Sun Compass Orientation Helps Coral Reef Fish Larvae Return to Their Natal Reef

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Abstract

Reef fish sustain populations on isolated reefs and show genetic diversity between nearby reefs even though larvae of many species are swept away from the natal site during pelagic dispersal. Retention or recruitment to natal reefs requires orientation capabilities that enable larvae to find their way. Although olfactory and acoustically based orientation has been implicated in homing when larvae are in the reef's vicinity, it is still unclear how they cope with greater distances. Here we show evidence for a sun compass mechanism that can bring the larvae to the vicinity of their natal reef. In a circular arena, pre-settlement larvae and early settlers (<24 hours) of the cardinal fish, *Ostorhinchus doederleini*, showed a strong SSE directional swimming response, which most likely has evolved to compensate for the locally prevailing large scale NNW current drift. When fish were clock-shifted 6 hours, they changed their orientation by ca. 180° as predicted by the tropical sun curve at One Tree Island, i.e. they used a time-compensated sun compass. Furthermore, the fish oriented most consistently at times of the day when the sun azimuth is easy to determine. Microsatellite markers showed that the larvae that had just arrived at One Tree Island genetically belonged to either the local reef population or to Fitzroy Reef located 12 kilometers to the SSE. The use of a sun compass adds a missing long-distance link to the hierarchy of other sensory abilities that can direct larvae to the region of origin, including their natal reef. Predominant local recruitment, in turn, can contribute to genetic isolation and potential speciation.

Introduction

The persistence of reef fish populations on isolated oceanic islands demonstrates that a significant number of their larvae return to their natal reef after their pelagic dispersal phase [1,2,3,4]. Such "self-recruitment" has been shown using transgenerational otolith tagging [5,6] and genetic markers e.g. [1,2]. Failure to recruit to a suitable reef is fatal and the only reef any larva can know initially –by smell, sound, and direction– is its natal reef. This suggests selection for natal homing abilities and there are several arguments that support this idea. Dispersal models better predict the observed recruitment when they include a behavioral component [7,8], i.e. sustained swimming in an appropriate direction. Swimming capabilities of larval reef fishes are surprisingly strong, particularly late in the pre-settlement period [9,10]; and sensory guidance based on acoustic [11,12,13] and olfactory [14,15] mechanisms have been suggested. However, both odor halos [14] and acoustic cues [16] become fainter with distance and are unlikely to provide useful directional information beyond a few kilometers. Genetic assignment (e.g. [2,17]) shows that a significant proportion of larvae end up ten kilometers or more away from their natal reef. Therefore, selection should strongly favor animals that can actively compensate for long distance drift. However, thus far, no plausible mechanism has been demonstrated that could allow pelagic larvae to locate the natal reef from distances beyond a few kilometers after their initial period of passive dispersal during which they are not capable of sustained directional swimming. For longer distance directional movements, animals typically use an innate or acquired compass [18,19,20,21,22,23,24]. Thus, the aim of the present paper is to investigate whether reef fish larvae have a compass mechanism that could be helpful for long-distance homing.

Results

We tested the directional preferences of just-settling fish (20/Jan and 01/Feb/2011) and pre-settlement fish (20–27/Jan/2012) at One Tree Island in the Capricorn Bunker reef group in the southern Great Barrier Reef, Australia (Figure 1). The directional preferences of each fish were measured by releasing it individually in the centre of a small swimming pool (bowl with diameter 17 cm). After ca. 60 sec, the fish's geographical position relative to the centre of the bowl was recorded in 30 sec intervals for the next 20 min. Based on the 40 recorded directions, the mean direction of the given fish and test was calculated. To allow for assessment of both intra and inter-individual variance each of 21 individual fish was retested 3–5 times under the natural sunny sky (maximum 75% cloud cover, mostly less than 25%). In total, 88 individual
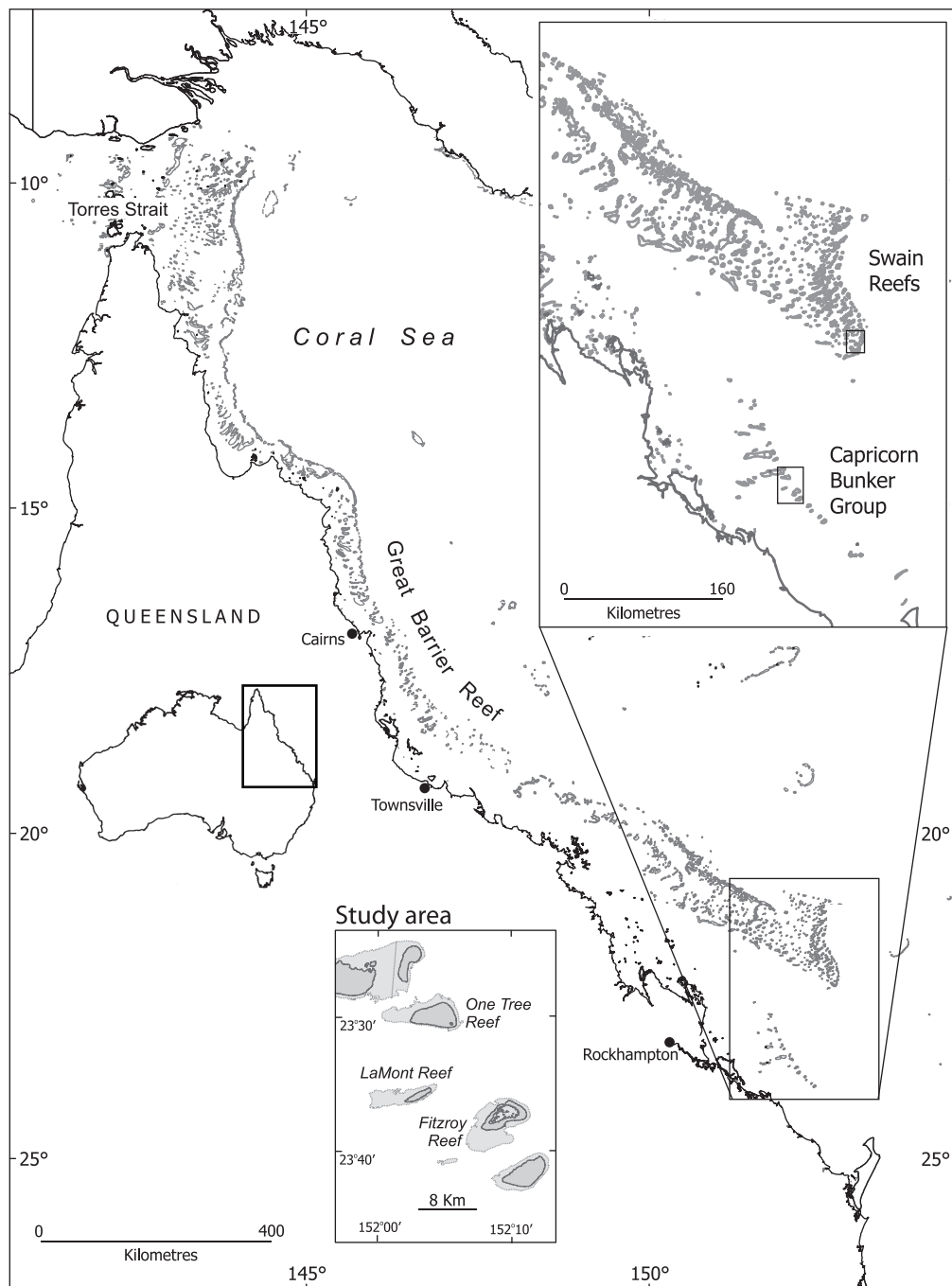
Figure 1. Location of One tree Island. One Tree reef (OTI, 23°30'S, 152°06'E) is one of fourteen reefs in the Capricorn Bunker Group in the southern Great Barrier Reef, Australia. OTI is situated 90 km from the Queensland coast and 5–10 km southeast of neighboring reefs Heron and Sykes.
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tests were performed under non-clock-shifted conditions (a complete raw data summary of these experiments can be found in the supplementary materials, Table S1–S4 in File S1).

The non-clock-shifted, just-settling fish tested in 2011 spontaneously and highly significantly oriented towards the SSE (Rayleigh Test: mean direction 152°, n = 14, r = 0.88, p<0.001; Figure 2A). This direction is opposite to the persistent NNW drift of the water masses around One Tree Island [2,25]. An internal replication using pre-settlement fish tested in 2012 showed the

same directional preference (Rayleigh Test: mean direction 161°, n = 7, r = 0.91, p<0.001; Figure 2C).

