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SHORT COMMUNICATION

Pre-settlement coral-reef fish larvae respond to magnetic field changes during the day

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ABSTRACT

Observations of coral-reef fish larvae have revealed remarkably consistent orientation behaviour while swimming offshore, requiring large-scale orientation cues. However, the mechanisms underlying this behaviour are still being investigated. One potential large-scale cue for orientation is the Earth's geomagnetic field. Here, we examined the effect of magnetic field manipulations on the orientation behaviour of coral-reef fish during the pelagic larval phase. In the absence of visual cues, individual larvae responded to a 90 deg shift of the horizontal component of the magnetic field within a Helmholtz coil with a comparable shift in orientation, demonstrating that they use a magnetic compass for orientation. Our findings suggest that geomagnetic field information guides swimming behaviour of larval fish in the pre-settlement phase. The ability to use large-scale sensory cues allows location-independent orientation of swimming, a behaviour that influences dispersal and connectivity of fish populations, which has important ecological implications for anthropogenic development of marine areas.

KEY WORDS: Magnetoreception, Larval ecology, Orientation behaviour, Dispersal

INTRODUCTION

The ability of animals to orient their movement during large-scale transitions between different habitats has long been one of the greatest mysteries of the natural world (Gould and Gould, 2012). Animals moving across large spatial scales use a variety of different environmental cues to derive directional information. Evidence of orientation of movement using directional information provided by the Earth's magnetic field is being found in an increasing number of taxa, including insects, crustaceans, amphibians, reptiles, birds and mammals (reviewed by Wiltschko and Wiltschko, 1995, 2005; Cain et al., 2005; Lohman et al., 2008; Wajnberg et al., 2010; Muheim et al., 2014). A growing number of studies also indicate that various fishes can perceive magnetic field information (Quinn and Brannon, 1982; Walker, 1984; Shcherbakov et al., 2005; Hellinger and Hoffmann, 2012; Putman et al., 2014). Such a sense can be useful in environments like the open ocean, which provide few navigational cues, in order to locate favourable habitat critical for success in recruitment or reproduction. Nevertheless, there are many species and life stages that remain to be investigated, including the pelagic larval dispersal phase of fishes.

The vast majority of marine organisms have a bipartite life cycle involving a period of development offshore as larvae, followed by movement to a benthic habitat on which to settle and complete their life cycle (Leis et al., 2011). Demersal marine species, particularly coral reef species, are commonly site-attached as adults, often remaining within a certain geographic range of their initial settlement habitat (Feary et al., 2014). Larval coral-reef fishes show an extraordinary ability to orient their swimming in the pelagic environment by the end of the dispersal phase (Leis et al., 2014), which increases the likelihood of encountering settlement habitat and in turn is critical for the survival and persistence of both individuals and populations (Caldwell and Nams, 2006). Fish larvae have been found to respond to a suite of sensory stimuli, such as visual, auditory and olfactory cues, providing directional information to potential habitat at a scale of less than a kilometre and eliciting behavioural responses from an early stage in development (Tolimieri et al., 2004; Dixson et al., 2011; Igulu et al., 2011). However, observations of coral-reef fish larvae *in situ* revealed that multiple species swam in a similar directional orientation independent of location with respect to coral reef habitat up to 1 km away (Leis and Carson-Ewart, 2003). This behaviour is consistent regardless of spatial or temporal differences and persists in the pelagic zone outside of the effective range of the visual, auditory and olfactory cues emanating from the habitat, suggesting the use of other larger-scale cues (Leis et al., 2014).

Coral-reef fishes collected shortly after settlement have recently been shown to respond to changes in the Earth's magnetic field at night (Bottesch et al., 2016), indicating that they use a magnetic compass at night in addition to the sun compass during the day (Mouritsen et al., 2013). Aspects that are yet to be explored include whether magnetic compass information is also used by pre-settlement larvae still making their way to reef habitat and whether this cue is also used during the day. Here, we aimed to test the behavioural response of coral-reef fish larvae at the pre-settlement stage to manipulations of the ambient magnetic field during the day and in the absence of other directional cues, comparing responses at both the group and individual level.

