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Divergent learning responses to a spatially consistent olfactory stimulus in two reef shark species

James P. Kilfoil¹, Gabrielle Krohn², Eric E. G. Clua^{3,4}, Serge Planes^{3,4},
Kirk R. Gastrich⁵, Michael R. Heithaus⁵, Aaron J. Wirsing^{2,*}

¹Marine Resources Research Institute, South Carolina Department of Natural Resources, Charleston, SC 29412, USA

²School of Environmental and Forest Sciences, University of Washington, Seattle, WA 98195, USA

³PSL Research University, EPHE-UPVD-CNRS, USR 3278 CRILOBE, Université de Perpignan,
58 Avenue Paul Alduy, 66860 Perpignan Cedex, France

⁴Laboratoire d'Excellence 'CORAIL', EPHE, PSL Research University, UPVD, CNRS, USR 3278 CRILOBE,
Papetoai, Moorea, French Polynesia

⁵Institute of Environment, Department of Biological Sciences, Florida International University, North Miami, FL 33181, USA

ABSTRACT: There is growing evidence of the important role learning plays in shark foraging, but few studies have examined the relationship between learning and foraging behavior in free-living settings. We addressed this knowledge gap by experimentally contrasting responses of blacktip reef *Carcharhinus melanopterus* and sicklefin lemon *Negaprion acutidens* sharks to an olfactory-only feeding stimulus — baited remote underwater video stations (BRUVS) — that was either spatially randomized (as a control) or offered repeatedly at the same location in the lagoon of Tetiaroa, French Polynesia. Relative to their response to the randomized BRUVS, blacktip reef sharks appeared to sensitize to the repeated treatment, exhibiting increasing relative abundance upon introduction of the cue (maximum number of individuals of a species observed on any frame of a video [MaxN] at deployment) and decreasing arrival times as the experiment progressed. By contrast, sicklefin lemon shark responses were either consistent across control and treatment BRUVS over time or suggested habituation (as evidenced by declining MaxN in response to the spatially repeated exposure). Accordingly, our findings advance our understanding of shark cognition by highlighting that sensitized learning responses to stable feeding cues can develop even when the olfactory attractant is not accompanied by a reward, while also indicating that shark responses to these cues can be species-specific. They also suggest that, for at least some shark species, olfactory cues alone could lead to learned responses that confound non-invasive efforts to monitor shark populations and communities (e.g. with BRUVS) and drive spatial behavior with the potential to affect both ecotourism and negative human-shark interactions.

KEY WORDS: Blacktip reef shark · BRUVS · Foraging · Habituation · *Negaprion* · Sensitization · Sicklefin lemon shark

1. INTRODUCTION

For many animals, learning shapes a variety of behaviors including dispersal (Clobert et al. 2009), foraging (Krebs & Inman 1992), habitat use (Wolf et al. 2009), migration (Couzin 2018), predator avoidance (Brown & Chivers 2005), and reproductive decisions

(Ryan et al. 2007). Our understanding of this cognitive process has contributed to innovative conservation efforts being developed and enacted (Berger-Tal et al. 2016), including management aimed at mitigating human–wildlife conflict (Blackwell et al. 2016, Snijders et al. 2019, Guomas et al. 2020). Much of what we know about animal cognition, or information

*Corresponding author: wirsinga@uw.edu

processing and associated learning capacity (Schluessel 2015), and its role in behavior comes from studies of birds and mammals (Shettleworth 2009, Matsubara et al. 2017). By contrast, research on cognition and learning in large-bodied fishes, including sharks, remains in its infancy (Brown & Schluessel 2023). Thus, studies of the potential for and implications of learning in these taxa, and especially those exploring learning in relation to natural behavior, would improve our understanding of their ecology and conservation (Guttridge et al. 2009).

There is growing evidence that elasmobranchs, including sharks, are on a par with other vertebrates in performing diverse cognitive tasks, ranging from discrimination and habituation to imitation in social settings, long-term memory, and even tool use, all of which presumably aid in making foraging, movement, and reproductive decisions (Guttridge et al. 2009, Brena et al. 2015, Schluessel 2015, Brown & Schluessel 2023). One dimension of shark learning that has recently received considerable attention is how they respond to feeding stimuli, including under natural conditions (Brown & Schluessel 2023). For example, sharks (most commonly the sicklefin lemon shark *Negaprion acutidens*) in Ningaloo Marine Park, Western Australia, exhibited significantly shorter arrival and first-feeding times in a fished area where depredation was known to occur relative to a no-take marine reserve (Mitchell et al. 2020). By implication, individuals in the former area had learned to associate the presence of boats (including the vessel used by the researchers) with food. Similarly, Heinrich et al. (2021) found that juvenile lemon sharks *N. brevirostris* in Bimini, Bahamas, learned to anticipate feeding opportunities, as evidenced by fine-scale spatial shifts toward the location where the feeding took place. Furthermore, Séguigne et al. (2022) found that anticipation of anthropogenic feeding by blacktip reef sharks *Carcharhinus melanopterus* in Moorea, French Polynesia, lasted through a 6 wk Covid-19 lockdown.

