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Orientation of fish larvae *in situ* is consistent among locations, years and methods, but varies with time of day

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ABSTRACT: Understanding larval dispersal requires knowledge of whether larvae *in situ* have orientated swimming, and how this varies temporally and spatially. Orientation of >300 settlement-stage larvae of *Chromis atripectoralis* (Pomacentridae) measured over 1998–2008 by divers near Lizard Island, Great Barrier Reef was consistent. All 10 data sets had southerly orientation at all locations; 94% of larvae swam directionally. Median bearings east and west of Lizard Island were 166° and 170°, respectively. Orientation precision was significantly higher under sunny than cloudy skies. Similar mean bearings were obtained in 2008 with more than 125 larvae observed in a drifting *in situ* chamber (DISC). Orientation varied with time of day. In sunny conditions, precision was weakly, significantly correlated with time of day, but not solar elevation; however, a greater proportion of larvae was significantly directional at low (<50°) than at high (>50°) solar elevation. Mean bearing and time of day were weakly, but significantly correlated. Bearings changed from SE during most of the day to SSW in the late afternoon, with distribution of bearings significantly different. Location-independent but diurnally-dependent orientation implies that larvae used celestial cues for orientation. Of 91 *Pomacentrus lepidogenys* larvae that were followed by divers, 89% swam directionally, but orientation differed among locations and years. DISC results with 20 larvae were similar. The similarity of orientation returned by different methods used on 2 fish species corroborates previous results using diver following. Both methods are useful for the study of larval-fish orientation *in situ*: each has advantages and limitations, and their use is complementary.

KEY WORDS: Pomacentridae · Reef-fish · Orientation · Larva · Solar · Celestial · Visual cue · Dispersal · Connectivity

INTRODUCTION

It has become increasingly obvious that larvae of many marine fishes have behavioural abilities sufficient to influence dispersal outcomes. The capacity of larvae of many species to swim at high speeds for long periods for much of their pelagic larval duration (PLD) is well documented (Fisher & Leis 2009, Leis 2010). Yet, regardless of swimming speeds or durations, unless larvae can swim in an orientated manner, behaviour will have limited direct influence on dispersal. Therefore, to understand and model larval

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dispersal, researchers need to learn more about orientation abilities in fish larvae, and the cues used for orientation (North et al. 2009, Leis et al. 2011, Staaterman & Paris 2013).

Little is known of how orientation behaviour of larval fishes *in situ* might vary spatially and temporally. Both location-dependent and location-independent orientation have been reported (Stobutzki & Bellwood 1998, Leis & Carson-Ewart 2003, Leis et al. 2006, 2007, 2009, Paris et al. 2013). In terms of temporal variation, attention has focused on ontogeny of orientation behaviour, showing that orientation varies ontogenetically in some species (Leis 2010). A few studies have examined changes in larval-fish orientation with time of day in tanks (Mouritsen et al. 2013) or *in situ* (Leis & Carson-Ewart 2003), but we are unaware of any studies that have studied variation in larval-fish orientation over longer time scales.

Evidence exists that larval fishes may use celestial cues for orientation (Waterman 1988, Leis & Carson-Ewart 2003, Mouritsen et al. 2013, Berenshtein et al. 2014), as is the case for many invertebrates and birds (Gould & Gould 2012). If celestial cues are indeed used for orientation by fish larvae, then several expectations follow including (1) orientation should be location-independent, (2) orientation precision and bearing should vary during the day, and (3) orientation should be poorer when the sky is obscured by heavy cloud cover because clouds can not only obscure a direct view of the sky and sun, but also alter the skylight polarization (Pomozi et al. 2001, Hegedüs et al. 2007). Herein, we test some of these expectations.

Orientation of larval fishes is difficult to study *in situ*. Until recently, the only practical way to study orientation behaviour of fish larvae in the ocean was for scuba divers to follow the larvae and to directly measure swimming direction with a hand-held compass (Leis et al. 1996). This approach, which we refer to as Following, has revealed that larvae of most studied species swim in a highly orientated manner in the ocean (Leis et al. 1996, 2009, Trnski 2002, Hindell et al. 2003, Leis & Carson-Ewart 2003). Circumstantial evidence has supported the validity of orientation data collected in this way (Leis et al. 1996, Leis & Carson-Ewart 1998). Yet, questions remain about the influence that the relatively large divers might have on behaviour of the larvae. Further, Following necessarily has limitations in terms of duration, depth of observations, and time of day.

A new methodology to study orientation of larvae *in situ* allows examination of some of these issues. It consists of a drifting, subsurface arena wherein behaviour of larvae can be imaged for later analysis (Paris et al. 2008, Irisson et al. 2009). The original hardware has been developed and refined, largely using clear acrylic in its construction, substituting a digital still camera for the video camera, reversing the camera to look upward, and adding sensors. With these changes, the new apparatus was renamed DISC (drifting *in-situ* chamber; see Paris et al. 2013). The DISC opens several lines of research that are not practical or possible with Following, including longer-term observations, observations at night or at depths greater than 20 m, and the manipulation of sensory cues. However, the data available from this new approach are as yet limited, and there has been no attempt to compare this new methodology to Following. Furthermore, a larva in a relatively small observation arena might not orientate in the same way as one free to swim in the pelagic water column.

Our purpose here is to address some of these issues with a unique and extensive *in situ* data set from the Lizard Island region of the Great Barrier Reef. First, we examine how orientation of settlement-stage larvae of 2 pomacentrid damselfish species varied over 10 yr, and among locations near Lizard Island. We address spatial variation with regard to distance from the reef (100–1000 m) and in areas about 4 km apart east and west of Lizard Island. Second, we examine how orientation in one of these species varies at different times during daylight hours, which allowed us to test whether the patterns of orientation are consistent with the use of celestial cues. Finally, we compare 2 methods for studying orientation of larval fishes in the sea: Following and the DISC.

MATERIALS AND METHODS

Study location

Orientation of larvae was studied 100–1000 m off the fringing reef edge in 2 locations near Lizard Island, Great Barrier Reef (14° 40′ S, 145° 27′ E; Fig. 1): off the east (windward) side, and B) off the west (leeward) side. Details of these 2 locations are available in Leis & Carson-Ewart (2003) and Leis (2004). All observations considered here were made from November to February (i.e. late Austral spring and summer).

Study species

Size at settlement, and hence size of larvae studied here, is 7–10 mm standard length for *Chromis atripectoralis* and 11–13 mm for *Pomacentrus lepi-*

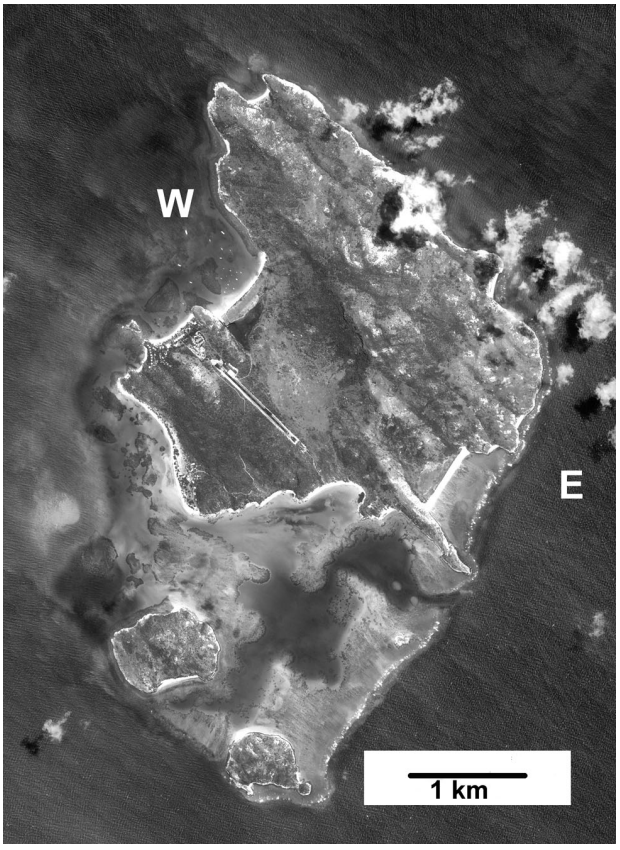

Fig. 1. Lizard Island, Great Barrier Reef, Australia. The study areas off the east and west sides of the island are indicated by E and W, respectively. North is toward the top of the figure. Satellite image ©GeoEye 2005, used with permission

dogenys. Both have a PLD of about 2–3 wk (Wellington & Victor 1989, Bay et al. 2006). Larvae were captured in light traps 50 to 100 m seaward of the reef edge, and observed *in situ* on the day of capture. Between capture and *in situ* observation, the larvae were kept in covered 15 l buckets, with frequent changes of sea water.

