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BRIEF COMMUNICATION

Recruiting a long way from home: Domino damselfish Dascyllus trimaculatus can detect new temperate coral habitat and conspecifics

Max J. O'Connell ¹ 💿 🛛	Ashley M. Fowler ^{1,2}	Ι	Sam J. Allan ¹	I	Giglia A. Beretta ¹
David J. Booth ¹					

¹School of Life Sciences, University of Technology Sydney, Sydney, New South Wales, Australia

²New South Wales Department of Primary Industries, Sydney Institute of Marine Science, Mosman, New South Wales, Australia

Correspondence

Max J. O'Connell, School of Life Sciences, Building 4, Level 6, University of Technology Sydney, NSW 2007, Australia. Email: max.oconnell@uts.edu.au

Funding information University of Technology Sydney

Abstract

Climate change is driving tropicalisation of temperate reefs, yet it is unclear how range-shifting tropical fishes locate suitable habitat. The authors tested whether juvenile tropical damsels *Dascyllus trimaculatus* could detect rare coral habitat (*Pocillopora aliciae*) and conspecifics on temperate rocky reefs using olfactory and visual preference experiments. For all cues, individuals selected and spent more time than expected in aquarium areas with the cue present, which included coral odour and conspecific odour and visual cues. This indicates that vagrant coral-reef fish can detect rare suitable habitat outside of their natal range.

KEYWORDS

Dascyllus trimaculatus, olfaction, sensory mechanisms, temperate coral habitat, tropicalisation

Strengthening and poleward penetration of Western Boundary Currents under climate change facilitate the movement of tropical propagules such as reef fish larvae into temperate areas (Stobutzki & Bellwood, 1994; Stobutzki & Bellwood, 1997). In eastern Australia, the strengthening East Australian Current (EAC) (Ridgway & Hill, 2009; Suthers *et al.*, 2011), in conjunction with warming ocean temperatures, has facilitated an increase in "vagrant" coral reef fish arrivals into temperate reefs (Booth *et al.*, 2007; Fowler *et al.*, 2018). Nonetheless, other factors such as habitat and local species interactions in the receiving environment (Harriott & Banks, 2002; Nakamura *et al.*, 2013) can limit the establishment and expansion of invading tropical populations.

Reef fish larvae have strong sensory capacity (Leis *et al.*, 2002), which can play an important role in location and selection of suitable habitat during the transitioning from a pelagic to benthic life stage. The use of olfactory and visual cues at various stages, to orientate and discern predators, has been demonstrated within and around natal reefs (Kingsford *et al.*, 2002; Lecchini *et al.*, 2014). Nonetheless, the ability of tropical reef fish to locate suitable habitat outside their natal range has received little attention. In this study, the authors examine the use of visual and olfactory sensory mechanisms to understand how such tropical vagrant fishes could locate suitable habitat in novel temperate ecosystems, where suitable habitat may be considerably scarcer and limiting than in natal regions.

Based on previous tropical studies, coral reef fish likely settle at night (Sweatman, 1988), suggesting that visual cues may be less effective and olfactory cues may play the key role in habitat detection. As a result of the differences in habitat structure and environmental factors compared to their natal locations, olfactory settlement cues may be atypical, and the normal settlement process for these individuals may be affected.

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The three-spot damselfish, *Dascyllus trimaculatus*, is a coral reef fish that is transported poleward as larvae on Australia's east coast and has previously been observed in Sydney's temperate rocky reefs (Fowler *et al.*, 2018). *D. trimaculatus* is known to inhabit anemones in its natal range (Schmitt & Holbrook, 2000), but in temperate rocky reefs *D. trimaculatus* use a sub-tropical branching coral species, *Pocillopora aliciae* (Schmidt-Roach *et al.*, 2013) which was first observed in Sydney in 2013 (Booth & Sear, 2018). It is the region's only known branching scleractinian coral and may also be expanding its range poleward, thereby facilitating range expansions of tropical fauna that use the species as habitat.

This study examined habitat and conspecific preferences, and their sensory mechanisms, in juvenile *D. trimaculatus*. Specifically, the authors asked: (a) Can *D. trimaculatus* differentiate and select *P. aliciae* through olfactory chemical cues alone? (a) Can *D. trimaculatus* differentiate and select for conspecific olfactory cues? (c) Do *D. trimaculatus* select conspecifics over heterospecifics using visual cues? Understanding the specific senses and preferences involved with habitat selection can help explain how tropical reef fish find suitable habitat, and the role of conspecifics in extra-natal environments, which will assist prediction of range shifts because of climate change.