The capacity of sharks to associate feeding with places and times has been leveraged by the diving and ecotourism industries for decades. For example, some dive-operators encourage the establishment of shark-viewing sites by repeatedly offering food sources at a location, so that sharks will learn to associate that place with a food reward, in a practice known as provisioning (Brena et al. 2015). Although this practice offers numerous potential conservation benefits, including promoting non-extractive value to sharks and unique educational opportunities, it is also capable of negatively affecting shark behavior and health (Gallagher et al. 2015, Gallagher & Huve-

neers 2018). These often-unintended consequences could include (but are not limited to) deviations from natural food sources (Abrantes et al. 2018), increased parasitic loads (Semeniuk & Rothley 2008), alterations to natural movement patterns and habitat use (Corcoran et al. 2013, Mourier et al. 2021), and enhanced likelihood of shark bites on tourists (Clua 2018). Consequently, several countries, states, and territories have enacted bans on shark-diving activities that include food rewards (e.g. Florida, Hawaii, New Caledonia, French Polynesia; Ward-Paige 2017). Other areas are promoting the use of 'olfactory-only' provisioning (e.g. bait plume with no feeding) as means to attract sharks without the need to feed (e.g. South Australian white shark cage-dive operators; Department of Environment, Water and Natural Resources 2016). However, the viability of olfactory-only stimuli as an effective alternative to food-based provisioning for dive operators remains largely unknown.

To date, what little research that has been conducted to address this question has largely relied on laboratory experiments. Of particular note is work by Heinrich et al. (2022), who tested for the potential of habituation to a food-related olfactory cue in captive juvenile Port Jackson sharks *Heterodontus portusjacksoni* in Jervis Bay, Australia. In this experiment, sharks were exposed to either an olfactory stimulus, feeding, partial feeding, or a control (no stimuli). The olfactory-only stimulus resulted in patterns consistent with learned habituation (loss of interest in the olfactory cue over time in the absence of food reward) compared to the feeding groups. If these results translate to non-captive environments, they raise questions about the viability of olfactory-only provisioning by the diving industry because sharks would likely lose interest in a dive site after a certain amount of time in the absence of food rewards. If confirmed in free-living situations, these results also have broader implications for the conservation and management of shark populations, particularly as they apply to non-invasive scientific sampling methods.

Non-extractive and non-destructive sampling techniques such as baited remote underwater video stations (BRUVS), which attract sharks with olfactory cues but use cages to prevent bait depletion, have gained traction in the last decade as a cost-effective method to survey marine species abundance, behavior, and diversity (Whitmarsh et al. 2017, Schramm et al. 2020). Compared to traditional sampling with fishing gears, these techniques offer numerous benefits, including a long-term record of the data (Harvey et al. 2013), exposure to behavior and habitat use of cryptic taxa (Langlois et al. 2010, Bacheler et al. 2013), and

reduced issues with size (Wells et al. 2008) and species selectivity (Bacheler et al. 2013). BRUVS also have a number of inherent limitations when studying elasmobranchs, however. First, in areas with high shark densities, traditional BRUV count metrics may be negatively biased, leading to underestimation of abundance (Kilfoil et al. 2017). Second, variation in environmental conditions, species morphology, and behavior can each influence shark counts from BRUVS footage (Kilfoil et al. 2021). The insights provided by BRUVS could also be biased if sharks can learn from the olfactory cues they generate. Namely, sharks could become habituated to the olfactory stimulus of BRUVS deployments given that no direct food reward exists, resulting in negatively biased counts. Conversely, if BRUVS are repeatedly deployed in close proximity and sharks are in some way sensitized or conditioned to their presence, increasing attraction over time could inflate estimates of local abundance.

