

Behavioural acclimation to cameras and observers in coral reef fishes

Gerrit B. Nanninga^{1,2,3}  | Isabelle M. Côté⁴ | Ricardo Beldade^{1,2} | Suzanne C. Mills^{1,2}

¹EPHE PSL Research University, USR 3278
CRIOBE CNRS-UPVD, Moorea, French
Polynesia

²Laboratoire d'Excellence "CORAIL", Moorea,
France

³Department of Zoology, University of
Cambridge, Cambridge, UK

⁴Earth to Ocean Research Group, Department
of Biological Sciences, Simon Fraser
University, Burnaby, BC, Canada

Correspondence

Gerrit B. Nanninga, Department of Zoology,
University of Cambridge, UK.
Email: gbn23@cam.ac.uk

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Abstract

Observer presence can bias behavioural studies of animals in both the wild and the laboratory. Despite existing evidence for significant observer effects across several taxa, little is known about the minimum periods of acclimation that should precede behavioural observations. To date, most studies either do not report any acclimation periods or include a non-specific period without empirically quantifying its appropriateness. Here, we conducted *in situ* behavioural observations of two species of demersal coral reef fishes using cameras and/or observers to examine the biases associated with either approach. For both treatments, we generated 25 min time series of a number of vigilance-associated behaviours (i.e., distance from shelter and mate, time out of shelter, swimming activity) and estimated the point of acclimation using changepoint analysis. In the camera trials, acclimation in both species appeared to occur between 2 and 7 min for different behaviours. When an observer was present, however, no apparent acclimation occurred until the observer left the area. Overall, our findings demonstrate that (i) behavioural studies of wild fishes conducted by an observer may be biased due to permanent observer effects, and (ii) when using video equipment, a species- and behaviour-specific acclimation period should precede behavioural scoring.

KEYWORDS

acclimation, anemonefish, beaugregory, changepoint analysis, *in situ* observations, observer effect

1 | INTRODUCTION

The observation of animals in their natural habitat is a critical component of the scientific study of behaviour. However, wild animals are known to react to humans, potentially exhibiting altered, non-natural behavioural patterns in the presence of an observer (e.g., Chapman, Johnstone, Dunn, & Creasey, 1974; MacFarlane & King, 2002). While they may sometimes be subtle in nature, such "observer effects" can significantly bias findings in behavioural studies (Iredale, Nevill, & Lutz, 2010).

The most common approaches in an attempt to circumvent the problem of observer effects in behavioural observations are (i) concealing the observer (e.g., behind a blind), (ii) allowing the focal animal

to acclimate or habituate to the observer and (iii) using a camera to replace the observer (Martin, Bateson, & Bateson, 1993; Setchell & Curtis, 2011). While the first approach may be suitable in the laboratory and most terrestrial habitats, it is unlikely to be practical in aquatic environments. Here, we focus on the latter two techniques.

It has long been postulated that, with sufficient "neutral exposure," animals should get accustomed to and eventually ignore a human observer in behavioural studies of wild animals (Carpenter, 1934); that is, the animals acclimate to observer presence. We define "acclimation period" as the time needed for an individual or group of individuals to become accustomed to the presence of an observer at first exposure. The focal animal is deemed to be acclimated when its behavioural patterns (or certain aspects thereof) have stabilized and are

assumed to be similar to pre-exposure levels. Note that acclimation should not be mistaken for “habituation”, where repeated exposure to human observers, typically over the course of weeks/months, results in a continuous reduction in the animals’ natural response (Williamson & Feistner, 2003). Hence, habituation presents a learned decrease in the response to observer presence during long-term, recurrent contact (i.e., the animal learns that the observer is not a threat), while acclimation, as defined here, represents a short-term adjustment in response(s) to an observer. Habituation and acclimation may overlap in certain studies and/or environments.

A non-exhaustive literature review of studies of *in situ* behavioural observations of fishes—our focal taxon—revealed that more than 65% of all papers did not report any acclimation period (Figure 1a, Table S1). Of the studies that did, over 75% allowed a maximum of 5 min of acclimation (Figure 1a). In no case was the lack or length of the chosen acclimation period empirically validated.