Because of research diving prohibition resulting from the COVID-19 pandemic, and a poor recruitment year for *D. trimaculatus* in south-eastern Australia, all fish used in experiments were sourced from an aquarium supplier (Cairns Marine Pty. Ltd.) that collected wild juveniles from coral reef located off Cairns, Australia. The authors sourced the smallest individual fish available, ranging from 27 to 49 mm in total length (L_T) (mean of 37.05 ± 1.5 mm). These represented young recruits, although they were larger than new settlers (*c.* 15 mm L_T).

An opaque circular aquarium (hereafter "arena"; 110 cm diameter \times 45 cm height) was used to test the selection of different olfactory and visual cues by *D. trimaculatus*. The arena was divided equally into four quadrants with an open centre area (30 cm diam.) for individuals to view quadrants before selection. An acclimation tube with a pulley system was used to release individuals without researcher interference. A video camera (GoPro Hero 7[®]) was positioned above the arena in a "birds-eye" field of view. Water height was 9 cm, and water temperature was 23°C. Artificial lights illuminated the arena on a diel cycle.

Several fragments of *P. aliciae* were collected from Sydney's Shelley Beach Headland Intertidal Protection Area (DPI Scientific Collection Permit No. P15/0042–2.0). Water quality in holding tanks (66 l) was maintained during the experiment through aeration, water flow, use of cultured live rock and daily water changes (75%). Water from a coral tank (hereafter "coral odour") was used to introduce a coral cue into the choice arena. Coral fragments were placed into a smaller holding tank (12 l) without live rock for 12 h before experimentation to isolate the coral cue [*sensu* Wright *et al.* (2005)].

Three experiments were used to test preferences of *D. trimaculatus* involving olfactory and visual cues: (a) a choice between coral odour (one quadrant) and sea water (three quadrants), (b) a choice between conspecific odour (one quadrant) and no conspecific odour (three quadrants) and (c) a choice between visual cues of conspecifics (one quadrant) and visual cues of heterospecific groups (three quadrants; Confamilial Pomacentrus coelestis) (n = 18, D. trimaculatus). Experiments were completed in succession, and 3 days were given between experiments. Olfactory cues were eliminated in the latter experiment by restricting treatment fish to plastic bags (1 l). Two trials of Experiment 1 were conducted to examine repeatability and were separated by 3 days.

For coral odour trial 1, 18 individuals were individually tested. For all the remaining experiments, 17 individuals were tested as one individual died between experiments. Each individual was placed into the acclimation tube for 1 min. The odour cue (coral or conspecific) was then released into a randomly selected quadrant at a rate of 2.5 ml s⁻¹ for 10 s. Spread of the cues was estimated by dispersal trials using coloured dye that indicated the water containing the cues would remain in the quadrant for up to 20 min. The individual was then released into the arena for 20 min and filmed, after which they were caught, measured for identification and then fed. The arena was rinsed and refilled between trials to remove trace odours.

The conspecific odour cue was obtained from tank water with conspecifics present for 12 h. Using the same arena configuration, two conspecific individuals were placed into a transparent sealed bag (1 l) with aerated sea water and placed into a randomly selected quadrant. The other three quadrants each contained a bag with two similarly sized confamilial individuals (*P. coelestis*), to provide a non-conspecific cue. The test individual was then acclimated and released into the arena. No ill effects of confinement were observed.

Preference for cues was examined from video using two response variables: (a) selection of the quadrant with a cue (*e.g.*, coral odour) following a period of initial exploration ("final selection") and (b) the proportion (%) of time spent in that quadrant during the experiment ("time spent"). The two metrics were used because of initial observations of fish roaming throughout the array before cueing towards a stimulus, along with an initial flight response that was unlikely related to cueing.

Linear mixed effect modelling was initially used to explore the effects of successive trials on the same individuals, release direction and body size [total length (L_T), cm] on time spent in coral odour. Results were used to inform subsequent analyses (see below) and assist in interpretation. Fixed effects were selected based on the significance of their *t*-value at the 0.05 level (Zuur *et al.*, 2009). Model validity was examined through a comparison of residuals to fitted values and comparison of standardised residuals to theoretical quantiles (Zuur *et al.*, 2009). Modelling was run using the "lme4" package (Bates *et al.*, 2014) in the R software environment (vers. 4.0.3).

Following initial model exploration, a one-sample *t*-test was used to examine whether time spent in the coral odour quadrant was greater than chance alone (expected 25% of the experimental duration). The values from Trials 1 and 2 for each individual were averaged [Kroon and Housefield (2003), James *et al.* (2008)], an approach supported by the lack of a trial effect in initial modelling.