Following by divers

The methodology of Following is described in previous publications (especially Leis et al. 1996, Leis & Carson-Ewart 1997, 1998). Briefly, larvae were released 5 m below the surface, usually one at a time, by a pair of SCUBA divers who followed them at 1–2 m distance, and who recorded depth (with 0.1 m precision) and orientation (degrees magnetic to nearest 5°) at 30 s intervals. Observation runs typically lasted 10 minutes, providing 21 measures of swimming direction. Within-run statistics refer to these 21 observations. Among-run statistics refer to the distribution of mean bearings of individual runs. Bearings are reported as degrees magnetic, which in the study area differs from degrees true by 7°.

Temporal and spatial variation

To examine temporal and spatial variation in orientation, 6 sets of Following data for *C. atripectoralis*, and 2 for *P. lepidogenys* were available from previous studies at Lizard Island (Leis et al. 2002, Leis & Carson-Ewart 2003; see Table 1). During the present study, we obtained 4 Following data sets for *C. atripectoralis*: one each from west and east of Lizard Island in January–February 2008, and 2 from west of the island in November–December 2008 (one of which involved the release of 10–12 larvae at a time). We obtained one Following data set for *P. lepidogenys* from west of the island in November–December 2008. The influence of distance from the reef edge (100, 500 and 1000 m) on orientation was assessed by Leis & Carson-Ewart (2003), and we report their assessment.

Diurnal variation

In order to examine variation in orientation during the day (hereafter, diurnal analysis), we utilized a reduced data set to avoid the possibility of confounding the diurnal analysis with influences involving different locations or experimental manipulations. Therefore, we included only data from west of Lizard Island, the location where we had the most data and the broadest coverage of times (see Table 1). We also excluded 2 data sets (CFW3 and 4; see Table 1) obtained when reef sounds were being broadcast (Leis et al. 2002) and a data set (CFW7) obtained when groups of larvae were being followed (J.-O. Irisson et al. unpubl.), as in both cases, orientation of the larvae had significantly different variability from that without the manipulations. An additional Following data set for *C. atripectoralis* was gathered in November–December 2011 from west of the island. These data were taken between 11:45 and 15:40 h local time (mean 13:10 h: all times are the start time of the run) to obtain information at times that were poorly represented in the other data sets. This provided 154 Following releases taken from 19 Nov to 22 Feb at starting times ranging from 08:36 to 18:02 h. For some analyses, these data were divided into 5×2 h bins, starting from 08:30 h, resulting in n = 24, 45, 33, 35 and 17, respectively. Of these, 114 were obtained in sunny

conditions (including scattered 'trade-wind clouds'), and 29 under full cloud cover. The field notes did not specify whether it was cloudy or sunny for 11 releases, so these were used only for analyses including all data. Insufficient diurnal coverage of *P. lepidogenys* data was obtained for diurnal analysis.

DISC

The DISC equipment and methodology are described by Paris et al. (2008, 2013) and Irisson et al. (2009). The DISC is a Lagrangian drifter with a circular, behavioural chamber with an upward looking camera positioned to capture time-lapse images of a larva placed within the chamber. A thin line links the DISC's primarily clear acrylic structure to a surface float, and a drogue underneath keeps it locked in the currents. A compass and sensors attached to the DISC's frame record environmental variables experienced by the fish larva in the chamber. The chamber is transparent to visible light, odour and sound but can be changed for cue manipulation.

In this study, a larva was inserted into the mesh chamber (40 cm diameter, 10 cm high), and the DISC launched to quickly reach the measurement depth. Based on the modal vertical distribution of *C. atripectoralis* from Following studies (Leis 2004), the DISC was suspended 9 m below the surface. It then drifted undisturbed, typically for 20 min (5 min of acclimation and 15 min observation), while the behaviour of the larva was captured by images taken every 2 s. The position of the larva in the image was subsequently digitized, and corrected for rotation of the DISC. Larval positions in the DISC were subsampled every 10 s, during which time the larva could easily swim across the chamber, creating an independent subset of 60 positions. 'Within-run' statistics refer to these 60 observations. 'Among-run' statistics refer to the distribution of mean bearings of individual runs. Data handling and analysis are detailed elsewhere (Paris et al. 2008, 2013, Irisson et al. 2009).

For *C. atripectoralis*, 4 data sets were obtained with the DISC: 3 east, and one west of the island. For *P. lepidogenys*, one DISC data set was obtained from west of the island. All DISC data were gathered in November–December 2008 (see Table 1).

Comparison of methods

We compared results of DISC and Following by contrasting the distribution of bearings from the DISC and Following data sets pairwise with appropriate statistical tests. We asked (1) if the differences between data sets involving between-method contrasts (i.e. Following vs. DISC) were different from those involving within-method contrasts, and (2) if within-method contrasts for DISC differed from those for Following. We made similar comparisons involving precision of directionality and orientation.

Data analysis

Circular statistics were used to analyze directional data (see Zar 1996 for details) and were done largely with Oriana software (Kovach Computing Services), including calculating mean direction, median and 95% confidence interval (95% CI) of the median direction, directional precision (length of the mean vector, **r**), Rayleigh Tests (R test) for single sample tests for uniformity of the distribution of angles, Watson U2 test (WU2) for multiple sample tests for differences in distribution of bearings, and circular-circular correlation (CC). Tests for differences of angular dispersion between samples were done with the 2-sample or multi-sample Walraff Test (W test, following Zar 1996): this was used to test for differences in variability in among-run (second-order) orientation. Tests involving **r** (which varies from a low of 0 to a high of 1) used the non-parametric Mann-Whitney test (MW test) or Kruskall-Wallis test (KW test), and included all runs. In all tests, a p-value < 0.05 was considered significant. No correction for multiple testing was done: rather, we provide actual p-values to allow the reader to assess if a Type I error due to multiple testing was likely.

For each run, the mean bearing was computed and its significance assessed with the R test. Significant runs are said to be directional. Only those significant means were used in among-run (second-order) analyses (using R or WU2 tests) to assess the overall orientation within each dataset and for comparisons between datasets.

In order to compare the observations made by Following and DISC, we were interested in both the direction and precision of orientation at both within-run and among-run levels. Comparisons of bearings and precision were first made pairwise between all datasets (i.e. individual data sets were compared in all combinations 2 at a time), and analysed in terms of location (East vs. West) and method (DISC vs. Following). Then, data were pooled by location and method into 4 categories (DISC West, DISC East, Following West, Following East), to highlight geograph-

ical or methodological effects. For *C. atripectoralis*, this resulted in DISC East (n = 3 data sets), DISC West (n = 1), Following East (n = 3), and Following West (n = 7). Observations of *P. lepidogenys* were made only west of the island (3 Following and 1 DISC data sets). Finally data were pooled by method only, to specifically test Following against DISC. These test results are summarized in the Results, and presented in more detail in the Supplement at www.int-res.com/articles/suppl/m505p193_supp.pdf.

In most cases, we present medians, rather than means, for **r** and among-run bearings. For **r** this is because the length of the mean vector is constrained between 0 and 1, so we used non-parametric statistical methods for its analysis. For bearings, non-parametric circular statistical tests were the most appropriate in many cases due to their less restrained assumptions. The median is the most appropriate measure of central tendency when using nonparametric tests. Further, with circular statistics, the 95% CI of the median provides a more readily understandable measure of error than either circular variance or circular standard deviation, which are not directly equivalent to their non-circular counterparts.