We have direct evidence that long distance NNW dispersal occurs at the testing site. Using DNA microsatellite markers, we could assign the individual larvae, which we had shown to swim SSE in 2011, to adult populations of OTI and to Fitzroy reef located 12 kilometers from OTI in the same SSE direction (Figure 1). None of the larvae were assigned to Heron or Lamont reef populations located to the NW or SW of OTI. Five (31%) larvae were assigned to the OTI population with a mean
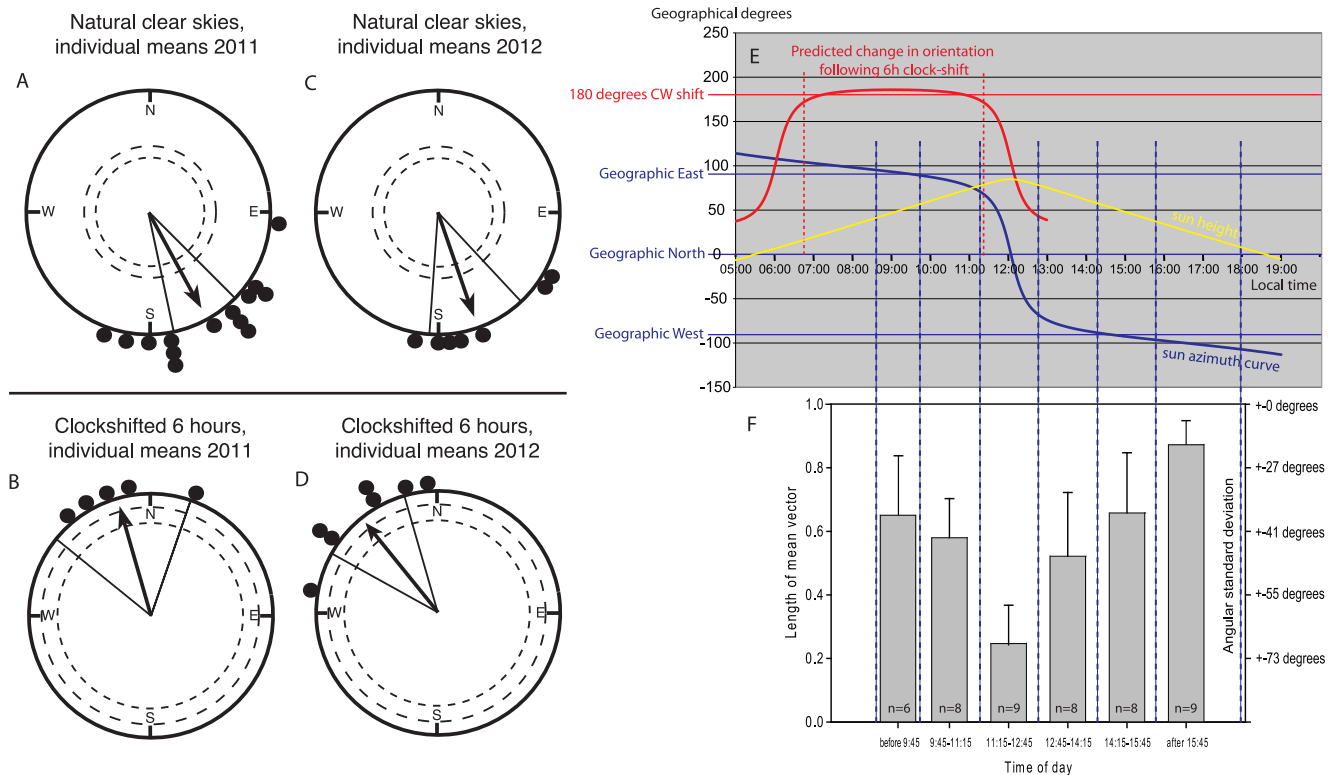
Figure 2. Settling stage *Ostorhinchus doederleini* use a time-compensated sun compass to orient towards SSE. A: Fourteen just-settled *O. doederleini* tested under natural sunny skies in 2011 showed a clear orientation towards SSE (mean direction: 152°, r = 0.88, n = 14, p<0.001). **B**: When five of these fish were clock-shifted 6 hours backwards, they turned their orientation by ca. 180° (mean direction: 344°, r = 0.94, n = 5, p<0.01). **C, D**: In January 2012, we repeated the experiments with pre-settlement fish and got very similar results. Seven pre-settlement *O. doederleini* tested under natural sunny skies showed a clear orientation towards SSE (**C**: mean direction: 161°, r = 0.91, n = 7, p<0.001). When all 7 fish were clock-shifted 6 hours backwards, they turned their orientation by ca. 180° (**D**: mean direction: 321°, r = 0.92, n = 7, p<0.001). Each dot at the circle periphery indicates the mean orientation chosen by each individual fish based on the second order average of all tests made with a given fish in the given condition. Arrows indicate the group mean vectors. Inner and outer dashed circles indicate the radius of the group mean vector needed for significance according to the Rayleigh Test (p<0.05 and p<0.01, respectively). Lines flanking the group mean vector indicate the 95% confidence intervals for the group mean direction. **E**: We performed all orientation tests between 20/Jan and 01/Feb. The yellow curve in **E** shows the height of the sun above the horizon at One Tree Island calculated for 25 January 2012 (90° means directly overhead, 0° means that the sun is at the horizon). The blue curve in **E** is the sun azimuth curve at One Tree Island calculated for 25 January 2012. Notice that in the morning until about 11:15, the sun azimuth is very consistently in the East (117°–77°). Likewise, in the afternoon from 12:45 onwards, the sun azimuth is very consistently in the West (293°–243°). In contrast, at noon between 11:15 and 12:45, the sun is more or less directly overhead (the sun is 78–86 degrees above the horizon, see yellow curve) and the sun azimuth changes by 139 degrees in just 90 minutes. **F** is showing how strongly oriented the individual fish were during tests in the different time intervals. The left y-axis is indicating the length of the mean vector, ''r'', calculated by vector addition of the 40 observed directions during a single test of a given individual. The greater the r, the more consistently the fish oriented. The mean vector length is inversely proportional to the angular standard deviation (s = (-ln(r)) ½) which is indicated on the right y-axis. Figure **F** is aligned exactly under Figure **E** so that the blue dashed lines identify the time range and sun azimuth positions that contributed data to each of the six time blocks. Notice that the fish oriented very poorly during the 11:15–12:45 time block, when a sun compass would be very difficult to use because the sun is almost directly overhead and shows an exceptionally rapid change in azimuth (139 degrees in just 90 minutes, i.e. 1.5 degrees/minute). Accurate orientation during this time would require a very precise synchronization of the animals' internal clock to the specific sun curve. In contrast, late in the afternoon when a sun compass would be particularly easy to use because the sun azimuth changes very slowly and because the sun is close to the horizon, the fish showed extremely directed orientation. The unusual sun curve also means that a 6 hour clock-shift where the animals wake up around midnight and are tested before noon, when they think it is afternoon, leads to an extremely consistent predicted change in orientation of 180 degrees. This is documented by the red curve in Fig. **E**, which shows the predicted clockwise shift in orientation following a 6 hour clock-shift as a function of the time of day during which the fish are tested after being clock-shifted 6 hours. The red curve was calculated as follows: the Sun azimuth at testing time - the sun azimuth 6 hours later. We tested our clock-shifted fish between 06:45 and 11:02 (as indicated by the dashed vertical red lines) when the expected orientation of the 6 hrs time shifted larvae predicts a 180 degree shift for this entire 4:17-hr observation window.
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probability of 78%; the other eight (69%) larvae were assigned to the Fitzroy population with a mean probability of 77% (Table 1). Thus, NNW larval dispersal beyond 10 kilometers (i.e. from Fitzroy) occurs regularly. Therefore, the SSE swimming direction could be an adaptation to counter large-scale dispersal away from the fishes' natal reef. This begs the question of how they know and maintain this SSE heading.

During orientation tests at different times of day (the time ranges are indicated by the dashed blue lines in Figure 2E–F and detailed information on each individual test is given in Table S1 in File S1) we noticed that the fish appeared very poorly oriented when tested around noon (Figure 2F). One Tree Island (23°30″ S, 152°05″ E) is part of the Capricorn group of islands, named so because these islands are located on the ''Tropic of Capricorn'', where the sun passes directly overhead on 21st of December. In

Table 1. Genetic assignment of recently settled larval *O. doederleini* to adult reef populations.