Binomial exact tests were used to test the significance of final quadrant selection at 20 min in all experiments relative to that expected by chance alone (expected: 25%). Results were considered significant if P < 0.05. One-sample t-tests were conducted using SigmaPlot 14.0 (Systat Software Inc., 2020), and binomial exact tests were conducted in the R software environment (RStudio Team, 2020).

TABLE 1 Results of linear mixed modelling of time spent in coral odour as a function of trial number, release direction and body size (mm, L_T)

Predictors	Estimates	C.I.	Р	df
Intercept	13.92	1.47-26.36	0.030	17.73
Trial 2	0.77	-2.88 to 4.43	0.659	15.58
Release	8.00	2.25-13.75	0.008	26.87
Size	-0.22	-0.54 to 0.10	0.169	15.80
Fish ID				
σ^2	23.11			
τ _{00 ID}	4.33			
ICC	0.16			
N _{ID}	17			

Note: Trial 1 was the reference category. Fish ID was included as a random effect. Bold indicates significance at P < 0.05.

Abbreviations: c.i.: confidence interval; ICC: Intraclass correlation coefficient.

Mixed modelling indicated that individuals released towards the coral odour quadrant spent significantly greater time in that quadrant compared to individuals not released towards it (Table 1). The result suggests that releases directed at the cue of interest must be balanced with releases not directed at the cue, even when direction of release is randomised. No difference in time spent in coral odour was found between Trials 1 and 2, supporting combination of values from both trials into a single average for subsequent comparison (Table 1). Body size also did not affect time spent (Table 1).

The combined results from the three experiments suggest D. trimaculatus can use olfactory and visual cues to select coral habitat and conspecific groups. Fish spent significantly more time in quadrants with coral olfactory cues present than the null-hypothesised average of 25% of the experimental duration [mean % of time: 37.24 \pm 4.79 (Figure 1a); one-sample *t*-test; t = 2.558, df = 16, P = 0.011]. Final selection of the coral odour quadrant occurred significantly more often than the null-hypothesised proportion of 25% in Trial 2 (binomial exact test; 59%, P = 0.003) (Figure 1c), but not in Trial 1 (binomial exact test; 44%, P = 0.057). The non-significant result during Trial 1 may have been caused by variability in behaviour over a timescale of days, or insufficient acclimation before the first trial. Nonetheless, as shown by the confidence intervals, there is a strong suggestion of the D. trimaculatus individuals selecting the coral odour. The preference for coral odour observed in the current study has also been observed for fish on coral reefs (Coppock et al., 2020; Feary et al., 2007; McCormick et al., 2010), suggesting general importance of olfactory mechanisms for locating coral habitat during early life.

Time spent by *D. trimaculatus* in the conspecific odour quadrant was greater than the null-hypothesised average of 25% of the experimental duration [mean % of time = 37.963 ± 6.43 one-sample t-test (Figure 1b); t = 2.017, df = 16, P = 0.03]. The same preference was not demonstrated with final quadrant selection [binomial exact test, 41%, P = 0.107; (Figure 1d)]; nonetheless, as shown by the confidence intervals, there is a strong suggestion of the *D. trimaculatus* individuals selecting the conspecific odour.

Time spent by *D. trimaculatus* in the conspecific quadrant (vs. the confamilial *P. coelestis* quadrants) was three times greater than the null-hypothesised average of 25% of the experimental duration [mean % of time: 76.23 ± 3.139 (Figure 1b); one-sample *t*-test; t = 16.320, df = 16, P < 0.001]. Final selection of the conspecific quadrants also occurred three times more often than the null-hypothesised proportion of 25% [binomial exact test; 76%, P = <0.001 (Figure 1d)].

Once fish approach a reef within sufficient distance for both visual and olfactory cues, the focus on habitat selection may change to visual cues for conspecifics (Lecchini & Nakamura, 2013). Both olfactory and visual cues (much stronger in the current study) of conspecifics could be key indicators for new recruits on whether or not a habitat is considered suitable, as it infers adequate shelter, food and/or an absence of predators (Booth, 1992; Vail & McCormick, 2011). The lack of conspecifics in temperate ecosystems may result in fewer available olfactory and visual cues for tropical vagrants, at least during the early stages of colonisation and extranatal population establishment. Potential future studies could combine conspecific and coral olfactory cues and determine how one may enhance the other for individuals finding new habitat.

