Received: 26 January 2020 | Accepted: 8 August 2020
DOI: 10.1111/jfb.14492

BRIEF COMMUNICATION

JOURNAL OF FISH BIOLOGY fsbi

Scaring Nemo: Contrasting effects of observer presence on two anemonefish species

Lena Trnski[1] | Armagan Sabetian[1] | Julian Lilkendey[1,2]

[1]Auckland University of Technology, School of Science, Auckland, New Zealand

[2]Leibniz Centre for Tropical Marine Research (ZMT), Bremen, Germany

Correspondence
Julian Lilkendey
Email: julian.lilkendey@icloud.com

Funding information
Deutscher Akademischer Austauschdienst

Abstract

Behaviours of Clark's anemonefish *Amphiprion clarkii* and the dusky anemonefish *Amphiprion melanopus* were studied in Vanuatu. Six anemones and their resident fish were observed for typical behaviours (hiding, watching, roaming, inter-, and intraspecific behaviour) with and without the presence of a snorkelling observer. Observer presence had significant but contrasting effects on hiding behaviour in *A. clarkii* and *A. melanopus*. Bolder anemonefish species may be able to outcompete other species in areas with high human presence.

KEYWORDS

anemonefish, behaviour, boldness, observer effect, Vanuatu

Observer presence has long been acknowledged as a bias-factor in reef fish behavioural studies (Pereira *et al*., 2016; Ross, 1978). Moreover, studies on the effects of human presence on fish species have concluded that fish tend to respond to humans as they do to predators (Frid & Dill, 2002) and thus hide more frequently. Human-induced changes, particularly in the reduction of assertive behaviours, could potentially impact the biological fitness of fish species (Tuomainen & Candolin, 2011), especially in areas with high human presence (*e.g.*, tourist destinations).

Anemonefishes are a particularly popular model organism for behavioural studies as their intrinsic symbiotic relationship with anemones causes them to display several aggressive and defensive behaviours in guarding their territory (Godwin, 1994; Godwin & Fautin, 1992). The aim of this study was to investigate the impact of observer presence/absence on behaviours of two anemonefish species: Clark's anemonefish *Amphiprion clarkii* (Bennett 1830) and the dusky anemonefish *Amphiprion melanopus* Bleeker 1852. The former is a generalist species, able to coexist with up to 10 anemone species, while the latter is more of a specialist, existing within only three species of symbiont anemones (Fautin & Allen, 1992).

This study was conducted in Vanuatu, a tropical archipelago in the Pacific Ocean. Port Vila (17°44′ 5″S, 168°19′19″E), the capital city of Vanuatu and located on the Island of Efate, is a tourism hotspot, particularly popular among SCUBA divers wishing to explore South Pacific coral reefs. Data collection occurred from 5 to 26 January 2019 between 11:30 and 17:30. During this time the average air and water temperatures were approximately 31 and 28°C, respectively. Anemones were sampled opportunistically at three sites on Efate: one in Havannah Bay and two in Mele Bay off Port Vila, the latter sites separated by 1.1 km (Appendix Table A1). Anemones observed within each site were at least 20 m from each other to ensure they were independent. We set up a GoPro Hero 3 silver video camera (GoPro Inc.) with a full view of the entire anemone of either *Entacmaea quadricolor* (bubble tip anemone) or *Heteractis crispa* (sebae anemone) that had target fishes within the colony. The first 2 min of video footage were disregarded due to the possible lingering influence of human presence while setting up; the subsequent 15 min were used for behavioural recordings. To simulate observer presence a snorkeler hovered 1–3 m over the anemone. No human observer was present during video footage recorded as control.

For each anemone we recorded depth (m), resident anemonefish species, the number of individuals in the anemone and fish size (cm total length) estimated by visual approximation by the same observer to obtain relative fish size. Fish behaviours displayed in observer presence and absence were recorded for a total of 21 fishes from six anemones (Appendix Table A1). Fish behaviour was noted at 15 s intervals for each anemone. In total, 60 behaviour events were recorded for each anemonefish in both observer presence and absence. Frequency of behaviour is the proportion of occurrence of each behaviour for each anemone and each treatment (presence/absence of observer). Full datasets were recorded for each anemone so data were not pooled.

