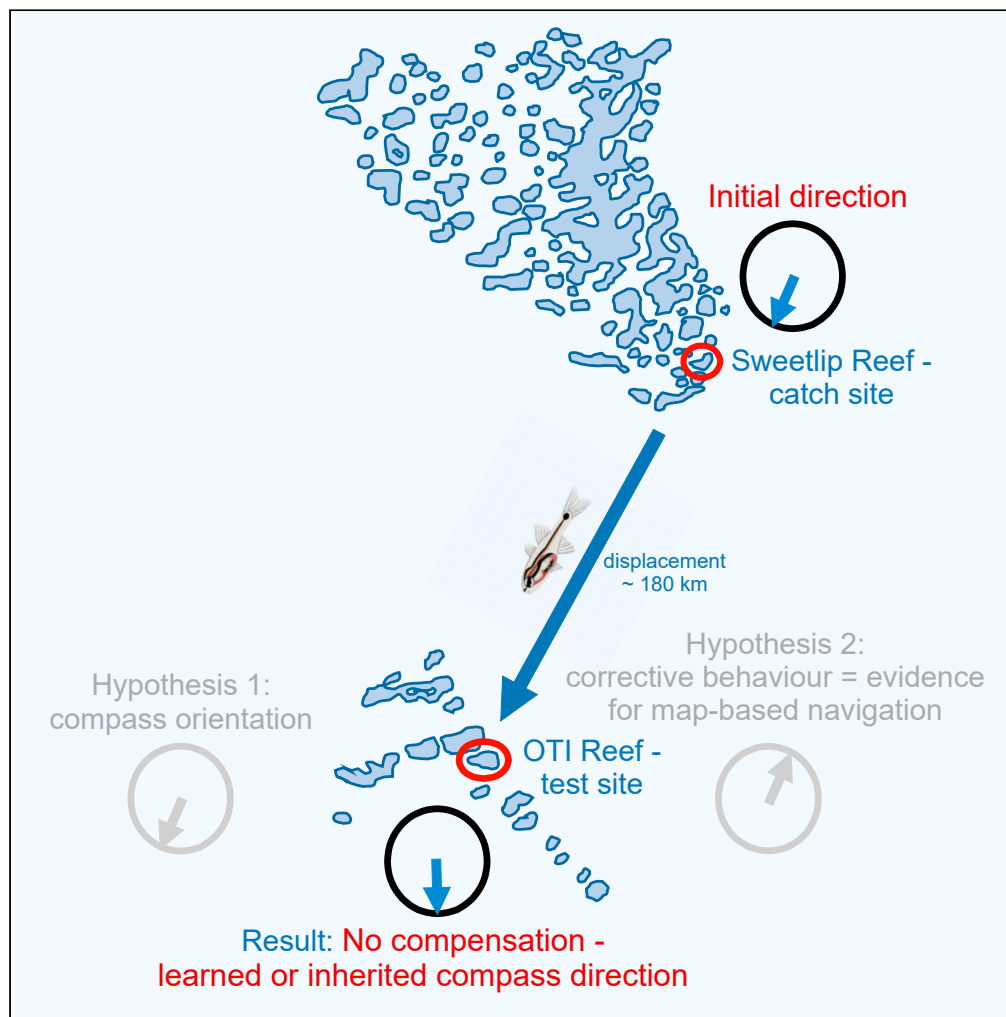


## Article

## Coral reef fish larvae show no evidence for map-based navigation after physical displacement



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**Highlights**

Physical displacement of *Ostorhinchus doederleini* juveniles over 180 km

Swimming direction at displacement site is indistinguishable from control direction

No signs of corrective orientation back toward the origin or disorientation

Coral reef fish show compass orientation rather than map-based navigation

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## Article

## Coral reef fish larvae show no evidence for map-based navigation after physical displacement

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## SUMMARY

Millions of minute, newly hatched coral reef fish larvae get carried into the open ocean by highly complex and variable currents. To survive, they must return to a suitable reef habitat within a species-specific time. Strikingly, previous studies have demonstrated that return to home reefs is much more frequent than would be expected by chance. It has been shown that magnetic and sun compass orientation can help cardinalfish maintain their innate swimming direction but do they also have a navigational map to cope with unexpected displacements? If displaced settling-stage cardinalfish *Ostorhinchus doederleini* use positional information during their pelagic dispersal, we would expect them to re-orient toward their home reef. However, after physical displacement by 180 km, the fish showed a swimming direction indistinguishable from original directions near the capture site. This suggests that the tested fish rely on innate or learned compass directions and show no evidence for map-based navigation.

## INTRODUCTION

Newly hatched coral reef fish larvae avoid predation by dispersing from their natal reef and spending several days to weeks in the open ocean before returning to a reef to settle. Larval dispersal routes have never been tracked, but modeling approaches assume dispersal distances in the range of a few to over 200 km depending on the species and ecological circumstances.<sup>1–3</sup> Dispersal distances for our study species, the fourline cardinalfish *Ostorhinchus doederleini* breeding at One Tree Island (OTI) were estimated to be 10–20 km for the first 8 days as planktonic larvae, based on the release of neutrally buoyant particles within an ocean circulation model.<sup>4,5</sup> Larval dispersal is the main driver of population connectivity and replenishment of reef fish populations.

