the mesh. The frame slowly reaches its final depth as tension in the line causes it to align vertically in the water column. After 3–5 min, the apparatus is stable within the current and is allowed to drift for a period of 20 min. The boat briefly motors a few hundred meters downstream from the surface float and the engine is shut off for the remainder of the experiment. After the experiment, the surface float is approached from upstream to pull the instrument aboard. The specimen is retrieved from the arena and preserved in 75% ethanol. The video camera batteries and remaining time on the tape are checked before starting a new experiment.

Characteristics of data: Typical evidence for orientation preference is directionality in the swimming bearings (Leis et al. 1996). In an enclosed circular arena, however, a larva is restricted by the boundary, and its orientation behavior may take two forms: (1) the larva may swim toward a preferred direction, as it would do in the open environment, then touch the boundary and swim in a non-oriented manner around the arena before heading toward its preferred direction again, in which case its average swimming *direction* is indicative of orientation; or (2) the larva may be less active and simply stay in the region of the arena corresponding to its preferred bearing, in which case its *positions* are indicative of orientation. To capture and statistically quantify these behaviors, a good representation of the trajectory of the larva is necessary.

Raw video recordings of larval positions are corrupted by several factors: in situ images are often noisy; unexpected events may occur during the recording (e.g., adult fish swimming around the arena disturbing the study specimen); the camera usually vibrates slightly with respect to the arena; and the whole device rotates on itself (ca. 360° per 20 min). Therefore, a series of processing steps are performed to mitigate these factors and yield accurate estimates of larval trajectories from video data: subsampling and enhancing of video data, acquisition and calibration of trajectories, and appropriate statistical analysis (Fig. 2). This whole process is achieved using a set of customized open source software.

Video processing: The raw video data comes encoded as a 30-images/s movie. The position of the specimen is detected manually (*see* below) with a mean imprecision of 1.7 mm (manual detection is repeated on several frames, and the mean range of the estimated positions is computed). Manual detection on all frames would be laborious and error prone, since 1.7 mm represents half the displacement of a larva swimming at 10 cm s^{-1} during 1/30 s. Instead, the video is resampled by keeping only one frame each 30 frames (i.e., one image per second). However, even when the trajectory curves during a 1-s interval, it is estimated as a straight line. The scenario leading to the largest resampling error would be a larva swimming in small circles around the center of the arena (the smaller the circles, the larger the angular speed and error). The theoretical case of a larva swimming regularly in a 15-cm-diameter circle suggests that a 1-s resampling period is virtually error free near mean cruising speeds (5 cm s^{-1}; Leis and Carson-Ewart 1997) and induces little relative error at higher speeds (10% at 20 cm s^{-1}; Fig. 3).

To minimize anomalous data, the video is analyzed only once the device drifts at the selected depth and the boat engine is turned off. Video frames are denoised using the high-quality denoise filter of MPlayer (hqdn3d; MPlayer development team, versions 0.90 to 1.0rc1; http://www.mplayerhq.hu/), and the contrast and luminosity are enhanced manually to facilitate the detection of the larva (Fig. 4). Individual frames are then exported as Portable Gray Map (PGM) images and stacked in a single Tagged Image File Format (TIFF) image sequence. PGM is an uncompressed grayscale image format that can be loaded very quickly for later analysis. Finally, the gray shades are normalized throughout the stack to dampen the variations in the lighting conditions: the brightest point of each image is scaled to white and the darkest to black.

Data acquisition and calibration: The position of the larva is recorded on each frame of the stack by clicking on it within a graphical user interface provided by the software ImageJ (W. S. Rasband, versions 1.34 to 1.42, NIH, Bethesda, MD; http://rsb.info.nih.gov/ij/). When other organisms, such as larger fishes, are visible in the frame, the position of the larva is simply discarded in the current, preceding, and following frames. This process outputs raw coordinates of the larva in pixel units, relative to the bottom left corner of the image, which need to be calibrated.

The center and diameter of the arena are recorded on the first image and provide both the scale and frame of reference for the raw coordinates. However, this frame of reference is still relative to the arena, which may vibrate relative to the

