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Long-term memory retention in a wild fish species *Labroides dimidiatus* eleven months after an aversive event

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Abstract

Memory is essential to enhance future survival and reproduction as it helps in storing and retrieving useful information to solve particular environmental problems. However, we lack quantitative evidence on how far animals in the wild can maintain given information for extended periods without reinforcement. Here, we document correlative evidence of cleaner fish *Labroides dimidiatus* remembering being caught in a barrier net for up to 11 months. In 2015, about 60% of cleaners from one large isolated reef had been used for laboratory experiments and then returned to their site of capture. Eleven months later, 50% of cleaners at the same site showed an unusual hiding response to the placement of the barrier net, in contrast to three control sites where no cleaners had been caught during the last 2 years. The results suggest that a single highly aversive event (i.e., being caught in a barrier net) resulted in cleaners storing long-term crucial information that allowed them to avoid being caught again. Our results further our knowledge of fish cognitive capacities and long-term memory retention.

KEYWORDS
cognition, escape behaviour, female cleaner fish, learning, memory retention

1 | INTRODUCTION

Solving a particular environmental problem may need the retrieval of learned and memorised information that is suitable for the specifics of the situation (Shettleworth, 1993). For instance, recalling mate location in a particular area or making the association between specific events and negative stimuli, such as predator presence, may help individuals to make beneficial decisions (Gerber et al., 2014). A memory to enhance future survival and reproduction is hence essential in a variable and challenging environmental conditions (i.e., food availability, predation risk, contextual information, etc.) (Brom, Both, Laan, Everaerd, & Spinhoven, 2014; Dunlap, McLinn, MacCormick, Scott, & Kerr, 2009; Templer & Hampton, 2013).

Long-term memories that can last at least a few months to decades have been documented in nonhuman animal species. As such, long-term memory in recognising conspecifics in songbirds can be retained at least 8 months to 3 years (Boeckle & Bugnyar, 2012), 1 year in hyena (Holekamp, Sakai, & Lundrigan, 2007), 3 years in monkeys (Murai, Tanaka, Tomonaga, & Sakagami, 2011), and for decades in elephants (Mccomb, Moss, Sayialel, & Baker, 2000) and dolphins (Bruck, 2013). As it has been shown that the neural basis of learning and memory is well conserved across vertebrate species (O'Connell & Hofmann, 2011; Salas et al., 2006), there are also widespread examples of learning and memory abilities in fish that are similar to other vertebrate species (see review by Brown, 2015).

Concerning long-term memory, various studies provided evidence that fish can retain information in their memory for a few weeks up to several months. As such, rainbow trout retrieved an information consolidated 3 months ago, wherein they had to press on a trigger to get food delivered to them (Adron, Grant, & Cowey, 1973); gobies returned to their home pools 40 days after being translocated to neighbouring pools (Aronson, 1971); anemonefish still remembered landmarks of their anemones' neighbourhood after 6 months (Fricke, 1974); tilapia responding to an acoustic stimulus up to 6 months after conditioning (Zion et al. 2011). What may strengthen memory retention capacities is the valence of the event, wherein long-term memories documented in the literature are often memories of adverse events (Brown, 2001, 2015; Gerber et al., 2014; Schafe, Nader, Blair, & LeDoux, 2001). For instance, salmon and carp that had been caught with hook and line showed hook-shyness 1 year later without any exposure in between (Beukema, 1970; Tarrant, 1964). Similarly, crimson spotted rainbowfish retrieved the memory of an escape strategy learned in a laboratory experiment 11 months earlier (Brown, 2001). Although, the author of the study acknowledged that familiarity with the test environment could have been a potential confounding factor (Brown, 2001).