To address these possibilities, we used the lagoon environment of Tetiaroa, French Polynesia, to experimentally compare responses of blacktip reef and sicklefin lemon sharks to an olfactory-only stimulus (BRUVS) that was either spatially randomized or offered repeatedly at the same location. Under this experimental setup, we assumed that spatiotemporally randomized BRUVS deployments provide no opportunity for sharks to demonstrate a learned response to the olfactory stimulus and hence serve as a control. In accord with the findings of Heinrich et al. (2022), we hypothesized that, without a paired feeding reward, spatially repeated olfactory cues alone should lead to habituation over time. Under this hypothesis, we predicted that both species would exhibit (1) decreasing maximum counts (relative abundance) overall and at the time of deployment and increasing (2) arrival times and (3) times to reach maximum relative abundance over time at repeated but not randomized BRUVS sampling sites.

2. MATERIALS AND METHODS

2.1. Study area and field sampling

Data for this study were collected as part of an ongoing investigation of blacktip reef shark and sicklefin lemon shark behavior from 18 July–9 August 2016 in Tetiaroa (17.000° S, 149.550° W), a small French Polynesian atoll in the Society Islands approximately 55 km from Tahiti. Tetiaroa experiences a tropical climate, with a rainy season spanning November to April and dryer weather prevailing from May through Oc-

tober. The tidal range within its lagoon is narrow (<0.5 m; Jeanson et al. 2014) and, notably, the brevity of the investigation minimized temporal variation in environmental conditions such as temperature and tidal state. BRUVS were used to survey blacktip reef sharks and sicklefin lemon sharks within the atoll's inner lagoon at depths ranging from 0.4 to 13.7 m and either at randomly chosen locations ($n = 42$ deployments) or repeatedly at a single location ($n = 30$ deployments). The lone location chosen for repeated BRUVS deployments was typical of those characterizing the random deployments in terms of substrate and surroundings; the depth of the repeated site was 1.98 m, whereas the median depth for the randomized sites was 2.98 m. The repeated BRUVS site was not used for any random deployments. All BRUVS deployments were undertaken according to the approach described in detail by Kilfoil et al. (2017). Briefly, BRUVS consisted of aluminum frames equipped with a single GoPro Hero4 camera and baited with 1 kg of thawed and crushed sardines (*Sardina pilchardus*, *Sardinops* spp.) and were set on sandy sediment throughout the day (~08:00–15:00 h) by snorkelers to ensure correct placement and orientation. Once settled on the sea floor, all BRUVS soaked for roughly 60 min; we set between 3 and 8 randomly located BRUVS (mean = 5.3) and 1 or 2 repeated BRUVS (at a single location; mean = 1.5) per day. Given that fewer BRUVS could be deployed at the repeated location per day, we deployed the repeated BRUVS over a longer duration than the randomly located BRUVS (25 July through 4 August) to achieve a sufficient sample of the former. All BRUVS sampling locations were a minimum of 1 km apart, and no randomized location was resampled over the course of the investigation. All fieldwork was non-invasive and conducted under University of Washington Institutional Animal Care and Use (IACUC) protocol #4226-08, a permit issued by the Territorial Government of French Polynesia (Délégation à la Recherche) and the Haut-commissariat de la République en Polynésie Française (DTRT) (Protocole d'Accueil 2016), and a permit (Arrete N° 9524) to work on reef sharks in French Polynesia issued by Direction de l'Environnement (DIREN).

2.2. Statistical analysis

After sampling, video files collected by the BRUVS were stitched, synchronized, and analyzed by trained, independent observers. We extracted 4 behavioral metrics from the BRUVS footage for each shark species: MaxN (the maximum number of individuals of a species observed on any frame of a video; Ellis &

DeMartini 1995) for the duration of the deployment; MaxN at the time of deployment (i.e. within the first 5 min; hereafter 'initial MaxN'); time to first arrival; and time to MaxN. MaxN is a proxy for relative abundance (Harvey et al. 2018) that is used widely to assess shark population status (e.g. MacNeil et al. 2020) as well as habitat use (e.g. Stoffers et al. 2021). We then examined temporal trends in each of the 4 metrics as a function of sampling date using Mann-Kendall (MK) tests in the R package 'trend' (Thorsten 2020). The MK test is a rank-based, non-parametric approach that is robust to outliers and commonly used to determine if there is a monotonic trend in time series data (Hipel & McLeod 1994). For all 4 metrics, we considered there to be evidence for a significant difference between behavioral responses to the randomized (control) and repeated (treatment) BRUVS deployments if either a significant temporal trend was observed for the repeated but not the randomized BRUVS, or significant temporal trends were observed for both deployment types but in opposite directions. Accordingly, we consider the habituation hypothesis to be supported if the MK test revealed a significant, negative trend in MaxN and initial MaxN with respect to sampling date for the repeated but not the random BRUVS deployments, and significant, positive trends in time to first arrival and time to MaxN with respect to sampling date for the repeated but not the random deployments. For all MK tests, we set the threshold for significance at $p \leq 0.05$.