Replacing the human observer with an immobile video camera is another approach to minimize observer effects in aquatic environments. The use of underwater cameras has increased markedly in the past decade, coinciding with the advent of small, relatively inexpensive video units. In our literature survey, we observed an increase in the use of cameras from 10% (of 29 studies) before 2006 to 20% (of 46 studies) thereafter. Despite the growing adoption of camera technology in aquatic behavioural studies, this approach has limitations. First, a stationary camera may miss certain aspects of behaviour (MacFarlane & King, 2002), specifically if the focal animal frequently leaves the field of view. Second, the camera must be positioned by a person, typically a snorkeller or diver, potentially entailing the stirring of sediments and the production of bubbles and noise. This process may result in lasting effects on the focal animal (Watson & Harvey, 2007), likewise necessitating an acclimation period.

In most cases, observer presence changes fish behaviour because humans are perceived as a threat (Frid & Dill, 2002). This explains the often-reported discrepancy in fish abundance estimates between underwater visual censuses carried out by divers/snorkellers vs. remotely

operated and/or stationary cameras (Dearden, Theberge, & Yasué, 2010; Usseglio, 2015). However, while large, mobile fish species can temporarily leave an area disturbed by humans, the same is not true for smaller, site-attached species. Their continued presence might nevertheless belie a range of behavioural shifts that entail potential fitness consequences. For instance, a fish that is hiding within the reef matrix in response to observer presence cannot feed, defend its territory, guard its eggs, or engage in courtship behaviour. While the magnitude of some of these shifts has recently been estimated for several coral reef fish species (Pereira, Leal, & Araújo, 2016), the existence and/or extent of behavioural acclimation to diver or camera presence remains unknown.

Here, we analysed time series of behaviour, measured *in situ*, of two species of small, site-attached coral reef fishes. Our objectives were to (i) assess evidence for an observer/camera effect on vigilance-related behaviours, (ii) identify the point at which individuals acclimate to the deployment and the presence of a camera and/or the presence of an observer, and (iii) to estimate differences in post-acclimation behavioural patterns between fish exposed to observers and those exposed to cameras. This study provides one of the first empirical examinations of the necessity of acclimation periods in behavioural observations of fishes in the wild.

2 | METHODS

2.1 | Study species

The two focal species of this study were the orange-fin anemonefish, *Amphiprion chrysopterus* (Cuvier 1830), and the beaugregory, *Stegastes leucostictus* (Castelnau 1855), both belonging to the family Pomacentridae. The two species were chosen because of their restricted home ranges, making them amenable to stationary video analysis. *Amphiprion chrysopterus* occurs in the central and western tropical Pacific and forms permanent breeding pairs, sometimes accompanied by one to six non-breeders (Beldade et al., 2012).