Assigned larva*	Rank 1	Score %	Rank 2	Score %
OTI11,HM1 (cs)	OTI	87.4	F	8.5
OTI11,HM3 (cs)	F	77.7	H	10.5
OTI11,HM4 (cs)	OTI	89.2	F	10.7
OTI11,HM5 (cs)	F	55.9	OTI	41.8
OTI11,HM9	F	92.4	L	7.0
OTI11,HM10 (cs)	OTI	86.3	F	13.1
OTI11,HM11	F	99.6	OTI	0.4
OTI11,HM12	OTI	55.6	F	44.2
OTI11,HM13	F	97.1	OTI	2.7
OTI11,HM19	OTI	80.5	H	14.6
OTI11,HM20	F	64.7	OTI	33.1
OTI11,HM21	F	55.7	OTI	34.1
OTI11,HM22	F	70.6	L	28.4

*One sample (OTI11, HM2) could not be used for genetics. "(cs)" means that this individual was clock-shifted after being tested under non-clock-shifted conditions. Genetic assignment of thirteen post-settlement larvae caught at the One Tree reef (OTI) tested for orientation in the sun compass using five microsatellite markers; the first two ranks (i.e. the two most likely origins of the larvae) and their probability score are shown. Statistical analysis was performed following the Bayesian approach by Rannala [52]. L (Lamont), H (Heron) F (Fitzroy).
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late January on One Tree Island, the sun still passes from very consistent easterly positions before noon, to being almost directly overhead at noon, and to very consistent westerly positions in the afternoon. This leads to a very unusual local sun azimuth curve (see Figure 2E), which is ideally suited to test for sun compass orientation. In the morning from 07:00 to 11:00, the sun azimuth is in the East moving only 25 degrees in 4 hours. Likewise, in the afternoon from 13:00 to 17:00, the sun azimuth is in the West moving only 28 degrees in 4 hours. In contrast, around noon, between 11:15 and 12:45, the sun is more or less directly overhead (78–86 degrees above the horizon) and the sun azimuth changes by 139 degrees in just 90 minutes. This causes problems for the use of a sun compass because the sun azimuth is difficult to determine: the sun is almost directly overhead and the exceptionally rapid change in azimuth would require very precise synchronization of the animals' internal clock to the local sun curve. The observed lack of orientation around noon suggests that the fish might use a sun compass at other times.

We therefore systematically investigated the consistency of the fish's directional choices as a function of time of day in 2011 (Figure 2F, a summary table listing the time and orientation result of each individual test contributing to Figure 2F is provided in supplementary materials, Table S2 in File S1). The fish's directedness, quantified as the length of the mean vector (r) of the 40 recorded headings in each single test, showed a clear dependence on time of day (One-way ANOVA; df = 47, F = 16.1, p<0.001, Figure 2F). In fact, the fish were randomly oriented around noon during the period when a sun compass would be very difficult to determine (11:15–12:45, mean r = 0.25; the average length of the mean vector was significantly smaller than in any other time interval, p<0.01 (One-way ANOVA followed by All Pairwise Multiple Comparison Procedures using the Student-Newman-Keuls Method). In contrast, the fish were exceptionally clearly oriented in the late afternoon when the sun is close to the

horizon and the sun azimuth is therefore particularly easy to determine (after 15:45, mean r = 0.87; the average length of the mean vector was significantly larger than in any other time interval, p<0.01). This is also reflected in the negative correlation between directedness of the fish and sun elevation (see Figure S2 in File S1). I.e. the lower the sun, the more directed the individual fish were during each single 20 min test session.

To sum up this section, the striking change in the fish's directedness as a function of time of day indicates that they use a sun compass (e.g. [22,26,27]), since the easier the sun azimuth angle could be determined, the better the fish were able to orient. Sun compass orientation has been considered in fish [28], particularly in mosquitofish [29] and migrating salmon [30], and even though it has been suggested as a potential mechanism [31,32], there has been no direct experimental demonstration for any reef fish larvae.

Since sun position can only be used as a compass cue if time of day is taken into consideration [22,26,27], the key experiment needed to prove whether an animal uses a sun compass to determine its orientation direction, is a clock-shift experiment. One Tree Island is ideally suited for such an experiment. When fish are clock-shifted 6 hour backward (light on at 23:15, light out at 13:15) and then tested between 06:45 and 11:15 in the morning (see red curve and red dashed lines in Figure 2E), the summer tropical sun azimuth curve means that the sun azimuth is consistently 180±10° different six hours later in the day. Thus, if our fish used a time-compensated sun compass to orient SSE, they should shift their orientation by 180° when clock-shifted 6 hours backward and should thus orient to the NNW.

We therefore clock-shifted five of the 2011 just-settling fish and all the 2012 pre-settlement fish 6 hours backwards (light on at 23:15, light out at 13:15). After being clock-shifted for 6 days, we re-tested their orientation under sunny skies before noon (06:45–11:02) when the sun was in the East. However, since their shifted inner clock told them it was afternoon, the fish should expect the sun to be towards the West. If the fish use a time-compensated sun compass, they should therefore make a ~180° orientation "mistake" (red curve in Figure 2E). We conducted 44 individual tests with the 12 clock-shifted fish (for detailed information about each single test, see supplementary Table S3 in File S1).

Indeed, the 6 hours clock-shifted, just-settling fish tested in 2011 oriented significantly towards the NNW (Rayleigh Test: mean direction 344°, n = 5, r = 0.94, p<0.01; Figure 2B), while the nine non-clock-shifted fish continued to orient to the SSE, (Results shown in Figure 2A include data from non-shifted control fish tested at the same post-capture time as the clock-shifted fish, for full details see Table S1 and Table S3 in File S1). The same switch of orientation from SSE to NNW in the clock-shifted condition was observed when we replicated the experiment with pre-settlement fish in 2012 (Rayleigh Test: mean direction 321°, n = 7, r = 0.92, p<0.001; Figure 2D). When the mean directions chosen by each individual fish tested both before and after the clock-shift were compared, it was clear that all individuals strongly shifted their mean orientation in response to the clock-shift (Figure 3). The tests with the clock-shifted fish demonstrate that the fish have an internal clock ("*zeitgeber*") that they use as part of a time-compensated sun compass to maintain their SSE heading.

Discussion

Although it is not known how far *O. doederleini* larvae disperse from the reef during their pre-settlement phase, genetic data presented here and in [2] indicate that distances beyond 10 kilometers occur regularly. Thus, we consider two likely situations:

Turn in response to the clock-shifts,
 comparison on the individual level
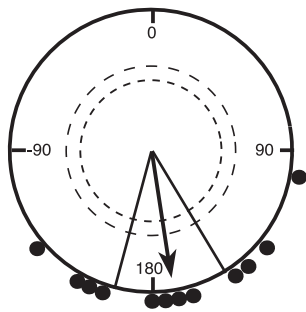
Figure 3. Relative orientation of the individual fish in the clock-shifted condition compared to the same fish's orientation the non-clock-shifted condition. The fish's orientation in the non-clock-shifted condition is defined as 0 degrees and the orientation of each individual fish after the clock-shift compared to before the clock-shift is indicated by the dots at the circle periphery (2011 and 2012 fish combined). Thus, a point in 0 degrees would mean no difference between an individual fish's orientation in the clock-shifted and non-clock-shifted condition. On average, the fish highly significantly shifted their orientation by 172 degrees clockwise in the clock-shifted condition compared to the same fish's orientation in the non-clock-shifted condition (Rayleigh Test, mean direction = 172, r = 0.83, n = 12, p<0.001. Furthermore, even 99.9% confidence intervals do not include 0 degrees), and the 95% confidence interval (150–195 degrees) amply includes the predicted 180 degrees shift. Raw data in Table S4 in File S1. For description of the circular diagram, see legend to Figure 2.
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(1) some larvae remain close to the reef, and (2) others drift further away on prevailing currents and may or may not return to the natal reef (see also Figure 4).

In the first case, OTI larvae would be retained in the reef vicinity within lee side eddies resulting from oscillating tidal currents [25,33] mostly north of the One Tree reef. For such larvae the sun compass might not be crucial, but it could still be useful to cross the reef crest and settle. In the second case, OTI larvae would be transported away from the tidal eddies by the weak but persistent and unidirectional NNW current resulting from the dominant SE trade winds. The observed SSE directional swimming would be an adaptation to counter long distance NNW drift. It is important to note that the much stronger tidal currents are oscillating East-West around the reef and thus contribute little to long-term, large-scale drift.

For larvae that drift farther away from their natal reef, such as our identified Fitzroy larvae, we suggest the following orientation-relevant chain of events: (I) a passive dispersal phase leading to drift away from the home reef; (II) an actively swimming, sun-compass guided phase to compensate for the drift; and (III) a multi-sensory settlement phase similar to the larvae that were retained near the reef.

We suggest the following pelagic dispersal and settlement scenario. Hatching, *O. doederleini* larvae leave the reef by tidal currents [34]. At the beginning of this passive dispersal phase, the larvae are immersed in natal reef water making imprinting on various home reef cues such as odor and sound possible. A dispersal model based on field-release of neutrally buoyant particles in the currents around the One Tree Reef [2] shows that the centre of the particle distribution on post-release day 8 has drifted to a location of ca. 20 kilometers NW of the One Tree Reef during flood tide and ca. 13 kilometers NNW during ebb tide, i.e. the drift current speed is ca. 13 km/8 days = 1.9 cm/s (Figure 4). Even though large-scale currents will vary with ocean basin

phenomena such as ENSO, this drift current is consistent with prevailing trade winds, which are remarkably stable around the study site; South-South Easterly 90% of the time. This predictability would facilitate the emergence of an innate mechanism for sun compass guided directionality by selection, year after year, in favor of the larvae that swim SSE. Since the time-compensation required for a sun compass needs to be learned (because the exact movement of the sun varies greatly with season and latitude), it is likely that this learning takes place during the early dispersal phase.