Especially at the beginning of their journey, larvae, being poor swimmers, are exposed to highly complex and variable currents.<sup>6</sup> Unlike many migratory birds,<sup>7,8</sup> dispersing larvae cannot “stopover” and interrupt their migratory movements, e.g., during storms or by unusual currents. It is unknown how marine larval organisms cope with dislocations and whether they can correct for large-distance displacements.

Coral reef fish have orientation abilities that can help them find their way back to a reef and even to their natal reef.<sup>4</sup> Over longer distances of tens up to thousands of kilometers, time-compensated sun compass<sup>9</sup> and magnetic compass orientation,<sup>5,10</sup> among others, have been found. For shorter (some meters up to few km) distances, juvenile fish can perceive olfactory,<sup>4,11</sup> acoustic<sup>12</sup> and visual<sup>13</sup> signals of a reef. Although compass orientation provides directional information which enables them to maintain a consistent bearing, only map-based orientation would offer positional information that is needed to elicit corrective behavior.<sup>14–16</sup> Because landmarks are not available in the open ocean, this would require, for example, a magnetic map like the one known from experienced birds.<sup>17–21</sup> However, other sensory inputs such as an olfactory based map might be used as well.

To test for corrective behavior, we conducted displacement experiments. Several studies were performed in which juvenile marine animals were displaced virtually by changing magnetic field conditions that simulated locations along their potential migration route.<sup>22–27</sup> In contrast, physical displacement of marine animals has been performed in the past only with adult animals, which could have already explored their

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spatial environment before displacement, similar to homing pigeons. Thus, displacement would have occurred in familiar terrain, which could have greatly facilitated their return to their ancestral place. Homing abilities to such sites later in life were observed in individually tagged adult *O. doederleini* displaced within the OTI lagoon over distances of 1 and 2 km from collection sites.<sup>28</sup> The majority of tagged fish returned to their original tag sites within 3 days after displacement. Adult spiny lobsters oriented toward their capture site after a displacement by 12–37 km<sup>29</sup> and adult green turtles returned to their nesting beach when displaced 100–120 km,<sup>30</sup> which are places that could have been explored by the animals earlier.

Here, we tested whether juvenile coral reef fish, the fourline cardinalfish *O. doederleini*, have positional information available during their pelagic dispersal: Do they compensate for displacement over 180 km from Sweetlip Lagoon (SL) to OTI (Australia, Great Barrier Reef) (Figure 1) by changing their orientation toward the original location?

## RESULTS AND DISCUSSION

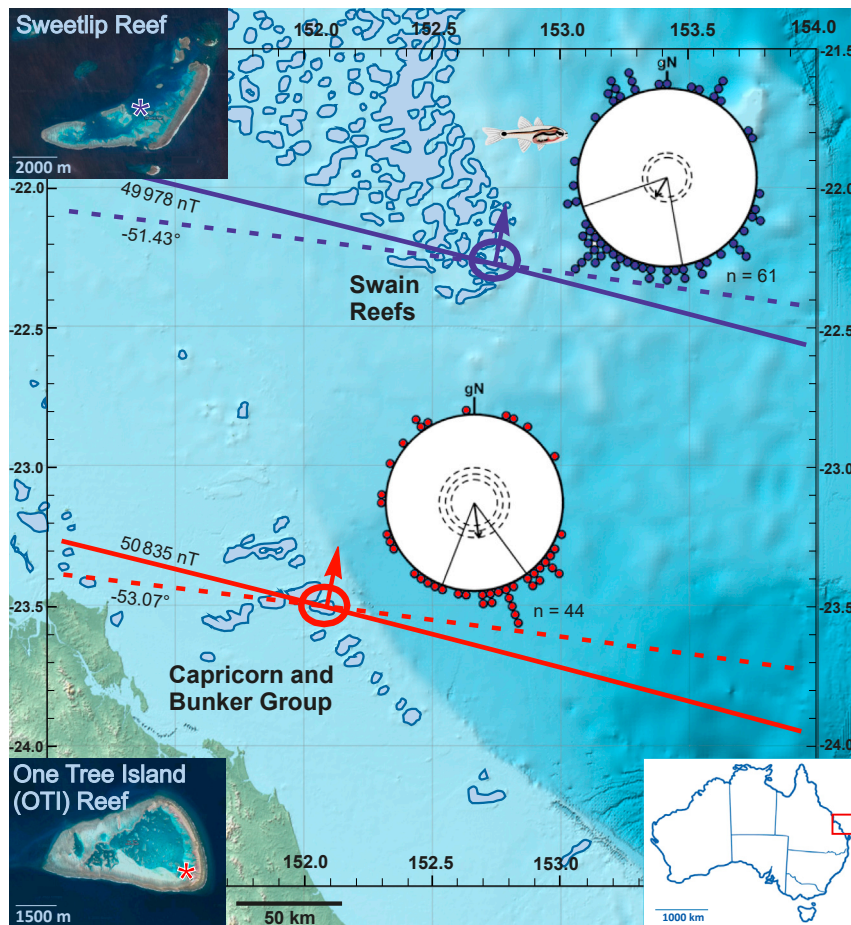
### No evidence of map-based navigation after displacement of juvenile *O. doederleini*