Given that repeated BRUVS were deployed over a longer span of time than the random BRUVS, we were concerned that our full analysis might be biased toward detecting temporal trends in shark responses to the former. To explore this possibility, we conducted an auxiliary analysis in which data from both deployment types were restricted to the first 10 d of deployment (Supplement; www.int-res.com/articles/suppl/m738p151_supp.pdf). The results of this truncated analysis generally matched those of the full analysis, particularly with respect to the significant temporal trends in initial MaxN and time to arrival in blacktip reef sharks for the repeated BRUVS (see Table S1, Fig. S1 in the Supplement). Thus, for the remainder of the paper we focus on the results of the analysis incorporating all BRUVS deployments.

Each random BRUVS was deployed in a distinct location. On days when 2 repeated BRUVS sets were achieved, however, the second set was, by definition, redeployed at the same location where the first one had been positioned. Though at least 3 h elapsed between deployments, this arrangement raises the possibility that sharks attracted to the earlier repeated set

might have been closer than at random to the location of the repeated BRUVS at the time of the second deployment. To explore this possibility, we used Wilcoxon signed-rank tests to compare MaxN, initial MaxN, and time to arrival in blacktip reef sharks, for which we found multiple significant temporal trends (see the Supplement), associated with the first and second deployments on the same day. Blacktip reef shark MaxN values were actually lower for the second set on the same day at the repeated site (Fig. S2), and initial MaxN (Fig. S3) and time to arrival (Fig. S4) did not differ markedly between the 2 sets, suggesting that any attraction bias stemming from using the same repeated site on a given day was minimal or, in the case of MaxN, ran counter to overall results.

3. RESULTS

3.1. MaxN

Over the course of the investigation, MaxN values for blacktip reef sharks averaged 1.71 ± 1.13 SD (range = 0–5) for randomly deployed BRUVS and 5.44 ± 1.72 SD (range = 2–9) for BRUVS deployed at the repeated site. For sicklefin lemon sharks, mean MaxN values were 0.36 ± 0.62 SD (range = 0–2) for the random BRUVS and 2.31 ± 1.15 SD (range = 0–4) for those deployed repeatedly at the same site. We found significant evidence that MaxN increased over time in blacktip reef sharks for both the randomly and repeatedly deployed BRUVS (Table 1, Fig. 1a). In sicklefin lemon sharks, there was no evidence that MaxN values varied with time for the random deployments, whereas MaxN values decreased significantly with time for the repeated deployments (Table 1, Fig. 1b).

3.2. Initial MaxN

There was significant evidence that MaxN at the time of deployment (first 5 min) increased over time in blacktip reef sharks for repeated but not randomized BRUVS deployments (Table 1, Fig. 1c). For sicklefin lemon sharks, we found no evidence that initial MaxN varied with time for either random or repeated BRUVS deployments (Table 1, Fig. 1d).

3.3. Time to arrival

The MK tests produced significant evidence that time to arrival decreased over time in blacktip reef

Table 1. Results of Mann-Kendall (MK) tests for monotonic trends in 4 behavioural metrics—MaxN (maximum number of individuals of a species observed on any frame of a video) for the duration of the deployment, MaxN at the time of deployment (i.e. within the first 5 min; ‘initial MaxN’), time to first arrival, and time to MaxN—extracted from baited remote underwater video surveys (BRUVS) deployments targeting blacktip reef *Carcharhinus melanopterus* (‘blacktip’) and sicklefin lemon *Negaprion acutidens* (‘lemon’) sharks and deployed either at random locations or repeatedly at the same location. Cases where there was significant evidence for a change in values over time (p -values ≤ 0.05) are presented in **bold**

Species	Treatment	Metric	Statistic	p-value
Blacktip	Random	MaxN	2.229	0.026
Blacktip	Random	Initial MaxN	0.437	0.662
Blacktip	Random	Time to arrival	−0.393	0.694
Blacktip	Random	Time to MaxN	1.493	0.135
Blacktip	Repeated	MaxN	2.894	0.004
Blacktip	Repeated	Initial MaxN	3.126	0.002
Blacktip	Repeated	Time to arrival	−4.307	<0.001
Blacktip	Repeated	Time to MaxN	−1.054	0.292
Lemon	Random	MaxN	0.275	0.783
Lemon	Random	Initial MaxN	−0.148	0.883
Lemon	Random	Time to arrival	1.375	0.169
Lemon	Random	Time to MaxN	1.303	0.193
Lemon	Repeated	MaxN	−2.076	0.038
Lemon	Repeated	Initial MaxN	−1.666	0.096
Lemon	Repeated	Time to arrival	−0.293	0.769
Lemon	Repeated	Time to MaxN	−1.064	0.287

sharks for repeated BRUVS deployments, whereas this metric did not change significantly with time for the random deployments (Table 1, Fig. 1e). There was no evidence to suggest that time to arrival changed with time for either BRUVS deployment type in sicklefin lemon sharks (Table 1, Fig. 1f).