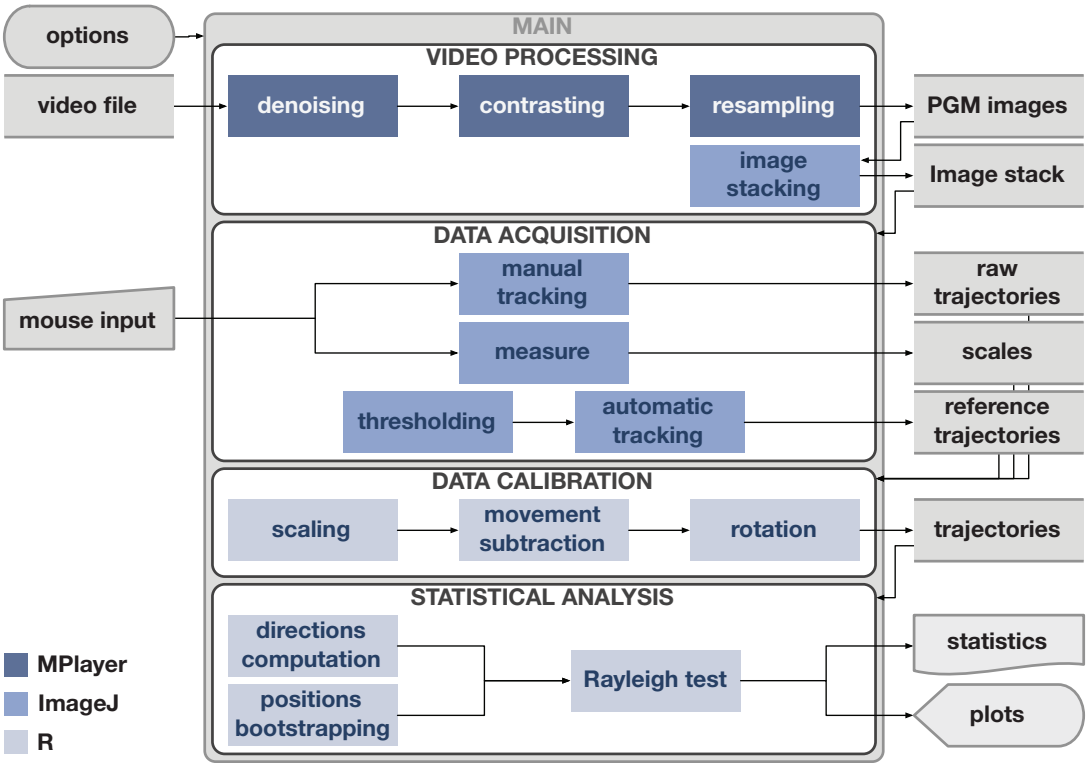

Fig. 2. Flowchart of the video analysis process. Input is on the left, output on the right. Action boxes are colored according to the external software on which they depend. The complete software environment used for the analysis is open source and documented: http://rsmas.miami.edu/personal/cparis/ownfor/.

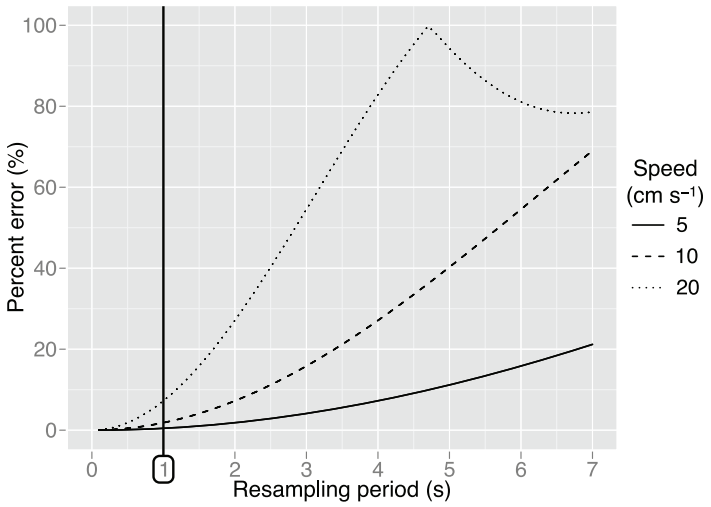

Fig. 3. When the resampling period increases, the difference increases between the distance traveled along the real, possibly curved, trajectory of the larva and the straight line distance estimated from the positions on the two resampled frames. Data are presented for the worst-case scenario of a larva swimming in circles of 15-cm diameter around the center of the arena at three different swimming speeds.

