Orientation with No Frame of Reference (OWNFOR): A Novel System to Observe and Quantify Orientation in Reef Fish Larvae

Claire B. Paris¹, Cédric M. Guigand¹, Jean-Olivier Irisson², Rebecca Fisher³, Evan D’Alessandro¹

¹Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, FL. cparris@rsmas.miami.edu
²University of Perpignan, France
³Townsville, Australia (rebecca_fisher76@yahoo.com.au)

Abstract
A central question in marine ecology today is to understand the spatial scales over which populations are connected by larval dispersal. Although coral reef fish larvae develop strong behavioral capabilities during the processes of dispersal (e.g., vertical migration, swimming), the influence of these capabilities on survival depends on the ability of larvae to orient in the open ocean. Yet, behavioral mechanisms by which reef fish larvae achieve successful recruitment from blue waters to coral reefs are still unknown. We describe a novel system designed to detect and quantify the orientation of larval coral reef fish in the pelagic environment, where they have no apparent frame of reference to navigate. The Orientation With No Frame Of Reference (OWNFOR) system is deployed at sea and drifts while videotaping the movement of a larva placed within a clear, circular arena. The data are then treated with a combination of open-source programs that track particles and analyze orientation using circular statistics. We demonstrate that (1) this system successfully detects orientation behavior and (2) the shape of the behavioral arena and appropriate statistical treatments minimize the impact of the semi-enclosure on quantifying larval bearing. This observational approach provides a means of assessing the abilities of larvae to orientate during ontogeny and of testing cues, representing a breakthrough in the field of larval ecology. Such behavioral data will provide critical inputs to a new generation of biophysical larval dispersal models that are vital to achieve a better understanding of larval connectivity in marine systems.

Introduction
Most coral reef fishes have a dispersive larval stage that might distance them from reef predators (Johannes 1978). This dispersive stage ends when the well-developed larva leaves the pelagic environment and settles onto coral reef habitat. The supply rate of larvae is critical for the structure of reef populations and is recognized as an important factor in their management (Sale and Kritzer this issue). With the increasing trend toward the implementation of a network of marine protected areas (MPAs) designed to enhance spawning production and to maximize the number of larvae settling within the network, an understanding of the connectivity of marine populations through larval dispersal and recruitment patterns has become an area of critical concern. Understanding population connectivity is one of the most important and challenging goals in ecology, evolution, and conservation (Steneck 2006). There has been considerable

investment in high-resolution numerical simulations of currents from basin-scale circulation to small hydrodynamic features around coral reefs (Wolanski and Sarneski 1997; Chassignet et al. 2006; Cherubin et al. 2007; Ezer et al. 2005). Taking advantage of these models, spatially explicit biophysical models have been used to determine the trajectories of individual larvae from a population in realistic flow fields (Werner et al. 2001; Paris et al. 2002, 2005; Cowen et al. 2000, 2006). Yet, a major question in marine ecology is how the larval stages of benthic marine organisms behave while in the pelagic environment, long prior to settlement, because their behavior could considerably influence their dispersal trajectories (see Cowen 2002, for review). Recent advances in the study of the sensory system of reef fish larvae (see Myrberg and Fuiman 2002; Montgomery et al. 2006, for review), of their vertical migration (Paris and Cowen 2004) and of their swimming behavior (Leis 2006, for review) have demonstrated that larval reef fishes have considerable capabilities that enable them to actively control their position in the ocean at relatively large scales. Late-stage larvae may navigate toward suitable settlement sites by responding to a variety of gradients of environmental stimuli (Kingsford et al. 2002) and even early-stage fish larvae can exhibit directed horizontal or vertical motion (Fisher et al. 2000; Paris and Cowen 2004). However, the realized impact of such behavior remains unclear. For larvae to have a significant impact on their dispersal they must be capable of orientation, possibly using a variety of different cues (Stobuski and Belwood 1998; Leis and Carson-Ewart 2003; Leis 2006).