RESULTS

Orientation patterns over years and locations

Following by divers

The southerly orientation in 278 runs of *Chromis atripectoralis* larvae as measured by Following was remarkably consistent (all data-set medians were SE to SSW) over both location and time (Table 1). In the 10 Following data sets, 82 to 100% (mean 93%) of larvae had significant within-run directionality, with a median **r** range of 0.67–0.95 (Fig. 2, Table 1). The median bearing of the 10 data sets was to the south, varying between 136 and 206°, and among-run orientation was significant ($p < 0.05$, R test) in 7 data sets. The median bearing of all 10 pooled data sets was 168°, and was significantly directional (Table 1). The median bearing of the 3 data sets from east of the island was 166°, and that of the 7 data sets from west the island was 170°: both were significant (Table 1).

In *C. atripectoralis*, variation in orientation with distance from the reef edge was considered by Leis & Carson-Ewart (2003), who found no significant difference in mean bearings ranging from 137° to 176° between 100, 500 and 1000 m from the reef east of the island. In the morning on the west side, orientation at all 3 distances from the reef was to the southeast, but orientation 500 m from the reef was significantly more easterly (105°) than it was either 100 or 1000 m from the reef (151 and 166°, respectively). However, in the afternoon on the west side, there was no significant difference with distance from the reef edge in mean bearings of 99° to 210°.

Fewer data were available for *Pomacentrus lepidogenys*, and only from the west side of the island, but orientation in 91 runs of this species was less consistent over time than that of *C. atripectoralis* (Table 1, Fig. 3). In the 3 Following data sets, 85.7 to 95.5% of *P. lepidogenys* larvae had significant within-run directionality, with median **r** range of 0.79–0.89. Only 1 of the 3 data sets (PFW2) had significant among-run orientation, and the median bearings ranged widely: 140°, 185° and 346°.

P. lepidogenys had significant differences in mean bearing among distances from the reef both morning and afternoon (Leis & Carson-Ewart 2003). In both morning and afternoon, only the 1000 m distance had a significant mean bearing. In the morning, mean bearing at 1000 m (93°) was significantly different from mean bearings at 100 or 500 m (200° and 266°, respectively), whereas the latter 2 did not differ. In the afternoon, the results were similar: 163° at 1000 m, 188° at 100 m and 249° at 500 m, with 1000 m significantly different from the other two.

DISC

The 4 DISC data sets of 126 *C. atripectoralis* runs (Table 1, Fig. 2) provided similar results to the Following data. Between 77 and 97% (mean 92%) of larvae had significant within-run directionality, with median **r** range of 0.60–0.83. Three of 4 data sets had southerly median bearings (2 were significant; Table 1), whereas the remaining data set (CDW1) had a non-significant north-westerly median bearing (318°). The median bearing for all pooled DISC *C. atripectoralis* was 173° and significant (Table 1).

In the single *P. lepidogenys* DISC data set of 20 runs (Table 1, Fig. 3), 95% of larvae had significant within-run directionality, but the north-westerly among-run median bearing (334°) was not significant.

Diurnal study: orientation in relation to time of day, solar elevation and sky conditions

Using the Following method, time-dependent changes in both direction and precision of orientation were

Table 1. Orientation of larvae of *Chromis atripectoralis* and *Pomacentrus lepidogenys* around Lizard Island using 2 techniques. The 4-symbol dataset codes indicate the species (C or P: *C. atripectoralis* or *P. lepidogenys*), method (F or D: following by divers, or drifting *in situ* chamber [DISC] observations), location (E or W: east or west side), and dataset number. Times are at the start of a run. Techniques were consistent, but data were recorded in several studies and conditions. Unless noted otherwise, observations were under ambient conditions, meaning individual larvae observed in an unmodified environment. Modifications include broadcasting of sound near the larvae (reef sound for CFE2 and CFW2, and 'white noise' for CFW4), partial shading of the observed individuals (CDE2), and observation of groups of 10–12 larvae released simultaneously (CFW7). For each dataset we report the proportion of directional larvae (n dir. / total) and the median within-run **r** (and its 95% CI), a measure of precision. Directional bearings were used in an among-run (i.e. second-order) analysis, for which we report the median bearing (with 95% CI: note that the CIs run in a clockwise direction), the per-dataset **r** and the p-value (R test, in **bold** if significant). In addition, data were pooled and summarized per location (East/West), method (Following/DISC), and species

Dataset code	Data source		Within-run		Among-run		
	Date	Time (h), mean (range)	n dir./ total	Median **r** [95% CI]	Median bearing [95% CI]	**r**	R test p-value
CFE1	Dec 98, Feb 99[a]	09:24 (08:23–12:05)	39 / 40	0.91 [0.84–0.95]	143° [111–179]	0.48	**$<10^{-4}$**
CFE2	Dec 99[b]	11:38 (09:23–14:20)	15 / 17	0.87 [0.78–0.96]	160° [38–247]	0.13	0.79
CFE3	Jan–Feb 08[c]	12:36 (09:52–15:05)	20 / 20	0.74 [0.68–0.86]	203° [152–258]	0.41	**0.03**
CFE All	All East		74 / 77	0.87 [0.80–0.92]	166° [150–202]	0.36	**$<10^{-4}$**
CFW1	Dec 98, Feb 99, Jan 00[a]	10:43 (08:36–13:45)	42 / 48	0.83 [0.69–0.88]	136° [116–166]	0.58	**$<10^{-6}$**
CFW2	Feb 99, Jan 00[a]	16:12 (14:46–18:02)	41 / 44	0.87 [0.45–0.94]	206° [170–234]	0.29	**0.03**
CFW3	Dec 99[b]	11:25 (09:07–13:40)	19 / 22	0.82 [0.45–0.94]	143° [81–195]	0.29	0.20
CFW4	Jan 01[b]	10:50 (09:45–13:26)	23 / 23	0.89 [0.80–0.95]	177° [162–216]	0.64	**$<10^{-4}$**
CFW5	Jan–Feb 08[c]	12:18 (10:57–13:25)	9 / 11	0.76 [0.11–0.88]	167° [133–197]	0.82	**0.001**
CFW6	Nov–Dec 08[c]	12:08 (09:54–14:54)	17 / 18	0.91 [0.64–0.96]	172° [126–237]	0.28	0.26
CFW7	Nov–Dec 08[c]	12:18 (09:20–15:21)	35 / 35	0.95 [0.92–0.97]	192° [158–215]	0.56	**$<10^{-5}$**
CFW All	All West		186 / 201	0.88 [0.84–0.90]	170° [162–180]	0.43	**$<10^{-6}$**
CF All	All Locations		260 / 278	0.87 [0.84–0.90]	168° [160–176]	0.41	**$<10^{-6}$**
CDE1	Nov–Dec 08[c]	12:38 (09:24–15:11)	33 / 34	0.67 [0.36–0.88]	164° [91–213]	0.09	0.78
CDE2	Nov–Dec 08[c]	12:20 (09:32–15:42)	23 / 30	0.60 [0.20–0.68]	144° [125–173]	0.62	**$<10^{-4}$**
CDE3	Nov–Dec 08[c]	12:32 (09:45–15:19)	32 / 33	0.83 [0.68–0.95]	203° [171–224]	0.53	**$<10^{-4}$**
CDE All	All East		88 / 97	0.68 [0.58–0.79]	173° [160–195]	0.34	**$<10^{-4}$**
CDW1	Nov–Dec 08[c]	11:53 (09:10–14:41)	28 / 29	0.61 [0.50–0.80]	318° [272–40]	0.08	0.83
CD All	All Locations		116 / 126	0.67 [0.59–0.77]	173° [160–196]	0.24	**0.001**
PFW1	Nov 96, Nov–Dec 99[a]	10:50 (09:08–13:39)	36 / 41	0.79 [0.68–0.86]	140° [86–200]	0.17	0.34
PFW2	Nov 00, Jan 01[a]	16:01 (14:59–17:06)	21 / 22	0.84 [0.73–0.92]	185° [157–240]	0.52	**0.003**
PFW3	Nov–Dec 08[c]	13:12 (10:17–15:48)	24 / 28	0.89 [0.72–0.95]	346° [273–59]	0.09	0.81
PFW All	All West		81 / 91	0.85 [0.79–0.88]	200° [159–226]	0.17	0.10
PDW1	Nov–Dec 08[c]	11:05 (08:59–15:49)	19 / 20	0.75 [0.54–0.87]	334° [275–62]	0.08	0.88

[a]Leis & Carson-Ewart (2003); [b]Leis et al. (2002); [c]Present study

found both within runs and among runs in *C. atripectoralis*. As this study was largely exploratory, we have subdivided and analyzed the data several ways to assess related, but somewhat different possibilities.

