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Santa Barbara

Reconstructing historical shark communities on coral reefs  
using fossil dermal denticle assemblages

A dissertation submitted in partial satisfaction of the  
requirements for the degree Doctor of Philosophy  
in Ecology, Evolution, and Marine Biology

by

Erin Mackenzie Dillon

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May 2022

Reconstructing historical shark communities on coral reefs  
using fossil dermal denticle assemblages

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by

Erin Mackenzie Dillon

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## ABSTRACT

Reconstructing historical shark communities on coral reefs  
using fossil dermal denticle assemblages

by

Erin Mackenzie Dillon

Shark populations worldwide have been depleted by overfishing, yet empirical evidence of shark abundance and diversity before human impact is scarce. Without long-term data to document the timing and magnitude of shark declines, it is challenging to understand how the loss of these high-level predators has reshaped ecosystems and set meaningful management targets informed by natural variability. In this dissertation, I use fossil shark scales (dermal denticles) to reconstruct shark communities on coral reefs over the last several millennia. After finding denticles to be well-preserved in reef sediments, I refined an extraction method and built a reference collection to facilitate denticle classification. By measuring denticle shedding in captive sharks, I showed that shedding rates vary across sharks with different life modes, thereby influencing how shark communities are represented as denticle assemblages in the fossil record. I then calibrated the denticle record by comparing denticle assemblages preserved in surface sediments with wild shark populations on a remote, unfished atoll. I found that denticle accumulation rates were positively correlated with shark abundance and that denticle assemblage composition was consistent with commonly observed shark species. Building on this foundation, I recovered denticles

from mid-Holocene and modern reef sediments in Caribbean Panama to define a local shark baseline before major human impact and quantify shifts in the modern shark community from this historical reference point. Denticle assemblages indicated that shark communities in this region declined threefold since the mid-Holocene and were functionally restructured by both long-term harvesting and habitat modification. Together, this work demonstrates that the denticle record can reveal changes in shark communities over long ecological timescales, helping to contextualize contemporary abundances and inform shark management and ecology.

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# CHAPTER 1: INTRODUCTION

## *1.1 Background*

Humans have transformed ecosystems on Earth over the previous centuries to millennia, but only a fraction of this past change has been recorded by ecological monitoring (Vitousek et al. 1997, Jackson et al. 2001, Lotze et al. 2006, Halpern et al. 2008). Human perturbations now rival environmental processes as the primary forces shaping ecosystem structure and function (Möllmann et al. 2009, Williams et al. 2015, Lyons et al. 2016). In the face of rapid anthropogenic change, scientists race to understand how intact ecosystems operate and trace the ecological legacy of human impacts, in hope of assessing the consequences and conserving what remains. Yet, ecological surveys are conducted over relatively short timeframes spanning months to years and began after human stressors (Jackson 2001, Estes et al. 2018). Although these surveys have documented the rate and ubiquity of recent change, their conclusions often overlook long-term perspectives on natural variability around pre-impact baselines or how those baselines are influenced by environmental conditions (Jackson et al. 2001, Lotze et al. 2006, McClenachan et al. 2012). Historical reference points are needed to evaluate the full extent of biotic responses to environmental change, disentangle the relative contributions of human and non-human drivers, and set meaningful management goals (Dietl & Flessa 2011, Dietl et al. 2015). Without this context, expectations about natural ecological states tend to drift through time, enabling further degradation (Pauly 1995). As we deliberate on the future fate and functioning of ecosystems, understanding their past dynamics is critical.

Incorporating retrospective data into ecological and conservation assessments is especially important for sharks given their long history of exploitation and strong top-down control on food webs. Throughout their >400-million-year evolutionary history, sharks have

shown remarkable resilience to extinction (Pimiento et al. 2017, Paillard et al. 2021). But, in the geological blink of an eye, humans have pushed many shark populations worldwide to the brink of collapse. Overfishing (Dulvy et al. 2021) and habitat degradation (Lotze et al. 2006) have caused shark abundance to decline by as much as 70–99% over the last half century (Ferretti et al. 2010, Pacoureau et al. 2021). These dramatic losses have heightened sharks' extinction risk, particularly in tropical coastal ecosystems such as coral reefs (Dulvy et al. 2021), where sharks are mobile high-level predators (Frisch et al. 2016, Roff et al. 2016). For example, coral reef-associated sharks structure food webs (Bascompte et al. 2005), influence prey behavior (Heithaus et al. 2008), and couple pelagic and coastal resource pools by foraging across multiple habitats (McCauley et al. 2012a). Sharks also have socioeconomic value as ecotourism attractions (Cisneros-Montemayor et al. 2013) and cultural symbols (Castro 2013), in addition to serving as charismatic flagship species in conservation. Consequently, their losses have resulted in complex and sometimes surprising consequences for both ecosystem functioning and human societies (Roff et al. 2016, Hammerschlag et al. 2019).