The physical displacement we performed corresponded to ca. 900nT difference in total magnetic field strength (49.978 nT SL vs. 50.853 nT OTI) and an inclination change of 1.64° (−51.43° SL vs. −53.07° OTI) (Figure 1), i.e., roughly comparable to the virtual magnetic displacement that in experienced songbirds had elicited re-orientation.<sup>31</sup> A total of 61 *O. doederleini* juveniles were tested for (sun) compass orientation under sunny skies (see STAR Methods) in SL (aboard a vessel), 44 of which were subsequently transported to OTI and retested there. In SL, the group mean orientation of the fish was directed toward south-southwest (210°, n = 61, r = 0.249, p = 0.023, 95% confidence interval of the mean: 165–255°, Figure 1, raw data see Tables S3 and S4). When retested at OTI, the orientation of the fish was toward south (173°, n = 44, r = 0.399, p < 0.001, 95% confidence interval of the mean: 143–203°, Figure 1, raw data see Tables S5 and S6) and statistically indistinguishable from the one displayed at the original location (Figure 1, the 95% confidence intervals of the two distributions overlap widely - range of overlap 165°–203°; Mardia-Watson-Wheeler W = 5.531, p = 0.063). We also found no signs of corrective orientation behavior back toward the origin when conducting a V-test with the prespecified azimuthal direction of 27° between OTI and SL (V test (u) = 3.097, V test (p) = 0.999). Thus, even though *O. doederleini* juveniles can use a magnetic compass for orientation,<sup>5</sup> they do not seem to possess a magnetic-map useful at the tested distance. It appears that not only does the magnetic change not elicit reorientation, neither did the other available cues such as inertial, sun and olfactory (the fish were tested in OTI water at OTI) cues. This indicates that the compass orientation in this species is rather robust and probably innate or acquired very early in development and that the fish at this life-stage are likely not capable of ‘true navigation’.

To be useful for orientation, the sensitivity thresholds of fish larvae to changes in the magnetic field must correspond to their potential migration distances. However, precise sensitivity thresholds in magnetic field perception have never been studied. The actual dispersal distance is also not known, but considering the dispersal duration of 3 weeks, it is likely that very few fish would be displaced more than 180 km in nature.<sup>3,32</sup> To estimate whether natural exchange between the two populations (SL and OTI) occur at all, we performed a population genetic analysis.

### Microsatellite analysis reveals genetic exchange between populations at SL and OTI

To identify the potential site of origin of 53 juvenile *O. doederleini* sampled at SL, we analyzed whether they were genetically most similar to the adult populations at OTI or SL. Considering 13 microsatellite loci (see Tables S1 and S2) we found a statistically significant genetic difference among adults from OTI (n = 43) and SL (n = 40) (Dest OTIad-SLad = 0.064, p = 0.001). (Dest values ranging from 0 to 1, with 0 no genetic difference; 1 = completely different). With more than 90% probability, 33 (62%) freshly settled fish from the SL could be assigned to the SL adults and 11 (21%) were more genetically similar to the OTI adults or to any genetically similar but non-sampled population. Owing to logistical constraints, we only genetically sampled the SL and OTI populations. For nine individuals (17%) an assignment of less than 90% indicated an uncertain place of origin. The low frequency of genetic assignments that could possibly have come from OTI or from genetically similar populations indicate that there is not a homogeneous population between the two studied reef sites and therefore not high mixing of populations from OTI and SL at SL, so the displacement distance we chose is at the upper realistic limit. Nevertheless, the data suggest that there could be some degree of genetic exchange between populations in the SL region and OTI region. An



**Figure 1. Juvenile *O. doederleini* did not significantly change their orientation after physical displacement**

Map of the southern great barrier reef (Australia) including the Swain Reefs (with Sweetlip Lagoon circled in dark blue -  $22^{\circ}16'25.0''S$   $152^{\circ}44'24.7''E$ ) and the Capricorn and Bunker Reef Group (with One Tree Island circled in red -  $23^{\circ}29'49.0''S$   $152^{\circ}04'46.4''E$ ). The displacement distance between the two experimental sites amounts to about 180 km. The two sites also differ in terms of geomagnetic total intensity (solid isolines) and inclination (dashed contour lines). The local magnetic North direction is indicated by an arrow at each site. The orientation of juvenile fish was tested in circular bowls under sunny skies. The fish's headings were recorded by GoPro cameras fixed underneath the bowls. Within the Sweetlip Lagoon, the fish were tested on board of the research vessel "Bindy", on OTI they were tested on the beach. The circular diagrams show the group compass orientation of all tested fish: mean direction SL:  $210^{\circ}$ ,  $r = 0.249$ ,  $p = 0.023$ ; mean direction OTI:  $173^{\circ}$ ,  $r = 0.399$ ,  $n = 44$ ,  $p = 7.18E-4$ . Each dot indicates the mean swimming direction of one individual tested at least twice in similar conditions between 8:00 and 11:00 a.m., and/or between 2:00 and 6:00 p.m. The black arrows indicate the group mean vectors. The solid lines flanking the mean vectors indicate the 95% confidence intervals for the mean direction. The dashed lines indicate statistical significance according to the Rayleigh test for  $p < 0.05$ ,  $p < 0.01$  (both in left and right diagram) and  $p < 0.001$  (only left diagram). gN = geographic north; ns = not significant.