To investigate the ability of reef fish larvae to orientate during ontogeny, we present a new concept for behavioral observations in the pelagic environment. The notion is that larvae have no apparent frame of reference to orientate in open waters. Presumably, larvae may not sense the direction of the water mass in which they are embedded (Galileo 1632) to avoid being swept downstream, nor are they able to see the coral reefs or the seafloor that would provide a visual frame of reference for navigation back home or to the nearest coral reef. Our main goal is to observe larvae in their natural settings and detect whether they actively swim in a non-random direction while the current moves them. This contribution describes the Orientation With No Frame of Reference (OWNFOR) system and tests its capacity to record data on orientation. The effectiveness of the system depends on the success in recording true orientation. Because fish larvae are known to display a rheotactic behavior (Stobutzki and Bellwood 1994; Hogan and Mora 2005), usually swimming against the current, it is important that the system drifts exactly locked in the current (i.e., with no drag). Furthermore, the larva’s movement is altered near the edges of the arena and might bias its orientation behavior by holding the larva in a specific location. Thus, in addition to demonstrate an orientation versus random movement, we need to show that the larva has no position preference within the circular arena.

Materials and Methods
OWNFOR
The observational system is designed to detect and quantify orientation behavior of larval fish in their natural settings using the concept of a float. The structural frame is equipped with drogue panels, a surface global positioning system (GPS), a compass, a reference marker, a digital video camera, a small environmental sensing system (i.e., conductivity-temperature-density, CTD), and a circular (38 cm $\Theta$, 10 cm deep), and a semi-enclosed behavioral arena (Fig. 1). A larva placed inside the arena is filmed while the system drifts at sea. The frame is half-submerged along the side of the boat to introduce the specimen before it is released (Fig. 1E). The boat motors downstream and the engine is turned off during the behavioral trial. The system is recovered and
the larva is replaced for the next deployment. The video recording system looks down on the arena, as well as on the reference marker to enable corrections on the recorded larval trajectory and the compass to obtain orientation data in a fixed cardinal reference (Fig. 1D). The recording is done in progressive scanning mode (30 frames s⁻¹) for maximum resolution per image (0.3 mm pixel⁻¹). The video data (i.e., Mini DV tape) are retrieved after a set of deployments (Fig. 2A, 3B). To test this novel system, pre-settlement reef fish were captured in light traps on French Reef (Key Largo), collected at sunrise and immediately used in the trials. We first deployed OWNFOR at 20 m below the surface, near the 50-m isobath, off Key Largo on June 8, 2006. After examination of the video, the seafloor was visible, providing the larva a visual frame of reference for navigation (Fig. 2B). For the purpose of testing larval fish orientation with no visual cue, the bottom depth of the test release sites was always ≥ 60 m as larvae may see the dark outline of reef patches from as far as 30 m (Lara 2001).

**Fig. 1.** Orientation With No Frame Of Reference (OWNFOR) system: A) schematic view of the system deployed at sea; B) the surface buoy is equipped with a GPS antenna and data logger to record the drifting track of the system; C) the underwater drifting unit is linked to the surface buoy via a line, a small sub-surface buoy, and three bridles and is made of four aluminum bars and three rings holding the drogues, a circular arena (made of optically clear acrylic sheets on the top and bottom and a 300 µm Nytex® mesh on the periphery, attached with velcro), and a digital video camera (Sony Handycam DCR-PC350®) housed in a pressure vessel (Ikelite Underwater Systems Inc.); D) view from the camera looking down on the behavioral arena with the larva (in the red circle), a compass and a reference marker; E) a larva is placed in the arena by opening the mesh, while the system is half-emerged along the boat and setup for a new trial.

**Movement Analysis**

In order to describe and compare orientation behaviors, the convention is directionality in the swimming bearings (Leis et al. 1996). However, in a circular arena the larva is bounded and orientation is associated with two types of
behavior: the larva will either (1) continue to swim along the edge then swim back toward its preferred direction, in which case swimming direction indicates orientation, or (2) stop and stay in the preferred direction, in which case the larval position indicates the orientation. To capture those behaviors, we need a good representation of the trajectory of the larva for each observation. The video is sub-sampled to 0.6 mm pixel⁻¹ and 1 image s⁻¹ because it is sufficient to resolve the movement of the pigmented post-flexion larvae used in these experiments (mean standard length = 4.5 mm) with typical mean swimming speed of 10-15 cm s⁻¹ (Leis 2006). The position of the larva is manually recorded with Image J®, an Open Source Software (OSS) for image analysis. Based on this OSS, we developed a program called BlueBidule (http://rsmas.miami.edu/personal/cparis/ownfor/doc/bluebidule.html) to automatically perform video processing (i.e., denoising, sub-sampling), tracking (i.e. tracks calibration, tracking of the larva, the fixed reference point and the compass, tracks corrections), the statistical analyses, and the visualization.