MATERIALS AND METHODS

Field collection

We collected pre-settlement stage larvae (7–10 mm standard length, pre-metamorphosis) of the coral-reef damselfish *Chromis atripectoralis*, Welander and Schultz 1951, at sunrise during seasonal recruitment in November 2013 and February 2014 using light traps off the fringing reefs surrounding Lizard Island (14°40′ 42.2″S, 145°25′43.4″E, Great Barrier Reef Marine Park, QLD, Australia). This species has previously demonstrated orientation ability *in situ* in the same region and life-history stage (Leis and Carson-Ewart, 2003; Leis et al., 2014). Each larva was tested on the day of capture. Prior to testing, we kept larvae in a temperature-controlled laboratory in aerated 10 l aquaria separated by opaque barriers and supplied with filtered seawater on a flow-through system. All experiments were approved by the University of

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Journal of Experimental Biology

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Experiments in a manipulated magnetic field

We used a single Helmholtz coil design (coil size 1.6 m, 0.65 m between coils) to manipulate the horizontal component of the magnetic field in the test arena (Fig. 1), shifting magnetic North within the coil by +90 deg (the coil itself was set up at an orientation of 135 deg to natural magnetic north), while maintaining the intensity and inclination of the natural local geomagnetic field (48,691 nT and 41.2 deg, respectively). We monitored magnetic field parameters within the coil using a high-speed 3-axis digital fluxgate magnetometer (Model 693, Applied Physics Systems, Mountain View, CA, USA). To minimise man-made electromagnetic field interference as much as possible, we conducted the orientation experiments in the magnetic coil ∼50 m from the research station. We tested damselfish larvae in a plastic circular arena (55 cm diameter) under a wooden pavilion to exclude visual stimuli from the environment and direct sunlight. The tank was also covered with an opaque, light-tight PVC plastic sheet of 5 mm thickness. A HD video camera (GoPro, Woodman Labs, CA, USA) was mounted centrally in the tank cover to record the movements of the fish larvae (Fig. 1). Filtered seawater was maintained at 20 cm depth in the arena and replaced between each fish larva. We introduced single larvae into the centre of the circular test arena using a PVC cylinder of 5 cm diameter. After we removed the cylinder, the larva had a 2 min acclimation period before its movement was recorded. Between treatments we moved the larvae to a holding tank while switching between conditions and to recover from handling for a period of 5 min before placing them back into the centre of the test arena. We tested each larva in two consecutive trials under the following conditions: natural magnetic field (control) and +90 deg-shifted magnetic field (Mag 90 deg shift). The order of treatments was randomly allocated, with N=16 larvae first tested under the control treatment and N=17 larvae first tested under the Mag 90 deg shift treatment.

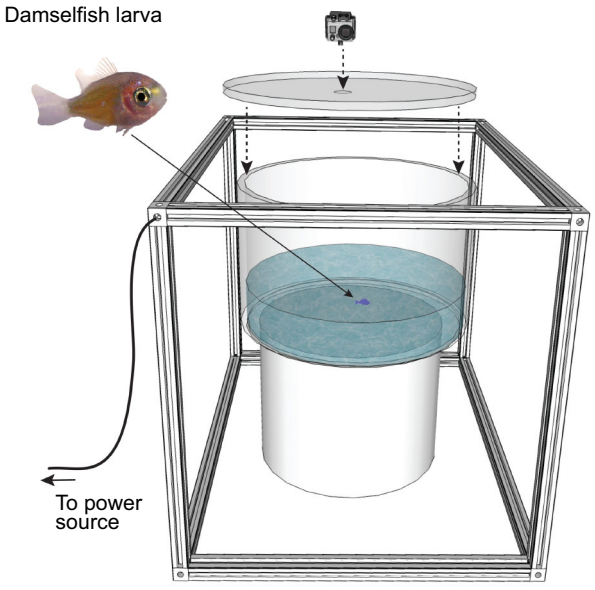

Fig. 1. Experimental setup used to study magnetic orientation in coral reef-fish larvae, *Chromis atripectoralis*. A Helmholtz coil design was used to shift the horizontal component of the magnetic field in the arena by +90 deg. Orientation behaviour and movement were recorded from above with a HD camera mounted in the tank cover.

Data analysis

From the video recordings we automatically sampled the position of larvae in the arena at 15 frames s^{-1} (9000 data points per treatment) with a custom-made video-tracking program written in MATLAB® 2013b (The MathWorks Inc., Natick, MA, USA). The program provided a complete track of the movement of each individual larva and calculated the mean direction and mean variation (length of the mean vector, **r**) using vector addition. For each individual, we calculated the mean orientation for both the unimodal and axial distributions (Batschelet, 1981), as the axiality of behaviour can relate to different cue responses and motivations (Dallimer and Jones, 2002). If the mean vector length of the axial distribution was larger than that for the unimodal distribution (i.e. $\mathbf{r}_{axial}>\mathbf{r}_{unidirectional}$), we regarded the orientation as axial (n=8 of a total of 54 experiments). For further calculations, we only included the mean direction of the side of the axis that was closer to the unimodal mean.