After about a week of dispersal, the larval swimming capabilities may now be sufficiently effective to start moving in a sun compass-based direction. In young birds, this direction is innate [35,36], and hatchling sea turtles show adaptive innate reactions to specific magnetic fields [23,37]). It is likely that the observed SSE swimming direction is innate in OTI *O. doederleini* larvae, but this has not yet been demonstrated.

Could these reef fish larvae realistically compensate for the drift current? Although this species has not been tested for swimming speed, other cardinal fish can maintain speeds of 2 cm/s at hatching and 10 cm/s at settlement [9], which is faster than the slow NNW drift current of ca. 1.9 cm/s observed around OTI [2]. Over a two-week period of perfectly directed sustained swimming the larvae could theoretically cover up to 100 kilometers. However, the larvae are certainly not swimming perfectly SSE and they do not swim all the time. It is nevertheless plausible that sun-compass directed swimming could, in principle, help them return from the 13–20 kilometer drift distance calculated for their early larval period back to the general vicinity of their natal reef. Homing may be facilitated further if the larvae choose favorable currents at different water depths and tidal periods [38,39]. The constant tidal oscillations generate a permanent odor halo extending several kilometers around each reef, which would further increase the chances of larvae detecting the vicinity of the natal reef. The suggested sun compass guided swimming response is likely to continue until the larvae recognize the odor halo and/or sound of their natal reef, which present a much larger homing target for returning larvae than the reef itself. A larger target allows for greater variance in large-scale currents and homing precision. Surely, the majority of larvae get lost or perish otherwise, but their sun compass directed swimming behavior observed here would increase the likelihood of relocating the area of origin including the natal reef and thus be of evolutionary advantage compared to fish not performing this behavior (Figure 4).

Finally, once the larvae are in the vicinity of their natal reef, we expect them to start using other more local homing cues such as sound [11,13,40] and/or odor [14]. The succession of orientation mechanisms suggested here for reef fish larvae is similar to the innate mechanisms used by birds and monarch butterflies during migration: first a compass mechanism based on global cues for the long-distance navigation phase followed by a homing process based on more local cues (e.g. [22,24,35,36]).

In conclusion, here we provide data documenting that reef fish larvae possess and can use a time-compensated sun compass. Our experiments include the critical clock-shifted condition and an internal replication performed in two different years. Our data show that both pre-settlement and recently settled larvae of *O. doederleini* can use a time-compensated sun compass to keep a consistent direction. This direction seems adapted to compensate for drift away from the natal reef and its surroundings. This orientation mechanism, well-known in various terrestrial animals, is well-suited to explain how dispersing fish larvae can avoid long-
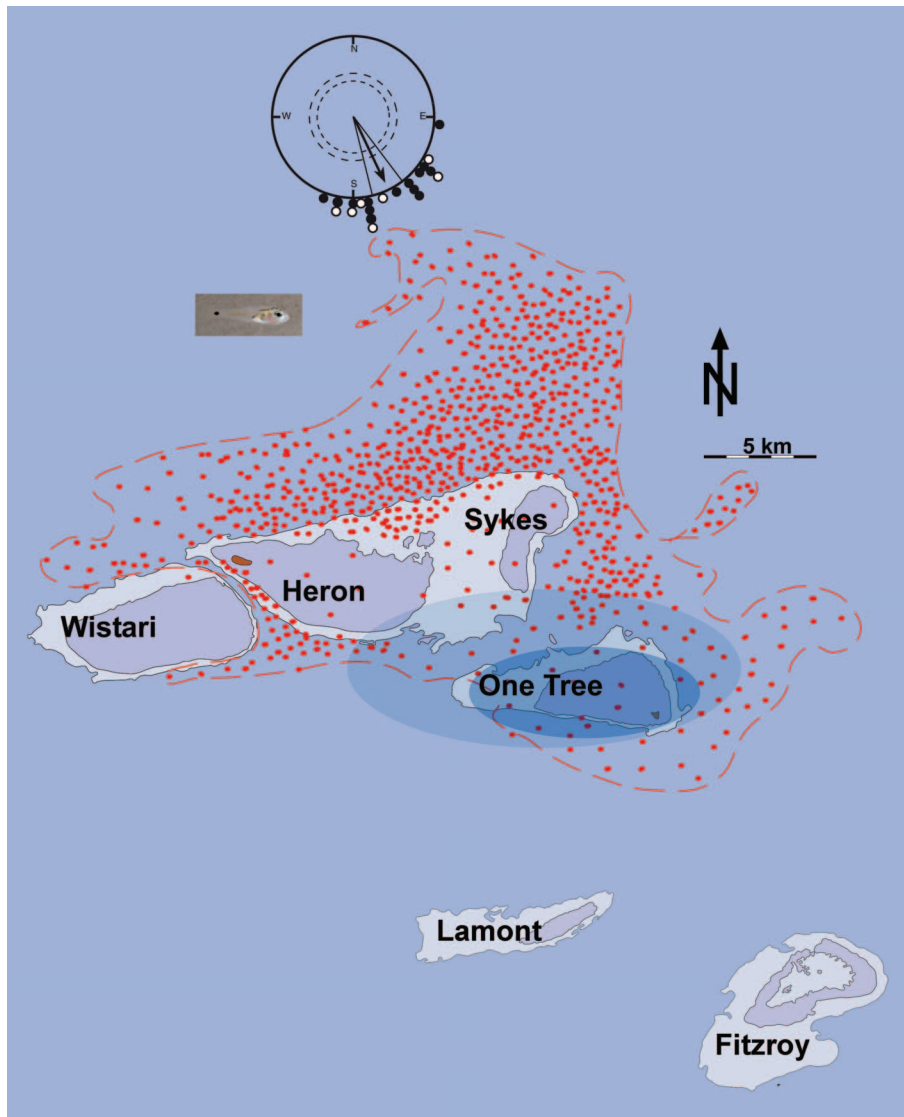
Figure 4. Model illustrating how a time-compensated sun compass could help passively drifted reef fish larvae to relocate their natal OTI reef. Red dots indicate the locations at ebb tide of passively dispersed particles 8 days after release from One Tree Reef according to the dispersal experiments and model calculations (Red dots drawn after Fig. 1C in reference [2]). At flood tide the dots will be displaced 5–7 km WNW. Notice that most larvae considered as passive particles for their first 8 days would be transported significantly to the NNW beyond the odor halo of OTI (idealized odor halos in decreasing intensity blue) and even beyond neighboring reefs to the NNW before they gain sustained swimming capabilities. The polar diagram shows the time-compensated sun compass orientation of just-settled (●) and pre-settlement (o) larvae (Figure 2A and 2C combined: mean direction 155°, n = 21, r = 0.89, p<0.001). One-week old *O. doederleini* larvae (symbolized by the dispersed cloud of red dots) would be more likely to relocate the OTI reef if they used a sun compass to swim actively toward SSE, than if they would swim in random directions. Picture shows settling stage *Ostorhinchus doederleini*.
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distance drift into unsuitable habitat and relocate their natal region from a greater distance.

The use of a time-compensated sun compass would be particularly valuable to species on isolated islands in steady ocean currents, where loss of sensory contact with the home reef is lethal and an innate swimming direction could easily evolve. We predict that fish tested at different locations with differently directed drift currents will show time-compensated, sun compass directed swimming in correspondingly different directions. We might even suggest that the combination of sun-compass-based directed swimming and natal reef recognition could be a significant factor in explaining the extraordinary biodiversity of coral reef fish species. Innate orientation supports homing, which can lead to

genetic isolation and potential speciation as observed in migratory birds [41].

Materials and Methods

Ethics Statement

Fish collection permits were obtained from the Great Barrier Reef Marine Park Authority (G10/33239.1) and Queensland Department of Primary Industries and Fisheries (103256). The research was carried out in accordance with the Australian Code of Practice for the care and use of animals for scientific purposes. The protocol was approved by the Ethics Review Committee of

James Cook University (Permit Number: A1614). All efforts were made to minimize suffering.

Experimental Animals

The experiments were performed at the Field Station on One Tree Island (23.30′00 S, 152.05′00 E), a reef in the Capricorn-Bunker group of Islands in the southern Great Barrier Reef, Australia (Figure 1). In 2011, we collected newly settled *Ostorhinchus doederleini* on "patch-reefs" that were constructed from small coral rubble piles equivalent to natural substrates for settling *O. doederleini* [42]. The patch reefs (dimensions ~1 m×1 m×0.5 m) were located on sand areas in one of the main entrances into the lagoon, 10 to 80 m from a main reef in 4–5 m water depth. Since settlement takes place at night [34] and we cleared the patch reefs early each day, the collected fish would only have been post-settlement for a few hours. They had pelagic (no-stripe) pigmentation. Using the otolith daily aging technique, we determined that the mean age of settlers that had arrived at the same time as the tested fish was 20.5 days, ranging from 16 to 35 days (n = 30). This overlaps with the youngest age of newly settled fish found at patch reefs in the lagoon [42].