3.4. Time to MaxN

In blacktip reef sharks, there was no evidence that time to MaxN varied over time for either the random or repeated BRUVS deployments (Table 1, Fig. 1g). There was also no evidence that time to MaxN changed over time in sicklefin lemon sharks (Table 1, Fig. 1h).

4. DISCUSSION

Evidence for learning in sharks is growing (Guttridge et al. 2009, Schluessel 2015, Brown & Schluessel 2023), but few studies have explored this process in open systems where cues that signal the potential

for feeding vary in space and time and are not paired with access to food. Here, we used a field experiment where sharks were presented with olfactory-only cues (in the form of BRUVS) either randomly or repeatedly in the same location within the lagoon of Tetiaroa, French Polynesia. We documented mixed support for the hypothesis that persistent exposure to olfactory cues alone fosters habituation. Namely, MaxN values for sicklefin lemon sharks declined over time in response to repeated but not randomized BRUVS. By contrast, blacktip reef sharks showed no evidence of habituation and instead appeared to sensitize (Lieberman 1990) to the olfactory cue. Their relative abundance upon the introduction of the olfactory cue (initial MaxN) increased with time at repeated but not randomized sampling sites, and their arrival time decreased over time at repeated but not randomized sampling sites. Our findings add to our understanding of shark learning, particularly by showing that sympatric species may exhibit divergent responses to olfactory-only stimuli and thus implying that shark monitoring approaches that rely on olfactory attractants may be subject to species-specific patterns of bias.

Studies are beginning to explore the relationship between learning and foraging behavior in free-living sharks. For example, Schleimer et al. (2015) showed that whale sharks *Rhincodon typus* in the waters of the Philippines exhibited anticipatory behaviour, with resighted individuals gradually arriving earlier in response to the presence of a feeder boat, and similar responses to provisioning locations have been documented in coral reef (Séguigne et al. 2022) and mangrove (Heinrich et al. 2021) ecosystems. All of these field studies have involved provisioning. By contrast, sicklefin lemon sharks in the present study appeared to lose interest in, or habituate to, the repeated olfactory-only cue (the bait plume from the BRUVS). Namely, although 3 of the 4 metrics for this species showed no change (in relation to the control BRUVS) over time, MaxN declined significantly for the repeated but not the randomized BRUVS. This pattern of habituation can be attributed to the absence of a food reward, rather than a property intrinsic to sicklefin lemon sharks, as this species was among several appearing to learn the location of and anticipate an anthropogenic feeding opportunity in Ningaloo Marine Park, Australia when given nutritional reinforcement (Mitchell et al. 2020). Thus, it builds on the captive work on juvenile Port Jackson sharks by Heinrich et al. (2022) to suggest that, for at least some free-living taxa, olfactory-only cues offer diminishing efficacy as an attractant.