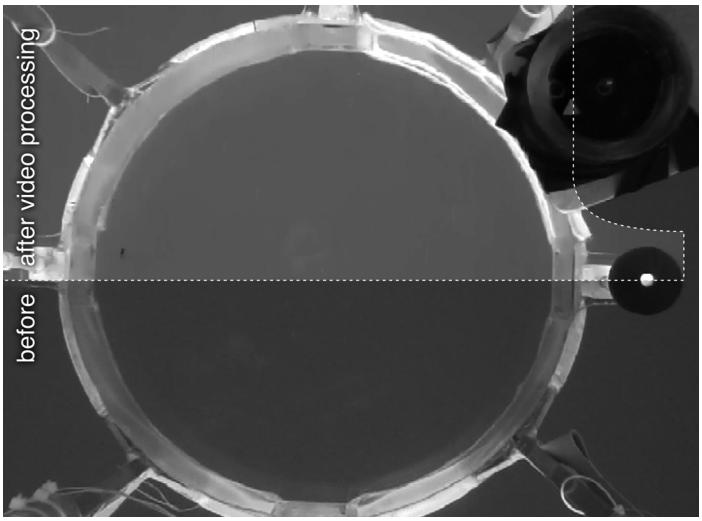

Fig. 4. A typical video frame before and after video processing. In the center is the circular arena, with the larva near the left side (dark blob) and the white reference mark on the right side. On the upper right side, but 60 cm above the arena, is the diving compass. Video processing removes background irregularities (some frames have more noise but more intense filtering achieves the same quality) and enhances contrast.

camera and rotate on itself; we are interested in the orientation of larvae in an absolute cardinal reference. To obtain this, the position of the white reference mark is automatically detected on every frame and its movement is subtracted from the larva's coordinates to suppress the vibration of the camera relative to the arena (Fig. 5). Most corrections are very small

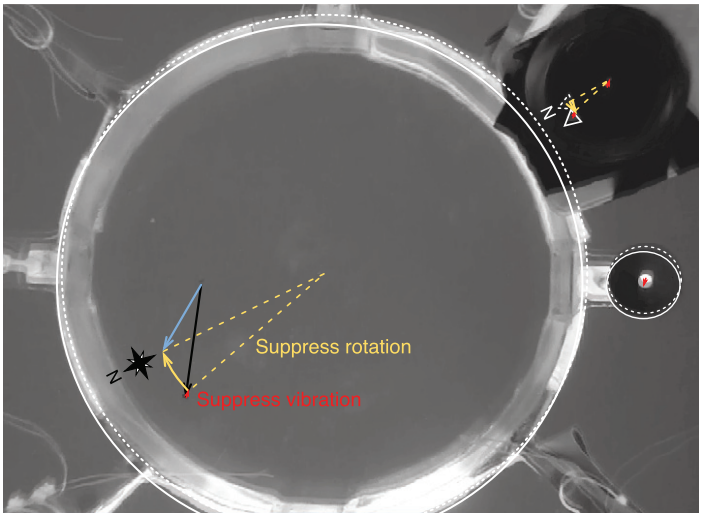

Fig. 5. Superposition of two frames explaining the process of data calibration (note: nonconsecutive frames are shown here to exaggerate the displacements and better visualize the corrections). The movement of the larva before calibration is the black arrow. The first frame is considered as the reference, and the shift of the second one with respect to the first is estimated from the white reference mark on the right (red arrow). Every displacement in the second frame is shifted back to suppress the effect of vibration. The rotation of the device is then estimated and subtracted from the trajectory (yellow lines), allowing the expression of both points in the same cardinal reference. The final trajectory in this absolute reference is the blue line.

(median 0.68 mm, mean 1.6 mm), but this helps correct occasional large vibrations (max 25 mm). The detection is performed with a custom version of the automatic tracking plugin of ImageJ. It outputs the coordinates of the centroid of the white reference mark on each frame, which are further manipulated in R (R Development Core Team 2009, versions 2.3 to 2.8, R Foundation for Statistical Computing; http://www.R-project.org). Owing to the changes in lighting conditions and the presence of planktonic particles in the water, the white reference mark can change in size and there can be several or no white blob detected on any particular frame. A size and distance filter is applied to make sure the correct blob is followed: the reference mark is considered to be the white blob whose size is within 40% of the size it had on the first frame and, if several satisfy this criteria, the one closest to the previous position, because the vibration causes only small displacements. When no, or no suitable, particle is detected, the point is considered not to have moved since the previous frame and a warning is issued.

Magnetic north, which appears as a white triangle on the compass's dark background, is also automatically detected and corrected with the same procedure. Even though the compass is closer to the camera than the reference mark, the same vibration correction applies because the camera vibrates on a horizontal plane (rather than tilts) so the whole frame is uniformly shifted. Next, the compass bearings are computed and subtracted from the positions of the larva represented in polar coordinates relative to the center of the arena (Fig. 5). At this point, north is consistent and the trajectories are available in real-world coordinates (centimeters).