Pilot studies indicated that the distribution of mean bearings of larvae across treatment groups became more dispersed if individuals with **r**<0.2 were included, but did not become more tightly oriented if only animals with **r**>0.3 or higher were included. Thus, a larva was included in further analysis when the individual mean vector lengths in both the control and Mag 90 deg shift treatments were **r**>0.2 (see Muheim et al., 2002, for a similar method). Of 33 larvae tested, 6 (18%) performed below this threshold and were therefore excluded from further analysis, leaving 27 replicates for group analysis.

To assess group orientation, we used Oriana 4.0 (Kovach Computing Services, Anglesey, UK) to calculate the mean direction (including 95% confidence interval, CI) and the length of the mean vector **r** as measure of group scatter for both the unimodal and axial distributions. The distribution with the larger **r** value was tested with a Rayleigh test for significance. We used a Watson's U^2 test to assess whether control and experimental treatments differed from each other. We calculated individual responses between the treatments for individual larvae by setting the mean bearing of the control treatment to 0 deg and calculating the deviation from the bearing of the experimental treatment (see Muheim et al., 2006). The mean angular response was then compared with the expected response of +90 deg shift using the 95% CI test (Batschelet, 1981).

RESULTS AND DISCUSSION

Damselfish larvae were generally directional at an individual level (median individual **r**=0.64). Groups of *C. atripectoralis* larvae showed a non-significant mean orientation to the southeast under the control condition, shifting to the southwest when magnetic north was shifted by +90 deg (Fig. 2A). The angular difference of +95 deg between these treatments agrees with the shift of the horizontal component of the magnetic field by +90 deg, but the distribution of bearings was not significantly different between the control and experimental treatment (Watson's U^2=0.11, P>0.2). However, the individual responses of the larvae to the shifted magnetic field compared with the control treatment were significantly clustered around a mean deviation of +125 deg (Fig. 2B). The 95% CI encompasses the expected +90 deg shift in orientation, demonstrating that the larvae responded to the +90 deg shift of the horizontal component of the magnetic field (95% CI 87–162 deg, **r**=0.39, Rayleigh test: P=0.015, n=27).

Our results demonstrate that pre-settlement *C. atripectoralis* larvae use directional information from the Earth's magnetic field for compass orientation. Individual larvae oriented in various

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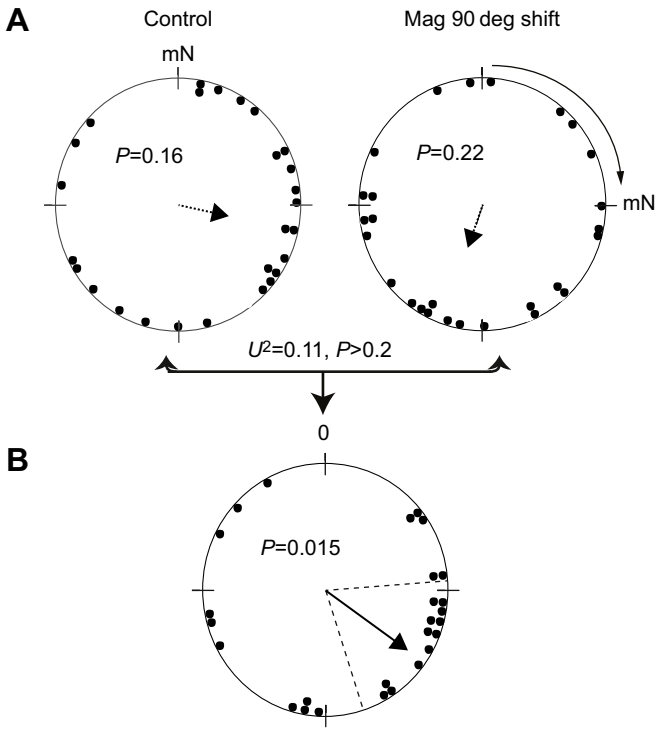