These surprises stem, in part, from gaps in our knowledge regarding the extent to which sharks on coral reefs have shifted from unfished baselines. Shark declines likely preceded even the longest time series of survey data due to historical overfishing (Jackson et al. 2001), coupled with sharks' biological vulnerabilities to harvesting (Dulvy et al. 2014). Although explorers' logs are replete with stories of seas teeming with sharks (Cook 1784, Bergreen 2011), empirical evidence of pre-exploitation shark abundance and diversity is scarce. We know little about how many sharks were present before human impacts became pervasive or how their losses have altered modern reef ecosystems, particularly those close to human population centers and markets. Instead, ecological studies often use remote,

uninhabited islands to shape general expectations about baseline shark abundance and ecological interactions (Sandin et al. 2008, Nadon et al. 2012, Bradley et al. 2017a), irrespective of any historical exploitation (Graham et al. 2010, Ferretti et al. 2018).

Yet, shark carrying capacities vary with environmental conditions, so universal baselines can be misleading. Modern spatial variation in reef shark abundance is correlated with bottom-up forces like primary productivity and sea surface temperature, indicating that all reefs cannot support uniformly high shark biomass (Nadon et al. 2012, Tickler et al. 2017, Valdivia et al. 2017). Superimposed on this biogeographic variation are human pressures, which obscure the natural biophysical controls on shark carrying capacity (Stallings et al. 2009, Williams et al. 2015) and create novel patterns of occurrence that track socio-economic aspects of resource extraction (MacNeil et al. 2020, Clementi et al. 2021). Because humans simultaneously deplete shark populations and transform their surroundings, it is difficult to distinguish between environmental and anthropogenic drivers of shark abundance (e.g. Jouffray et al. 2019) or make predictions about their range of variability before human disturbance (Valdivia et al. 2017).

The recent fossil record can be used to reconstruct location-specific ecological baselines (Dietl et al. 2015, Barnosky et al. 2017, O’Dea et al. 2017). Coral reefs archive their histories as they accrete, providing powerful insight into ecological and environmental processes that play out beyond the timespan of direct human observation. Paleontological studies have often focused on corals and reef framework (Aronson et al. 1997, Pandolfi & Jackson 2006), yet reef sediments preserve a diversity of skeletal elements, such as mollusc shells (Fredston-Hermann et al. 2013, Cramer et al. 2015), fish ear bones (Lin et al. 2019) and teeth (Cramer et al. 2017), and shark dermal scales (Dillon et al. 2017). By illuminating long-term community dynamics, these fossil assemblages can help contextualize modern

change and inform conservation decisions by envisioning a range of future scenarios that restoration could plausibly achieve (Hobbs et al. 2009, Dietl 2019, O’Dea et al. 2020).

Although the fossil record of sharks has traditionally been studied using teeth due to their prevalence in fossil deposits, sharks possess multiple orders of magnitude more dermal denticles. These microscopic (<2 mm) tooth-like scales cover shark skin, where they improve hydrodynamic performance and provide protection (Reif 1978, 1985, Raschi & Musick 1986, Raschi & Tabit 1992, Lauder et al. 2016, Ferrón & Botella 2017). Denticles are shed and replaced continuously over a shark’s life span (Reif 1985), producing denticle accumulations in marine sediments. Denticles have been found to be well-preserved over millions of years (Helms & Riedel 1971, Sibert & Norris 2015, Dillon et al. 2017) because of their durable calcium phosphate composition (Doyle & Riedel 1979), and they represent the oldest known shark remains in the fossil record (Sansom et al. 1996). Due to their high preservation potential and diagnostic characteristics, fossil denticle assemblages should archive information about the abundance and composition of shark communities through ecological and evolutionary time.