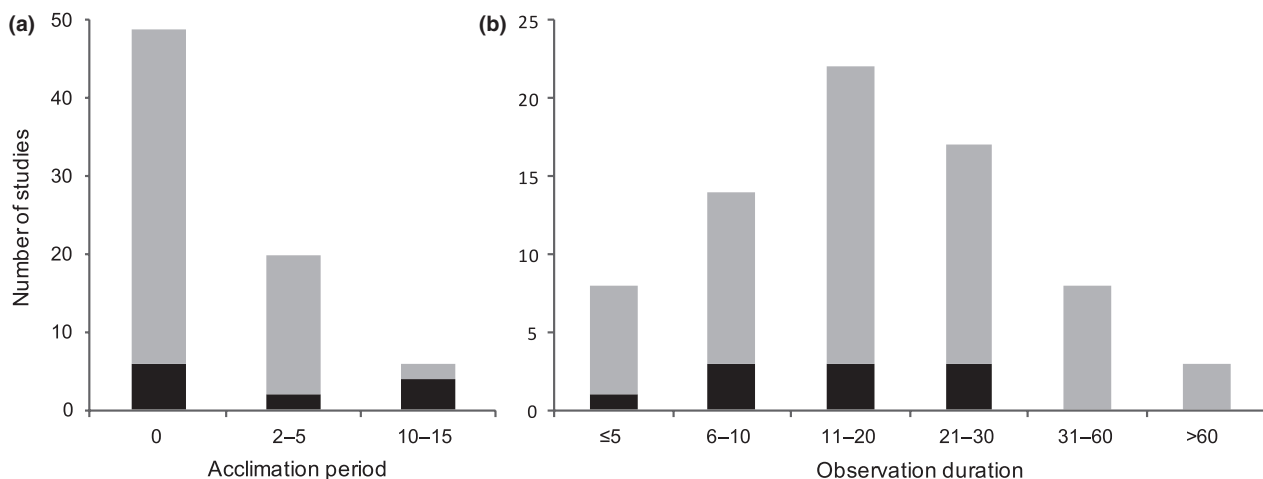


FIGURE 1 Results of a non-exhaustive Web of Science literature survey showing (a) acclimation periods (0 = none reported) and (b) observation durations reported in studies of *in situ* marine and freshwater fish behaviour; grey = observer, black = camera (refer to supplementary material for search criteria)

Each group lives in facultative symbiosis with one or several host anemones, which the fish use as nesting site and shelter against predation. *Stegastes leucostictus* inhabits the shallow tropical waters of the western Atlantic. Males and females hold individual territories of 1–2 m² over substrates of mixed sand and coral rubble, which they defend against conspecifics and other species (Horne & Itzkowitz, 1995; Itzkowitz, 1985). Beaugregories usually take shelter in a crevice in or under an isolated coral colony or coral rock, located near the centre of their territory.

2.2 | Video collection

Videos were taken *in situ* in the fishes' natural habitats. Data for *A. chrysopterus* were obtained in February 2016 at sheltered inshore reefs around the island of Moorea, French Polynesia, at depths between 1 and 2 m. A GoPro (Hero 4) camera equipped with dive weights was placed on the sea floor by a snorkeller at a distance of 1–2 m from a cluster of anemones hosting a breeding pair. We left the camera to film for 30 min, but excluded the final 5 min from analysis to avoid disturbance of the fish by the returning snorkeller. An observation duration of 25 min is consistent with the majority (~81%) of studies in our literature review (Figure 1b). For the anemonefish, two treatments were employed: (i) Camera only (Cam) in which the snorkeller left the area immediately after starting the video and returned only to retrieve the camera ($N = 10$ breeding pairs), and (ii) Camera and observer present (Obs) in which the snorkeller stayed closely behind the camera for 25 min ($N =$ five different breeding pairs). In the Obs treatment, four videos included an additional 5 min period without observer (i.e., 30 min total). In these instances, the observer departed after the 25-min period, leaving the camera to film for an additional 5 min without an observer present.

Data for *S. leucostictus* were collected in July 2015 in Rock Sound, a large shallow bay at the southern end of Eleuthera Island, The Bahamas. For this species, only the Cam treatment was implemented. Upon encountering a beaugregory, a snorkeller observed the fish for approximately 2 min prior to deploying the camera to make sure that it was territorial and to identify the shelter location. A weighted GoPro (Hero 3) camera was then placed 1–2 m from the shelter and the snorkeller left the area. Cameras were retrieved after 30 min ($N = 13$ individuals).

2.3 | Video analysis

We quantified four behavioural variables, each relating to the level of vigilance and/or fearfulness and stress: (i) distance from mate (only for *A. chrysopterus*), (ii) distance from shelter, (iii) proportion of time spent outside of shelter, and (iv) swimming activity. These behaviours were scored every 5 sec for *A. chrysopterus* and every 10 sec for *S. leucostictus* during each 25 min video. For this purpose, the video was paused and measurements estimated from the still image (individually for both fish in each breeding pair of *A. chrysopterus*). Preliminary analysis showed that scoring behaviours every 5 and every 10 s yielded similar results (data not shown).