In 2012, true pre-settlement *O. doederleini* were caught with channel nets that were set to fish on the flood tides only at night on 20th and 21st of January 2012; current speeds were 35 cm/sec or more. The nets (n = 2) were square mouthed (0.75×0.75 m), the mesh was 500 μm, organized as a box/pyramid with an efficiency of 1:12 (mouth area: open sifting surface; full procedures see [43]). Pre-settlement *O. doederleini* were easily identified by morphology and a black dot on the caudal peduncle.

The collected fish measured 10–13 mm at the beginning of the experiments and 11–15 mm at the end of the experiments. The fish were kept in individual tanks made of plastic or glass. Each tank contained one piece of dead coral and fresh OTI Reef water, which was oxygenated with a bubble stone and partly changed once every 24 hours. The fish tanks were kept in a wet lab with an opening pointing towards N and translucent plastic "windows" on parts of the east and west sides allowing light to come in but through which no clear contours could be observed. The fish never had access to direct views of the sun from within their holding tanks but the non-clock-shifted fish will in some cases have been able to see parts of blue sky towards the North. Once per day, the fish were fed plankton caught during night-time plankton tows.

Orientation Tests

Orientation tests with these ~12 mm larvae were performed in a clear plastic circular bowl (17 cm [diameter] × 12 cm [depth]) allowing the fish a good view of the sky all the way down to the horizon. This bowl was placed on a leveled wooden platform on which a sundial of black lines was drawn in 22.5 degree segments relative to magnetic North. A finer angular resolution for recording fish position does not make sense given the size of the fish and the size of the bowl, but with 40 directions recorded, this resolution is more than sufficient to record the fish's preferred direction. Each fish was tested separately. A given test was performed as follows: The fish was transferred from its home aquarium in the wet lab to the testing bowl on the beach with a small glass jar. It was carefully released into the middle of the bowl. After ca. 60 sec of acclimation, its geographic position relative to the centre of the bowl was recorded in 30 sec intervals for the next 20 min. The observer simply noted down, which of the black lines in the sun dial was closest to the head of the fish.

The fish spent the vast majority of their time hovering somewhere near the edge of the bowl. The fish position relative to the centre of the bowl was used as the directional measure

rather than their heading, since they can move no further in any given direction once it reaches the edge of the bowl. Most of our fish clearly showed a preferred direction by either hovering fairly stationary or by slowly swimming back-and-fourth along the edge of the bowl around their preferred direction, or by moving away and quickly returning to the edge near the preferred direction. This behavior confirms that position in the bowl relative to the centre of the bowl is the most relevant orientation measure.

The observer sat on a stool next to the bowl. The observer systematically rotated position relative to the bowl (N, E, S, or W) between tests, so that the observer sat north of the bowl during 25% of the tests, south of the bowl in 25% of the tests, east of the bowl in 25% of the tests, and west of the bowl in 25% of the tests. The consistency of the fish's orientation (Figure 2A–D and Figure S1 in File S1) shows that the position of the observer had no significant effect on the directional choices of the fish (see Figure S3 in File S1). If a strong observer bias would have existed, the distributions in Figure 2A–D should have been random or quadrimodal since the observer position was balanced between N, S, E, and W. In sum, observer bias could never have *improved* the clarity of the results. In contrast, any slight observer bias would have added a symmetrical distribution component to the results and therefore *reduced* the clarity of the observed directional responses.

At the end of the test, the fish was transferred back to its holding tank. Between tests, the water in the test bowl was replaced by fresh OTI reef sea water.

Individual fish were tested at least three times (3–5 times) in each condition (for details see supplementary information, Tables S1–S4 in File S1). It is known from the vast literature on orientation tests with migratory birds that repeated tests are needed to determine the intended mean orientation direction of an individual with reasonable accuracy (e.g. [44,45,46,47,48]). By using average directions based on repeated tests of the same individuals, the number of experimental animals can also be significantly reduced, which is an important ethical consideration in modern biology.

Clock-shifting

Five newly settled fish in 2011 and all 7 pre-settlement fish in 2012 were clock-shifted after they had been tested and shown clear orientation in three control tests under non-clock-shifted conditions. The fish were clock-shifted 6 h backwards by placing them in a windowless room, where the light (5–7 lamps fitted with a mix of incandescent and energy-saving bulbs and connected to an automatic timer) went on at 23:15 and off at 13:15. Water changes and feeding (plankton, see above) took place in the morning between 7–10. While the five 2011 fish were being clock-shifted and then tested outdoors under natural sunny skies, we continued to keep and test the other nine fish from the same cohort under non-clock-shifted conditions. Thereby, we ensured that there were no time-in-captivity effects on the orientation direction of the larvae. Also, we took care to perform the first tests with the clock-shifted fish quite late in the morning (details in Table S3 in File S1), so that even if the fish's clock was not yet shifted the full 6 hours (we expect the clock-shift to have been complete after 6 days, but just in case), the predicted change in orientation would still be close to 180 degrees (compare sun azimuth position late in the morning, e.g. between 09:00 and 11:00 with the sun azimuth 5 hours later in Figure 2E). Finally, we took care to test the clock-shifted fish outdoors under the natural sunny sky at the exact same location where we performed the non-clock-shifted tests. Since all individual fish clearly shifted their orientation by ca. 180° in response to the clock-shift (Figure 3), the orientation of the fish

were not affected by local landmarks visible through the sides of the bowl.

Evaluation of Results and Circular Statistics

The evaluation of the orientation data was done using the standard methods applied in animal orientation studies [49]. The circular statistics program Oriana was used to calculate the mean and concentration of the 40 observed directions. All orientation directions recorded were corrected for the declination at One Tree Island (by turning the results 10 degrees counter-clockwise; e.g. an orientation towards 167 degrees relative to magnetic North is equivalent to 157 degrees relative to geographic North at One Tree Island) so that North in all figures and calculations refers to geographical North, not magnetic North, as geographical North is the relevant reference for a sun compass.

Since we cannot completely exclude that each individual observation may be somewhat dependent on the previous location of the fish, for the circular diagrams, we only included the results of single tests where the orientation of the fish was very clearly directed (very clearly directed = tests where the directionality was significant at the $p < 0.001$ level according to the Rayleigh test; [49]). This criterion was reached in 114 out of 132 individual tests (86%, see Table S1 and Table S3 in File S1). Of the 18 tests that did not reach this very conservative significance level, 10 were performed between 11:15 and 12:45, when the sun is so high in the sky and the sun azimuth changes so fast that it is unlikely that a sun compass would work, see Figure 2E. Thus, only 8 individual tests (6% of the 132 tests), done when the sun azimuth was likely to have provided useful information, resulted in disoriented behavior.

To give the reader the clearest impression of the variability in the test results, in the supplementary materials (File S1), we depict the data in two different ways. In Figure S1A, S1C, S1E and S1G in File S1, we include the results of all individual tests. In this kind of illustration, several separate tests of the same fish are included and thus the stated "n" is pseudo-replicated. However, it is known from orientation experiments with birds that the inter- and intra-individual variation in orientation are similar [45]. The fact that the 95% confidence intervals in our experiments remained almost identical regardless whether all tests or individual means are depicted (compare Figure S1A with S1B, S1C with S1D, S1E with S1F, and S1G with S1H, all in File S1) shows that intra- and inter-individual variation are also similar for our fish. Even though some readers may find this additional graph superfluous, we find it important to also present the results of all individual tests in a circular diagram, since it gives the reader a good impression of the spread in orientation observed between tests.

The second order mean directions of 3–5 tests per individual (Figure 2A–D, and Fig. S1B, S1D, S1F, and S1H in File S1) were calculated by vector addition of unit vectors in each of the mean directions from the individual tests (see Table S4 in File S1). This is the standard procedure used in the literature on migratory birds tested in orientation cages for the last four decades (e.g. [44,46,47,48,49]).

Time-of-day effects (Figure 2F) on the directedness of the individual tests were evaluated post hoc resulting in slightly different sample sizes. As a measure for directedness we use the length of the mean vector, "r", for each individual test. The r-value is a measure of how consistently a given fish oriented in its chosen mean direction during a single 20-minute test session. This analysis is based on the logic that if a fish has access to good orientation cues, it should orient more consistently in whatever direction it chooses than if a fish has access to poor orientation relevant information, e.g. because the azimuth angle is difficult to determine due to the sun being very close to the zenith. To avoid any concern about pseudoreplication in this post hoc analysis, the average r-value of the two tests was entered into the calculations if a given fish had been tested twice during the same time-period. The data leading to Figure 2F are provided in supplementary materials (Table S2 in File S1).

Genetic Analysis

Tissue of test animals was stored in 99% ethanol until DNA extraction. DNA was isolated from the samples using Chelex chelating resin (BioRad Chelex 100 Resin). We used five previously described polymorphic DNA microsatellite markers: Ad65.2, Ad67 Ad70 Ad86.2, Ad94 Table 1 [50]. PCR was carried out using approximately 100 ng of template DNA and the following cycle treatment; initial step of 5 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 44–46°C, and 1 min at 72°C, with a final extension step of 5 min at 72°C. Total reaction volume was 10 µL and contained 2.5 µL 10 X RED Taq Polymerase Buffer (Sigma, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.1 mM MgCl2 and 0.01% gelatin), 100 µM of each dNTP (Promega), 0.5 µM of both forward and reverse primer, and 0.5 U Taq polymerase (Sigma) 0.25 U RED Taq DNA Polymerase (Sigma). PCR fragments were separated and scored on a Beckman-Coulter CEQTM 2000XL DNA analysis system.