Recorded behaviours were similar to those described in previous studies on anemonefishes (Cohn *et al*., 2019; Iwata & Manbo, 2013): Hiding, fish retreating to and hiding deep within the tentacles of the anemone; Watching, fish hovering and occasionally posing (45° snout-up angle pectoral sculling) above or just inside of anemone tentacles; Roaming, fish outside the tentacles of the anemone, often covering a distance of up to 1 m; Intraspecific interactions, fish chasing or escaping from another individual of the same species; Interspecific interactions, fish chasing or escaping from an individual of a different species. Behaviours do not overlap and a single behaviour was recorded at each 15 s interval. The data recorded for this study are available in Trnski (2020).

Principal component analysis (PCA) with covariance matrix on relative occurrence of all behaviours was computed for both treatments. We only extracted PCs with eigenvalues >1 (Budaev, 2010).

We used a linear mixed effects model with restricted maximum likelihood (REML) in JMP 14.3 (SAS Institute Inc., Cary, NC, USA, www.jmp.com) to account for repeated measures on the same individual. To compare the entire observed suite of behaviours, we tested each behaviour against "species", "observer (presence / absence)", "anemone depth", "number of fish in anemone" and "fish total length" as main effects, and "individual ID" as a random effect nested within "anemone ID" (Wong *et al*., 2013, 2017). Relevant interaction terms were tested one after the other to avoid overfitting the model. No interaction term was significant. The resulting minimal adequate models were visually tested for the constancy of variances (residuals against fitted values) and the normal distribution of their residuals (normal quantile-quantile plot).

In *A. clarkii* Watching was the most recorded behaviour during observer absence, while Hiding was the predominant behaviour during observer presence. In *A. melanopus* Watching was the most recorded behaviour during both treatments (Figure 1a). In examining the relationships among behaviours, PCA during observer absence revealed that PC1 and PC2 explained 68.6% of the total variance, with strong component loadings for Roaming and Watching. During observer presence PC1 and PC2 explained 67.8% of the variances and with Hiding and Roaming exhibiting the strongest component loadings (Table 1).

The random effect "individual ID" accounted for 0% and 41.5% of the REML variances in the models describing Hiding and Interspecific behaviour, respectively. The variables "species" and "fish total length" exhibited positive effects on Hiding behaviour (Figure 1b), whereas "anemone depth", "number of individuals in anemone" and "observer (presence/absence)" constituted negative effects (Appendix Table A2). The model explained 49% of the observed variances. The number of individual fish in the anemone exhibited negative effects on the frequency of Interspecific behaviours. Overall, 70% of the variances were explained by our model.

