



BRIEF COMMUNICATION

Response to underwater laser pointer in the Orange-finned Anemonefish *Amphiprion chrysopterus* and three-spot damselfish *Dascyllus trimaculatus*

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Abstract

Response of orange-finned anemonefish *Amphiprion chrysopterus* and three-spot damselfish *Dascyllus trimaculatus* to red laser-pointer light was studied in Mo'orea, French Polynesia. Four magnificent anemones *Heteractis magnifica* and their resident fish were observed for typical behaviours (biting, chasing, hiding, posing, lunging and retreating) with and without exposure to laser-pointer light. Lunging behaviour increased significantly for both fish species upon exposure to laser-pointer light; none of the other behaviours changed significantly. We advance the hypothesis that orange-finned anemonefish and three-spot damselfish interpret laser pointer stimulation as a territorial threat.

KEYWORDS

anemonefish, boldness, French Polynesia, laser pointer, Moorea

Underwater laser pointers are popular scuba diving accessories, as they precisely and easily indicate features of interest to dive partners (e.g., can be used to point out animals in refugia to dive partners). As a consequence of their use, divers documented that reef fish respond to the spot of laser light with chasing and biting (YouTube, 2015). In order to study this behaviour, we observed orange-finned anemonefish *Amphiprion chrysopterus* (Cuvier 1830) and three-spot damselfish *Dascyllus trimaculatus* (Rüppell 1829) resident on magnificent anemones *Heteractis magnifica* in Mo'orea, French Polynesia. Anemonefish offer several advantages to characterise novel behaviour in that the same individual fish can be observed repeatedly on easily recognised sessile anemones (Fautin, 1991), they have well-established behavioural repertoires (Chen & Hsieh, 2017; Mills *et al.*, 2018; Nanninga *et al.*, 2017), and they respond to laser-pointer light (this study). Using fish resident on anemones, we tested the hypothesis that *A. chrysopterus* and *D. trimaculatus* perceive the artificial stimulus of laser-pointer light as a territorial threat.

A cluster of 4 *H. magnifica*, within a 2 × 6 m area at 1–2 m depth, were observed while snorkelling in Oponoho Bay, Mo'orea French Polynesia (17° 29'43.7" S, 149° 51'06.8" W) 16–28 May 2019. Each

anemone was c. 40 cm in diameter and had a single resident adult anemonefish with 2–4 resident three-spot damselfish (juveniles and adults) except for anemone #2 with only damselfish. Anemones and resident fish were chosen after consultation with local researchers, so as not to be included in several long-term anemonefish studies on the island (Mills *et al.*, 2018; Nanninga *et al.*, 2017; Norin *et al.*, 2018). Thus, these sites and these resident fish should have less history with researchers than other individuals on Mo'orea (although we cannot account for tourist interactions). Water temperature during the observations was 28–30°C and although some other anemones in the bay were bleached, anemones in this study were not. All observations were conducted soon after sunrise (06:30–08:30 h) and at dusk (16:00–17:30 h) to maximise laser contrast.

All observations were made by two observers while snorkelling, with a third snorkeler manipulating the laser pointer. Snorkelers swam to 3 m from the anemones, waited 6 min for the fish to acclimate to their presence (Nanninga *et al.*, 2017), then recorded numbers of behaviours per fish (averaged for 2 observers per fish) for 2.5 min. Immediately after observation without laser pointer stimulation, a red-light laser pointer (Trident DL70; www.scuba.com) was aimed at

the base of the anemone in question, with 10 s revolutions repeated over the 2.5 min observation trial. A red-light laser pointer was chosen as the most inexpensive colour (and probably the most common among recreational divers). This protocol was repeated for each of the 4 anemones twice daily during the study. One set of observations at one anemone, with and without laser pointer, constituted one trial. Over the course of 13 days, 50 total trials were conducted for three-spot damselfish and 39 trials for anemonefish (fewer trials for anemonefish due to their absence on anemone #2 and some morning or evening sessions abandoned due to heavy rain that hindered visibility).