even higher degree of genetic exchange among reef populations around OTI and the Swains reefs over several years was suggested in a previous publication.<sup>33</sup>

### Currents seem to be the most important factors in connecting reef populations

Summarizing all obtained data (<sup>5,9</sup> and the present study), we conclude that, similar to inexperienced juvenile solitary migratory birds,<sup>15,34–36</sup> cardinalfish, at least during their pelagic phase and shortly thereafter, lack corrective orientation that would indicate map-based navigation. This suggests that only those larvae that find a window of time when the currents match the normal pattern to which their innate or learned compass orientation has evolved can find their way back to a reef or even their home reef. Apparently, a high reproductive output of thousands of eggs and larvae can produce enough successful settlers to replenish

local populations through compass orientation alone. Orientation using the sun and magnetic compasses, as well as olfactory and acoustic preferences, can help guide fish to reefs, but currents probably remain the most important factor in connecting reef populations.<sup>37</sup> If the flow regime changes because of climate and temperature effects, fish larvae may not respond to major positional changes, which could negatively affect metapopulation structure, replenishment, and connectivity of fish populations in coral reefs.

### Limitations of the study

Our studies, as well as all other studies of larval fish dispersal, are limited by the small size of the animals, which does not allow for the attachment of tracking devices or markers. We have attempted to compensate for this by using genetic markers that characterize the genetic fingerprint of populations; however, the power is limited because it was not possible to analyze all reef populations.

### STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
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- EXPERIMENTAL MODEL AND SUBJECT DETAILS
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- METHOD DETAILS
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  - Data analysis and statistics

### SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2023.106950>.

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### AUTHOR CONTRIBUTIONS

G.G., H.M., M.W., and L.S. designed the study; G.G., A.B., L.S., F.C., and M.K. organized the logistics and collected the experimental animals; G.G., A.B., L.S., and F.C. performed the experiments. L.S., F.C., and B.L. analyzed the data. P.K. developed microsatellite markers; N.J. tested microsatellite markers and N.J. and G.G. performed the population genetic analysis. L.S. and G.G. wrote the article, which all authors commented on.

### DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<i>Biological samples</i>		
Sw.P1ad.1.101	Sweetlip Lagoon, P1 adult	–
Sw.P1ad.1.102	Sweetlip Lagoon, P1 adult	–
Sw.P1ad.1.103	Sweetlip Lagoon, P1 adult	–
Sw.P1ad.1.104	Sweetlip Lagoon, P1 adult	–
Sw.P1ad.2.105	Sweetlip Lagoon, P1 adult	–
Sw.P1ad.2.106	Sweetlip Lagoon, P1 adult	–
Sw.P1ad.2.107	Sweetlip Lagoon, P1 adult	–
Sw.P1ad.2.108	Sweetlip Lagoon, P1 adult	–
Sw.P1ad.3.109	Sweetlip Lagoon, P1 adult	–
Sw.P1ad.3.110	Sweetlip Lagoon, P1 adult	–
Sw.P1ad.3.111	Sweetlip Lagoon, P1 adult	–
Sw.P1ad.3.112	Sweetlip Lagoon, P1 adult	–
Sw.P1ad.4.113	Sweetlip Lagoon, P1 adult	–
Sw.P1ad.4.114	Sweetlip Lagoon, P1 adult	–
Sw.P1ad.4.115	Sweetlip Lagoon, P1 adult	–
Sw.P1ad.4.116	Sweetlip Lagoon, P1 adult	–
Sw.P1ad.5.117	Sweetlip Lagoon, P1 adult	–
Sw.P1ad.5.118	Sweetlip Lagoon, P1 adult	–
Sw.P1ad.5.119	Sweetlip Lagoon, P1 adult	–
Sw.P1ad.5.120	Sweetlip Lagoon, P1 adult	–
Sw.P2ad.6.121	Sweetlip Lagoon, P2 adult	–
Sw.P2ad.6.122	Sweetlip Lagoon, P2 adult	–
Sw.P2ad.6.123	Sweetlip Lagoon, P2 adult	–
Sw.P2ad.6.124	Sweetlip Lagoon, P2 adult	–
Sw.P2ad.7.125	Sweetlip Lagoon, P2 adult	–
Sw.P2ad.7.126	Sweetlip Lagoon, P2 adult	–
Sw.P2ad.7.127	Sweetlip Lagoon, P2 adult	–
Sw.P2ad.7.128	Sweetlip Lagoon, P2 adult	–
Sw.P2ad.8.129	Sweetlip Lagoon, P2 adult	–
Sw.P2ad.8.130	Sweetlip Lagoon, P2 adult	–
Sw.P2ad.8.131	Sweetlip Lagoon, P2 adult	–
Sw.P2ad.8.132	Sweetlip Lagoon, P2 adult	–
Sw.P2ad.9.133	Sweetlip Lagoon, P2 adult	–
Sw.P2ad.9.134	Sweetlip Lagoon, P2 adult	–
Sw.P2ad.9.135	Sweetlip Lagoon, P2 adult	–
Sw.P2ad.9.136	Sweetlip Lagoon, P2 adult	–
Sw.P2ad.10.137	Sweetlip Lagoon, P2 adult	–
Sw.P2ad.10.138	Sweetlip Lagoon, P2 adult	–
Sw.P2ad.10.139	Sweetlip Lagoon, P2 adult	–
Sw.P2ad.10.140	Sweetlip Lagoon, P2 adult	–
Sw.eher.s.141	Sweetlip Lagoon, juvenile	–