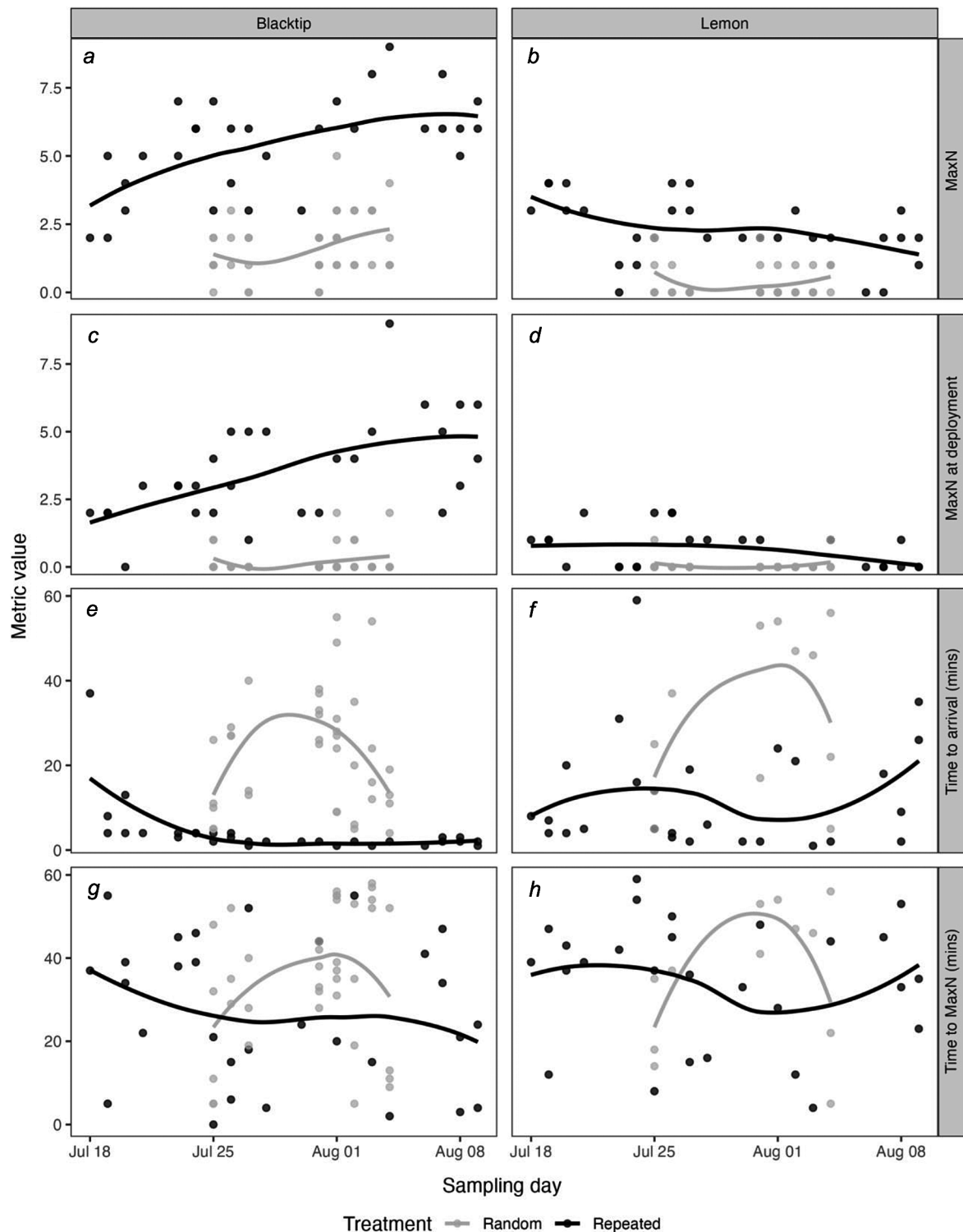


Fig. 1. Responses of blacktip reef *Carcharhinus melanopterus* ('Blacktip') and sicklefin lemon *Negaprion acutidens* ('Lemon') sharks to spatially randomized baited remote underwater video surveys (BRUVS) (grey) versus BRUVS deployed repeatedly at the same location (black). Temporal trends in each of the 4 metrics — (a,b) MaxN (maximum number of individuals of a species observed on any frame of a video), (c,d) MaxN at deployment (initial MaxN; first 5 min), (e,f) time to arrival, and (g,h) time to MaxN — were analyzed as a function of sampling date (x-axes) using Mann-Kendall tests. Dots represent individual BRUVS deployments; trend lines were created using the `geom_smooth` function with a loess smoother in R

Given that congeneric juvenile lemon sharks *Negaprion brevirostris* exhibited anticipation with fine-scale spatial shifts toward a feeding location (Heinrich et al. 2021), we might have expected declining sicklefin lemon shark MaxN (i.e. habituation) in the present study to be accompanied by reduced spatial attraction over time, as evidenced by diminishing initial MaxN and increasing time to MaxN and arrival times. Yet, for sicklefin lemon sharks, these other behavioral metrics did not vary temporally for either the randomized or repeated BRUVS. Because our study did not involve provisioning, it is possible that, unlike in the earlier study by Heinrich et al. (2021), the initial attractiveness of the olfactory-only cue was not accompanied by changes to spatial behavior, perhaps because such spatial shifts depend on the olfactory cue being paired with a nutritional reward. If so, then the MaxN decay we observed likely reflected habituation by individuals already local to the area where the repeated BRUVS were deployed, minimizing the likelihood of changes to space use driving trends in the timing of MaxN and arrival times. Moreover, Heinrich et al. (2023) found that *N. brevirostris* juveniles offered spatiotemporally predictable food in semi-natural pens in the Bahamas manifested distributions resembling those of control conspecifics and thus failed to show evidence of time-place learning, or the ability to link locations and timing of an event or stimulus. The authors speculated that their findings may have been confounded by the influence of tides. By implication, it is also possible that environmental conditions associated with working in the field (e.g. turbidity, wave activity) hindered our ability to detect temporal trends with respect to these other behavioral metrics. Although the brevity of our investigation (23 d within one season) and relative stability of some of these conditions (e.g. tidal state) within Tetiaroa's lagoon reduced the chances of our inferences being confounded by environmental volatility, we nevertheless encourage further work that more explicitly addresses the impacts of environmental covariates on shark learning.