The denticle record’s utility for reconstructing shark communities, however, has just begun to be explored. Seminal work by Wolf-Ernst Reif documented variation in denticle morphology across a shark’s body and between taxa, creating a framework for interpreting fossil denticles and delimiting the taxonomic boundaries of inference (Reif 1978, 1985). He described several denticle “functional morphotypes” after observing that sharks with similar ecological traits, such as swimming speed or habitat preference, had denticles with similar morphology (Reif 1985). Quantitative studies using museum specimens quantified the occurrence of these functional morphotypes across ecological and taxonomic groups of sharks, thereby linking denticle morphology to shark life modes and facilitating denticle

classification (Ferrón et al. 2014, Dillon et al. 2017, Ferrón & Botella 2017). Methods now exist to recover denticles from various sediment types and quantify them as a proxy for shark abundance (Dillon et al. 2017, Sibert et al. 2017). These methods have been applied in deep-sea cores, showing that denticles are abundant enough in the sediment record to permit analyses of relative shark abundance and community structure through deep time (Sibert et al. 2014, Sibert & Norris 2015, Sibert et al. 2016, Sibert & Rubin 2021). Collectively, this body of research has set the stage for me to extend the application of the denticle record over more recent, conservation-relevant timescales.

### ***1.2 Dissertation outline***

In this dissertation, I develop and calibrate a technique to interpret shark dermal denticles accumulating in the recent fossil record. I then apply this approach to reconstruct shark abundance and community structure on coral reefs over the last several millennia.

In **Chapter 2**, I measure denticle shedding rates in a controlled aquarium setting to assess the relationship between absolute shark abundance and denticle accumulation. Shedding rates differed across shark species with different life modes and denticle morphologies. One implication is that shark taxa contribute unevenly to the denticle record, suggesting that shedding rate measurements can help inform and constrain ecological interpretations of denticle assemblages (Dillon et al. 2022).

In **Chapter 3**, I test the ecological fidelity of the denticle record in a wild setting by comparing denticles accumulating in surface sediments with contemporary shark survey data on a remote, unfished atoll. I find a significant positive correlation between denticle accumulation rates and shark abundances derived from underwater visual census, baited remote underwater video, and hook and line surveys. Furthermore, the rank abundance of denticle functional morphotypes corresponded with those found on the shark species that

were commonly observed in the ecological surveys. These patterns indicate that denticle assemblages can reflect a signal of relative shark abundance and functional diversity in low-energy coral reef habitats (Dillon et al. 2020).

In **Chapter 4**, I present a first application of the near-time denticle record on coral reefs. I reconstruct an empirical pre-exploitation shark baseline on a Caribbean coral reef and quantify how much the modern shark community shifted from this historical reference point. Denticle assemblages revealed that shark communities on these reefs not only experienced severe declines but had been functionally restructured since the mid-Holocene, likely because of long-term harvesting and habitat modification (Dillon et al. 2021).

Together, these chapters demonstrate the utility of the near-time dermal denticle record for answering long-standing questions about the baseline conditions of reef shark communities, the drivers of shark declines over millennia, and their ultimate ecological and conservation implications.

### ***1.3 Permissions and attributions***

Chapter 2 was previously published as “**Dillon EM**, Bagla A, Plioplys KD, McCauley DJ, Lafferty KD, O’Dea A (2022). Dermal denticle shedding rates vary between two captive shark species. *Marine Ecology Progress Series*, 682, 153–167.” It is reproduced in this dissertation with permission from Inter-Research (© Inter-Research 2022).

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Chapter 3 was previously published as “**Dillon EM**, Lafferty KD, McCauley DJ, Bradley D, Norris RD, Caselle JE, DiRenzo GV, Gardner JPA, O’Dea A (2020). Dermal denticle assemblages in coral reef sediments correlate with conventional shark surveys. *Methods in Ecology and Evolution*, 11(3), 362–375.” It is reproduced in this dissertation with permission from the British Ecological Society (© British Ecological Society 2020);



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Chapter 4 was previously published as “**Dillon EM**, McCauley DJ, Morales-Saldaña JM, Leonard ND, Zhao J-x, O’Dea A (2021). Fossil dermal denticles reveal the preexploitation baseline of a Caribbean coral reef shark community. *Proceedings of the National Academy of Sciences USA*, 118(29), e2017735118.” It is reproduced in this dissertation with permission from the National Academy of Sciences (© National Academy of Sciences 2021). <https://doi.org/10.1073/pnas.2017735118>