(Continued on next page)



*Continued*

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Sw.eher.s.142	Sweetlip Lagoon, juvenile	–
Sw.eher.s.143	Sweetlip Lagoon, juvenile	–
Sw.eher.s.144	Sweetlip Lagoon, juvenile	–
Sw.eher.s.145	Sweetlip Lagoon, juvenile	–
Sw.eher.s.146	Sweetlip Lagoon, juvenile	–
Sw.eher.s.147	Sweetlip Lagoon, juvenile	–
Sw.eher.s.148	Sweetlip Lagoon, juvenile	–
Sw.sued.149	Sweetlip Lagoon, juvenile	–
Sw.sued.150	Sweetlip Lagoon, juvenile	–
Sw.sued.151	Sweetlip Lagoon, juvenile	–
Sw.sued.152	Sweetlip Lagoon, juvenile	–
Sw.sued.153	Sweetlip Lagoon, juvenile	–
Sw.sued.154	Sweetlip Lagoon, juvenile	–
Sw.sued.155	Sweetlip Lagoon, juvenile	–
Sw.sued.156	Sweetlip Lagoon, juvenile	–
Sw.eher.n.157	Sweetlip Lagoon, juvenile	–
Sw.eher.n.158	Sweetlip Lagoon, juvenile	–
Sw.eher.n.159	Sweetlip Lagoon, juvenile	–
Sw.eher.n.160	Sweetlip Lagoon, juvenile	–
Sw.eher.n.161	Sweetlip Lagoon, juvenile	–
Sw.eher.n.162	Sweetlip Lagoon, juvenile	–
Sw.nord.163	Sweetlip Lagoon, juvenile	–
Sw.nord.164	Sweetlip Lagoon, juvenile	–
Sw.nord.165	Sweetlip Lagoon, juvenile	–
Sw.nord.166	Sweetlip Lagoon, juvenile	–
Sw.nord.167	Sweetlip Lagoon, juvenile	–
Sw.untest.168	Sweetlip Lagoon, juvenile	–
Sw.untest.169	Sweetlip Lagoon, juvenile	–
Sw.untest.170	Sweetlip Lagoon, juvenile	–
Sw.untest.171	Sweetlip Lagoon, juvenile	–
Sw.untest.172	Sweetlip Lagoon, juvenile	–
Sw.untest.173	Sweetlip Lagoon, juvenile	–
Sw.untest.174	Sweetlip Lagoon, juvenile	–
Sw.untest.175	Sweetlip Lagoon, juvenile	–
Sw.untest.176	Sweetlip Lagoon, juvenile	–
Sw.untest.177	Sweetlip Lagoon, juvenile	–
Sw.untest.178	Sweetlip Lagoon, juvenile	–
Sw.untest.179	Sweetlip Lagoon, juvenile	–
Sw.untest.180	Sweetlip Lagoon, juvenile	–
Sw.untest.181	Sweetlip Lagoon, juvenile	–
Sw.untest.182	Sweetlip Lagoon, juvenile	–
Sw.untest.183	Sweetlip Lagoon, juvenile	–
Sw.no.cl.ch.184	Sweetlip Lagoon, juvenile	–
Sw.no.cl.ch.185	Sweetlip Lagoon, juvenile	–
Sw.no.cl.ch.186	Sweetlip Lagoon, juvenile	–

(Continued on next page)