Contrary to our expectations, blacktip reef sharks exhibited increasing initial MaxN and declining time to arrival when olfactory-only cues were offered repeatedly at the same location but showed no such responses to similar cues that were introduced randomly throughout the lagoon. There are several possible reasons for this divergent pattern of behavior relative to that displayed by sicklefin lemon sharks. First, blacktip reef sharks may have sensitized to the olfactory-only cue through non-associative learning (Lieberman 1990), leading to spatial shifts that would

result in quicker response times to the introduction of the attractant. This scenario is unlikely, however, given previous work showing that olfactory cues alone diminish in attractive efficacy in other reef shark species (Heinrich et al. 2022) and that the bait species we used to create the olfactory plume (sardines) are not considered to be part of the regular diet for blacktip reef sharks (Esposito et al. 2022). Second, blacktip reef sharks may have gained access to small amounts of bait during visits to the BRUVS, creating a reinforcing reward that promoted associative learning. We also consider this scenario to be improbable because the mesh on the bait cages used in our study was too fine to allow anything more than tiny pieces of tissue to escape, providing a trivial nutritional reward, and because sicklefin lemon sharks were not similarly affected. Third, blacktip reef sharks visiting the repeated BRUVS could have fed on teleosts attracted to the olfactory cue and thus learned associatively from an unintentionally paired reward. Addressing this post-hoc hypothesis is beyond the scope of the present study, partly because there are no data on the diets of blacktip reef sharks in Tetiaroa's lagoon, but it is plausible given the diversity of fish species that typically visit BRUVS (Schramm et al. 2020), including those deployed in our study area (Kilfoil et al. 2017). Fourth, blacktip reef sharks are competitively subordinate to sicklefin lemon sharks when targeting shared food resources (Weideli et al. 2023). Thus, it is possible that this competitive asymmetry influenced our findings. Indeed, as sicklefin presence (in terms of MaxN) at the repeated BRUVS waned over the course of the investigation, competitive pressure potentially excluding or inhibiting blacktip reef sharks would also presumably have declined, perhaps providing elevated opportunity for interaction with the bait cages and, therefore, contributing to if not driving the appearance of sensitization. Irrespective of the underlying mechanism, we only tested for temporal changes in behavior for a relatively short duration (23 d), leaving open the question of whether the apparent sensitization to the repeated site that we observed in blacktip reef sharks would have persisted over a longer interval.

As the taxonomic breadth of animal cognition and learning research has expanded, studies have begun to address interspecific differences in the same system. For example, Odling-Smee et al. (2008) showed that benthic threespine stickleback species within the *Gasterosteus aculeatus* complex performed a spatial learning task more quickly than limnetic species. Similarly, White & Brown (2014) found that 2 intertidal rock pool gobies (*Bathygobius cocosensis*, *B. krefftii*)

outperformed 2 sympatric sand-dwelling gobies (*Favonigobius lentiginosus*, *Istigobius hoesei*) in a spatial learning task, consistent with the hypothesis that species from rock pools require superior spatial learning skills to avoid being stranded at low tide. Here, we present the results of a comparison involving 2 reef shark species, finding evidence for habituation to an olfactory-only cue in sicklefin lemon sharks but sensitization to the same cue in sympatric blacktip reef sharks. This disparity is an early indicator that learning responses to anthropogenic cues in free-living systems, and thus any broader impacts of these responses on shark populations and ecology, can be species-specific. Looking ahead, there remains a need for studies asking if this putative learning difference between sicklefin lemon and blacktip reef sharks is conserved when repeated BRUVS are deployed at multiple locations within a system, to better account for environmental heterogeneity, as well as across ecosystems and over longer periods of time. Furthermore, we encourage future work in our system, and others, incorporating telemetry in conjunction with olfactory cue deployment to allow for before-after-control-impact (BACI) designs focused on patterns of individual behavior that can clarify the mechanism(s) underlying this disparity and more rigorously assess the consequences of each species' response.