Distances were measured in body lengths. The measurements were estimated by eye by the observer (GBN for *A. chrysopterus*, IMC for *S. leucostictus*). A distance from shelter of 0 indicated that the individual was within its shelter (i.e., either fully immersed in the tentacles of the anemone for *A. chrysopterus*, or completely hidden in the shelter for *S. leucostictus*), while a distance of 1 indicated that the focal fish was at the periphery of the shelter (i.e., merely touching the tentacles, or partly exposed at the shelter entrance). A score of 2 indicated a distance of one body length from shelter, a score of 3, two body lengths and so forth. At times where visibility of an individual was obstructed by a structure (e.g., a rock or coral), we averaged the last score before disappearance and the first score after reappearance. These two scores were usually only 1–2 body lengths apart. Where an individual swam out of the field of view of the camera (mean occurrence: 8.7% \pm 3.0 [SE] of sampling points), distance from shelter was recorded as the minimum distance from shelter according to the field of view in each video (i.e., depending on the maximum distance from shelter that the video frame captured). In these instances, distance from mate was estimated as the minimum distance of the missing individual from the present individual. Where both individuals swam out of view simultaneously, distance from mate was left blank (mean occurrence: 5.5% \pm 2.8). Distance from mate was always estimated in terms of the number of body lengths of the larger individual (i.e., the female).

Time out of shelter was scored as a binary factor (0 = in anemone/shelter, 1 = outside of anemone/shelter). This measure complements rather than duplicates the information yielded by "distance to shelter." As a fish can spend a long time at a very short distance from its shelter, the two measures are not necessarily correlated. Similarly, without "time out of shelter" information, very low averaged distances across individuals can mean either that time was spent within or merely near shelter.

Swimming activity was scored as 0 (motionless), 1 (slow swimming: ≤ 1 BL /sec), or 2 (fast swimming: ≥ 2 BL /sec) at the point of pausing the video. Whenever an individual swam out of the field of view of the camera, no swimming scores were recorded.

2.4 | Statistical analysis

For each behaviour, we tallied 300 (scored every 5 s) and 150 (scored every 10 s) behavioural scores for each individual of *A. chrysopterus* and *S. leucostictus*, respectively. Raw distance and swimming activity scores were averaged over 30-s intervals, and time out of shelter scores were converted to "proportion of time out of shelter per 30 s," to create a time series of 50 mean behavioural scores throughout each 25-min video for each individual.

We conducted preliminary analyses to determine the effect of gender on *A. chrysopterus* behaviour. We used a mixed-effects model (LME) for each behavioural measure and treatment (Cam, Obs), using sex as a covariate, time [min elapsed in video] as predictor, and breeding pair [pair] as random variable to account for non-independence of pair members ($y \sim \text{time} + \text{sex}$, random = pair). We determined that sex had no significant effect on any of the behavioural measures (distance from mate, Cam: $p = .66$, Obs: $p = .66$, proportion of time out

of shelter: $p = .70$ and 0.89 , swimming activity: $p = .15$ and 0.68). We thus combined male and female individuals to generate an average time sequence of behavioural scores across all breeding pairs for *A. chrysopterus*. Similarly, we created an average time series across all individuals for *S. leucostictus*.

We assumed that, at the point of acclimation to an observer or camera, we should be able to detect a stable change in mean behaviour within the time series (i.e., a point at which the behaviour becomes more or less pronounced). To assess potential acclimation points, we employed changepoint analysis in R using the package “changepoint” (Killick & Eckley, 2014). Changepoint analysis allows for the identification of shifts in mean of a process within a time series. We implemented the BinSeg method, allowing for the specification of the maximum number of changepoints. As we were looking for a single, stable change in behaviour, we set the number of allowed change points to 1 for all sequences, except for the “Obs” treatment in *A. chrysopterus* (i.e., when the observer was present for 25 min and then absent for another 5 min). In this instance, the number was set to 2 to allow for a potential change within the first 25 min and thereafter. Changepoint analysis typically includes a penalty that controls the sensitivity of the analysis. The penalty value may range from zero (maximum number of possible changepoints) to infinity (no changepoints). We assumed that changes in behaviour due to acclimation to an observer or camera would be subtle. To maximize sensitivity of changepoint detection, we chose a manually set penalty value of 1. To confirm statistical significance of the detected changepoints, we conducted paired-sample *t* tests comparing mean behaviours of the time intervals before and after a changepoint. We used *t* tests in the same way to compare mean behaviours between the time intervals of the Cam treatments before acclimation (i.e., omitting the intervals after acclimation had occurred according to the changepoint analysis) and the time intervals of the Obs treatments after the observer had left (i.e., the last five minutes in the 30 min videos).