The automatic tracking routine proceeds by isolating the two regions of interest in the image (the dark reference disk and the compass), thresholding the grayscale data to pure black and white, and computing the positions of the centroids of white blobs (the reference mark and the compass's triangle) on their black background. The same technique could theoretically be used to track the larva in the arena. However, the background is not a uniform color that can be thresholded to black: the structure of the instrument is visible and has to be erased. Background subtraction techniques proceed by suppressing the pixels that do not change through time (i.e., the background), hence leaving only the moving particles (i.e., the foreground) on a uniform black fill. But the background also changes through time because of the vibration of the camera and variations in the natural lighting conditions. The vibration can be corrected on each frame once it is quantified using the reference mark, but the correction is not pixel accurate. Gradual light variations on a fixed background can be detected and eliminated using several time-averaging techniques (Piccardi 2004). However, quick variations in illumination, such as the ones observed in clear shallow waters because of the heterogeneous refraction of light on waves, are more difficult to cope with: they make the background itself appear to move. The bright areas, such as the mesh on the side of the arena (Fig. 4), are particularly sensitive. They appear to flicker between a well-exposed tone and pure white (that even "glows" on the surrounding pixels) when a light beam reaches them directly and the diaphragm of the camera has not adjusted yet. Both vibration and flickering complicate background subtraction. Considering that even advanced automatic tracking could be unreliable and that these two problems would be better solved in hardware than in the analysis software, a manual tracking approach is preferred. Even though it seems more tedious, the detection of the positions of larvae can still be done in near real time (25–30 min to process a 20-min clip).

Statistical analysis: Circular statistics treat data as independent unit vectors pointing toward recorded angles (Batschelet 1981). The sum of these vectors gives information on directionality in the data set. If angles are uniformly distributed, all vectors cancel out and their sum vector is short. Conversely, if some vectors point in the same direction, the sum vector length is $\gg 0$. This technique removes noise and extracts the information we are interested in. Therefore, we reduce our data to bearings of vectors between the center of the arena and the position of the larva (discarding the length of such vectors) or swimming directions (discarding swimming speed). The sum vector is tested for significant directionality for each larva with the Rayleigh test. However, whereas swimming directions are independent (lags ≥ 1 s show autocorrelation $<10\%$), positions are not. A bootstrap-like technique is then used, resampling randomly 5% of the position data. The Rayleigh test is computed

on the subset of independent data, and the process is repeated 1000 times. Directionality in the data set is assumed if >95% of the 1000 sum vectors are significant and point toward a similar bearing. Using this technique, angles are treated as independent records, regardless of their sequence or frequency. This allows us to freely skip frames on which the larva is disturbed or undetectable with little impact on the data. All analyses are performed using the circular package available for R (C. Agostinelli and U. Lund, version 0.3-8, Comprehensive R Archive Network; http://cran.r-project.org/web/packages/circular).

Assessment

To be considered successful, the OWNFOR method must meet two criteria. First, the device needs to be locked in the water mass and drift without drag. This is necessary to ensure that larvae experience environmental conditions similar to those of free larvae, having no frame of reference for the direction of the current in which they are embedded. Second, the system must be able to capture nonrandom movement of larvae and differentiate orientation behavior from artifacts potentially caused by the enclosure.

The system was tested off Key Largo and Miami (Florida, USA) during 6 days of calm weather (wind speed <5 kt, wave height <1 m) in the summer of 2005 and spring of 2006. Settlement-stage larvae were captured at night, near the reef margin, with light traps retrieved at dawn, on the day of the experiments (Sponaugle and Pinkard 2004). The device was deployed in water with a depth >60 m and drogued at ca. 20 m below the surface.

In all deployments, the system drifted northward or northeastward with the Florida Current, generally following the isobaths (Fig. 6). Mean drifting speeds were 0.56 m s^{-1} off Key Largo and 1.12 m s^{-1} off Miami, well in agreement with the rapid surface-current speeds measured in those locations at similar distances from the reef edge (Lee et al. 1992). Further corroborating the device's effectiveness as a drogue, there was little to no displacement of planktonic particles between the camera and the arena.

Of the 18 fish larvae observed, 16 showed significant directionality in their positions and none showed significant directionality in their swimming bearings (Table 1). The absence of directionality in the swimming direction was to be expected for such late-stage larvae, because of the relatively small size of the arena. The cruising speed of late-stage larvae is fast enough (10–15 cm s^{-1}; Leis 2006) to force them to turn very often and lead to vectors in almost every direction, although four larvae showed bidirectional swimming patterns. As such, orientation was detected through the positions of the larvae rather than in their swimming directions.