*Continued*

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Sw.no.cl.ch.187	Sweetlip Lagoon, juvenile	–
Sw.no.cl.ch.188	Sweetlip Lagoon, juvenile	–
Sw.no.cl.ch.189	Sweetlip Lagoon, juvenile	–
Sw.no.cl.ch.190	Sweetlip Lagoon, juvenile	–
Sw.no.cl.ch.191	Sweetlip Lagoon, juvenile	–
Sw.no.cl.ch.192	Sweetlip Lagoon, juvenile	–
Sw.no.cl.ch.193	Sweetlip Lagoon, juvenile	–
OTI.ad.194	One Tree Island Reef, adult	–
OTI.ad.195	One Tree Island Reef, adult	–
OTI.ad.196	One Tree Island Reef, adult	–
OTI.ad.197	One Tree Island Reef, adult	–
OTI.ad.198	One Tree Island Reef, adult	–
OTI.ad.199	One Tree Island Reef, adult	–
OTI.ad.200	One Tree Island Reef, adult	–
OTI.ad.201	One Tree Island Reef, adult	–
OTI.ad.202	One Tree Island Reef, adult	–
OTI.ad.203	One Tree Island Reef, adult	–
OTI.ad.204	One Tree Island Reef, adult	–
OTI.ad.205	One Tree Island Reef, adult	–
OTI.ad.206	One Tree Island Reef, adult	–
OTI.ad.207	One Tree Island Reef, adult	–
OTI.ad.208	One Tree Island Reef, adult	–
OTI.ad.209	One Tree Island Reef, adult	–
OTI.ad.210	One Tree Island Reef, adult	–
OTI.ad.211	One Tree Island Reef, adult	–
OTI.ad.212	One Tree Island Reef, adult	–
OTI.ad.213	One Tree Island Reef, adult	–
OTI.ad.214	One Tree Island Reef, adult	–
OTI.ad.215	One Tree Island Reef, adult	–
OTI.ad.216	One Tree Island Reef, adult	–
OTI.ad.217	One Tree Island Reef, adult	–
OTI.ad.218	One Tree Island Reef, adult	–
OTI.ad.219	One Tree Island Reef, adult	–
OTI.ad.220	One Tree Island Reef, adult	–
OTI.ad.221	One Tree Island Reef, adult	–
OTI.ad.222	One Tree Island Reef, adult	–
OTI.ad.223	One Tree Island Reef, adult	–
OTI.ad.224	One Tree Island Reef, adult	–
OTI.ad.225	One Tree Island Reef, adult	–
OTI.ad.226	One Tree Island Reef, adult	–
OTI.ad.227	One Tree Island Reef, adult	–
OTI.ad.228	One Tree Island Reef, adult	–
OTI.ad.229	One Tree Island Reef, adult	–
OTI.ad.230	One Tree Island Reef, adult	–
OTI.ad.231	One Tree Island Reef, adult	–

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
OTI.ad.S.A. 232	One Tree Island Reef, adult	–
OTI.ad.S.A. 233	One Tree Island Reef, adult	–
OTI.ad.S.A. 234	One Tree Island Reef, adult	–
OTI.ad.S.A. 235	One Tree Island Reef, adult	–
OTI.ad.S.A. 236	One Tree Island Reef, adult	–

**Chemicals, peptides, and recombinant proteins**

Chelex 100 Resin	BioRad	Cat# 1422842
Dye (D2-PA, D3-PA, D4-PA)	Sigma Aldrich	N/A

**Critical commercial assays**

HotStarTaq	Quiagen	203203
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**Deposited data**

Microsatellites	–	<a href="https://www.ebi.ac.uk/ena/browser/view/PRJEB55917">https://www.ebi.ac.uk/ena/browser/view/PRJEB55917</a> ; Accession number: PRJEB55917
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**Experimental models: Organisms/strains**

<i>Ostorhinchus doederleini</i>	Juveniles SL: Wild caught, Sweetlip reef, Australia	N/A
<i>Ostorhinchus doederleini</i>	Adults SL: Wild caught, Sweetlip reef, Australia	N/A
<i>Ostorhinchus doederleini</i>	Adults OTI: Wild caught, One Tree Island reef, Australia	N/A

**Oligonucleotides**

Primers		
Od.DN412; F: TGTAACACGACGGCCAGTCCACAG ACAGAAAACAGCGT, R: GACCCAGAACCTGCTAACAC	this paper	N/A
Od.DN20096; F: TGTAACACGACGGCCAGTACATTTTCC CAATTCGCAATAGA, R: GAACTGGTGCTGCTTCGTAC	this paper	N/A
Od.DN20612; F: TGTAACACGACGGCCAGTGCCTCA GTCTCTATTTGGC, R: GAACTGGTGCTGCTTCGTAC	this paper	N/A
Od.DN22986; F: TGTAACACGACGGCCAGTTGAAAGGG TGAGAGGTCGTC, R: GGAAGACAAAGCTGGTGGTG	this paper	N/A
Od.DN9619; F: TGTAACACGACGGCCAGTTTTTCAGTCA CAGTACCGGCT, R: AGAGTGACGGGATTGTGAG	this paper	N/A
Od.DN16204; F: TGTAACACGACGGCCAGTAGTTGCAT GCACATACACACT, R: AGCAGGCTGTTTTGGTTGAT	this paper	N/A
Od.DN20621; F: TGTAACACGACGGCCAGTACCCAAA TGAAAGCAGCTCA, R: CATGGAGAGAATGTGTGTTTT	this paper	N/A
Od.DN20664; F: TGTAACACGACGGCCAGTTGTTGTGA GTGTGATGTCCTG, R: AGGAGGGAGGTGGGACTAAT	this paper	N/A
Od.DN20695; F: TGTAACACGACGGCCAGTTGGTGACT CTGAACAAGCCT, R: AAAGGAAAACACGTGTGAGC	this paper	N/A
Od.DN21433; F: TGTAACACGACGGCCAGTCTTCGCCT TGTTTCATCACCC, R: AAAGGAAAACACGTGTGAGC	this paper	N/A
Od.DN51627; F: TGTAACACGACGGCCAGTGCTCAGTC ACTGCAGATGTT, R: TCCTGCCTGATATGTGTCTGA	this paper	N/A