To assess potential observer effects on behaviour in *A. chrysopterus*, we compared the behavioural time series between the Cam and Obs treatments for the section after which acclimation had occurred. More specifically, we omitted the first minutes in both treatments according to the longest time to acclimation in either treatment as determined by the changepoint analysis. We then employed ANCOVA in R to compare slopes and intercepts of LMEs of each behavioural variable ($y \sim \text{time}$, random = ID) between treatments.

3 | RESULTS

3.1 | Acclimation periods

In both focal species, we detected analogous changepoints in mean behaviour within several of the time series in the Cam treatments. For *A. chrysopterus*, we identified significant changepoints in three behavioural measures occurring between 6.5 and 7.0 min after the departure of the snorkeller (paired *t* test: distance from partner: $t_{12} = -5.01$, $p < .001$; distance from shelter: $t_{13} = -5.87$, $p < .001$; time out

of shelter: $t_{12} = -2.86$, $p = .01$) and one changepoint in mean swimming activity occurring after 2 min ($t_3 = 5.3$, $p = .01$) (Figure 2a, blue).

In *S. leucostictus*, we detected mean changepoints in two behavioural measures between 3 and 4 min after the snorkeller left (distance from shelter: $t_5 = -9.12$, $p < .001$; swimming activity: $t_7 = 2.38$, $p = .049$) (Figure 2b). There was no detectable changepoint in the time series of proportion of time spent out of shelter (Figure 2b).

In the Obs treatment (*A. chrysopterus* only), there were no detectable changepoints in any of the behavioural measures while the observer was present (Figure 2a, red). However, all but one behavioural measure increased significantly immediately upon the departure of the observer (distance from shelter: $t_9 = -6.75$, $p < .001$; proportion of time out of shelter: $t_9 = -4.33$, $p = .002$; swimming activity: $t_9 = -4.36$, $p = .002$) (Figure 2a, green). The only exception was distance to mate, which only increased marginally ($t_9 = -2.18$, $p = .057$) after the snorkeller left. The means of all behavioural measures in these final intervals of the Obs treatment (Figure 2a, green) were not statistically different from those of the pre-acclimation intervals of the Cam treatments (Figure 2a, blue) ($p > .05$ for all variables).

3.2 | Observer effect

The post-acclimation time series of *A. chrysopterus* in the Obs and Cam treatments exhibited parallel slopes, but significantly different intercepts in all measured behavioural variables (slopes: $p > .05$, intercepts: $p < .001$ for all variables) (Figure 2a, blue vs. red).

4 | DISCUSSION

Our findings reveal subtle but significant effects of observer and camera presence on the behaviour of wild coral reef fishes. We found evidence for temporary behavioural alterations due to the deployment and/or presence of a camera, indicating the need to incorporate acclimation periods when analysing recordings of *in situ* fish behaviour. Moreover, we found that, in the presence of a human observer, these behavioural alterations may outlast the duration of most commonly employed observation periods (i.e., 10–30 min; Figure 1b). Our findings have important ramifications for future behavioural studies of fishes in the wild.

4.1 | Acclimation periods

Our analyses indicate that the presence of a camera and/or its deployment by a snorkeller may result in a period of altered behaviour before the focal fish acclimate. Anemonefish stayed closer to their host anemone and their mate (by ~1 body length) and spent approximately 16% more time sheltered within the anemone for the first 6 to 7 min following camera deployment than after this period (Figure 2a). Levels of swimming activity were elevated for the first 2 min (Figure 2a). Similarly, beaugregory stayed closer to their shelter (by ~2 body lengths) and exhibited elevated swimming activity levels for the first 3 to 4 min following camera deployment (Figure 2b).