Although the arena is symmetrical, it is critical to verify that the concentration of positions is not an artifact caused by preference for a feature of the arena. Such behaviors can be discerned from true orientation when correcting for the rotation of the device. When a larva artifactually follows a feature of the arena,

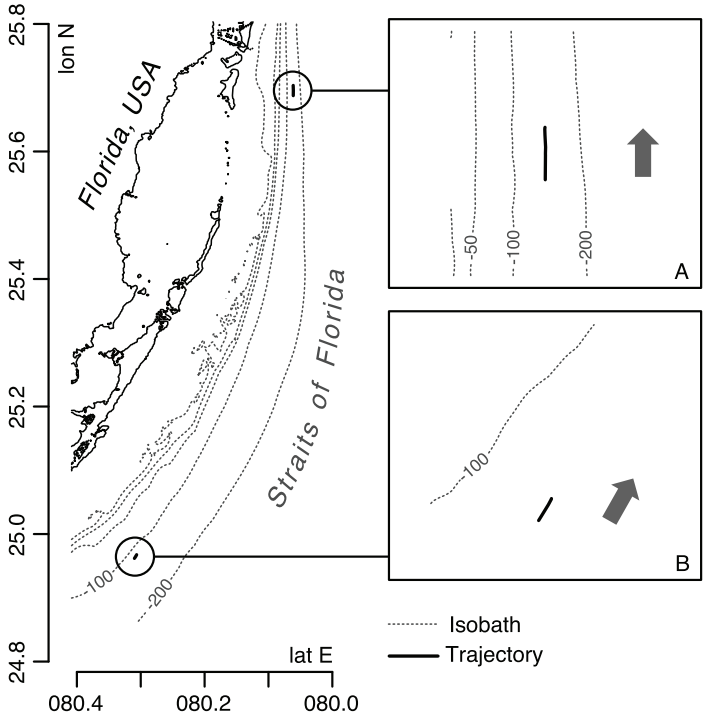

Fig. 6. Two characteristic trajectories of the OWNFOR device, which drifted along the isobaths and remained fully entrained in the current. Regional map on the left, detail and drifting direction arrows on the right: A, off Miami; B, off Key Largo.

its positions aggregate around this point when related to the device itself but are scattered around the arena when observed in a cardinal reference, due to the rotation of the device. Conversely, when a larva has preference for a course rather than for a feature of the arena, its trajectory is more coherent after correction by compass readings than before (Fig. 7). Thus, series of comparisons before and after correction are carried out. The concentration of positions indicates true orientation if three criteria are met: (1) the proportion of significant sum vectors of the bootstrap procedure is larger after correction, (2) the circular dispersion of those significant vectors is smaller, and (3) the mean circular dispersion of position angles is reduced. For half the specimens, these three criteria were all met, illustrating that these larvae oriented despite the enclosure. Alternatively, only two larvae had preference for a section of the arena. For the rest of the larvae, only two of the three criteria were met because the amount rotation of the drifting system was not enough. Orientation was unequivocally detectable (i.e., all three criteria agreed) when the apparatus rotated.

In summary, the OWNFOR system drifted correctly and remained embedded within its surrounding water mass, and larvae of various coral reef fish species displayed orientation through their positions in the arena. More deployments with larvae of the same species are necessary before we can relate orientation results to the literature. However, our goal to provide a means of observing orientation in pelagic fish larvae

Table 1. Orientation of larvae according to positions and swimming directions.

Family	Position bearing	%	Orientation	Direction bearing	P
Apogonidae	279.5	97.40	+	274	0.65
Apogonidae	218.4	96.90	+	168.7	0.99
Balistidae	293	96.30	–	36.8	0.18
Monacanthidae	189.9	100.00	NA	145	0.26
Pomacentridae	64.8	100.00	+	26.8	0.66
Pomacentridae	32.9	100.00	+	325.1	0.60
Pomacentridae	19	100.00	+	222.6	0.76
Pomacentridae	199.1	100.00	NA	130.1	0.42
Pomacentridae	82.7	99.90	NA	112.5	0.80
Pomacentridae	263.6	100.00	NA	178.5	0.12
Pomacentridae	54	99.70	–	8.5	0.81
Pomacentridae	38.1	72.80		302.6	0.31
Pomacentridae	332	100.00	NA	319.2	0.92
Pomacentridae	181.7	11.40		193.4	0.94
Pomacentridae	226.2	100.00	+	180.3	0.90
Pomacentridae	80.1	100.00	+	70.5	0.74
Pomacentridae	82.6	100.00	+	195.9	0.17
Pomacentridae	61.8	100.00	NA	357.5	0.66

For each larva (n = 18) mean position bearing (mean of the significant sum vectors among the 1000 computed during the bootstrap procedure) and mean direction bearing are reported. The directionality of positions is quantified by the proportion of the 1000 tests that were significant (directionality if >95%). When directionality is detected, three criteria are used to determine whether it is real orientation or not (+ for orientation: all criteria met; – for artifact: no criterion met; NA: criteria do not concord). The directionality in directions is quantified by the P value of the Rayleigh test.

was met. Furthermore, the design of the device made it easy to build and to deploy at any depth for any period. The use of free, open source software further reduced the cost, and tailoring the programs to our use made them more efficient and transparent than other software solutions.