Within-run directionality

A total of 138 of the 154 *C. atripectoralis* individuals (89.6%) in the Diurnal study had significantly directional trajectories (R test, $p < 0.05$). Under sunny conditions, 105 of 114 individuals (92.1%) were significantly directional. Under cloudy skies, 24 of 29 individuals (82.8%) were significantly directional. The proportion of directional individuals did not differ significantly between sunny and cloudy conditions (χ^2, $p = 0.14$). However, precision of directionality (**r**) was significantly greater under sunny (median **r** = 0.87) than cloudy (median **r** = 0.70) conditions (2-tailed, MW test, $p = 0.0011$, $n_{sunny} = 114$, $n_{cloudy} = 29$), which is consistent with use of celestial cues for orientation.

Under sunny skies, within-run precision of directionality increased during the day. Precision (**r**) and time of day were significantly, although weakly, correlated (**r** = 0.043 × time + 0.559, $R^2 = 0.043$, $p = 0.026$, $n = 114$, units of time as proportion of 24 h; Fig. 4). The correlation became non-significant when all data were included ($R^2 = 0.01$, $p = 0.23$, $n = 154$). Although median precision was greater in the mid and late afternoon (**r** = 0.88, 0.93, respectively) than for mid-morning, late morning and early afternoon

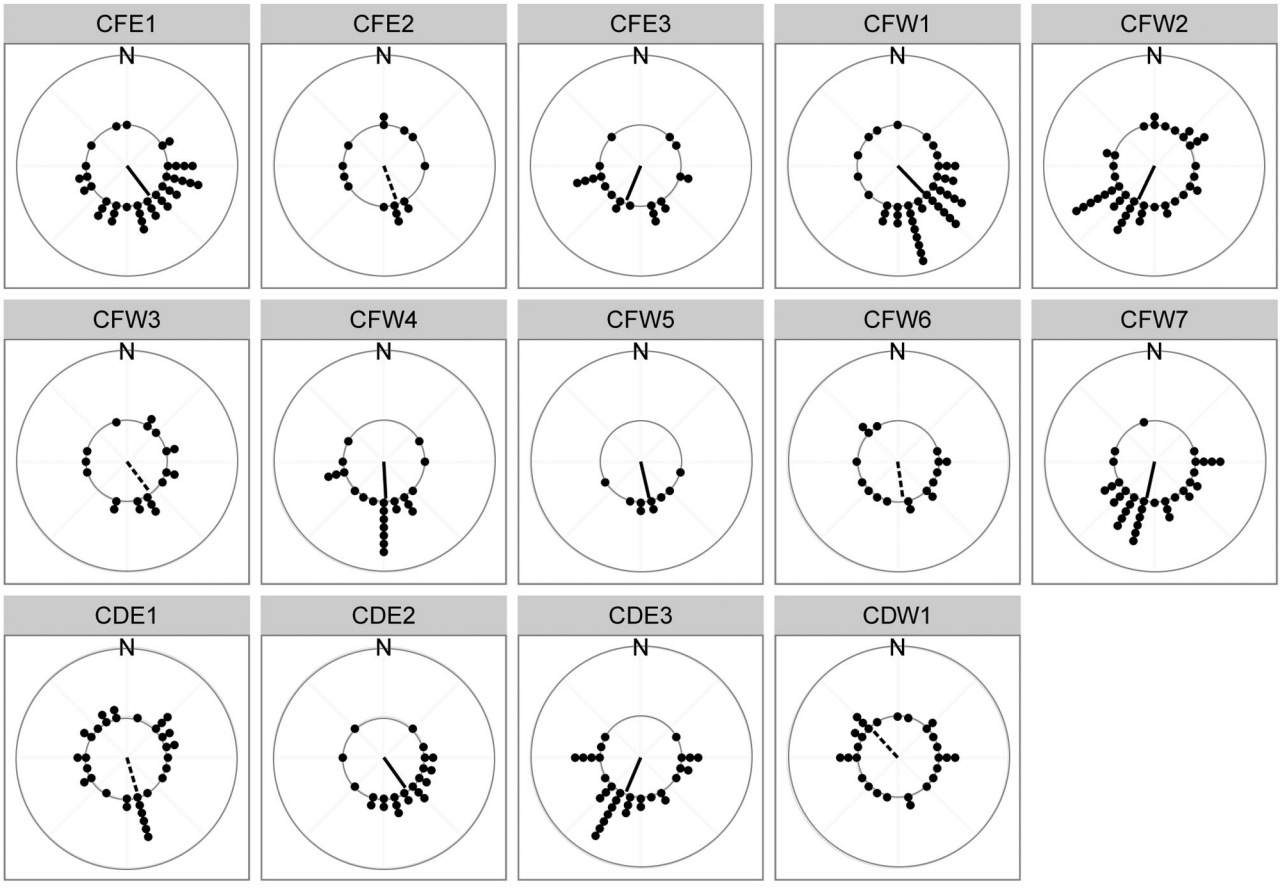

Fig. 2. Distribution of median bearings of directional runs of *Chromis atripectoralis* using Following and drifting *in situ* chamber (DISC) methodology. Points: mean bearings of directional runs; black radii: 2nd-order mean bearing (solid if $p < 0.05$, dashed if $p > 0.05$). See Table 1 for statistics for each plot. The 4-symbol dataset codes indicate the species (C: *C. atripectoralis*), method (F: Following; D: DISC), area (E: East; W:West), and data-set number

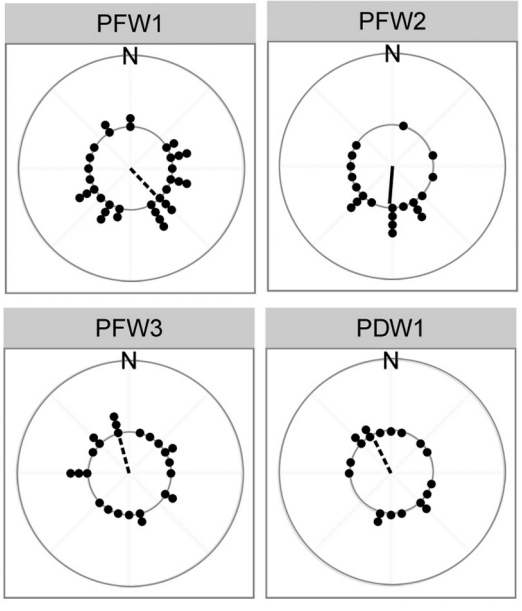

Fig. 3. Distribution of median bearings of directional runs of *Pomacentrus lepidogenys* using Following and DISC methodology. 'P' in dataset code: *P. lepidogenys*; all other details as in Fig. 2

(**r** = 0.76, 0.87, 0.85), there was no significant difference in within-run precision among the 5 time periods: 08:30–10:30, 10:30–12:30, 12:30–14:30, 14:30–16:30, 16:30–18:02 h (KW test, $p = 0.20$ for sunny periods, and $p = 0.48$ for all data). This provides weak support for the use of celestial cues in orientation.