**Fig. 2.** OWNFOR deployments: A) drift trajectories of the system deployed at 20 m from the surface during June 16, 2005, offshore Biscayne Bay (Miami) and during September 9, 2005, offshore Key Largo (Florida Keys); Snapshots of the arena during a deployment in ca. 40 m (B) and 100 m (C) waters. Note that the coral reef is visible in (B), giving a visual point of reference to the larva on its drift and, in this case, a direct visual cue of settlement habitat.

**Circular Statistics**
The circular shape and symmetrical design of the arena is ideal to perform circular statistics (Batschelet 1981). The bearings of vectors between the center of the aquarium and the position of the larva (or the swimming direction) are extracted from the trajectory data. Because we are interested in the orientation in a cardinal reference, magnetic north as determined by the compass is automatically recorded to correct the larval trajectory from warp caused by changes in the float direction and/or rotation. The angular component of the compass position is subtracted from the angular component of the position of the larva, on each frame. Swimming distances are then computed between two successive frames only when the larva is detected on both, and converted to instantaneous swimming speeds. Those bearings (positions
and directions) are regarded as unit vectors and their sum vectors are computed. Their bearings are the mean angle of each dataset and their norm describes the strength of the directionality of the data. We can compute:

\[ r = \frac{|\vec{V}|}{N} \]

where N is the number of observations and \(\vec{V}\) the sum vector. If the positions are evenly distributed in the aquarium or if swimming direction is random, the sum vector will be small. In contrast, if the larva is more frequently present in one portion of the arena or if it swims more often toward one direction, the sum vector will be longer and pointing in this direction. As a direct consequence, the closer it is to one, the less random the observations. Hence, the sum vector captures the behaviors we want to detect and quantify. The mean bearing is then tested for directionality with Rayleigh's test for uniformity and the null hypothesis is rejected if the sum vector (\(r\)) is not significantly different from one. As in all statistical frameworks, this technique assumes that observations are independent from each other. This may be true for swimming directions but not for position (Fig. 4). Therefore, we use partial bootstrapping by computing statistic descriptors on a subset (i.e., 5\%) of independent position data and repeat this process 1000 times for each larva (Fig. 3D). The percentage of sub-sampling is chosen as the largest percentage for which independent data are obtained.

**Fig. 3.** Detection and quantification of orientation behavior: **A**) Depth, salinity and temperature profile of a series of five deployments of the OWNFOR system offshore Key Largo, Florida Keys; **B**) trajectories of larva A and B before and after compass correction, showing that larva A orientate while larva B does not (the analysis of larva B is stopped); **C**) displacement vector of larva A plotted from the center of the arena with mean direction indicated by the red dashed line; **D**) mean direction associated with the bootstrapped positions of the random subset of positions of larva A; **E**) rose diagram of the current direction. The Florida Current flowed mostly northward at 1.6 m s\(^{-1}\) on June 16, 2005.
Results

Detection of Orientation Behavior

A total of eighteen 20-minute trials with larvae from four families (Apogonidae, Balistidae, Pomacentridae, Monacanthidae) were used for the analyses. Based on the analyses of positions, sixteen out of eighteen trials showed significant concentration of larvae positions. From those, eight were identified as true orientation, two as artefactual concentrations and the rest could not be discriminated. There seemed to be a taxonomic effect on orientation, but we did not have enough larvae from each taxon to robustly document this effect nor to document differences in bearings between species. An example of discrimination between orientation versus non-orientation is given in Fig. 3. A larva that orients keeps a steady position relative to a cardinal reference, regardless of the direction of the drift or of the rotation of the system (e.g., larva A, Fig. 3B). Alternatively, a larva that does not orient shows either a random position with regard to a bearing, and/or preference for a section of the arena (e.g., larva B, Fig. 3B). The drift of the apparatus revealed the steady direction of the Florida Current during the trials (Table 1, Fig. 2). However, none of the larvae oriented directly opposed to this drift (i.e., rheotaxis behavior), indicating that there was no detectable flow in the arena and that the device was well locked in the current, a fundamental criterion to provide the natural settings of the pelagic larva (i.e., no frame of reference in relation to the direction of the current).