Discussion

The OWNFOR system is a hybrid between conventional laboratory experiments and free, in situ methods. Indeed, in situ observations are performed in an environment that can be controlled by the observer to some extent. As revealed in our work, the enclosure causes swimming bearings of fast-moving larvae to be uniformly distributed in all directions. Yet this does not prevent the detection of orientation through the positions of the larvae. Additionally, this is likely to be less of a problem for younger larvae or other taxa that are less-capable swimmers. The enclosure also limits the vertical movement of larvae. In consequence, vertical swimming behavior and cues that would trigger a response by vertical positioning, such as light intensity, water density, or concentration of chlorophyll (Job and Bellwood 2000; Cowen et al. 2003), cannot be investigated with this device. Its purpose is to explore the horizontal (i.e., cardinal) orientation of larvae. In addition, to test for effects of vertical position on cardinal orientation, the system can be deployed at various depths where navigational capabilities can be tested and related to environmental data recorded along with the trajectories. Finally, when the intensity of the cue is very low, the searching animal detects it sporadically and its search path is likely to display some frequent casting or zigzagging events in the quest for information (Vergassola et al. 2007). Such cases are likely to arise for chemical cues far downstream of reefs. Because the device's movement, rather than the larva itself, determines the large-scale trajectory of the larva, our system is inappropriate to detect these types of behavior. However, until we can use acoustic telemetry tags on individual larvae, these cases are likely to remain unexplored.

The proof-of-concept trials presented here show that larvae orient in the arena and that, similarly with the method of Leis et al. (1996), their orientation can be detected in situ. The immediate advantages of the OWNFOR device are to (1) limit human presence, (2) increase the spatiotemporal scales of the observations (e.g., further offshore, deeper in the water column, at night using far red lighting), and (3) observe larvae at earlier stages and throughout ontogeny. However, the full potential of this system resides in the fact that it enables testing of the influence of individual cues on orientation behavior directly in situ. For example, larvae can easily be isolated from ambient chemicals in a hermetically closed arena made of acoustically clear plastic film so that it still lets sound through. High-frequency sound can be reduced to inaudible levels using two nested arenas isolated by a layer of air. A polarizing acrylic filter placed over the chamber can change the polarization of light. Eventually, even the magnetic information could be altered using a solenoid coil placed around the arena (Lohmann pers. comm.).

Compared to the experimental methods used on the reef or in the laboratory (manipulated light traps [Tolimieri et al.

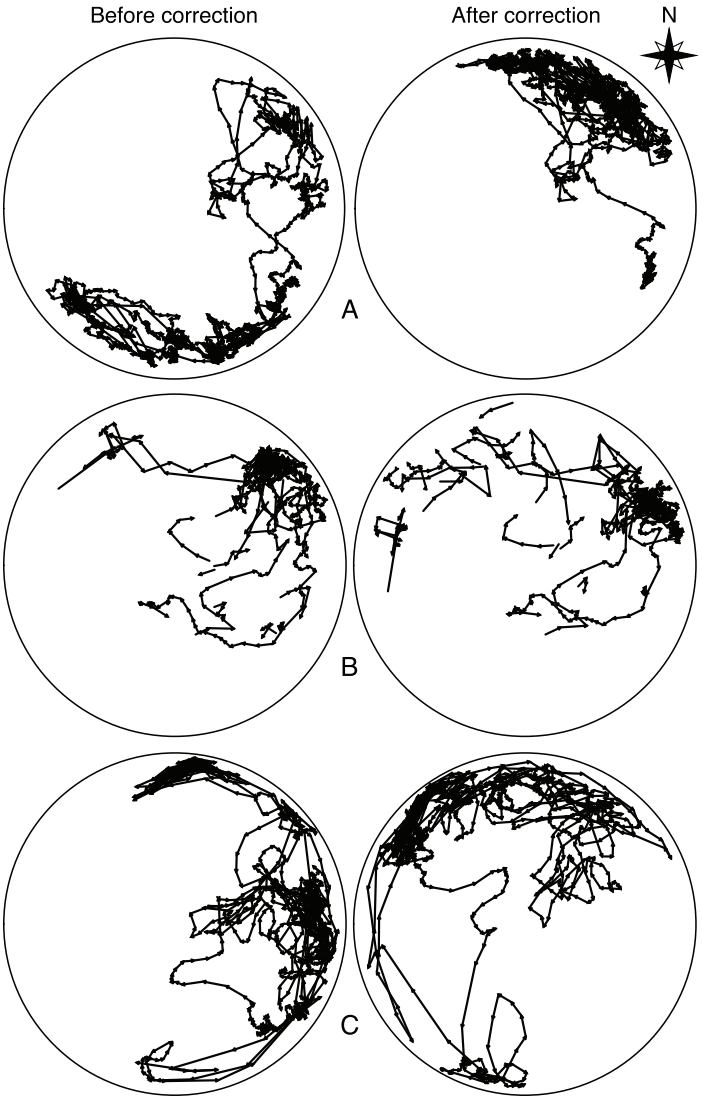

Fig. 7. Recorded trajectories of three damselfish (Pomacentridae) larva plotted before (left) and after (right) correction by compass readings. The right column only is in a cardinal reference. Positions of larva A are more concentrated after correction to a cardinal reference, indicating orientation (+ in Table 1). For larva B, positions are slightly more concentrated before correction (less excursions outside the main concentration zone), hence indicating a possible artifact (– in Table 1). The status of larva C is unclear and criteria do not concord (NA in Table 1).