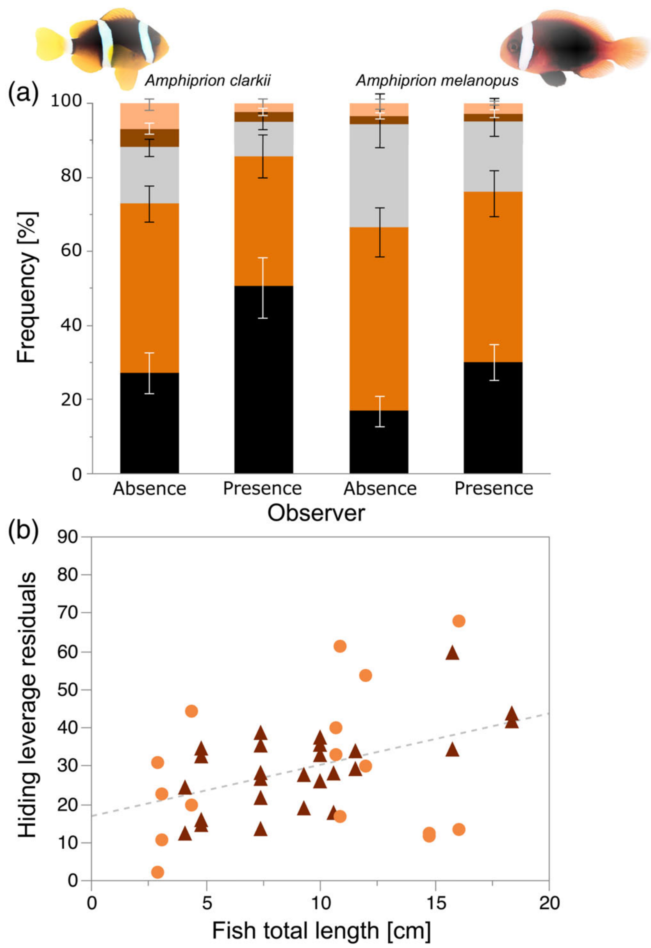

FIGURE 1 Stacked bar chart of mean (±S.E.) frequency of behaviours observed in *Amphiprion clarkii* (*n* = 8) and *Amphiprion melanopus* (*n* = 13) during observer presence and absence in (a) (■) Intraspecific, (■) Interspecific, (■) Roaming, (■) Watching, and (■) Hiding; linear relationships between individual fish total length (cm) and the partial residuals of the mixed effects model with restricted maximum likelihood for the behaviour Hiding in (b) (●) *Amphiprion clarkii* and (▲) *Amphiprion melanopus*

In situ field data for fish is generally collected *via* snorkelling or SCUBA diving. Both methods are invasive and produce low-frequency sound waves, bubbles, and a large visual cue. Anemonefishes are known to regard divers as an immediate threat and respond by exhibiting agonistic behaviours (Hayashi *et al.*, 2019). Our findings compliment these observations by illustrating a significant effect of snorkelling observer presence on the suite of expressed behaviours in anemonefishes. Since this study only observed anemones from shallower depths, our results are not representative of anemonefishes resident in deeper anemones.

Uneven numbers of anemones *H. crispa* and *E. quadricolor* (4 and 2, respectively) from different sampling locations were used in this study, with the *E. quadricolor* anemones only hosting

TABLE 1 Component loadings of each behaviour, the variance explained and the total variance explained by the first two principle components (eigenvalues >1)

	Observer absence		Observer presence	
	PC1	PC2	PC1	PC2
Eigenvalue	1.80	1.63	2.03	1.36
Variances explained	36.0	32.6	40.7	27.2
Total variances explained	36.0	68.6	40.7	67.8
Hiding	0.73	0.52	−0.95	0.14
Watching	0.16	−0.95	0.65	−0.76
Roaming	−0.85	0.51	0.47	0.80
Interspecific	0.69	0.37	−0.47	−0.19
Intraspecific	0.22	−0.24	0.52	0.29

A. melanopus individuals (Appendix Table A1). Since neither sampling location nor anemone species were significant explanatory factors in our models, statistical analysis suggests that they are not confounding variables influencing fish behaviour in this study.

Increased assertiveness is an advantage in outcompeting other species, particularly given the reward of greater reproductive success (Ballew *et al*., 2017). In this study, we show that the frequency with which individual fish were hiding during observer presence and absence was species specific. Because *A. melanopus* is a specialist species that has a smaller range of anemones to inhabit, they may behave more assertively in finding food, interacting socially, responding to threats and guarding territory. Such bolder behaviour will likely compensate for a lack of biological fitness due to the reduced chances of finding a suitable host anemone as larvae. In the long term, species abundances may lean towards those that are better adapted to overcoming human disturbances, especially in areas of high tourism (Robertson, 1996).

Anemonefish have strict social hierarchies in which one sexually functional adult pair is often accompanied by several smaller, immature males (Moyer & Nakazono, 1978; Ross, 1978). The adult pair is dominant to any immature males that compete to be the next breeding male. When the female is lost from the colony, the largest immature male becomes the female after protandrous sex reversal (Ross, 1978). We found that smaller fish (juvenile males) were hiding less frequently than bigger fish and thus can be regarded as bolder. A juvenile male must be bolder and more aggressive than its conspecifics in order to acquire more food and grow faster to secure its position in line as part of the next breeding pair.

Juvenile males also compete against other fishes to increase their biological fitness and thus their rank (Buston, 2003) while defending the anemone from predators (Porat & Chadwick-Furman, 2004). In our study, the number of individual fish in an anemone had a significant effect on the frequency of interspecific and hiding behaviours. Increased densities of aggressive juvenile males may have elevated the chances of interacting with other fish species in proximity of the anemone as a means of anemone defence (Srinivasan *et al*., 1999).

In the present study individual fish may have become accustomed to observer presence as they hid more frequently at the beginning of observations, but tended to become more assertive as time passed (personal observation). This is likely a reaction to acclimation time and perceived predation risk, which may last up to 7 min after observer departure (Nanninga *et al*., 2017). Long term, this could explain why fish hid less frequently at shallower depth, as these individuals have habituated to human visitation by swimmers, divers and snorkelers, or disturbance by anthropogenic water-based activities, whereas fish at greater depths encounter humans less frequently (Titus *et al*., 2015). Our results also show that in anemones that are likely to have been subjected to continued human presence (*i.e*., in areas of high tourism) assertive fish behaviours may be altered, resulting in less foraging time, diminished reproductive success and, ultimately, reduced biological fitness.