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**Continued**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Od.DN28813; F: TGTA AACGACGGCCAGTTGCCCCAC AATTGATGCTG, R: AAGGGGATGGGAGACTCTT	this paper	N/A
<b>Software and algorithms</b>		
Demetics	R Foundation for Statistical Computing, Vienna, Austria	<a href="https://rdrr.io/cran/DEMEtics/">https://rdrr.io/cran/DEMEtics/</a>
RStudio	RStudio, Inc. Boston, MA, US	<a href="https://www.rstudio.com/">https://www.rstudio.com/</a>
Oriana 4	Kovach Computing Services	<a href="https://www.kovcomp.co.uk/oriana/">https://www.kovcomp.co.uk/oriana/</a>
Mathematica 13.0	Wolfram Research	<a href="https://www.wolfram.com/mathematica">https://www.wolfram.com/mathematica</a>
<b>Other</b>		
Fluxgate Magnetometer (FVM-400)	Meda Inc., Dulles, VA, USA	–
CEQ™ 2000XL DNA Analysis System	Beckman Coulter	Cat# 718286-AA

**RESOURCE AVAILABILITY****Lead contact**

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Lisa Spiecker ([lisa.spiecker@uni-oldenburg.de](mailto:lisa.spiecker@uni-oldenburg.de)).

Alternative contacts: [gabriele.gerlach@uni-oldenburg.de](mailto:gabriele.gerlach@uni-oldenburg.de), [michael.winklhofer@uni-oldenburg.de](mailto:michael.winklhofer@uni-oldenburg.de), [henrik.mouritsen@uni-oldenburg.de](mailto:henrik.mouritsen@uni-oldenburg.de).

**Materials availability**

This study did not generate new unique reagents.

**Data and code availability**

All original code is available from the [lead contact](#) upon request. Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

**EXPERIMENTAL MODEL AND SUBJECT DETAILS****Ethics statement**

The fish were collected legally for scientific purposes utilizing the Great Barrier Reef Marine Park Authority permit number G18/38392.1 and the Queensland Department of Agriculture and Fisheries (DAF) General Fisheries Permit No 2111760.

**Experimental areas**

Experimental locations were chosen according to the main currents present in the selected areas. Within the Swain Reefs, currents are complex, and on the eastern side the main current points southerly while it points in the opposite direction, to the north-northwest, at OTI. However, reefs as well as tides are influencing local currents.

**Experimental animals**

Newly settled *O. doederleini* juveniles were collected on small reef patches in the Sweetlip Reef in January 2020 at a maximum depth of about 10 m. Because juveniles settle at night, we collected them early in the morning and kept them until testing in 20 L buckets on board of a research vessel. Testing was performed with each fish individually in small tanks made of plastic and filled with oxygenated, fresh seawater. Once per day, the juveniles were fed *Artemia* sp. shrimp or plankton that had been caught in plankton traps during the night.

*O. doederleini*, also known as the fourline cardinalfish, are paternal mouthbrooders that release their larvae into the water when hatching. Directly thereafter, they are taken away from the reef by the currents. Drawn into the open ocean, they spend 15.7 to 26.0 days (mean = 19.9)<sup>38</sup>; for the first 8 days as planktonic larvae a

dispersal distance of approximately 10–20 km has been extrapolated.<sup>4,5</sup> Population genetic studies performed by Gerlach et al.<sup>4</sup> have shown that up to 60% of juvenile fourline cardinalfish originated from the reef in which they were about to settle.

## METHOD DETAILS

### Sun compass orientation tests

For testing sun compass orientation, each fish was transferred from its plastic tank into the round testing bowl. Each fish was tested individually in one of the 9 experimental bowls (20 cm in diameter, 8 cm in height) made of plastic, with a base made of glass, and filmed using a GoPro camera (GoPro Hero 4 silver) fixed in place on a rail underneath the bowls. When working on the boat, a compass was placed in the center of the experimental setup and its directional reading was also recorded so that the orientation data of the fish could be subsequently compensated for boat movements. Each recording was a time-lapsed video, obtained by taking a picture every 10 s. After 3 min of acclimatization, each fish was filmed for a total of 20 min. In intervals of 30 s, we recorded the geographic position of the fish's head relative to the center of the bowl by overlaying the video with a compass (printed on a transparent plastic film) and on the boat additionally comparing it to the recorded magnetic compass direction within the setup. This way, 40 data points were collected for each experimental run.

Thereafter fish were transferred back to their individual plastic tanks and the test bowls were rinsed with fresh ocean water before starting a new set of experiments. Each fish was tested at least twice.