2000] or patch reefs [Simpson et al. 2005] and choice chambers [Stobutzki and Bellwood 1998; Tolimieri et al. 2004; Atema et al. 2002]), this device greatly broadens the scope of the experiment. It makes it possible to study early-stage as well as competent larvae within pelagic waters—their natural environment—possibly at night, instead of restricting the study to settlement-stage larvae near the reef and during daytime.

Previous experimental methods investigated only the possibility for young larvae to detect a particular cue, without any information about whether it was actually used for orientation. In contrast, in situ methods showed that larvae orient, but allow only speculation regarding the cues involved. The OWNFOR method could bring together those two types of results and allow for an in situ investigation of the influence of environmental cues in the orientation behavior of all larval stages. Great efforts have been directed toward modeling larval trajectories and incorporating larval behavior in dispersal models (*see* Werner et al. 2007 for a review). The success and effectiveness of this new device in investigating both orientation and related cues opens new possibilities for such models and for the understanding of larval ecology in general.

Comments and recommendations

Great care was taken to make the device perfectly symmetrical so that it offers no point of reference, particularly when it rotates. However, the frame was still made of conspicuous aluminum bars and the arena was enclosed with white mesh fabric. Although the field of view of fish larvae is probably limited (Galbraith et al. 2004), some features of the device may explain the artifactual concentration of positions recorded in at least two larvae (Table 1). Using materials such as acrylic for the frame and transparent plastic mesh for the arena should help diminish these artifacts. Indeed, such transparent materials disappear almost completely once placed in water. In addition, aluminum proved to be very sensitive to vibrations which transmitted from the frame to the camera and eventually affected the images. Plastic materials should also improve this aspect and help in developing a fully automated analysis solution.

The orientation of the sun and the polarization of light could be orientation cues for larval fish (Kingsford et al. 2002). In this device, the light shield atop the frame blocks such information for the sake of video quality. However, it should be possible to turn the whole system upside down. In such a configuration, the larva would receive direct light information and would be visible as a shadow against the light coming from the surface. Sound waves, which also are an important orientation cue for marine larvae (Simpson et al. 2005), may have been modified by the two flat acrylic discs that constitute the arena. Their replacement by thin films of acoustically clear plastic should avoid such disruption.

In the end, the rotation of the device was required to discern between true orientation and artifactual concentrations of positions. Therefore, any new drogue design that would facilitate a slow rotation of the device would be a necessary improvement for its deployment in currents less intense than the Gulf Stream.

Although the current device proved sufficient to detect orientation in half of the study specimens, such modifications are being tested now to further enhance the possibilities of this observation system.

References

Armsworth, P. R. 2000. Modelling the swimming response of late stage larval reef fish to different stimuli. Mar. Ecol. Prog. Ser. 195:231–247.

Atema, J., M. J. Kingsford, and G. Gerlach. 2002. Larval reef fish could use odour for detection, retention and orientation to reefs. Mar. Ecol. Prog. Ser. 241:151–160.

Batschelet, E. 1981. Circular statistics in biology. Academic Press.

Codling, E. A., N. A. Hill, J. W. Pitchford, and S. D. Simpson. 2004. Random walk models for the movement and recruitment of reef fish larvae. Mar. Ecol. Prog. Ser. 279:215–224.

Cowen, R. K., S. Sponaugle, C. B. Paris, J. L. Fortuna, K. M. M. Lwiza, and S. Dorsey. 2003. Impact of North Brazil Current rings on the local circulation and coral reef fish recruitment to Barbados, West Indies, p. 443–462. *In* G. Goni and P. Malanotte-Rizzoli [eds.], Interhemispheric water exchange in the Atlantic Ocean. Elsevier.

Doherty, P. J. 2002. Variable replenishment and the dynamics of reef fish populations, ch. 15, p. 327–355. *In* P. F. Sale [ed.], Coral reef fishes: Dynamics and diversity in a complex ecosystem. Academic Press.

Galbraith, P. S., H. I. Browman, R. G. Racca, A. B. Skiftesvik, and J. F., Saint-Pierre. 2004. Effect of turbulence on the energetics of foraging in Atlantic cod (*Gadus morhua*) larvae. Mar. Ecol. Prog. Ser. 281:241–257.