Sun compass tested juveniles were statistically assigned to adult populations of adjacent reefs (OTI, Lamont, heron, Fitzroy reefs) using Geneclass2 [51].

Supporting Information

File S1 Supporting Figures S1, S2, S3, and Supporting Tables S1, S2, S3, and S4.
(PDF)

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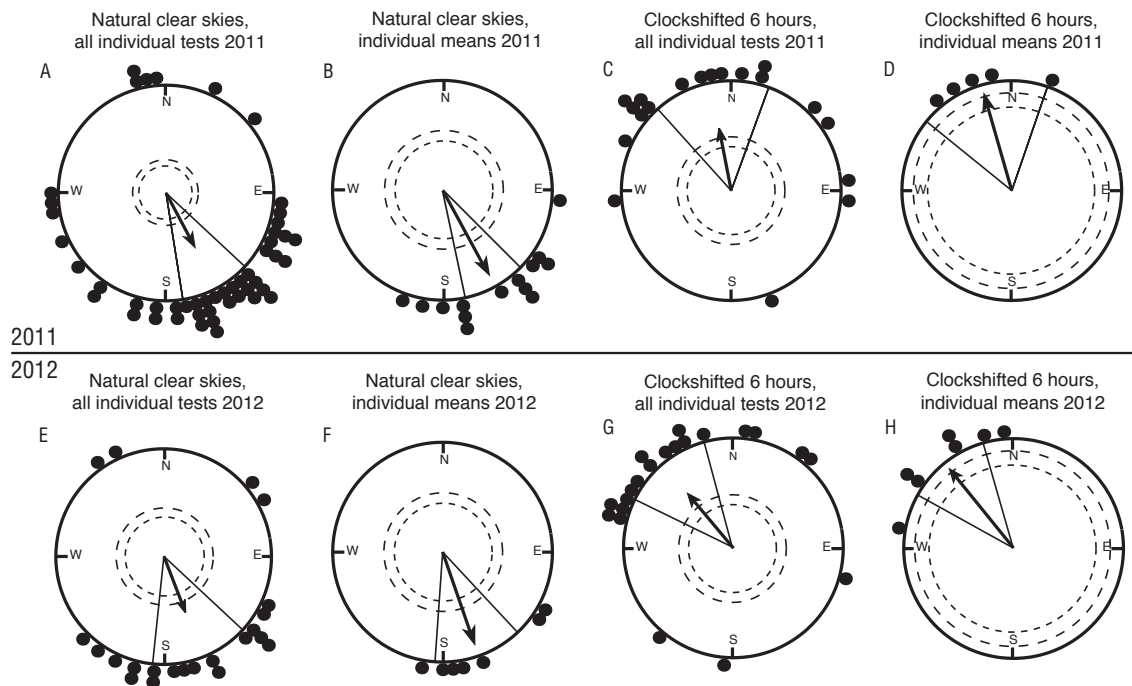
Figure S1. Inter- and intra-individual variation in orientation is similar in our settling stage *Ostorhinchus doederleini*. A: Forty-nine single tests under natural sunny skies with 14 just-settled *O. doederleini* tested in 2011 showed a clear orientation towards SSE, both when all the individual tests were considered (A: mean direction: 162°, r=0.56, n=49, p<0.001) and when only the second order means of each of the tested individuals were considered (B: mean direction: 162°, r=0.88, n=14, p<0.001). When five fish were clock-shifted 6 hours backwards, they turned their orientation by ca. 180° (C: all individual tests, mean direction: 359°, r=0.58, n=19, p<0.001; D: individual second order means, mean direction: 354°, r=0.94, n=5, p<0.01). Twenty-two single tests under natural sunny skies with 7 pre-settlement *O. doederleini* tested in 2012 also showed a clear orientation towards SSE, both when all the individual tests were considered (E: mean direction: 169°, r=0.57, n=22, p<0.001) and when only the second order means of each of the tested individuals were considered (F: mean direction: 171°, r=0.91, n=7, p<0.001). When all 7 fish were clock-shifted 6 hours backwards, they turned their orientation by ca. 180° (G: all individual tests, mean direction: 330°, r=0.62, n=21, p<0.001; H: individual second order means, mean direction: 331°, r=0.92, n=7, p<0.001). Notice that the 95% confidence intervals (lines flanking the group mean vector) in our experiments remained almost identical regardless whether all tests or individual means are depicted (compare Fig. S1A with S1B, S1C with S1D, S1E with S1F, and S1G with S1H). This is only to be expected if the intra- and inter-individual variation in orientation is very similar for our fish. Each dot at the circle periphery indicates the mean orientations observed in each of the individual tests done under the given condition (A, C, E, G) or the mean orientation chosen by each individual fish based on the second order average of all tests of the given fish in the given condition (B, D, F, H). Arrows indicate the group mean vectors. Inner and outer dashed circles indicate the radius of the group mean vector needed for significance according to the Rayleigh Test (p<0.05 and p<0.01, respectively).
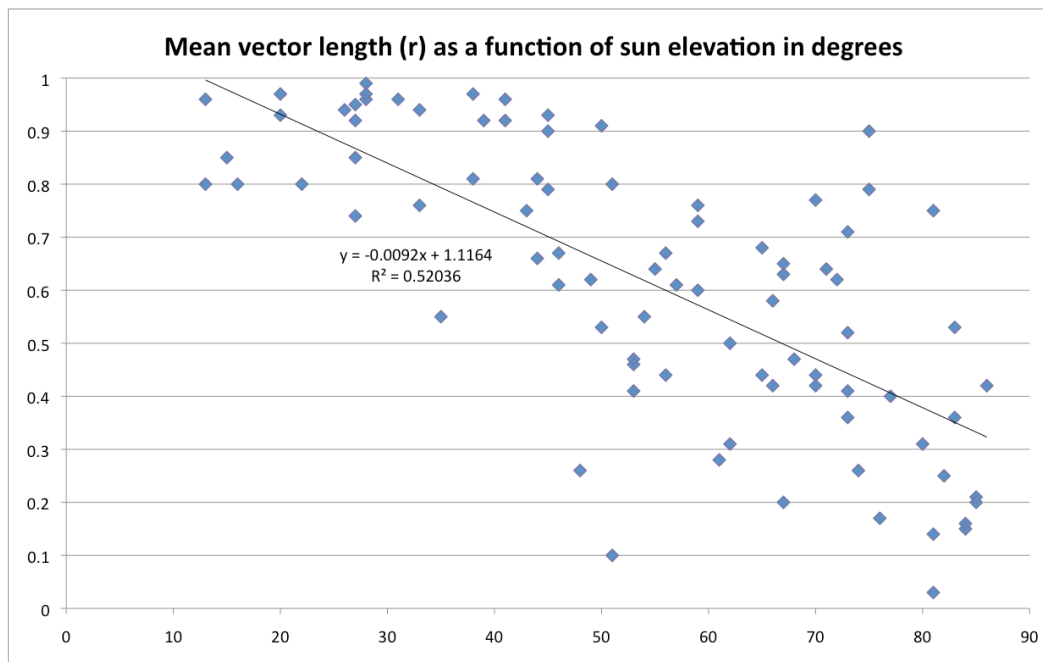
Figure S2. The lower the sun elevation, the better the fish were able to keep their chosen heading. The y-axis indicates the r-value for each individual test (the length of the mean vector of the 40 recorded single direction in a given single test). The x-axis indicates the sun elevation in degrees. There is a highly significant negative correlation between the r-value and the sun elevation, which means that the higher the sun elevation is, the poorer the fish are able to keep a chosen direction.
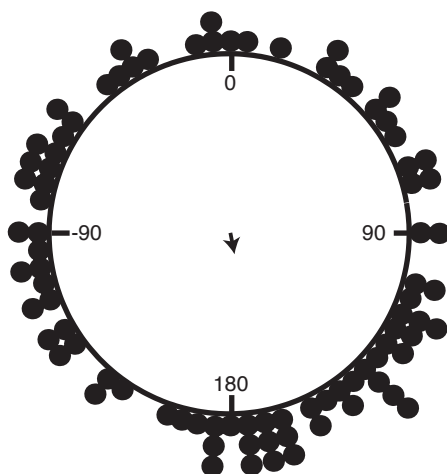
Figure S3. Evidence for no significant observer bias. The observer's position is defined as 0 degrees and the orientation of each individual fish in the non-clock-shifted condition is indicated by the dots at the circle periphery (2011 and 2012 fish combined). Thus, a point in 0 degrees would mean that the fish oriented towards the observer and a point in 180 degrees would mean that the fish oriented away from the observer. The distribution is random (mean direction 169°, r=0.12, n=87, n.s.). For description of the circular diagram, see legend to Figure S1.