## **CHAPTER 2: DERMAL DENTICLE SHEDDING RATES VARY BETWEEN TWO CAPTIVE SHARK SPECIES**

### ***2.1 Abstract***

Shark dermal scale (denticle) accumulation in the fossil record can provide information about the abundance and composition of past shark communities. Denticles are shed continuously, such that a single shark leaves a scattered composite of many isolated denticles in sediments. However, the rate of denticle shedding as well as how these rates vary among shark species with different life modes and their consistency over time are unknown, limiting the interpretation of denticle assemblages. To better understand the process of denticle shedding and calibrate the relationship between absolute shark abundance in the environment and denticle deposition in sediments, we captured denticles shed by two shark species in a large aquarium over nine months. We then simulated how these aquarium-derived shedding rates shape the relationship between shark abundance and denticle accumulation. Bonnethead sharks *Sphyrna tiburo*, a more active, benthopelagic species with small, thin denticles, shed 3.6 times faster on average than zebra sharks *Stegostoma fasciatum*, a more sedentary, demersal species with large, robust denticles. This pattern persisted when shedding rates were corrected by estimated denticle quantities, shark space use, and methodological factors (2.2- to 3.8-fold difference). Over the study, bonnet-head shark shedding rates declined while zebra shark shedding rates increased slightly. Finally, denticle assemblage composition corresponded with the relative abundance of denticles on the body of each species, consistent with natural shedding rather than selective loss. Overall, we show that shark taxa contribute unevenly to the denticle record, indicating

that shedding rate measurements can help inform and constrain ecological interpretations of denticle assemblages.

## ***2.2 Introduction***

Sharks have an extensive fossil record that chronicles how these mobile predators have shaped ecosystem structure and function over millions of years (Maisey 1984, Capetta 2012). Fossil marine deposits contain large numbers of shark teeth and microscopic, tooth-like dermal scales (denticles) due to their durability and continuous production through ontogeny (Owen 1866, Zangerl 1981, Maisey 1984, Capetta 2012). Teeth and denticles preserve patterns of shark abundance and diversity over geological time, providing a means to catalogue shark occurrences (Helms & Riedel 1971, Pla et al. 2013, Carrillo-Briceño et al. 2018), reveal how sharks responded to past global change (Sibert et al. 2014, 2016, Sibert & Norris 2015, Pimiento et al. 2017, Villafaña & Rivadeneira 2018, Ferrón et al. 2019, Wynd et al. 2020), guide paleoenvironmental inferences (Ferrón et al. 2014, Martínez-Pérez et al. 2018), and inform benchmarks for shark management amid recent declines (Drew et al. 2013, Dillon et al. 2017, 2021, Paillard et al. 2021). Denticles are several orders of magnitude more abundant than teeth, and their prevalence in sediments can facilitate statistical analyses of shark abundance and community structure over unprecedented reaches of space and time (Sibert et al. 2017, Dillon et al. 2021, Sibert & Rubin 2021). Yet, denticles are often overlooked in paleontological studies, and their potential for paleocommunity reconstruction has just begun to be explored (Ferrón et al. 2014, Dillon et al. 2017, Sibert et al. 2017).

One major methodological challenge when using the denticle record to reconstruct shark paleocommunities is that interpretations often rely on the assumption that the amount of denticle accumulation correlates with shark abundance (Sibert et al. 2017, Dillon et al.

2020). This assumption is complicated by the disarticulated, multi-element nature of denticle assemblages. Denticles accumulate in sediments after being shed through either the natural resorption of anchoring fibers on the skin, abrasion, predation, or movement (Reif 1985, Popp et al. 2020), such that a single shark leaves a scattered composite of many isolated denticles in sediments. Assemblages like these can obscure counts of individuals and skew assessments of absolute abundance in the fossil record (e.g. Badgley 1986, Shackleton 1988), limiting the ecological inferences that can be made.

To refine these inferences, it is important to understand how denticles enter the fossil record and test how well shark abundances can be estimated from denticle assemblages. Denticles are preserved over millions of years in a variety of marine sediment types owing to their robust calcium phosphate composition (Doyle & Riedel 1979, Sibert et al. 2017). Previous work has demonstrated a positive correlation between denticle accumulation in low-energy surface sediments and relative shark abundance for reef shark communities in aggregate across a spatial gradient of shark density (Dillon et al. 2020). Similarly, denticle-based reconstructions of shark community composition during the Middle Triassic are congruent with shark teeth recovered from the same localities, demonstrating the ecological fidelity and low taphonomic bias of the denticle record over million-year timescales (Ferrón et al. 2014). However, the role of denticle shedding in mediating how species counts are represented in these fossil assemblages has yet to be determined.