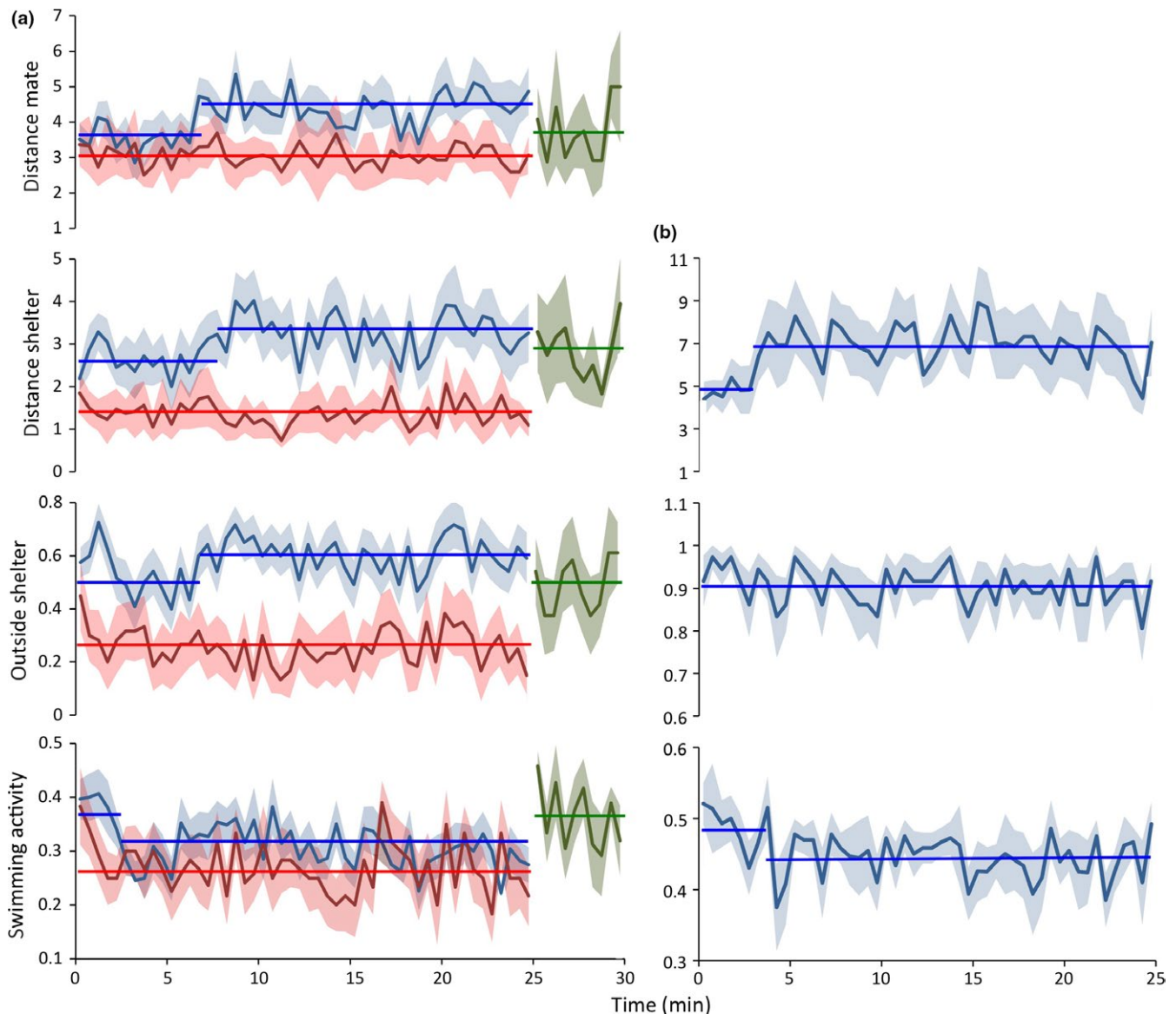


FIGURE 2 Behavioural time series including mean changepoints for (a) *Amphiprion chrysopterus* and (b) *Stegastes leucostictus*. Plots show mean variation (± 1 SE) in behaviours across all individuals over the observation period; red: observer and camera present ($N = 10$ individuals), blue: only camera present ($N = 20$ for *A. chrysopterus*, $N = 13$ for *S. leucostictus*), green: observer absent, after being present for 25 min ($N = 8$); horizontal lines indicate stable means determined using changepoint analysis. [Colour figure can be viewed at wileyonlinelibrary.com]

Both fish species displayed responses largely consistent with a relaxation of perceived predation risk occurring 2 to 7 min after the departure of snorkellers. Staying close to or hidden within shelter and seeking the proximity of conspecifics are behaviours that are expected in situations of elevated fear (Frid & Dill, 2002; Stankowich & Blumstein, 2005). The initial increase in swimming activity levels (first 2–4 min) on the other hand is somewhat inconsistent with previous findings of decreased activity levels in situations where predation risk is high (Lind & Cresswell, 2005). In this instance, it is important to note that the perceived threat (i.e., the snorkeller) had already left the area. While continued proximity to refuge and conspecifics may be a precautionary strategy, activity levels may have risen briefly after disappearance of the threat as fish left their shelters briefly to assess the situation, or in response to altered stress hormone levels that are

predicted to increase foraging and escapelike behaviours (Wingfield et al., 1998). This effect may be particularly marked in territorial individuals, such as damselfish, who must balance territory protection and sheltering. This idea is corroborated by studies on the behavioural responses of Australian sleepy lizards, *Tiliqua rugosa*, and Magellanic penguins, *Spheniscus magellanicus*, to human disturbance, where short periods of observer presence resulted in subsequent temporary increases in average activity levels (Kerr, Bull, & Mackay, 2004; Walker, Dee Boersma, & Wingfield, 2006).

Overall, we assume that the shifts in behaviour observed here reflect a return or convergence to “normal,” pre-observer levels. While we cannot make inferences about the exact cause(s) of the shifts (i.e., whether they are caused by the temporary presence of a snorkeller deploying the camera, the presence of the camera itself, or a combination