Table S1. Orientation results for non-clock-shifted larvae. "Time tested" indicates the start of the given 20 minute test. "r" is the length of the mean vector calculated from the 40 individual directions recorded during the individual test. "p" is the p-value resulting from a Rayleigh Test. However, since each individually recorded direction is unlikely to be completely independent, to be conservative, we demanded a p<0.001 for inclusion of the direction in the circular diagrams (Figure 1A-D and Figure S1).

Table S1.

Fish ID	Date tested (D.M.Y)	Time tested	Orientation relative to magnetic North in degrees	Orientation relative to geographic North in degrees	r	"p"	Sun height above horizon in degrees	Sun azimuth in degrees	Comment (NS = considered non-significant)
HM4	20.1.2011	10:17	119	109	0.63	0.001	67	86	
HM5	20.1.2011	12:00	187	177	0.42	0.001	86	331	
HM2	20.1.2011	13:07	169	159	0.79	0.001	75	279	
HM1	20.1.2011	15:34	154	144	0.96	0.001	41	263	
HM3	20.1.2011	16:34	180	170	0.99	0.001	28	259	
HM4	21.1.2011	08:51	132	122	0.67	0.001	46	95	
HM2	21.1.2011	09:22	3	353	0.47	0.001	53	93	
HM5	21.1.2011	12:36	256	246	0.75	0.001	81	293	
HM1	21.1.2011	13:10	201	191	0.26	0.07	74	279	NS
HM3	21.1.2011	15:03	29	19	0.26	0.06	48	266	NS
HM3	22.1.2011	8:44	107	97	0.81	0.001	44	95	
HM1	22.1.2011	9:17	326	316	0.10	0.68	51	93	NS
HM5	22.1.2011	9:48	128	118	0.60	0.001	59	90	
HM2	22.1.2011	10:52	149	139	0.71	0.001	73	81	
HM4	22.1.2011	13:48	270	260	0.68	0.001	65	274	
HM3	22.1.2011	15:49	273	263	0.81	0.001	38	263	
HM1	22.1.2011	16:38	147	137	0.85	0.001	27	259	
HM1	23.1.2011	8:42	147	137	0.75	0.001	43	95	
HM10	24.1.2011	10:43	129	119	0.64	0.001	71	81	
HM11	24.1.2011	11:11	121	111	0.40	0.001	77	74	
HM12	24.1.2011	11:39	201	191	0.36	0.01	83	55	NS
HM13	24.1.2011	14:04	268	258	0.31	0.02	62	273	NS
HM9	24.1.2011	14:43	203	193	0.46	0.001	53	269	
HM12	25.1.2011	14:39	117	107	0.55	0.001	54	270	
HM13	25.1.2011	15:11	281	271	0.61	0.001	46	267	
HM9	25.1.2011	15:50	355	345	0.97	0.001	38	263	
HM10	25.1.2011	16:17	174	164	0.96	0.001	31	261	
HM11	25.1.2011	16:43	357	347	0.94	0.001	26	259	
HM10	26.1.2011	10:24	37	27	0.42	0.001	66	84	
HM12	26.1.2011	10:52	61	51	0.36	0.001	73	78	
HM13	26.1.2011	11:33	338	328	0.14	0.44	81	59	NS
HM11	26.1.2011	12:00	258	248	0.21	0.18	85	12	NS
HM9	26.1.2011	12:26	224	214	0.53	0.001	83	311	
HM11	26.1.2011	15:33	359	349	0.92	0.001	41	265	
HM13	26.1.2011	16:02	160/340	-	0.55	0.001	35	263	bimodal
HM9	26.1.2011	16:36	186	176	0.92	0.001	27	260	
HM12	26.1.2011	17:30	163	153	0.85	0.001	15	256	
HM9	27.1.2011	11:08	317	307	0.17	0.3	76	72	NS
HM12	27.1.2011	11:35	188	178	0.25	0.09	82	56	NS
HM10	27.1.2011	12:02	165	155	0.20	0.19	85	6	NS
HM10	27.1.2011	16:34	161	151	0.97	0.001	28	261	

HM13	28.1.2011	16:30	171	161	0.96	0.001	28	261	
HM20	28.1.2011	16:58	207	197	0.80	0.001	22	259	
HM19	28.1.2011	17:26	171	161	0.80	0.001	16	257	
HM19	29.1.2011	11:31	193	183	0.31	0.02	80	57	NS
HM20	29.1.2011	11:55	97	87	0.15	0.42	84	23	NS
HM13	29.1.2011	12:18	307	297	0.16	0.37	84	330	NS
HM19	29.1.2011	13:16	224	214	0.52	0.001	73	285	
HM20	29.1.2011	13:45	241	231	0.58	0.001	66	278	
HM9	29.1.2011	14:55	194	184	0.91	0.001	50	270	
HM11	29.1.2011	15:18	174	164	0.93	0.001	45	268	
HM12	30.1.2011	9:12	160	150	0.62	0.001	49	90	
HM19	30.1.2011	9:45	147	137	0.61	0.001	57	87	
HM13	30.1.2011	10:33	124	114	0.47	0.001	68	80	
HM22	31.1.2011	11:56	18	8	0.03	0.96	81	50	NS
HM21	31.1.2011	13:11	169	159	0.41	0.001	73	288	
HM20	31.1.2011	13:40	121	111	0.20	0.22	67	281	NS
HM22	31.1.2011	14:28	177	167	0.67	0.001	56	273	
HM12	31.1.2011	14:51	147	137	0.80	0.001	51	271	
HM20	31.1.2011	15:22	153	143	0.66	0.001	44	268	
HM11	1.2.2011	8:55	148	138	0.90	0.001	45	91	
HM22	1.2.2011	9:54	152	142	0.73	0.001	59	85	
HM21	1.2.2011	14:02	109	99	0.50	0.001	62	278	
HM22	1.2.2011	14:32	121	111	0.64	0.001	55	273	
HM21	1.2.2011	15:15	195	185	0.79	0.001	45	269	
OTI20	20.1.2012	10:37	193	183	0.77	0.001	70	85	
OTI21	20.1.2012	11:01	138	128	0.90	0.001	75	80	
OTI22	20.1.2012	13:17	203	193	0.62	0.001	72	277	
OTI23	20.1.2012	13:42	333	323	0.65	0.001	67	273	
OTI24	20.1.2012	14:05	180	170	0.28	0.04	61	271	NS
OTI20	20.1.2012	14:30	71	61	0.44	0.001	56	268	
OTI21	20.1.2012	14:53	180	170	0.53	0.001	50	266	
OTI22	20.1.2012	15:18	194	184	0.79	0.001	45	264	
OTI23	20.1.2012	15:42	213	203	0.92	0.001	39	262	
OTI24	20.1.2012	16:08	142	132	0.94	0.001	33	261	
OTI20	20.1.2012	16:08	129	119	0.76	0.001	33	261	
OTI21	20.1.2012	16:37	237	227	0.95	0.001	27	258	
OTI22	20.1.2012	16:37	60	50	0.74	0.001	27	258	
OTI24	20.1.2012	17:09	126	116	0.93	0.001	20	256	
OTI23	20.1.2012	17:09	173	163	0.97	0.001	20	256	
OTI23	20.1.2012	17:38	147	137	0.80	0.001	13	254	
OTI24	20.1.2012	17:38	138	128	0.96	0.001	13	254	
OTI26	21.1.2012	9:23	166	156	0.47	0.001	53	93	
OTI25	21.1.2012	9:23	164	154	0.41	0.001	53	93	
OTI26	21.1.2012	9:50	206	196	0.76	0.001	59	90	
OTI25	21.1.2012	10:14	184	174	0.44	0.001	65	88	
OTI26	21.1.2012	10:38	225	215	0.42	0.001	70	84	
OTI25	21.1.2012	10:38	346	336	0.44	0.001	70	84	

Table S2. Mean vector lengths (r) in individual orientation tests performed in different time of day intervals for the non-clock-shifted larvae tested in 2011. Data originates from Table S1 (column 6). The mean time for any given 20 min test (i.e. "Time tested" + 10 min) decided in which time interval the test was considered. The third column in this table gives the mean r-value for each individual fish that was tested in a given time interval, and these values were used in the calculations of the means and standard deviations as indicated on Figure 1F and for the subsequent ANOVA.