Denticle shedding rate measurements can help guide ecological interpretations of the denticle record by calibrating the relationship between shark taxa in a community and their resultant denticle deposition in sediments. Little is known about the factors governing denticle shedding or the extent to which shedding rates vary among shark species and individuals with different life modes. Interspecific or temporal variation in denticle shedding

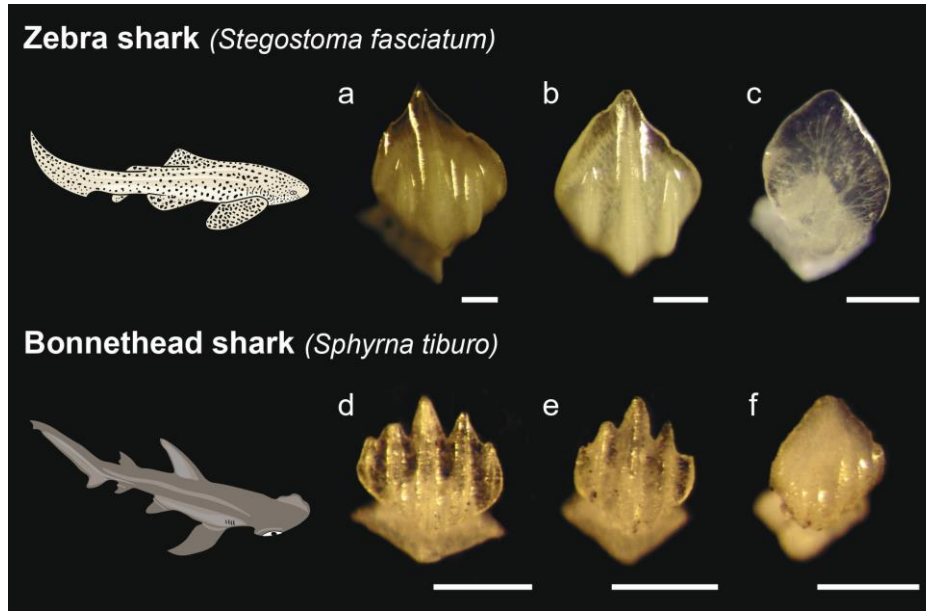
rates would cause different shark species to contribute disproportionately to the fossil record relative to their absolute abundances. For instance, a higher frequency of denticles from pelagic versus demersal species might reflect differences in relative abundance, differences in shedding rates, or both. Shark tooth replacement rates vary among species and seasons (Strasburg 1963, Moss 1967, 1972, Reif et al. 1978, Luer et al. 1990, Overstrom 1991, Correia 1999, Botella et al. 2009), but only speculative descriptions of denticle shedding rates exist at present (Märkel & Laubier 1969, Reif 1974, 1985, Jagt & Jagt-Yazykova 2017).

Here, we provide a first empirical estimate of denticle shedding rates in a controlled aquarium environment with a known shark density to examine variation in shedding between shark species with different life modes and denticle morphologies. Denticle accumulation into substrate-filled trays was recorded over nine months for zebra sharks *Stegostoma fasciatum* and bonnethead sharks *Sphyrna tiburo*, two warm-water, coastal species which differ in ecological traits such as habitat, body size, and activity patterns (Fig. 2.1 and Table 2.1). We recorded denticle accumulation at multiple time points to measure and incorporate any sub-annual temporal variability into the shedding rate estimates for each species. Behavioral surveys were conducted to assess how patterns of shark activity, space use, and interactions with the tank environment varied between species and, in turn, might have influenced denticle shedding. Lastly, we applied the aquarium-derived shedding rates in a computer simulation to explore how interspecific variation in denticle loss shapes the relationship between absolute shark abundance and denticle accumulation.