All 33 larvae observed in sunny conditions when solar elevation (SoEv, i.e. the angle of the sun above the horizon) was less than 50° had significant directionality. In contrast, 72 of 81 larvae observed when SoEv exceeded 50° in sunny conditions had significant directionality. These 2 frequencies were significantly different (χ^2, $p = 0.046$). In sunny conditions, when SoEv was used to partition the data into 4 groups (10–30°, 30–50°, 50–70°, 70–90°), median precision (**r**) was highest in the 10–30° group (0.928,

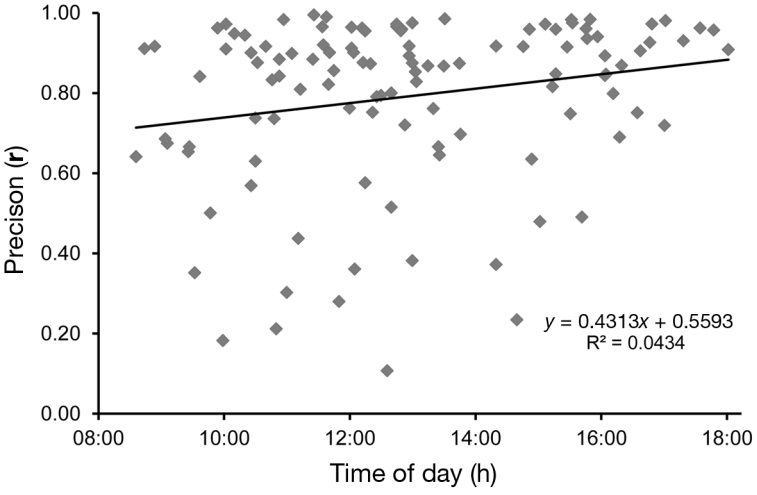

Fig. 4. Relationship between within-run **r** (i.e. directional precision), and time of day for *Chromis atripectoralis* on the west side of Lizard Island using Following methods under sunny conditions

n = 8). However, there was not a significant correlation between SoEv and precision (**r**): R^2 = 0.02, p = 0.11. Under sunny skies, there was not a significant difference in precision between the 4 SoEv groups (p = 0.182, KW test; Table 2), which varied in median **r** from 0.85 to 0.86, nor was there a significant difference when observations were pooled into 2 groups: <50° and >50° (MW test, p = 0.42). With one exception, similar results were obtained when all data were included (p = 0.51, KW test), although median **r** values were lower in all groups by about 0.02. The exception was that when all data were included, the frequency of larvae with significant directionality did not differ between the <50° and >50° groups: 51 of 55, and 87 of 99, respectively (χ^2, p = 0.34). The use of some sorts of celestial cues for orientation is thought to be easier when the sun is low in the sky, and these results provide limited support for that kind of use.

There was a significant, but weak correlation between time of day and within-run mean bearings during sunny periods (CC correlation, r_{CC} = 0.057, p < 0.05, n = 105): bearings tended to be more easterly in the morning and more westerly in the mid to late afternoon. Unfortunately, CC correlation is difficult to portray in a 2-dimensional figure, especially when many values are involved (but see Fig. 5 for the relationship between among-run median bearing and time of day). Similar results were obtained when all data were included (r_{CC} = 0.086, p < 0.05, n = 138), but with a slightly higher correlation coefficient. This provides somewhat equivocal support for the use of celestial cues for orientation (see 'Discussion' for more detail).

When SoEv was used as the independent variable, rather than time of day, a non-significant negative correlation between SoEv and **r** was found. This was the case both when all data were used (R^2 = 0.01, p = 0.228, n = 154), and when only observations taken under sunny skies were used (R^2 = 0.02, p = 0.114, n = 114). These results provide no support for the use of celestial cues.

Among-run orientation

Only data from the 138 directional runs were used in analyses of among-run orientation.

Among-run mean bearings were southerly overall, regardless of whether it was cloudy or sunny. When all data were included, median bearing was 166° (**r** = 0.32, p = 0.65E-7, R test, n = 138). Under sunny conditions, median bearing was 170° (**r** = 0.35, p = 2.06E-6, R test, n = 105). Under cloudy skies, median bearing was 188°, although this was not significant (**r** = 0.20, p = 0.395, R test, n = 24). The distribution of mean bearings was not significantly different between sunny (n = 105) and cloudy (n = 24) conditions (WU2 test, p > 0.5). This was not consistent with the use of celestial cues.

Orientation data for *C. atripectoralis*, when divided into five 2 h periods, revealed time-dependent changes in both precision and direction of orientation. When all data for directional individuals were used, significant orientation was found in all but 1 of the 5 time periods (Fig. 6, Table 2). The 14:30–16:30 h period had a non-significant median bearing to the SE (Table 2). The mid-morning, late morning and early afternoon periods had significant orientation with median bearings between ESE and SSE (Table 2). In contrast, the late afternoon period had a significant mean bearing to SSW (Table 2), and its distribution of directions was significantly different from the other 4 periods (p < 0.02 to 0.001, WU2 test), being more westerly and more precise (**r** = 0.67 vs. 0.14 to 0.48). In addition, the mid-afternoon period was significantly different from the late morning period (p < 0.05, WU2 test), being both less southerly and more variable (**r** = 0.16 vs. 0.44). No other significant differences were found. These results are consistent with use of celestial cues for orientation.

When consideration was limited to the 105 sunny period runs, a similar, although less resolved picture

Table 2. Diurnal orientation of larvae of *Chromis atripectoralis* using Following by divers west of Lizard Island in relation to time of day and solar elevation. Two sets of values are reported: all data, and sunny conditions. For each data set we report the proportion of directional larvae (n dir. / total) and the median within-run **r**, including the 95% confidence interval of the median. Directional bearings were used in an among-run (second-order) analysis, for which we report the median bearing [and 95% CI], the per-data set **r** and its significance (R test). Significant R test results given in **bold**

Starting conditions	All data				Sunny			
	Within-run		Among-run		Within-run		Among-run	
	n dir./ total	Median **r** [95% CI]	Median bearing [95% CI]	**r**, p (R test)	n dir./ total	Median **r** [95% CI]	Median bearing [95% CI]	**r**, p (R test)
Time (h)								
08:30–10:30 Mid morning	22 / 24	0.72 [0.65–0.91]	113° [85–172]	0.48, **0.007**	16 / 18	0.76 [0.64–0.96]	132° [80–176]	0.37, 0.12
10:30–12:30 Late morning	38 / 45	0.86 [0.78–0.88]	169° [144–197]	0.44, **0.0005**	31 / 35	0.87 [0.79–90]	180° [144–230]	0.41, **0.005**
12:30–14:30 Early afternoon	31 / 33	0.83 [0.72–0.88]	150° [101–199]	0.31, **0.05**	25 / 27	0.85 [0.72–0.92]	155° [123–223]	0.33, 0.07
14:30–16:30 Mid afternoon	30 / 35	0.85 [0.69–0.92]	130° [90–206]	0.14, 0.54	23 / 24	0.88 [0.80–0.96]	143° [101–232]	0.25, 0.24
16:30–18:02 Late afternoon	17 / 17	0.90 [0.81–0.96]	212° [190–234]	0.67, **0.0002**	10 / 10	0.93 [0.75–0.97]	204° [165–234]	0.76, **0.002**
Solar elevation (°)								
10–30°	14 / 14	0.90 [0.72–0.96]	209° [183–234]	0.63, **0.002**	8 / 8	0.93 [0.91–0.96]	204° [65–233]	0.75, **0.007**
30–50°	37 / 41	0.84 [0.69–0.91]	134° [109–186]	0.29, **0.048**	25 / 25	0.87 [0.75–0.94]	165° [122–225]	0.34, 0.058
50–70°	32 / 37	0.85 [0.70–0.90]	134° [88–169]	0.26, 0.11	29 / 33	0.85 [0.65–0.92]	134° [88–172]	0.26, 0.13
70–90°	55 / 62	0.83 [0.79–0.88]	164° [133–180]	0.37, **0.0005**	43 / 48	0.86 [0.76–0.90]	168° [133–205]	0.38, **0.002**

emerged (Table 2). Only 2 of the 5 periods (late morning and late afternoon) had significant orientation (Table 2). The late afternoon distribution of directions was significantly different ($p < 0.02–0.05$, WU2 test) from the other 4 periods, being more westerly and more precise (Table 2). There were no other significant differences.