In a previous anemonefish behaviour study the use of remotely operated video surveys reduced the bias of observer presence by increasing the activity level and the number of assertive behaviours displayed (Nanninga *et al*., 2017). While the use of a camera appeared to promote "normal" behaviours, there is still potential for behavioural bias, particularly in relation to acclimation time. Given the rather small acclimation time employed by us it is likely that even brief deployment of the camera biased fish behaviours over the entire course of recordings. In future behavioural studies we recommend the use of remote video surveys in conjunction with longer acclimation times (>15 min) over sampling techniques involving human presence, as this limits observer bias.

ACKNOWLEDGEMENTS

We are grateful to and wish to thank all of the people that helped with the data capture and completion of this report. Lisa Dartnall and Tilly Gibb contributed to the idea and methodology of this study. Maddie Smyth, Sonja Lukaszewicz and Rahul Sripathy-Cody helped to locate suitable anemones and set up Go Pros in Vanuatu. Thomas Trnski aided with GoPro set ups in Vanuatu and read and commented on several drafts.

AUTHOR CONTRIBUTION

L.T. undertook fieldwork and background research, J.L. led the statistical analysis and all authors made an equal contribution to writing this work.

ETHICS STATEMENTS

Fish used in the study were neither collected nor killed and did not experience significant distress during this experiment.

ORCID

Armagan Sabetian https://orcid.org/0000-0001-6690-6903
Julian Lilkendey https://orcid.org/0000-0003-3165-1079

REFERENCES

Ballew, N. G., Mittelbach, G. G., & Scribner, K. T. (2017). Fitness consequences of boldness in juvenile and adult largemouth bass. *American Naturalist*, *189*, 396–406.

Budaev, S. V. (2010). Using principal components and factor analysis in animal behaviour research: caveats and guidelines. *Ethology*, *116*, 472–480.

Buston, P. M. (2003). Mortality is associated with social rank in the clown Anemonefish (*Amphiprion percula*). *Marine Biology*, *143*, 811–815.

Cohn, E., Cole, P., Haymaker, A., Garner, A. M., & Londraville, R. L. (2019). Response to underwater laser pointer in the orange-finned Anemonefish *Amphiprion chrysopterus* and three-spot damselfish *Dascyllus trimaculatus*. *Journal of Fish Biology*, *96*(1), 274–277.

Fautin, D. G., & Allen, G. R. (1992). *Field guide to Anemonefishes and their Host Sea Anemones*. Perth, Australia, Western Australian Museum.

Frid, A., & Dill, L. (2002). Human-caused disturbance stimuli as a form of predation risk. *Conservation Ecology*, *6*, 11.

Godwin, J. (1994). Behavioural aspects of protandrous sex change in the Anemonefish, *Amphiprion melanopus*, and endocrine correlates. *Animal Behaviour*, *1994*, 551–567. https://doi.org/10.1006/anbe.1994.1275.

Godwin, J., & Fautin, D. G. (1992). Defense of host actinians by anemonefishes. *Copeia*, *1992*, 902–908.

Hayashi, K., Tachihara, K., & Reimer, J. D. (2019). Species and sexual differences in human-oriented behavior of Anemonefish at Okinawa Island, Japan. *Marine Ecology Progress Series*, *616*, 219–224.

Iwata, E., & Manbo, J. (2013). Territorial behaviour reflects sexual status in groups of false clown Anemonefish (*Amphiprion ocellaris*) under laboratory conditions. *Acta Ethologica*, *16*, 97–103.

Moyer, J. T., & Nakazono, A. (1978). Protandrous hermaphroditism in six species of the Anemonefish genus Amphiprion in Japan. *Japanese Journal of Ichthyology*, *25*, 101–106.

Nanninga, G. B., Côté, I. M., Beldade, R., & Mills, S. C. (2017). Behavioural acclimation to cameras and observers in coral reef fishes. *Ethology*, *123*, 705–711.

Pereira, P. H. C., Leal, I. C. S., & de Araújo, M. E. (2016). Observer presence may Alter the behaviour of reef fishes associated with coral colonies. *Marine Ecology*, *37*, 760–769.