of both), our findings clearly demonstrate the need to incorporate an acclimation period when analysing recordings of *in situ* fish behaviour.

Acclimation periods varied both between the two species and among different behaviours within each species. Interspecific differences may be related to general differences in territoriality and habitat type. Beaugregory typically guard a larger territory than anemonefish (*personal observation*) and accordingly may need to resume patrolling their territorial boundaries (i.e., larger distance to shelter) more rapidly. The intraspecific differences in acclimation times among behavioural measures are intriguing because they may be relevant for animal personality research. Our results suggest that often-measured behavioural traits, such as boldness (distance from shelter, time outside shelter), activity levels (swimming activity) and sociability (distance from mate), may require different acclimation times. This variability may also pertain to laboratory and experimental settings, which are typically more often employed in personality research.

It is difficult to put our result in context because there are few data on acclimation times for any aquatic taxa. In fact, until now, there appeared to be no dedicated study aimed at quantifying minimum acclimation periods for behavioural observations in wild animals. Thompson et al. (2012) recently investigated optimal acclimation periods for laboratory experiments on oysters. However, it is important to differentiate between field- and laboratory-based studies, or pure observational vs. experimental research, as acclimation periods are unlikely to be analogous among these types of investigations. Animals should require longer periods to resume normal behaviour after being handled or placed into a novel situation rather than merely observed in their natural habitat (e.g., Kerr et al., 2004).

In our literature review of behavioural observations of fishes, 50% of the studies conducted with cameras did not report any acclimation period. Among the ones that did, one-third allowed an acclimation period of a maximum of 5 min (Figure 1a). According to our findings, however, acclimation to video deployment may last up to 7 min depending on the focal species and behaviours.