A significant relationship between time of day and median bearing emerged when among-run median bearings were calculated for each one-hour period between 08:00 and 18:00 h using all data from directional individuals. Fig. 5 depicts the among-run (second-order) median bearings for each of the 10 × 1 h periods (n ranged from 4 to 28, total = 138; Table 3). A linear regression fitted to these 10 mean values ($R^2 = 0.53$, $p = 0.016$) estimates an increase in mean bearing of roughly 10° per hour from 110° at 08:00–09:00 h to 203° at 17:00–18:00 h (Fig. 5). These results are consistent with use of celestial cues for orientation.

There were too few observations under fully cloudy conditions for a full statistical analysis, but the 12 mid to late afternoon bearings were significantly different from the 12 morning to early afternoon bearings (WU2 test, $p = 0.0004$). The morning to early afternoon median bearing was 124° ($p = 0.009$, R test), whereas the mid to late afternoon median bearing was 271° ($p = 0.17$, R test), which is consistent with the patterns found during sunny periods. These results are consistent with use of celestial cues for orientation.

Bearings taken when SoEv was <30° were significantly different ($p < 0.01$, WU2 test) from those at higher SoEv values, being more westerly and more precise (Table 2) both with all data included, and under sunny skies. However, this was primarily because the 14 low SoEv values were all from late in the afternoon (16:30–18:02 h), when orientation was both more westerly and more precise than earlier in the day. At higher SoEv values (30–50°, 50–70° and 70–90°), median bearings were southerly (134–164°), and the distribution of bearings did not differ among the 3 SoEv groupings that were >30° ($p > 0.2$, WU2 test). These results are consistent with use of celestial cues for orientation.

Following and DISC compared

When DISC and Following were compared, differences in precision between methods were found, but differences in orientation between methods were no more likely than differences within methods.

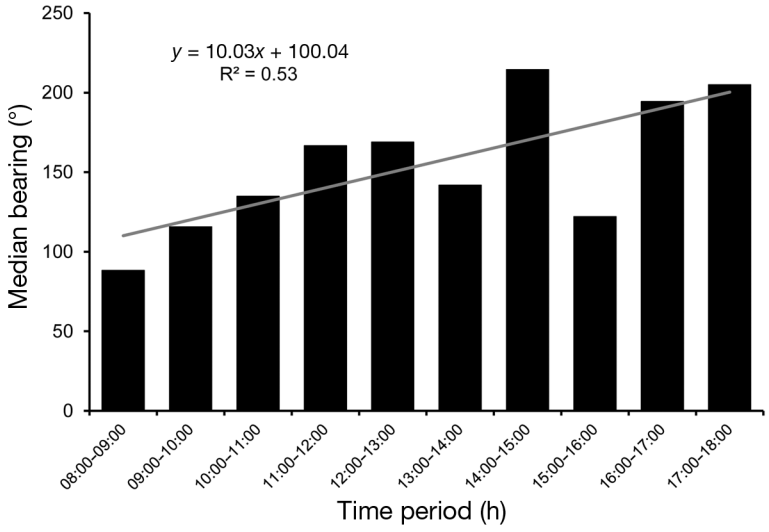

Fig. 5. Among-run (2nd order) median bearings of *Chromis atripectoralis* larvae as measured by Following on the west side of Lizard Island during one-hour time periods between 08:00 and 18:00 h. The regression line has p = 0.016. Table 3 provides details of the median bearings

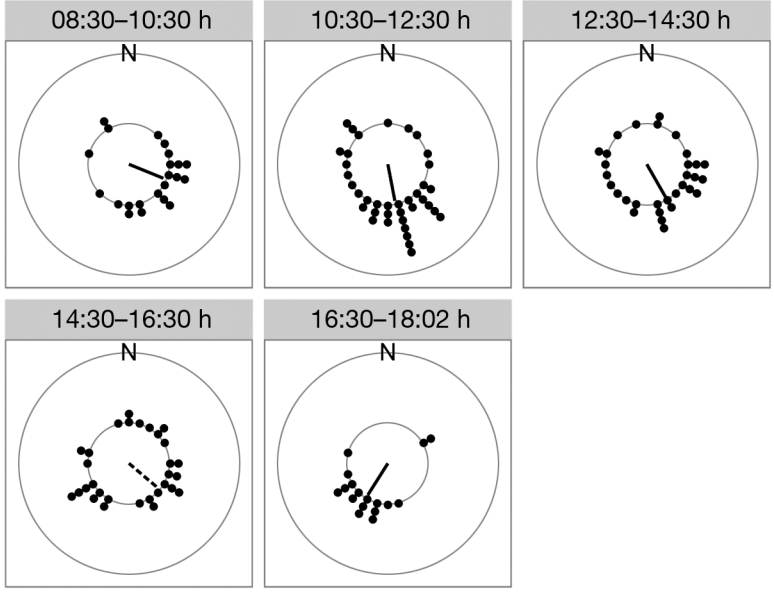

Fig. 6. Distribution of median bearings of directional *Chromis atripectoralis* during 5 diurnal periods on the west side of Lizard Island using Following methods. Details as in Fig. 2. See Table 2 for statistics of each plot

Within-run precision

Chromis atripectoralis (Table S1A in the Supplement). Among the 91 meaningful pairwise comparisons of within-run precision (**r**) among all data sets (MW test), there was not a significant difference in the proportion of significant differences between within-method (43.1%) and cross-method comparisons (57.5%) (χ^2, p = 0.25). However, pairwise MW tests within the 4 method-by-location categories revealed that precision was always significantly lower for DISC than for Following (MW, all $p < 10^{-5}$) regardless of location. Finally, when data were pooled by method, within-run precision was significantly lower for DISC (median **r** = 0.64) than for Following (median **r** = 0.87; Table 1; MW test, $p < 10^{-7}$).

Pomacentrus lepidogenys (Table S1B in the Supplement). Pairwise comparison of DISC within-run precision with the 3 Following data sets, revealed DISC median **r** (0.75) was significantly lower than Following PFW2 and PFW3 median **r** (0.84, 0.89, p = 0.03–0.04 MW test), but not different from Following PFW1 (0.79, p = 0.65). Note that for the same month and location, precision was lower for DISC than Following (PFW3). In contrast, the only difference in median **r** among the 3 Following data sets was that PFW1 was significantly different from PFW2 (0.79 v. 0.89, p = 0.02). However, when all Following data were pooled (median **r** = 0.77) and compared to the DISC data (median **r** = 0.69), there was not a significant difference in precision between methods (MW, p = 0.095).

Orientation

Chromis atripectoralis (Table S2A in the Supplement). Of the 91 meaningful pairwise comparisons, 30 returned a significant difference in distribution of bearings (WU2 test, p < 0.05). Of the 40 cross-method comparisons (DISC vs. Following), 15 were significant (37.5%). Five of 6 (83%) DISC vs. DISC comparisons were significant, whereas only 10 of 45 (22.2%) Following vs. Following comparisons were significant. This difference in proportions for within-method comparisons is significant (Fisher's Exact test, p = 0.006), implying that DISC data sets were less consistent in orientation than Following ones.