![Graph showing autocorrelation values for direction (independent), positions (strongly dependent), and bootstrapped position (independent).](image)

Quantification of Orientation

If the larva swims directionally, the displacement vectors are concentrated around the sum vector of directions (red dotted line, Fig. 3C) and Rayleigh's test for direction data is likely to reject the null hypothesis of lack of orientation. However, the swimming directions of most larvae (similar to larva A, Fig. 3C) did not differ from a uniform distribution of swimming directions. This is explained by the relatively small size of the arena in comparison with the average cruising speed of a reef fish post-flexion larva (e.g., 15 cm s⁻¹, Stobuski and Bellwood 1994; Leis and Carson-Ewart 1997, Bellwood and Fisher 2001, Fisher and Wilson 2004). Alternatively, orientation was detectable in position data for at least

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50% of the trials, where many mean angles calculated from bootstrapped positions are close to each others (Fig. 3D). Examples of significant bearings of larvae and the direction of the current in which they were set to drift are given in Table 1.

**Table 1.** Examples of the direction of the OWNFOR drift in the Florida Current and the corresponding bearings of damselfish (Pomacentridae) larvae. The influence of each larva orientation behavior on its trajectory is shown in Fig. 5.

<table>
<thead>
<tr>
<th>Deployment Date / Time</th>
<th>Frequency Distribution of Current Direction [range of bearing]</th>
<th>Probability Distribution of Larval Position [range of bearing]</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 16, 2005 / 14:21</td>
<td>[353° - 3°]</td>
<td>[34.5° - 99.5°]</td>
</tr>
<tr>
<td>Offshore Miami, Biscayne Bay</td>
<td>Fish larva A</td>
<td>![Diagram of Fish larva A]</td>
</tr>
<tr>
<td>Sep. 2, 2005 / 10:02</td>
<td>[351° - 10°]</td>
<td>[190.3°-257.4°]</td>
</tr>
<tr>
<td>Offshore Key Largo, Florida Keys</td>
<td>Fish larva B</td>
<td>![Diagram of Fish larva B]</td>
</tr>
</tbody>
</table>

**Orientation and Dispersal**

The influence of orientation behavior for the two larvae in Table 1 is shown based on the observed range of swimming speeds measured in the laboratory (Fisher 2005). The trajectory of larva A departs quickly from the passive drift, with a zonal displacement ranging from 100-450 m in ca.15 minutes (or 10-43 km in 24 hours, Fig. 5). This deviation is even more remarkable as this larva orientated generally in the current with only a small angular deviation of 30-70 degree from the strong northward flow (ca. 160 cm s⁻¹). The faster the larva swims, the larger the deviation from the passive trajectory, but the distance traveled by the larva relative to the bottom increases (i.e., dispersal distance increases). Alternatively, for larva B released off Key Largo (in slower current ca. 90 cm s⁻¹) and orienting by and large against the current, a small difference in bearing has a large effect on its deviation from the passive trajectory. In this case, the greater the
swimming speed, the smaller the dispersal distance. For larva B, a combination of both higher swimming speed and angular deviation from the current axis can bring larva B onto the reef in less than 24 hours.

**Fig. 5.** Influence of orientation behavior on larval trajectory depicted by the passive and active trajectories of two damselfish larvae (from Table 1), A) larva A released offshore Miami in a 1.6 m s⁻¹ northward flowing Florida Current, and B) larva B released offshore Key Largo in a slower current (ca. 0.9 m s⁻¹). When larval orientation is mainly with the current, swimming speed becomes important to deviate from the passive trajectory, even with a small angular deviation from the current axis. When orientation is largely against the current, the angular deviation from the current axis becomes as critical as swimming speed. In both scenarios, we used the critical speed of a pre-settlement damselfish (i.e. Ucrit 37.6 cm s⁻¹, Fisher 2005). The passive drift indicates the direction of the current and the pie chart the larva’s bearing during a 15-minute trial.