The individuals used for this experiment were not pre-settlement larvae, but young post-settlement individuals acquired through the aquarium trade. Olfactory abilities may change ontogenetically, as the size of the olfactory organ and its amount of lamellae increase with age in certain fish (Zeiske et al., 1992). Nonetheless, Wright et al. (2005) observed that pre- and post-settlement confamilials (Pomacentrus nagasakiensis) displayed similar olfactory responses, suggesting that the results of this study may be applicable to new settlers. Furthermore, Kavanagh and Alford (2003) found that olfactory development in D. trimaculatus began later and developed at a slower rate than other damselfish species that exhibit an affinity for specialist live habitat. Therefore, despite not using pre-settlement individuals, the results obtained from the current study still provide insights into the ability of D. trimaculatus to use chemical and conspecific cues to find habitat. The lack of a size effect on time spent in coral cues from the mixed modelling further suggests that larger individuals used can provide insights into the sensory abilities of smaller individuals, closer to settlement size. Nevertheless, conclusions regarding settlement abilities of D. trimaculatus are made cautiously here, and research on settlement-stage larvae is ultimately required to verify the results of this study here. In practice, research on settlement cues of late-stage larvae is rarely feasible, because of the difficulty in locating such individuals in sufficient abundance and the challenge of observing larvae while they make settlement choices in the field (Lecchini et al., 2005; Leis et al., 2002).

Conspecific olfactory and visual cues are likely to become a more integral part of the recruitment process for tropical vagrant species migrating to temperate SE Australia. As vagrant coral-reef fish species can overcome the bottleneck of overwintering (Figueira & Booth, 2010), the establishment of conspecific and congeneric communities which will provide a greater source of olfactory and visual cues to assist in recruitment than currently exists during the initial

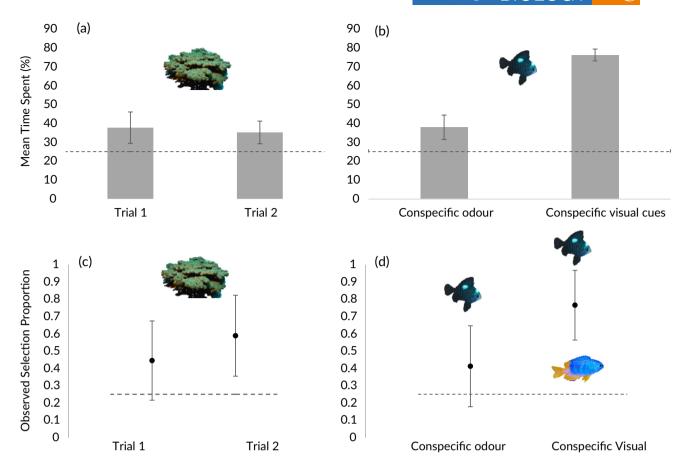


FIGURE 1 (a) Mean time spent (%) in coral odour quadrant during two trials (Trial 1: n = 18, Trial 2: n = 17). (b) Mean time spent (%) in conspecific odour quadrant and conspecific visual quadrant (n = 17 for each experiment). Each bar indicates one experiment. One-sample *t*-test used average against null hypothesis of 25% (dashed line). Error bars indicate standard error. (c) Final selection of coral odour quadrant during two trials ($\pm 95\%$ c.i.) (Trial 1: n = 18, Trial 2: n = 17). (d) Final selection for conspecific olfactory and conspecific visual cues (*Dascyllus trimaculatus* vs. *Pomacentrus coelestis*) (n = 17 in each) ($\pm 95\%$ c.i.). Binomial exact tests used against null hypothesis of 25% (horizontal line: a-d).

colonisation. The isolation of the various cues outlined in this study would be impractical *in situ*, and the use of laboratory techniques allows for the study of individual sensory cues to be measured and to determine which were involved in habitat selection. This study has shown the potential for poleward migrating coral reef fishes to detect scarce stands of hard branching corals that are expanding throughout south-eastern Australia as water temperature increases. The findings of this study note a wider relevance of general olfactory abilities in novel environments, as a result of active (*e.g.*, movement of juveniles between habitats after settlement) or reactive (*e.g.*, storm displacement) movement. Booth and Sear (2018) have recorded several vagrant reef fish species only found on the coral bed, suggesting further understanding of sensory cues and mechanisms specifically in relation to coral detection is an important part of revealing the processes behind tropicalisation of temperate ecosystems.

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AUTHOR CONTRIBUTIONS

All the authors contributed towards data generation, data analysis, manuscript preparation, and the overarching concepts and structure of the paper.

ETHICS AND PERMITS

This research was conducted under UTS Animal Ethics ETH19-4536. Coral collected was conducted under NSW Department of Primary Industries scientific collection permit P15/0042–2.0 and OUT18/14560.

CONFLICTS OF INTEREST

On behalf of all authors, the corresponding author states that there is no conflict of interest.

ORCID

Max J. O'Connell D https://orcid.org/0000-0002-6373-4365

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