Behaviours recorded were similar to what is described for similar reef studies (Hamb, 2011; Iwata & Manbo, 2013): lunging, resident fish quickly moves toward invading fish (in the control treatment) or toward laser/invading fish (in the treatment-with laser); chasing, resident fish pursues invading fish/laser past boundary of anemone; posing, resident fish adopts 45° snout-up angle, maintaining position with pectoral sculling; hiding, resident fish moves deep within anemone and may be out of site; retreating, resident fish slowly moves

backward into centre of anemone with pectoral sculling; biting, resident fish directing bite toward intruder.

We investigated how the total behaviours per fish per trial for each species was affected by laser pointer stimulation, and which behaviour was driving this effect, using a series of nonparametric statistical analyses. Nonparametric analyses were used because data violated one or both of the following assumptions of parametric statistical analyses: (a) variance between treatment groups is homogeneous and (b) residuals are normally distributed. Assumption (a) was tested using Levene's test, while assumption (b) was tested using the Shapiro-Wilk's test. When assumptions (a) and (b) were violated, Welch's *t*-test was used to compare the frequency of the behaviour as a function of treatment (with or without laser pointer). When assumption (b) was violated, Wilcoxon rank-sum test was used to examine how treatment affect the frequency of the particular behaviour. In all cases, individual anemone was modelled as a block to account for multiple measurements on the same anemone. A sequential Bonferroni correction was performed to account for multiple comparisons.