This implied method-dependent difference in orientation was primarily due to a single DISC data set, CDW1. Six of the 15 significant cross-method differ-

Table 3. Median bearings of *C. atripectoralis* for 1 h time periods between 08:00 and 18:00 h west of Lizard Island

Time period (h)	n	Bearing (°) Median	95% CI	p (R test)	r
08:00–09:00	5	88	57–176[a]	0.045	0.764
09:00–10:00	11	116	80–193	0.067	0.492
10:00–11:00	16	135	39–172	0.213	0.312
11:00–12:00	14	167	123–192	0.004	0.611
12:00–13:00	28	169	123–223	0.120	0.275
13:00–14:00	16	142	87–199	0.043	0.439
14:00–15:00	4	214	126–317[a]	0.938	0.134
15:00–16:00	19	122	43–232	0.816	0.105
16:00–17:00	14	194	101–274	0.209	0.336
17:00–18:00	11	205	65–234	0.013	0.609

[a]Range: 95% CI cannot be calculated if n < 6

ences involved the CDW1 data set, which had a median bearing of 318° (140–200° for other DISC sets, and 136–206° for Following sets). The size of the difference in median bearing (median 55°) for the 11 significant within-Following comparisons was not significantly different from that of the 8 cross-method significant comparisons involving the 3 CDE data sets (median 56°) (MW test, p = 0.91). In contrast, the 8 cross-method comparisons involving CDW1 had significantly larger differences in median bearing (median = 122°) than did the 11 within-Following comparisons (MW test, p = 0.0003).

For *C. atripectoralis* there was no meaningful difference between methods in variability in among-run orientation (Table S2A in the Supplement). None of the pairwise contrasts between data sets revealed a significant difference in angular dispersion (W test). When the comparison was based on data grouped by method and location (4 groups, based on DISC, Following, East and West), no significant difference in angular dispersion was found (W test, p = 0.21). When only the 2008 data sets (4 each DISC and Following) were included, a significant difference was found (W test, p = 0.007), but it was entirely due to the contrast between the most and least variable datasets (CDE1 and CFE5, respectively). The other data sets did not differ significantly in angular dispersion from each other or from either the most variable or the least variable sets. Therefore, for *C. atripectoralis*, variability in among-run orientation does not appear to differ between DISC and Following.

Pomacentrus lepidogenys (Table S2B in the Supplement). Only one of the *P. lepidogenys* data sets, PFW2, had a significant among-run mean orientation (Table 1). There were significant differences in distribution of bearings among data sets collected at different times, but no differences were found between data sets collected at the same time (all based on WU2). Thus, the distribution of bearings of the DISC data (median bearing of 334°, all taken in 2008) was not significantly different from that of the 2008 Following data (median direction 346°), nor did morning Following data (PFW1, median bearing 151°) differ from afternoon Following data (PFW2, median bearing 185°). So, the key cross-method comparison did not detect any difference in distribution of bearings between DISC and Following data. In contrast, all 4 comparisons involving data collected at different times returned significant differences in distribution of bearings. It seems that orientation of *P. lepidogenys* larvae varies on a temporal basis, but that both methods give similar estimates at any one time.

There was no difference in angular dispersion among the 4 *P. lepidogenys* data sets (p = 0.10–0.71, W tests; Table S2B in the Supplement) or between DISC data (PDW1) and Following data (PFW3) from the same time period (p > 0.20, W test). Therefore, for *P. lepidogenys*, variability of among-run orientation does not appear to differ between DISC and Following.

DISCUSSION

Settlement-stage larvae of both study species have mean swimming speeds greater than mean current speeds in the study region, so both have the ability to greatly influence their dispersal given sufficient orientation abilities. The 8–10 mm larvae of *Chromis atripectoralis* have average *in situ* swimming speeds of 22–25 cm s^{-1}, whereas the larger (11–13 mm) larvae of *Pomacentrus lepidogenys* are slower at 15–17 cm s^{-1} (Leis & Carson-Ewart 2003). In comparison, average current speeds in the Lizard Island region are 11–17 cm s^{-1}, depending on location (Frith et al. 1986, Leis & Carson-Ewart 2003). The large majority (89.0–93.5%) of individuals of both study species swam in a significantly directional manner, regardless of the method of observation, location or time.

Orientation variability among locations and years

Larvae of *C. atripectoralis* had very consistent orientation within runs, among runs within data sets, among data sets, among years, among distances from the reef edge and between sides of the island. All Following data sets collected over 10 yr and involving over 300 larvae and 3 of 4 DISC data sets had a

median bearing to the south: 136–206° for Following and 144–203° for DISC. Further, in 7 of 10 Following and 2 of 4 DISC data sets, the southerly median bearings were significantly directional. Virtually identical median bearings were found on both sides of Lizard Island, and swimming direction was southerly at all distances from shore. Two of the 3 Following data sets without significant orientation were from experiments when underwater broadcasts of nocturnal reef sound were thought to have disrupted the normal orientation of the larvae (Leis et al. 2002).

In fact, some significant differences in median direction between data sets could be identified, but these were relatively small differences within the general envelope of southerly swimming. The sole exception was the DISC data set from west of the island (CDW1), which had non-significant northwest orientation. This DISC dataset was obtained during the same period (Nov–Dec 2008) when CFW6, the only non-significant Following data set not manipulated by sound, was obtained. The distribution of bearings in the these Nov/Dec 2008 Following and DISC data sets were not significantly different.

At present, it is unknown whether location independence in orientation of *C. atripectoralis* larvae extends beyond the Lizard Island region to other locations. The species has a wide range, extending from the western Indian Ocean to the Central Pacific, but behaviour of its larvae has not been studied elsewhere. Regardless, the location-independent orientation of this species in the vicinity of Lizard Island has implications for the cues used to achieve it. Location independence of orientation implies use of location-independent cues such as celestial or magnetic cues (Leis et al. 2011). In larval marine fishes, there is some evidence for use of celestial cues in 2 families that inhabit tropical reefs, Pomacentridae and Apogonidae (Leis & Carson-Ewart 2003, Mouritsen et al. 2013), but none for use of magnetic cues. If orientation is location-independent, modelling larval dispersal will be more straightforward than if orientation changes with time or location (Staaterman et al. 2012, Staaterman & Paris 2013).

The reasons for the consistent southerly orientation of *C. atripectoralis* larvae (and apparently, larvae of other pomacentrids; Leis et al. 2007), regardless of location or time, are not clear. In an earlier study, using a subset of the data summarized here, involvement of solar cues was suggested (Leis & Carson-Ewart 2003). This suggestion has received some support from Mouritsen et al. (2013) and the diurnal portions of the present study, but this only addresses the proximate means by which orientation might be accomplished, not why larvae might prefer to swim to the south near Lizard Island during the day. In the Lizard Island region, the most common current direction is from south to north (Frith et al. 1986, Leis & Carson-Ewart 2003, Choukroun et al. 2010). All else being equal, larvae that swim primarily to the south in the Lizard Island region, would, on average, remain closer to their natal reef than larvae that did otherwise, resulting in limited dispersal (Leis et al. 2007), which has clear advantages. We do not suggest that the larvae directly detect the current and swim into it. Indeed, the available evidence is that larvae have the same swimming direction regardless of the current at any particular time (Leis & Carson-Ewart 2003). It is more likely is that a 'regional swimming direction' has evolved to counteract the prevailing non-tidal current. Alternatively, individuals without a southerly swimming orientation would be advected from Lizard Island by the prevailing current because it would not be counteracted by swimming. But, if the latter alternative were the case, one would expect larvae which did not swim to the south to be advected into the Lizard Island region from the south, resulting in a range of swimming directions near Lizard Island.

In contrast, orientation behaviour of *Pomacentrus lepidogenys* larvae was less precise among runs, and seemed to change over time as well as with distance from shore. Two of 3 *P. lepidogenys* Following data sets had a median bearing to the south, but only the late afternoon data set had significant orientation, again to the south. One Following and the sole DISC *P. lepidogenys* data set had non-significant median bearings to the north. Clearly, the 2 species have different orientation behaviour.