Table S2		
Fish ID	r-value in single tests	Mean r-value used for Figure 1F
8:00-9:45		
HM1	0.10	0.43
	0.75	
HM2	0.47	0.47
HM3	0.81	0.81
HM4	0.67	0.67
HM11	0.90	0.90
HM12	0.62	0.62
9:45-11:15		
HM2	0.71	0.71
HM4	0.63	0.63
HM5	0.60	0.60
HM10	0.64	0.53
	0.42	
HM12	0.36	0.36
HM13	0.47	0.47
HM19	0.61	0.61
HM22	0.73	0.73
11:15-12:45		
HM5	0.42	0.42
HM9	0.53	0.35
	0.17	
HM10	0.20	0.20
HM11	0.40	0.31
	0.21	
HM12	0.36	0.30
	0.25	
HM13	0.14	0.15
	0.16	
HM19	0.31	0.31
HM20	0.15	0.15
HM22	0.03	0.03

Table S2 (continued)		
12:45-14:15		
HM1	0.26	0.26
HM2	0.79	0.79
HM4	0.68	0.68
HM5	0.75	0.75
HM13	0.31	0.31
HM19	0.52	0.52
HM20	0.58	0.39
	0.2	
HM21	0.41	0.46
	0.5	
14:15-15:45		
HM3	0.26	0.26
HM9	0.46	0.68
	0.91	
HM11	0.92	0.93
	0.93	
HM12	0.55	0.67
	0.80	
HM13	0.61	0.61
HM20	0.66	0.66
HM21	0.79	0.79
HM22	0.67	0.66
	0.64	
after 15:45		
HM1	0.96	0.90
	0.85	
HM3	0.99	0.90
	0.81	
HM9	0.97	0.94
	0.92	
HM10	0.96	0.97
	0.97	
HM11	0.94	0.94
HM12	0.85	0.85
HM13	0.55	0.75
	0.96	
HM19	0.80	0.80
HM20	0.80	0.80

Table S3. Orientation results for clock-shifted larvae. "Time tested" indicates the start of the given 20 minute test. "r" is the length of the mean vector calculated from the 40 individual directions recorded during the individual test. "p" is the p-value resulting from a Rayleigh Test. However, since each individually recorded direction is unlikely to be completely independent, to be conservative, we demanded a p<0.001 for inclusion of the direction in the circular diagrams (Figure 1A-D and Figure S1).

Fish ID	Date tested	Time tested	Orientation relative to magnetic North in degrees	Orientation relative to geographic North in degrees	r	"p"	Sun height above horizon in degrees	Sun azimuth in degrees	Sun azimuth 6 hours later in degrees	Predicted clockwise shift in degrees	Comment
HM1 (cs)	29.1.2011	8:19	5	355	0.50	0.001	37	95	276	179	
HM3 (cs)	29.1.2011	8:48	318	308	0.56	0.001	44	93	270	183	
HM4 (cs)	29.1.2011	9:17	168	158	0.43	0.001	51	90	268	182	
HM5 (cs)	29.1.2011	9:50	106	96	0.57	0.001	58	87	265	182	
HM4 (cs)	29.1.2011	10:26	26	16	0.91	0.001	66	81	262	179	
HM5 (cs)	30.1.2011	8:24	23	13	0.75	0.001	38	95	273	182	
HM4 (cs)	30.1.2011	8:51	93	83	0.11	0.61	45	92	270	182	NS
HM3 (cs)	30.1.2011	11:02	17	7	0.55	0.001	74	72	259	173	
HM1 (cs)	31.1.2011	9:39	64	54	0.54	0.001	55	87	268	179	
HM4 (cs)	31.1.2011	10:03	323	313	0.44	0.001	61	84	264	180	
HM5 (cs)	31.1.2011	10:28	325	315	0.84	0.001	67	80	262	178	
HM3 (cs)	31.1.2011	10:52	93	83	0.43	0.001	72	74	260	174	
HM4 (cs)	1.2.2011	6:45	306	296	0.66	0.001	16	102	302	160	
HM1 (cs)	1.2.2011	7:09	1	351	0.90	0.001	21	100	290	170	
HM5 (cs)	1.2.2011	7:34	277	267	0.66	0.001	27	98	283	175	
HM3 (cs)	1.2.2011	8:04	319	309	0.60	0.001	34	96	277	179	
HM10 (cs)	1.2.2011	8:30	353	343	0.83	0.001	40	94	274	180	
HM10 (cs)	1.2.2011	9:19	347	337	0.62	0.001	51	89	269	180	
HM10 (cs)	1.2.2011	10:17	318	308	0.50	0.001	64	81	263	178	
HM1 (cs)	1.2.2011	10:43	55	45	0.72	0.001	70	76	261	175	
OTI20 (cs)	25.1.2012	10:25	324	314	0.56	0.001	67	84	261	183	
OTI21 (cs)	25.1.2012	10:25	299	289	0.57	0.001	67	84	261	183	
OTI22 (cs)	25.1.2012	10:50	195	185	0.46	0.001	72	79	259	180	
OTI23 (cs)	25.1.2012	10:50	325	315	0.57	0.001	72	79	259	180	
OTI24 (cs)	26.1.2012	7:16	303	293	0.90	0.001	24	101	282	179	
OTI20 (cs)	26.1.2012	7:16	49	39	0.94	0.001	24	101	282	179	
OTI21 (cs)	26.1.2012	7:43	298	288	0.95	0.001	30	99	277	182	
OTI22 (cs)	26.1.2012	7:43	344	334	0.71	0.001	30	99	277	182	
OTI23 (cs)	26.1.2012	8:11	309	299	0.95	0.001	36	97	273	184	
OTI24 (cs)	26.1.2012	8:11	17	7	0.91	0.001	36	97	273	184	
OTI20 (cs)	26.1.2012	8:37	239	229	0.19	0.24	42	95	270	185	NS
OTI21 (cs)	26.1.2012	8:37	353	343	0.65	0.001	42	95	270	185	
OTI23 (cs)	26.1.2012	9:04	232	222	0.79	0.001	48	93	268	185	
OTI22 (cs)	26.1.2012	9:04	335	325	0.96	0.001	48	93	268	185	
OTI20 (cs)	26.1.2012	9:35	297	287	0.88	0.001	55	90	265	185	
OTI24 (cs)	26.1.2012	9:35	44	34	0.91	0.001	55	90	265	185	
OTI25 (cs)	26.1.2012	10:00	115	105	0.54	0.001	61	87	263	184	
OTI26 (cs)	26.1.2012	10:00	297	287	0.78	0.001	61	87	263	184	
OTI26 (cs)	26.1.2012	10:26	22	12	0.67	0.001	67	83	261	182	
OTI25 (cs)	26.1.2012	10:26	342	332	0.56	0.001	67	83	261	182	
OTI26 (cs)	27.1.2012	8:05	344	334	0.89	0.001	34	97	274	183	

OTI25 (cs)	27.1.2012	8:05	314	304	0.78	0.001	34	97	274	183	
OTI25 (cs)	27.1.2012	10:05	354	344	0.71	0.001	62	86	263	183	
OTI26 (cs)	27.1.2012	10:05	339	329	0.77	0.001	62	86	263	183	
											Mean 180, r=1.00(!), P<0.001

Table S4: Summary table for calculations of individual mean orientation before and after clock-shift. The individual mean directions were calculated by vector addition of unit vectors in each of the mean directions shown in the individual tests of the given individual (Batchelet 1981).

	Table S4.					
Fish ID	Means orientation of individual non-clock-shifted tests	Mean orientation of the individual in the non-clock-shifted tests	Clock-shifted from date (D.M.Y)	Mean orientation of individual clock-shifted tests	Mean orientation of the individual in the clock-shifted tests	Clockwise deviation in the clock-shifted condition compared to the orientation of the same individual in the non-clock-shifted condition
HM1	144, 137, 137	139	23.1.2011	355, 54, 351, 45	21	200
HM2	159, 353, 139	128	22.1.2011	Died during clock-shifting	-	-
HM3	170, 97, 263	172	22.1.2011	308, 7, 83, 309	349	177
HM4	109, 122, 260	142	22.1.2011	158, 16, 313, 296	320	178
HM5	177, 246, 118	179	22.1.2011	96, 13, 315, 267	342	163
HM9	193, 345, 214, 176, 184	200	-	Not clock-shifted	-	-
HM10	119, 164, 27, 151	124	27.1.2011	343, 337, 308	329	205
HM11	111, 347, 349, 164, 138	94	-	Not clock-shifted	-	-
HM12	107, 51, 153, 150, 137	123	-	Not clock-shifted	-	-
HM13	271, 161, 114	170	-	Not clock-shifted	-	-
HM19	161, 214, 137	170	-	Not clock-shifted	-	-
HM20	197, 231, 143	191	-	Not clock-shifted	-	-
HM21	159, 99, 185	149	-	Not clock-shifted	-	-
HM22	167, 142, 111	140	-	Not clock-shifted	-	-
OTI20	183, 61, 119	120	21.1.2012	314, 39, 287	329	209
OTI21	128, 170, 227	174	21.1.2012	289, 288, 343	306	132
OTI22	193, 184, 50	160	21.1.2012	185, 334, 325	303	143
OTI23	323, 203, 163, 137	181	21.1.2012	315, 299, 222	281	100
OTI24	132, 116, 128	125	21.1.2012	293, 7, 34	354	229
OTI25	154, 174, 336	172	21.1.2012	105, 332, 304, 344	344	172
OTI26	156, 196, 215	189	21.1.2012	287, 12, 334, 329	331	142

Batchelet (1981). Circular Statistics in Biology. London: Academic Press.