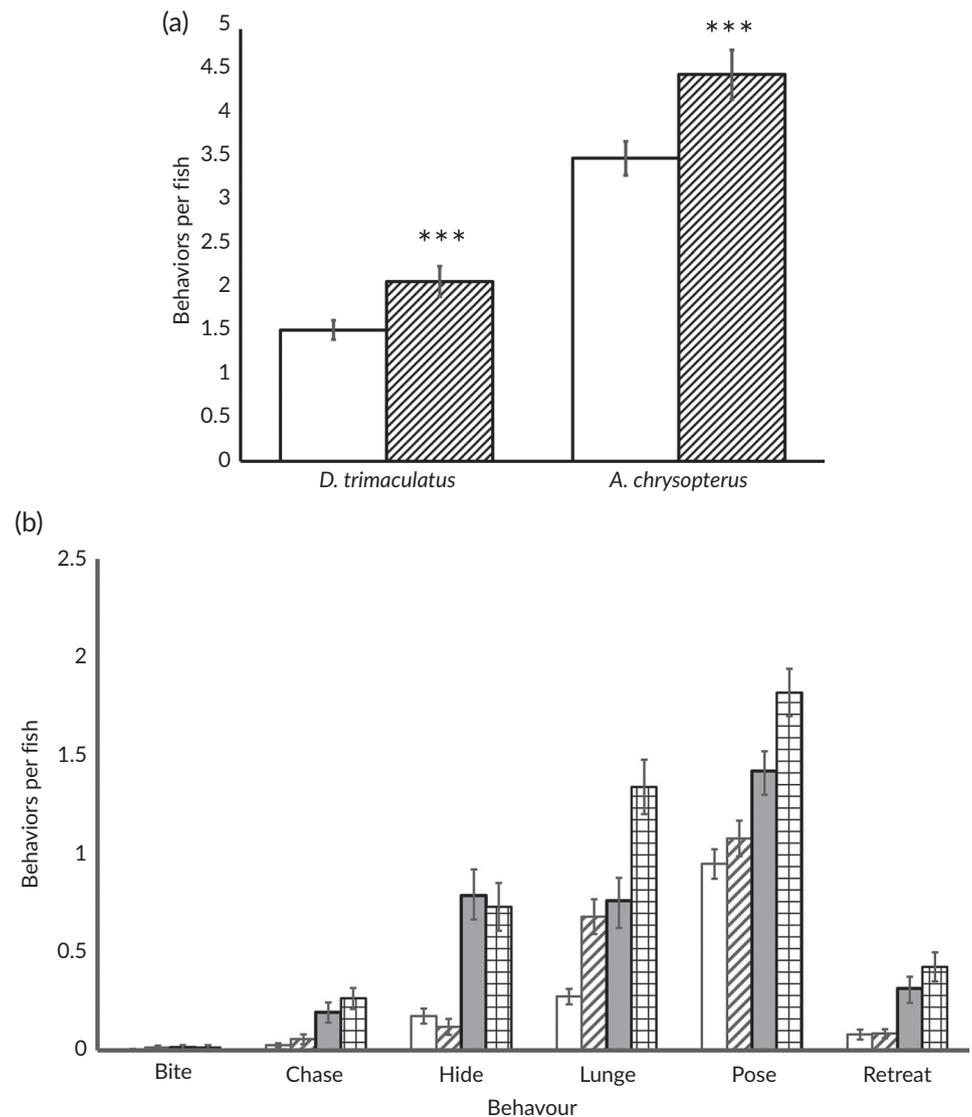


FIGURE 1 (a) Mean (\pm SE) total behaviours aggregated per fish (\square) without and (hatched) with laser-pointer. (b) Individual behaviours for *Amphiprion chrysopterus* ($n = 39$) and *Dascyllus trimaculatus* ($n = 50$) \square **, $P < 0.01$; ***, $P < 0.001$ (\square) *D. trimaculatus* without, (hatched) *D. trimaculatus* with, (solid grey) *A. chrysopterus* without, and (grid) *A. chrysopterus* with

There was no significant difference between morning and night observations, thus they were not parsed in the analysis. Laser pointer stimulation significantly increased overall number of behaviours per fish, combining all observations (Figure 1a). This was true for both species of fish. This significant overall effect was driven largely by lunging, which was significantly greater with laser-pointer exposure, and other recorded behaviours were not significantly different with laser-pointer exposure (Figure 1b). Although each species responded to the laser pointer with significantly more lunging, lunging was not the highest frequency behaviour (Figure 1b).

The use of artificial stimuli has its origins with the very founders of animal behaviour study (Pavlov, 1927; Tinbergen, 1976). Some artificial stimuli even elicit exaggerated responses, such as preference for an artificial egg over natural by gulls, or extremely aggressive attacks on models in sticklebacks *Gasterosteus aculeatus* (L. 1758) i.e. Staddon's (1975) 'supernormal stimuli'. We do not consider the response to the laser pointer by anemonefish and damselfish a supernormal response, given how our frequency data compared with similar studies (Iwata & Manbo, 2013), but our data do suggest that each species is indeed responding to the laser pointer (Figure 1). Also, we assert that the laser-pointer stimulation in this study is not a general attractant via increasing ambient light, (such as used in some commercial applications; Marchesan *et al.*, 2005), because the lumens added to the environment by the laser pointer during daylight are negligible. Further, we interpret the response as territorial and thus more on the boldness end of the boldness v. sociability behavioural spectrum (Wong, *et al.*, 2013). We advance the hypothesis that these two species responded to the laser pointer as a territorial threat, rather than a predator (no increased hiding or retreat) or prey (no increased biting). We recognise, however, that the number of individual fish sampled was small and that response may differ with how the stimulus is presented, sampling more individuals and locations, and among species. Indeed, anecdotal observation of other reef fish indicate the laser-pointer response is variable among fishes. The Pacific Gregory *Stegastes fasciolatus* (Ogilby 1889) consistently retreated from the laser pointer and the six-bar wrasse *Thalassoma hardwicke* (Bennett 1830) consistently bit at the laser-pointer light during the study, while many species (labrids, damselfishes, jacks, parrotfishes) had no discernible response.

We recognise that overall response rate may have been reduced by the presence of snorkelers (Nanninga *et al.*, 2017), but assert that the highly significant increases in lunging was a response to the laser-pointer stimulus. This artificial stimulus may be a mechanism to elicit a controlled territorial response for studies investigating hormone status (Mills *et al.*, 2018), stress (Norin Tommy *et al.*, 2018), recruitment (Buston, 2004) or general territorial behaviour (Iwata and Manbo, 2013).

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AUTHOR CONTRIBUTIONS

E.C., P.C. and A.H. made an equal contribution to this work.

ETHICS STATEMENT

Fishes were not collected or killed and did not experience significant distress during this experiment. No chemical agents were used and no long-term harm was caused to the fishes in this experiment. The experimental protocol was reviewed and approved by the Ministère De La Culture Et De L'Environnement, French Polynesia.

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