While the observed behavioural changes upon acclimation here were subtle, the omission of an acclimation period could significantly bias study results, depending on the types of questions asked and the behavioural variables investigated. Future studies investigating this phenomenon should aim to include more behavioural factors (e.g., foraging, aggressiveness) across a variety of species/taxa to determine more general recommendations for acclimation periods in behavioural studies on wild animals.

4.2 | Observer effect

Our findings further demonstrate that, in the presence of a human observer, wild anemonefishes may show altered behavioural patterns lasting up to 25 min and probably longer. Similarly to the camera treatment, individuals tended to exhibit behavioural responses consistent with the perception of an elevated threat, that is, they stayed closer to their breeding partner, spent more time closer to or hidden within the host anemone and exhibited decreased swimming activity levels in the presence of an observer compared to just a camera (Figure 2a,

red vs. blue) (Frid & Dill, 2002; Lind & Cresswell, 2005; Stankowich & Blumstein, 2005).

These findings concur with previous research demonstrating observer effects on behavioural patterns in different taxa, such as invertebrates (Baker & McGuffin, 2007; MacFarlane & King, 2002), reptiles (Kerr et al., 2004) and mammals (Iredale et al., 2010). Until recently, however, evidence for observer effects in behavioural studies of wild fishes has been sparse (but see Chapman et al., 1974). Watson and Harvey (2007) reported changes in average distances to a stationary camera in reef fishes between treatments with a SCUBA diver present and absent. In a more recent study on behaviour in coral reef fish communities, Pereira et al. (2016) demonstrated that several behaviours varied significantly depending on whether they were recorded by divers or cameras. Analogous to our findings, they observed an increase in time spent sheltering among coral branches and a decrease in swimming activity in the presence of a diver compared to a camera.

Interestingly, all behaviours increased rapidly immediately after the observer left the area (Figure 2a, green). Mean levels in these last 5 min without an observer corresponded markedly with pre-acclimation levels exhibited by individuals in the camera treatment (Figure 2a, blue). This pattern does not only confirm that the observed differences between camera and observer treatments were in fact related to observer presence, but also it indicates that the duration of observer presence (< 1 min in the camera treatment vs. 25 min in the observer treatment) has no effect on the initial behavioural reaction after the observer leaves (i.e., both periods resulted in equal levels of behavioural expression).

In our literature survey, more than 68% of the studies conducted by an observer did not report any acclimation period and only two allowed for more than 5 min of acclimation (Figure 1a). According to our findings, however, the required acclimation period when an observer is present may exceed 25 min; in fact, acclimation perhaps never occurs for some behaviours. Considering that the majority of reported observations lasted less than 20 min (Figure 1b), this is a worrisome result.

Observer effects may not be relevant to the conclusions of studies in which all treatments or individuals are affected equally by the bias introduced by observer presence. However, this will not be the case for all experimental designs. For instance, individuals in different habitats or areas visited by variable numbers of marine tourists may show variable reactions to observer presence because of habituation to divers and snorkellers (Titus, Daly, & Exton, 2015). Yet, in most fieldwork situations, it is clearly unfeasible to incorporate acclimation periods of ≥ 30 min per observation. Therefore, where possible, we advocate the use of remote camera equipment with a suitable delay between the onset of recording and the beginning of data collection.

5 | CONCLUSIONS

This study substantiates previous findings of observer effects on behavioural patterns in wild demersal coral reef fishes. Moreover, we demonstrate the need for an acclimation period of several minutes preceding analysis of behaviour scored using video equipment.

While our findings may be pertinent to other species with similar biological characteristics, we cannot make generalizations on such patterns in more mobile species with different habitat requirements. We advocate the use of our approach to empirically assess appropriate acclimation periods prior to behavioural analyses across a range of species/taxa in different investigational settings.

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DATA ACCESSIBILITY

Raw behavioural scores for both species are available from the Pangeae Data Repository (<https://doi.org/10.1594/PANGAEA.876132>).

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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