There are only a few studies on long-term memory retention in wild species (see above). Here, we add to the scarce literature on long-term memory in wild fish species by providing correlative evidence of cleaner fish *Labroides dimidiatus* (hereafter "cleaner") remembering a highly aversive event (i.e., being caught in a barrier net) 11 months later. We know that catching the same cleaner fish twice with a barrier net within relatively short periods (i.e., within 4 weeks) is rather difficult because targets alter their behaviour to avoid being caught again (R.B., personal observation). Initially, cleaners do not perceive divers as a threat, and the lines making up the net are thin and somewhat difficult to see (net size: 2 m × 1 m, 5 mm mesh). To catch a cleaner in the wild, the diver swims typically after the target and tries to guide it towards the barrier net. Once closer to the barrier net, the diver accelerates and causes the cleaner to get entangled in the net in its attempt to escape. However, cleaners that had been captured, marked and released, largely avoided the barrier net during an attempt of recapture 4 weeks later, and they opted to hide in small crevices instead (Wismer, 2017). Marking cleaners in that study was part of the experimental design, and it was necessary to facilitate the identification and recapture of the same individuals; an approach that is rarely needed in other studies performed on cleaner fish. However, as cleaners are highly territorial fish (Losey 1979; Bshary, 2001) and remain on their home territory even after being removed and returned to the reef (Wismer, 2017), location emerges as a good correlate of cleaner fish identity. Therefore, we started collecting data at capture sites more systematically once we had been aware of the apparent effects of catching. If cleaners have the capacity of long-term memory, then they should avoid the barrier nets after a significant retention interval.

2 | MATERIAL AND METHODS

2.1 | Study site and animals

Data were collected in July-August 2015 and June 2016 at Lizard Island, Great Barrier Reef, Australia, (14.6682°S, 145.4604°E). Data collection was part of ongoing projects at the time. In the projects, we only studied female cleaner fish as they are much more abundant than males (Triki & Bshary, 2018; Triki, Wismer, Levorato, & Bshary, 2018; Triki et al., 2019). Cleaners are protogynous hermaphrodites, that is they start their life as females and only switch sex to become a male harem holder when they reach a critical size (Robertson, 1972). Female cleaners hold small territories so-called "cleaning stations" where they are visited by a variety of "client" fishes to get their ectoparasites removed (Losey 1979). Precise data on various life-history parameters are missing, like age when reaching maturity and when changing sex. Cleaners supposedly reach a maximal age of 5 years, with a low estimate in yearly mortality rate (11%) at least during the early stages of adulthood (Eckert, 1987).

2.2 | Data collection

In 2015, we studied a large isolated reef site (Site 1 in Figure 1) harbouring about 65 adult cleaners (i.e., females and males). Cleaners numbers on Site 1 were estimated by two divers by swimming 2 m-wide circular transects from the outside of the reef towards its centre. The study aimed to test whether substantial reduction (i.e., 50%) in cleaner densities would affect female cleaner cleaning interactions (Demairé, Triki et al. *unpublished*). Therefore, we initially caught 31 female adult cleaners on the 23rd of July 2015, and later another ten female cleaners on the 28th of August 2015 to test for any changes in laboratory experiments as a consequence of the reduction in cleaner densities. Thus, a total of 41 adult female cleaners had been