Job, S. D., and D. R. Bellwood. 2000. Light sensitivity in larval fishes: Implications for vertical zonation in the pelagic zone. Limnol. Oceanogr. 45:362–371.

Kingsford, M. J., J. M. Leis, A. Shanks, K. C. Lindeman, S. G., Morgan, and J. Pineda. 2002. Sensory environments, larval abilities and local self-recruitment. Bull. Mar. Sci. 70:309–340.

Lee, T. N., C. Rooth, E. Williams, M. McGowan, A. F. Szmant, and M. E. Clarke. 1992. Influence of Florida Current, gyres and wind-driven circulation on transport of larvae and recruitment in the Florida Keys coral reefs. Cont. Shelf Res. 12:971–1002.

Leis, J. M. 2006. Are larvae of demersal fishes plankton or nekton? Adv. Mar. Biol. 51:57–141 [doi:10.1016/S0065-2881(06)51002-8].

———, H. P. A. Sweatman, and S. E. Reader. 1996. What the pelagic stages of coral reef fishes are doing out in blue water: Daytime field observations of larval behavioural capabilities. Mar. Freshw. Res. 47:401–411 [doi:10.1071/MF9960401].

———, and B. M. Carson-Ewart. 1997. *In situ* swimming speeds of the late pelagic larvae of some Indo-Pacific coral-reef fishes. Mar. Ecol. Prog. Ser. 159:165–174.

Montgomery, J. C., N. Tolimieri, and O. S. Haine. 2001. Active habitat selection by pre-settlement reef fishes. Fish Fisheries 2:261–277 [doi:10.1046/j.1467-2960.2001.00053.x].

———, A. Jeffs, S. D. Simpson, M. G. Meekan, and C. Tindle. 2006. Sound as an orientation cue for the pelagic larvae of reef fishes and decapod crustaceans. Adv. Mar. Biol. 51:143–239.

Paris, C. B., R. K. Cowen, R. Claro, and K. C. Lindeman. 2005. Larval transport pathways from Cuban snapper (Lutjanidae) spawning aggregations based on biophysical modeling. Mar. Ecol. Prog. Ser. 296:93–106 [doi:10.3354/meps296093].

———, C. M. Guigand, J.-O. Irisson, R. Fisher, and E. D'Alessandro. 2008. Orientation With No Frame of Reference (OWNFOR): A novel system to observe and quantify orientation in reef fish larvae. *In* R. Grober-Dunsmore and B. Keller [eds.], Caribbean connectivity: Implications for marine protected area management. Proceedings of a Special Symposium, 9-11 November 2006, 59th Annual Meeting of the Gulf and Caribbean Fisheries Institute, Belize City, Belize. Silver Spring (MD): U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Office of National Marine Sanctuaries. p. 52–62.

Piccardi, M. 2004. Background subtraction techniques: A review. *In* 2004 IEEE International Conference on Systems, Man & Cybernetics. V. 4. Piscataway (NJ): IEEE. p. 3099–3104. [doi:10.1109/ICSMC.2004.1400815].

Simpson, S. D., M. Meekan, J. Montgomery, R. McCauley, and A. Jeffs. 2005. Homeward sound. Science 308:221.

Sponaugle, S., and D. R. Pinkard. 2004. Lunar cyclic population replenishment of a coral reef fish: Shifting patterns following oceanic events. Mar. Ecol. Prog. Ser. 267:267–280.

Stobutzki, I. C., and D. R. Bellwood, 1998. Nocturnal orientation to reefs by late pelagic stage coral reef fish. Coral Reefs 17:103–110.

Tolimieri, N., A. Jeffs, and J. C. Montgomery. 2000. Ambient sound as a cue for navigation by the pelagic larvae of reef fishes. Mar. Ecol. Prog. Ser. 207:219–224.

———, O. Haine, A. Jeffs, R. McCauley, and J. Montgomery. 2004. Directional orientation of pomacentrid larvae to ambient reef sound. Coral Reefs 23:184–191 [doi:10.1007/s00338-004-0383-0].

Vergassola, M., E. Villermaux, and B. I. Shraiman. 2007. 'Infotaxis' as a strategy for searching without gradients. Nature 445:406–409 [doi:10.1038/nature05464].

Werner, F. E., R. K. Cowen, and C. B. Paris. 2007. Coupled biological and physical models: Present capabilities and necessary developments for future studies of population connectivity. Oceanography 20:54–69.

Wolanski, E., P. J. Doherty, and J. Carleton. 1997. Directional swimming of fish larvae determines connectivity of fish populations on the Great Barrier Reef. Naturwissenschaften 84:262–268.

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