Diurnal variation in orientation

The diurnal changes in *C. atripectoralis* orientation precision found here are consistent with the use of a celestial orientation cue or cues, as is the lower within-run precision under cloudy conditions, but there were some inconsistencies. Within-run precision was weakly positively correlated with time of day, increasing from mid-morning to late afternoon, but there were no significant differences in precision among the 5 diurnal time periods. Similar results were obtained when solar elevation (SoEv) was used to partition the data, rather than time, except that during sunny periods there was a higher proportion of significantly orientated individuals when the sun angle was low (<50°) than when it was high. If celes-

tial cues were being used by larval *C. atripectoralis* for orientation, precision would be expected to be lowest in the middle of the day (high SoEv), and higher in early morning and late afternoon when SoEv was low. In other words, one should expect a negative correlation between SoEv and precision, but no correlation between time of day and precision. In contrast, we found a significant, weak positive correlation with time of day, and a non-significant negative correlation with SoEv. One possible explanation for this is that we had no observations earlier than 08:30 h (when SoEv would have been about 40°). In contrast, we had 20 observations after 16:00 h, when SoEv would have been <40°). In large part this was due to the fact that the 360 m high peak of Lizard Island to the east blocks direct view of the sun in the early morning from much of the western (leeward) study area. Thus, the weak correlation between precision and time of day (between 08:30 and 18:00 h) is perhaps not unexpected if precision is highest at low SoEv. However, the lack of a significant correlation between precision and SoEv is not consistent with use of celestial cues. Based on the results presented here, there is, at best, only a weak connection between time of day (and SoEv) and orientation precision.

The change in direction of *C. atripectoralis* orientation from SE in mid-morning to SW in late afternoon is consistent with use of celestial cues for orientation. This is reflected in a significant, but weak, correlation between within-run mean bearing and time of day, and a significant difference in the distribution of bearings between the late afternoon period and the earlier periods. The increase in median bearing of about 10° per hour from 08:00 to 18:00 h is also consistent with use of a celestial cue.

There were too few observations on *P. lepidogenys* for a full examination of diurnal variation in orientation, but previous observations west of Lizard Island showed differences in orientation between morning and late afternoon that are consistent with use of celestial cues (Leis & Carson-Ewart 2003). Within-run precision during mid-morning to early afternoon (09:00–13:40 h, mean $\mathbf{r} = 0.72$) was lower than during mid to late afternoon (15:00–17:00 h, mean $\mathbf{r} = 0.82$), although the difference was of marginal significance ($p = 0.055$; Leis & Carson-Ewart 2003). Further, it was only during the afternoon period that among-run orientation in *P. lepidogenys* was significantly directional (Leis & Carson-Ewart 2003), and the late afternoon mean bearing was more westerly than the morning mean bearing. In the late afternoon *P. lepidogenys* larvae also had a shallower vertical distribution than they did in the morning, and this was also true for *C. atripectoralis* at 100 and 1000 m from shore (but not at 500 m; Leis 2004). In contrast, vertical distribution of *C. atripectoralis* did not differ significantly between cloudy and sunny periods (Leis 2004, unpubl. data), which might have been expected if larvae ascended in cloudy conditions to more readily detect downwelling, polarized light. If pomacentrid larvae use celestial cues for orientation, the underwater polarized light field would provide a stronger signal when the sun was lower in the sky in the late afternoon and it would be more readily detected at shallower depths (Novales Flamarique & Hawryshyn 1997).

The results presented here are largely consistent with the use of celestial cues for orientation by *C. atripectoralis* larvae, and to a lesser extent by *P. lepidogenys* larvae. Diver following observations are not likely to lead to more definitive conclusions. Manipulative field and laboratory experiments, including use of DISC methodology, will be necessary.

If larval fishes use celestial cues for orientation as implied by our results, there are several interesting implications. (1) Celestial cues allow orientation over large spatial scales (Waterman 1988) and without regard to distance from particular sources of cues, such as scents or sounds emanating from coral reefs. (2) One would expect that orientation would be adversely affected by cloud cover because clouds obscure some or all of the celestial cue. The fact that within-run precision in *C. atripectoralis* was lower under cloudy conditions than sunny conditions is consistent with this expectation. However, importantly, orientation abilities did not disappear under cloudy conditions. The sun position is discernible through cloud cover in many situations, and the skylight polarization pattern under heavy cloud, although reduced, is not qualitatively different from that under clear skies (Hegedüs et al. 2007). Therefore, orientation using celestial cues in cloudy conditions may still be possible, albeit more difficult. Many tropical areas have distinct rainy or monsoon seasons, and the increased cloud cover associated with these seasons could result in poorer orientation, and perhaps ultimately, poorer settlement, on a seasonal basis. (3) Wind causes surface waves and these alter the underwater light field in several ways (e.g. Stramska & Dickey 1998, Stramski & Tegowski 2001, Hieronymi & Macke 2012) including focusing/defocusing by waves, reflectance by bubbles caused by breaking waves, turbulence, and altered surface reflectivity. Potentially, these light field alterations could interfere with the ability of larval fishes to

detect celestial cues, causing difficulties for orientation when winds are high. (4) Certain anthropogenic activities such as eutrophication, increased turbidity due to run-off or dredging, or pollution such as oil spills can decrease the strength and visibility of celestial cues when viewed from underwater. These would also be likely to decrease orientation precision. (5) Celestial cues should be strongest, and therefore easiest to use, in the upper portions of the water column, so one would expect animals that utilize celestial cues for orientation would be shallow-living, as are the larvae of the 2 pomacentrid species studied here (Leis 1991, 2004).

Following compared to DISC

Following and DISC methods provided very similar orientation results for the 2 study species. In both methods, the large majority of individual runs were directional. Within-run precision was somewhat greater in Following data sets. However, this might be due to the somewhat different way that orientation is measured by the 2 methods, and the tendency of larvae in the DISC to move back and forth along the perimeter of the observation arena when their swimming trajectory is blocked. Between-method differences in median direction were found, but similar differences were found in within-method contrasts.

Aside from one DISC *C. atripectoralis* data set from west of Lizard Island, the magnitude of the cross-method differences in median bearings was nearly identical to that of within-Following differences. We can identify no obvious reason why the orientations estimated in that single DISC data set were so different from most of the Following data and the other DISC data. It was, however, the only *C. atripectoralis* DISC data set from west of Lizard Island, which is shallower than the east side of the island, and that may have influenced the results. We maintained the same DISC depth on both sides of the island for consistency, but *C. atripectoralis* larvae prefer somewhat shallower depths west of Lizard Island than off the east side (Leis 2004), although how this might influence horizontal orientation is not clear. It is also noteworthy that the Following data set from the same location during the same 2008 field trip was the only Following data set not manipulated by underwater sound broadcasts that did not have significant orientation to the south, and that its distribution of bearings was not significantly different from the DISC data set in question. In contrast, for *P. lepidogenys*, DISC and Following results from west of the island during the same 2008 field trip were nearly identical (non-significant, median orientation to NW).

SCUBA divers are very noisy (Radford et al. 2005), so it is noteworthy that the Following method, which includes the noise of the divers and the attendant support boat, provided results similar to those of the DISC, which involve neither diver nor boat noise. In contrast, the broadcasting of reef sounds — but not 'white noise' — during Following experiments did influence orientation of larvae (Leis et al. 2002). These observations firstly alleviate potential concerns that the noise associated with Following might result in misleading orientation data, and secondly, demonstrate that *C. atripectoralis* larvae can distinguish among different sounds even when other potentially masking sounds are present.

Thus, it seems that Following and DISC can both be used to study orientation of larval fishes in the ocean, and will provide similar results. In many ways the 2 methods are complementary, and use of both can increase confidence in the resulting data. Alternatively, if differences in orientation between the 2 methods are found, this would highlight the need for caution, and perhaps lead to better understanding of just what determines orientation. Each approach has strengths and weaknesses. For example, swimming speed and depth selection are readily studied by Following, but would be difficult to study with DISC. In contrast, DISC can be used at any depth, both day and night (with an infrared camera), and over long periods, which Following cannot. The lack of information on what larvae do at night is perhaps the largest gap in knowledge of larval behaviour, and DISC offers interesting possibilities in this area. Perhaps most important, DISC can be used to manipulate sensory cues to help delineate the sensory basis of orientation in ways that Following cannot. For example, a filter or opaque shade over the DISC can be used to study how altering the underwater light field affects orientation (e.g. Berenshtein et al. 2014). It is only through such experimental manipulations in the ocean that the implications of observations like those from the present study, suggesting that fish larvae use celestial cues for orientation, can be tested.

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