Porat, D., & Chadwick-Furman, N. E. (2004). Effects of Anemonefish on Giant Sea anemones: expansion behavior, growth, and survival. *Hydrobiologia*, *530–531*, 513–520.

Robertson, D. R. (1996). Interspecific competition controls abundance and habitat use of territorial Caribbean damselfishes. *Ecology*, *77*, 885–899.

Ross, R. M. (1978). Territorial behavior and ecology of the Anemonefish *Amphiprion melanopus* on Guam. *Zeitschrift für Tierpsychologie*, *36*, 71–83.

Srinivasan, M., Jones, G. P., & Caley, M. J. (1999). Experimental evaluation of the roles of habitat selection and interspecific competition in determining patterns of host use by two Anemonefishes. *Marine Ecology Progress Series*, *186*, 283–292.

Titus, B. M., Daly, M., & Exton, D. A. (2015). Do reef fish habituate to diver presence? Evidence from two reef sites with contrasting historical levels of SCUBA intensity in the Bay Islands, Honduras. *PLoS One*, *10*, 1–13.

Trnski, L. (2020). Recorded Behaviours by GoPro of Anemonefish species *Amphiprion clarkii* and *Amphiprion melanopus* in observer presence and observer absence, Vanuatu, 2019. PANGAEA, doi:https://doi.org/10.1594/PANGAEA.914226.

Tuomainen, U., & Candolin, U. (2011). Behavioural responses to human-induced environmental change. *Biological Reviews*, *86*, 640–657.

Wong, M. Y. L., Medina, A., Uppaluri, C., Arnold, S., Seymour, J. R., & Buston, P. M. (2013). Consistent behavioural traits and behavioural syndromes in pairs of the false clown Anemonefish *Amphiprion ocellaris*. *Journal of Fish Biology*, *83*, 207–213.

Wong, M. Y. L., Beasley, A. L., Douglass, T., Whalan, S., & Scott, A. (2017). Some Anemonefish lack personality: a comparative assessment of behavioral variation and repeatability in relation to environmental and social factors. *Coral Reefs*, *36*, 1307–1316.

How to cite this article: Trnski L, Sabetian A, Lilkendey J. Scaring Nemo: Contrasting effects of observer presence on two anemonefish species. *J Fish Biol*. 2020;1–5. https://doi.org/10.1111/jfb.14492

APPENDIX A

TABLE A1 List of anemones and resident anemonefishes sampled at Efate, Vanuatu

Anemone species	Anemonefish species	Number of fish in anemone	Location
Heteractis crispa	*Amphiprion clarkii*	3	Paradise Cove, Port Vila
Heteractis crispa	*Amphiprion clarkii*	5	Gideon's Landing, Havannah Bay
Heteractis crispa	*Amphiprion melanopus*	2	Paradise Cove, Port Vila
Heteractis crispa	*Amphiprion melanopus*	1	Coco's Resort, Port Vila
Entacmaea quadricolor	*Amphiprion melanopus*	5	Paradise Cove, Port Vila
Entacmaea quadricolor	*Amphiprion melanopus*	5	Coco's Resort, Port Vila

TABLE A2 Linear mixed effects model results for "hiding" and "interspecific behaviours" against the categorical predictors anemone fish "Species (*Amphiprion clarkii* / *Amphiprion melanopus*)" and "Observer (presence / absence)", as well as against the continuous predictors "Anemone depth (m)", "Number of individual fish in anemone" and individual "Fish total length (cm)". Estimates (Est.) of the parameters for the least adequate model are shown, together with their standard errors (S.E.) and *P* values

	Hiding			Interspecific behaviours		
	Est.	**S.E.**	***P***	**Est.**	**S.E.**	***P***
Intercept	76.8	18.0	0.0006*	9.23	1.91	0.0001*
Species	12.8	2.51	0.0001*			
Observer (presence / absence)	−8.51	2.29	0.0014*			
Anemone depth (m)	−8.70	3.09	0.0124*			
Fish total length (cm)	1.34	0.60	0.0392*			
Number of individuals in anemone	−9.22	2.41	0.0015*	−1.53	0.43	0.0022*
R^2		0.49			0.70	
RMSE		14.9			2.28	

Note: The model's correlation coefficients (R^2) and root mean square error (RMSE) are also indicated.