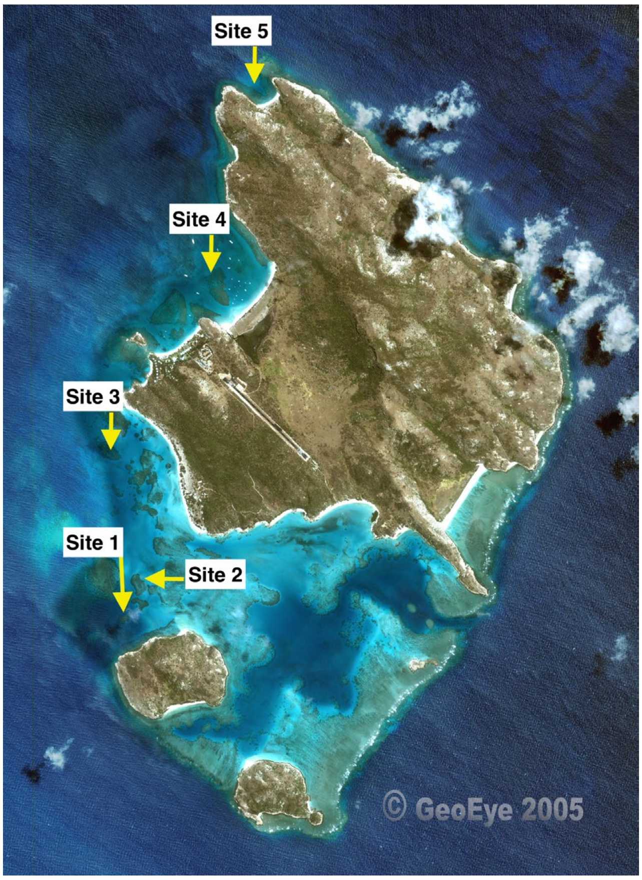

FIGURE 1 Map of the Lizard Island Group. Data were collected on the sites indicated by filled arrows: (Site 1) the treatment site Deep Vickie; and the four control sites: (Site 2) North Horseshoe, (Site 3) Casuarina beach, (Site 4) Watson's bay and (Site 5) Mermaid Cove [Colour figure can be viewed at wileyonlinelibrary.com]

caught in 2015 (63% of adult cleaners). All cleaners were brought to the laboratory facilities at the Lizard Island Research Station (LIRS) for experiments, and they were eventually released to their respective capture locations at the study reef site in mid-September 2015.

On the 25th of June 2016, we visited the same reef to collect cleaners for another project. To do so, we followed the usual procedure of cleaner fish catching methods: RB placed the barrier net (net size: 2 m × 1 m, 5 mm mesh) near the first cleaning station detected. The female cleaner, however, went into hiding immediately. The catching attempt was repeated three times for the same fish, and every time the target kept hiding for a while before reappearing relatively far away from the barrier net (~5 m). Upon such observations, we decided to take systematic notes of the capture attempts. To do so, the two divers first agreed on a fish for capture. Then one diver would place the net at a location where the cleaner had just passed. The net placing takes about 30 s; meanwhile, the second diver would observe the behaviour of the targeted cleaner. More precisely, the second diver noted whether the cleaner went into hiding, either in small crevices in the reef structure or below corals. In a single dive, we encountered a total of 14 individuals, nine females and five males. Given that the larger females caught in 2015 were likely to have changed sex in the meantime, we scored the response of all individuals. At Site 1, two males disappeared without us being able to determine whether they went into hiding or whether they moved on to visit another female. We, therefore, excluded these two males from the analysis. Furthermore, we encountered two very small females that did not hide. We caught them and measured their total length, which was 6.4 cm and 6.5 cm, respectively. These individuals must have been juveniles in 2015 (Gingins & Bshary 2016) and hence were also excluded from the analysis. As a control, we tested 42 female cleaners at four different reef sites (Site 2 to Site 5 Figure 1), using the same approach as above. None of the control sites had been exposed to capture attempts of cleaners between 2015 and 2016, yielding a minimum of 22 months since last attempts in 2014 (information from the archives of projects compilation forms at Lizard Island Research Station).

2.3 | Ethical note

Fish capture received ethical approval from the Animal Ethics Committee of the Queensland Government (DAFF) (permit numbers: CA 2015-06-869; CA 2016/05/97; CA2016/05/972 and CA 2016/06/974).

2.4 | Data analysis

Data were analysed in the free statistical software R version 3.5.3. We fitted a Bayesian generalised linear model `bayesglm()` from the package `arm` in R language. The model was chosen due to the presence of perfect separation in the data distribution, wherein the four control reef sites had zero hiding response. *Post hoc* analysis was run with the `emmeans` package in R language to help in generating an estimate. Statistical script and data are accessible at the public repository Figshare: https://doi.org/10.6084/m9.figshare.8248142

3 | RESULTS

We recorded five fish that went into hiding (4 females, 1 male) while five did not respond to the net (3 females, 2 males). At the control sites, not a single female went into hiding (Bayesian GLM: $\chi^2 = 24.026$, $p < .0001$). The only site where hiding had occurred had an estimate of 1.170, with $p = .0008$. The control sites had zero hiding response (Site 2: estimate = −0.425, $p = .725$; Site 3: estimate = 0.042, $p = .940$; Site 4: estimate = −0.139, $p = .940$; Site 5: estimate = −0.647, $p = .575$) (Figure 2).