**Discussion**

Although preliminary, this dataset provided substantial evidence that the novel concept and system (OWNFOR and BlueBidule Software) successfully captures and measures *in situ* orientation behavior in reef fish larvae with minimal disturbance. In effect, orientation was detected in the symmetrical arena using circular statistics, an efficient tool in dispersal ecology (Bullock et al. 2001). Independent orientation measures such as those obtained from divers following late-stage larvae (Leis et al. 1996) should be carried in parallel to check the constancy of results. For these trials, we used settlement stage larvae caught in light traps, but in the future we intend is to use pelagic larvae captured in the open ocean, as well as reared larvae to describe the ontogeny of orientation in the early life history of reef fish.

Swimming behavior has been measured in flume tanks where larvae swim against a current (Fisher and Bellwood 2003; Fisher et al. 2000; Hogan and Mora 2005; Stobutzki and Bellwood...
1997). However, Fisher and Bellwood (2003) have come closer to measuring undisturbed swimming speeds (or routine speed) in a still tank. Although larvae were more active during the day than at night, they swam at speeds far less than their critical speeds (Fisher and Wilson 2004). Similarly, the OWNFOR system may allow measurements of *in situ* swimming speeds of the earlier stages, when swimming behavior might not be affected by the enclosure. Vertical swimming behavior has also been observed in reef fish larvae (Cowen 2002; Paris and Cowen 2004; Leis and Carson-Ewart 2001). However the time scales at which vertical swimming behavior occurs is in the order of several hours (i.e., diel vertical migration) or days and weeks (i.e., ontogenetic vertical behavior). OWNFOR was deployed at depths corresponding to observed depth ranges at developmental stage (Paris-Limouzy 2001; Cowen 2002). The effect on orientation of OWNFOR deployment depth relative to the depth of the thermocline and pycnocline should be further tested.

Fish larval swimming capabilities hint at an active behavioral influence on recruitment. Yet without environmental awareness (e.g., direction of the land masses in relation to their position) and orientation, this potential cannot be materialized into successful recruitment. It is therefore crucial to identify the cues involved in orientation (Montgomery et al. 2000, 2001). This new system brings a platform to test a series of isolated and combined sensory cues such as solar compass, sound (Tolimieri et al. 2004; Simpson et al. 2004), magnetic field (Lohmann et al. 2004), and chemicals (Atema et al. 2002; Gerlach et al. 2007). The device can also be deployed at different distances from the reef to measure the sensory envelope of pelagic larvae, which represents an important parameter in larval dispersal and recruitment models (Paris et al. 2005; Cowen et al. 2006). Here we demonstrate that orientation has profound implications for modeling larval dispersal (Fig. 5), most likely changing patterns of population connectivity (Cisco et al. 2007) and conservation strategies (Leis 2002).

There is room for improvement of this first, yet successful OWNFOR version. For example, the entire system can be built in clear polycarbonate (transparent to visible light) to eliminate any possible visual signal that would have a positive (i.e., attract) or negative (i.e., scare) effect on behavior; infrared lights can be implemented for night-time experiments for the study of orientation in late-stage larvae settling at night (Stobutzki and Bellwood 1998; Sponaugle et al. 2002). This observational system can also be applied to a large array of marine organisms. The success of this exploratory approach, together with rapid development of remote sensing and camera technologies, will help to catalyze innovative advances in the field of larval ecology. This system provides information vital to our understanding of the potential for these larvae to influence their dispersal patterns using active behavior.

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Literature Cited


Galileo, G. 1632. The dialogue concerning the two chief world systems (Dialogo sopra i due massimi sistemi del mondo). University of California Press. 496 pp.


Montgomery JC, Tolimieri N, Haine O (2001) Active habitat selection by pre-settlement reef fishes. Fish and Fisheries 2:261-277