Fig. 2. Group and individual magnetic orientation. (A) Orientation of fish larvae (*N*=27) tested in the ambient magnetic field (control) and in a 90 deg clockwise-shifted magnetic field (Mag 90 deg shift). (B) Individual responses of the larvae between the control (set to 0 deg) and Mag 90 deg shift conditions. Each dot represents the orientation of one fish larva. Dashed arrows indicate the mean orientation of the group of larvae; solid arrow with dashed radial lines indicates significantly directional orientation (Rayleigh test, $P<0.05$) with 95% confidence intervals. mN indicates magnetic North. *P*-values are given inside each diagram; Watson's U^2 test statistic compares bearing distributions between treatments.

directions in the absence of any directional cues other than magnetic field information, but they consistently responded to a change in the magnetic field polarity with a comparative change in magnetic orientation (see also Lohmann et al., 1995; Muheim et al., 2006). Magnetic compass orientation has recently been demonstrated in another coral reef fish (cardinalfish, *Ostorhinchus doederleini*) in the post-settlement phase (Bottesch et al., 2016). Together, these findings indicate that the ability to use directional information from the Earth's magnetic field may be shared between various species and life history stages, and influences behaviour both before and after settlement. Coral reef fishes around the settlement stage have also been shown to use a sun compass for orientation (Mouritsen et al., 2013). As solar cues may not be consistently available during inclement weather patterns or at night, larvae can therefore switch cues and use a magnetic compass to maintain directional swimming.

Larvae of coral-reef fishes observed in the field have shown remarkably consistent location-independent orientation (Leis et al., 2015). The mean orientation of our sample population was not significantly directed at the group level under control conditions, which could be beneficial to a model of dispersal across varying spatial scales, as opposed to the limiting extent predicted by a singular orientation across all individuals of a given subpopulation. Still, our larvae tended to orient towards the southeast, similar to the southerly directions seen in individuals of *C. atripectoralis* observed *in situ* in the vicinity of Lizard Island (Leis and Carson-Ewart, 2003). Interestingly, this is also the direction in which post-larval cardinalfish were found to orient among individuals (Bottesch et al., 2016). Significant directionality of swimming in the pelagic dispersal phase has now been reported in larvae of multiple coral-reef fish species using various observational methodologies (Mouritsen et al., 2013; Leis et al., 2014; Faillettaz et al., 2015). Maintaining high levels of individual directionality despite being tested in an unfamiliar location may indicate that coral-reef fish larvae use available orientation cues to maintain a straight heading, increasing the chances of finding habitat in a patchy environment, such as a coral reef (Zollner and Lima, 1999; Caldwell and Nams, 2006). The capacity for common orientation among individuals has been proposed as an adaptive response to prevailing current conditions, providing a mechanism to minimise dispersion and transport of fish larvae (Mouritsen et al., 2013; Leis et al., 2015). Indeed, several studies have suggested the use of magnetic map information in guiding animals during their migration through ocean currents (Boles and Lohmann, 2003; Putman et al., 2011, 2014; Naisbett-Jones et al., 2017). This highlights the potential importance of geomagnetic field information to dispersal-relevant behaviours in pre-settlement stage coral-reef fishes.

The finding that information from the Earth's magnetic field plays an important role in orientation of larval fishes has ecological implications for the anthropogenic development of marine areas. Increasing interest in renewable energy sources of hydrokinetic energy and offshore wind power has led to research into the potential effects of anthropogenic electromagnetic fields on marine organisms due to submarine electrical infrastructure (Öhman et al., 2007; Inger et al., 2009; Bevelhimer et al., 2013). Electromagnetic field disturbance has the potential to disrupt the orientation of animals that use a magnetic sense for orientation (Engels et al., 2014; Muheim et al., 2016). For individuals that move across large spatial scales, such as coral-reef fish during the pelagic larval phase, changes to the local geomagnetic field could significantly influence spatial patterns of dispersal. Future work should further investigate (1) the magnetic response of individuals across different subpopulations and spatial scales and (2) the physiological mechanisms and ontogenetic development of the magnetic sense. In this way, we can gain a better understanding of how this sensory modality affects spatial and temporal patterns of fish populations.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: J.O., R.M.; Methodology: J.O., R.M.; Software: R.M.; Validation: J.O.; Formal analysis: J.O., R.M.; Investigation: J.O.; Resources: J.O.; Data curation: J.O., R.M.; Writing - original draft: J.O.; Writing - review & editing: J.O., R.M.; Visualization: J.O.; Supervision: R.M.; Project administration: J.O.; Funding acquisition: J.O., R.M.

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Journal of Experimental Biology

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