The evidence for learning in blacktip reef and sicklefin lemon sharks produced by this study has important implications for efforts to monitor shark populations and communities. Namely, BRUVS, which have become a popular tool for sampling sharks, and other taxa, across an array of aquatic environments (Schmid et al. 2017, Whitmarsh et al. 2017, Schramm et al. 2020), rely on olfactory-only cues to attract animals. Yet, in our system, blacktip reef sharks sensitized to the repeated BRUVS, whereas sicklefin lemon sharks showed evidence of habituation to the same stimulus. These divergent responses would increasingly bias detection rates and relative abundance estimates associated with BRUVS deployed consistently in the same area in either a positive or negative direction, respectively, underscoring the need to spatially randomize BRUVS sets for monitoring purposes. Other studies have also illustrated how learning could affect the inferences drawn from shark research. For example, individual catchability of blacktip reef sharks in Moorea, French Polynesia, decreased as a function of experience (prior capture with rod and reel), potentially biasing efforts to estimate the abundance of this species with capture-mark-recapture (CMR) models, which assume equal catchability (Mourier et al. 2017). By implication, studies using attractants or captures

to sample blacktip reef and sicklefin lemon sharks should account for possible bias stemming from learning. More broadly, there is need for additional work examining the consequences of learning for shark population and community assessments.

Our findings also offer new insights regarding olfactory-only cues as a potential substitute for provisioning as a shark-based tourism practice. Provisioning can improve local livelihoods while also promoting opportunities for shark conservation (Siddiqi et al. 2024). However, when sharks, like other taxa, learn to exploit anthropogenic changes to the environment, including the provisioning of resources, they may exhibit altered patterns of individual behavior (Gallagher et al. 2015) that can affect demography and cascade through marine communities (Brena et al. 2015). These effects may be persistent in species capable of retaining memory of associations for extended intervals; for example, spatial memory lasts for at least 6 wk in grey bamboo sharks *Chiloscyllium griseum* (Schluessel & Bleckmann 2012). Moreover, cases where sharks are attracted to anthropogenic subsidies may promote conflict with humans. For instance, shark feeding in Moorea led to increased residency and heightened rates of intra- and interspecific aggression, setting up the possibility of higher rates of aggression toward humans (Clua et al. 2010), and feeding in Ningaloo Marine Park fostered depredation by creating associations between sharks and hooked fish (Mitchell et al. 2020). Olfactory cues that are not paired with feeding could offer an alternative means of attracting sharks for tourism purposes with reduced potential for associative learning and any associated consequences for sharks, their ecosystems, and people. However, our findings suggest that, for some shark species (sicklefin lemon sharks), the attractiveness of olfactory-only cues will diminish rapidly, whereas for others (blacktip reef sharks) sensitization, and any associated potential for effects on sharks and their environments, may occur despite the absence of a reward. By implication, olfactory-only cues are probably not a panacea for the negative effects that can stem from provisioning-based shark tourism. Recent research has shed light on reducing potentially harmful associations that can arise from tourism-related feeding operations. For example, Heinrich et al. (2020) found that reducing reinforcement frequency was more likely to be more effective in reducing the chances of Port Jackson sharks forming associations with human foods than minimizing the amount of food offered. Similarly, future studies exploring how different delivery methods and intensities influence shark responses to olfactory cues

could identify approaches that minimize habituation or sensitization and thus make the use of these cues a more realistic alternative to provisioning.

Although our understanding of shark learning is growing, few studies to date have explored this process in field locations where lack of true experimental control and the vagaries of environmental heterogeneity and interference from non-target species can obscure inference. Given these challenges, it is perhaps not surprising that some studies of free-living sharks have revealed evidence of learning as a result of conditioning with a food reward (e.g. Heinrich et al. 2021) whereas others have not (e.g. Séguigne et al. 2023). Our findings add to our understanding of shark learning under free-living circumstances by revealing that olfactory-only cues can promote learning, but with species-specific responses manifesting as habituation or sensitization. They also raise questions about the reason(s) for this species disparity, and particularly why blacktip reef sharks appeared to gravitate toward the repeated BRUVS over the course of the experiment, that merit further investigation in our system and beyond. Indeed, such inquiry is critical given the growing interest in olfactory cues as a replacement for provisioning as a shark attractant, as well as the possibility that unintended attraction to persistent olfactory cues could enhance the potential for human–shark conflict.

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