4 | DISCUSSION

Our data provide correlative evidence that cleaner fish can remember a single negative experience (i.e., being caught in a barrier net) about 11 months later in the wild. Although we were not able to identify the fish to the individual level, the 50% (five out of ten fish) hiding response at Site 1 suggests that this particular behaviour is the result of long-term memory retention of being caught in a barrier net. We consider it most likely that fish that went into hiding reacted to the barrier net instead of to the mere presence of divers. This is because when a fish went into hiding, the very same individual re-emerged when the barrier net had been removed. However, it is possible that the combination of the presence of a barrier net and the close proximity of the diver triggered the hiding response. Only more detailed future experiments, with larger sample size, may hence allow determining how previously caught individuals respond to either a diver, to a net or to the combination of the two to infer what cues cleaners remember.

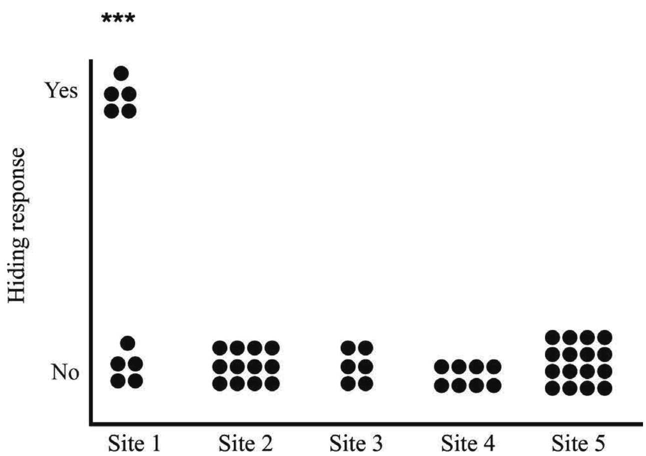

FIGURE 2 Cleaners' response to a barrier net (hiding "yes" or "no." (Site 1) Deep Vickie; (Site 2) North Horseshoe; (Site 3) Casuarina beach; (Site 4) Watson's bay; (Site 5) Mermaid Cove. Site 1 is the treatment site, while the other four sites served as a control. ***: $p < .001$

We suggest that the five fish showing a hiding response to the barrier net are more likely to be part of the group of cleaners caught in 2015 on the same site, for several reasons. As it stands, cleaners have relatively low mortality rate, estimated by Eckert (1987) to be around 11% in adults per year, and they show high site fidelity (Losey 1979; Bshary, 2001; Wismer, 2017). Furthermore, it should be noted that a hiding response in the wild is extremely unusual behaviour in cleaner fish (personal observations, RB and Alexandra Grutter). Cleaners even approach and enter predators mouth without getting eaten (Grutter, 2004; Potts, 1973). In addition, there are various pictures and movie clips on the web showing that cleaner fish can even approach and enter the mouth of snorkelers and divers. Taken together, the available knowledge about the cleaner life-history and the circumstantial evidence regarding their usual interactions with divers support the view that the previous capture had been memorised and hence caused the cleaners' change in behaviour.

The ability of cleaners to remember for at least 11 months a single event of being caught in a barrier net is similar to long-term memory retention proposed to cause escape response from a trawl in crimson spotted rainbowfish (Brown, 2001), and hook-shyness in salmon and carp (Beukema, 1970; Tarrant, 1964). These memories, being caught in a barrier net, trawl or a fishhook, have in common that both are consequences of highly aversive events linked to life-threatening events. It is hence crucial to retain information after a single exposure and to adjust behaviour in a similar future event. A recent study on sharks, for instance, has shown that individuals may learn to avoid being caught after the catch-and-release experience (Mourier, Brown, & Planes, 2017). Indeed, catching a cleaner that goes into hiding is much more difficult for a diver than to catch a cleaner that swims in the open (personal observation).

An interesting fact about our control data is that none of the 42 cleaners from the control reefs went into hiding and hence were captured for laboratory experiments. The control reef sites 2, 3, 4 and 5 had been visited to collect cleaners in 2014 (archives of LIRS). The lack of any indication for long-term memory of being caught before could be due to various factors. First, at least site 2 and site 5 are larger, and hence we may have, by chance, missed previously captured individuals. Second, with a delay of almost 2 years, most individuals may have been either dead or transformed into males and not subjected to capture attempts. Third, it could be that long-term memory retention is shorter than 2 years in cleaner fish. Only further research may help to determine long-term memory retention potential in cleaners. In general, more studies on wild species in their natural habitats are needed to understand the memory capacities of wild animals better.

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CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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