Previous displacement experiments with newts<sup>39</sup> suggest that accurate homing orientation after displacement requires that the animals are held overnight at the testing site, and since this is also standard procedure for previous experiments with birds (e.g.<sup>20,40</sup>), we gave the fish at least one night to acclimatize after being transported from SL to OTI before we started the orientation tests.

### Magnetic field data

The geomagnetic field parameters total intensity *F*, inclination *I*, and declination *D* in the study region for Jan 2020 were predicted on the basis of the World Magnetic Model (WMM), using the online calculator hosted by the National Centers for Environmental Information of the United States government, <https://www.ngdc.noaa.gov/geomag/calculators/magcalc.shtml#igrfgrid>, for a geographic grid (151°E to 154°E, 24.5°S to 21.5°S, each in steps of 0.1°) covering the study region. The selected pairs of isolines of *F* and *I* depicted in Figure 1, were computed with Mathematica 13 (Wolfram Research) from the data grid. The relief data of the study region were produced with GeoGraphics Wolfram Language function (Mathematica 13, Wolfram Research). Local magnetic field readings at the study sites at Sweetlip Lagoon and One Tree Island were taken with a three-axis fluxgate magnetometer (FVM-400, Meda Inc., Dulles, VA, USA) and found to agree with the WMM predicted values within 100 nT and 0.2 deg in intensity and inclination, respectively. The magnetic compass deviations measured on the ship deviated less than 1° from parallel measurements of the field off the boat, which indicates that the disturbance of the magnetic field by the boat was minimal. Furthermore, since the same fish were tested on the boat and on the beach and showed the same directed orientation in both cases, it is very unlikely that the minimal magnetic disturbances on the boat will have had any effects on their behavior.

### Population genetic analysis

DNA was isolated from tissue using chelating resin following the manufacturer's protocol (BioRad, Chelex 100 Resin). We used two previously described polymorphic DNA microsatellite markers: Ad65, Ad94.<sup>41</sup> Further microsatellite loci were identified from a transcriptome analysis (see Table S1).

PCR was carried out as described in Miller-Sims et al.<sup>41</sup> PCR fragments were separated and scored by eye by the same person (GG) on a Beckman-Coulter CEQTM 2000XL DNA analysis system. The amplification of microsatellites per sample was repeated until the fragment length could be determined unequivocally.

1 μL of DNA was used as a template for PCR with a universal M13 sequence to add a dye (D2-PA, D3-PA, D4-PA; Sigma Aldrich) to each locus-specific primer pair via an additional chimeric forward primer 20. PCR was performed in a total volume of 10.3 μL and contained 0.7 μL template DNA, 2.95 μL ddH<sub>2</sub>O, 2 μL 5x Q-Solution, 1 μL Coral Red Buffer including MgCl, 2 μL MgCl 25 nM, 0.25 μL of universal forward dye primer

and reverse locus-specific primer (10  $\mu\text{mol}$ ), and 0.1  $\mu\text{L}$  of locus-specific forward primer. Finally, 1  $\mu\text{L}$  dNTP (2.5 nM) and 0.05  $\mu\text{L}$  Hot Star taq (5 U/ $\mu\text{L}$ , Quiagen) were added. PCR conditions were set as follows: Pre-denaturation at 94°C for 5 min, followed by 20 cycles of denaturation at 94°C for 30 s, annealing at the locus-specific annealing temperature (Table S1) for 45 s, and elongation at 72°C for 45 s. In a second cycle, the dyed universal primer was then added to the product with 20 cycles of denaturation at 94°C for 30 s, annealing at 53°C for 45 s, and elongation at 72°C for 45 s. The final annealing was performed at the location-specific annealing temperature. Final elongation at 72°C was performed for 10 min. Finally, the products were run on the Beckman CEQ 2000 XL capillary sequencer. Allele fragment length was evaluated by eye using a 400 bp size standard on the Beckman CEQ Workbench. As a measure of genetic differentiation, *Dest*-values were calculated using the DEMETics package in R.<sup>42</sup>

We derived the probability density of allele frequencies in each population by using a Bayesian approach<sup>43</sup> implemented in the program GeneClass2.<sup>44</sup> The test has power to detect immigrant ancestors, for these data, up to two generations in the past even though the overall differentiation of allele frequencies among populations is low.

## QUANTIFICATION AND STATISTICAL ANALYSIS

### Data analysis and statistics

Using the program Oriana 4,<sup>45</sup> we calculated the mean bearing of the fish's position every 30 s. We only used tests in which the fish showed a significant bearing at the  $p < 0.05$  level according to the Rayleigh test calculated in Oriana. Second order mean directions were calculated from the 2–4 tests per individual. This procedure is standardly used in the literature describing behavioral tests in orientation cages performed with migratory birds (e.g.<sup>17,20,31,40</sup>) as well as in previous publications reporting sun compass as well as magnetic compass orientation in *O. doederleini*.<sup>5,9</sup> A Mardia-Watson-Wheeler test was performed to compare the datasets from the 2 locations. The V-test was performed with a prespecified direction of 27.111°, which was calculated with the Mathematica function GeoDirection[].