## 2.3 Methods

### 2.3.1 Setting

Denticle shedding was measured at the Aquarium of the Pacific (Long Beach, California, USA) in the Tropical Pacific Gallery. The tank was ~1,325,000 l in volume and housed five captive sharks of two species: three zebra sharks and two bonnethead sharks (Fig. A.1). All sharks were mature females apart from one juvenile female zebra shark, and they had been in captivity for ~5–15 y. The tank also housed numerous other bony and cartilaginous (ray) fish species. Seawater was maintained between 23.2 and 24.7°C ( $M \pm SD$ ;  $23.9 \pm 0.1^\circ\text{C}$ ), and water quality was monitored to ensure animal health. The water turnover rate was approximately one hour, and seawater was pumped in and out of the tank through a diffuse network of pipes, resulting in low flow conditions. The tank was illuminated with natural sunlight via skylights, and additional artificial light was provided in the morning and evening using metal halide fixtures (photoperiod of ~10–14 h light and ~14–10 h dark, with the amount of natural light varying seasonally). Shark diets remained consistent throughout the study, with zebra sharks receiving 0.45 kg d<sup>-1</sup> and bonnethead sharks receiving 0.2 kg d<sup>-1</sup> of a mix of clam foot, sardines, mackerel, mahi mahi, capelin, herring, and market squid.



**Figure 2.1** Variation in denticle morphology between the two shark species in the Tropical Pacific Gallery at the Aquarium of the Pacific on the (a,d) body, (b,e) fins, and (c,f) fin edges. Zebra sharks *Stegostoma fasciatum* are characterized by ridged abrasion strength and abrasion strength denticles, which provide this demersal species with protection during contact with sandy, hard, or coral-rich substrates. In contrast, bonnethead sharks *Sphyrna tiburo* are covered almost entirely by hydrodynamic drag reduction denticles, with only a small proportion of abrasion strength and ridged abrasion strength denticles found along the leading edges of the fins and around the snout. Shark illustrations are courtesy of Ashley Diedenhofen (artist). Scale bars = 200  $\mu\text{m}$ .

**Table 2.1** Ecological traits (life mode) and denticle characteristics of the two shark species in the Tropical Pacific Gallery at the Aquarium of the Pacific. The ecological traits and denticle characteristics varied between species, allowing us to examine how they affect shedding rates.

Trait or characteristic	Zebra shark ( <i>Stegostoma fasciatum</i> )	Bonnethead shark ( <i>Sphyrna tiburo</i> )
Habitat <sup>a</sup>	Over sand, rubble, and coral bottoms	Over sand, mud, seagrass, and coral bottoms
Relative location to the seafloor <sup>a</sup>	Demersal	Benthopelagic
Behavior <sup>a</sup>	Sluggish during the day; rests on the seafloor while buccal pumping; nocturnal hunter	Continuous swimming (obligate ram-ventilator); social
Total length <sup>a</sup> [mean length in Tropical Pacific Gallery] (cm)	~150-350 [210]	~50-150 [100]
Estimated body surface area (m <sup>3</sup> ) <sup>b</sup>	1.03	0.37
Denticle density (denticles mm <sup>-2</sup> )	3-10 (Raschi & Tabit 1992)	20-31 (Creager & Porter 2018)
Proportional cover of drag reduction denticles (%) <sup>c</sup>	0.0	93.8
Proportional cover of abrasion strength denticles (%) <sup>c</sup>	5.3	2.8
Proportional cover of ridged abrasion strength denticles (%) <sup>c</sup>	86.4	2.4
Proportional cover of generalized functions denticles (%) <sup>c</sup>	8.3	1.0

Source: <sup>a</sup>Compagno et al. (2005); <sup>b</sup>Modeled as an ellipse, using estimated body measurements from sharks in the Tropical Pacific Gallery; <sup>c</sup>Mean proportional cover data were obtained from published morphometric studies of museum specimens (described in Section 2.3.3)

### 2.3.2 Denticle shedding rate measurements

To measure denticle shedding rates, we deployed six Pyrex® trays ( $38.6 \times 26.5 \times 4.8$  cm, 4.5 l volume) containing 1.2 kg of CaribSea® aragonite sand substrate (Fig. A.1) for intervals of 25–42 d at six time points between June 2018 and March 2019 ( $n = 190$  d). No measurements were taken in November and December 2018 due to construction in the tank. The trays were enclosed in separate 26 l Sterilite® containers ( $58.4 \times 41.3 \times 15.2$  cm) covered with a mesh baffle ( $\sim 2$  cm<sup>2</sup> openings) to prevent disturbance from animals and reduce turbulence that could displace the substrate (Gardner 1980). We deployed the trays in three locations within the tank, with two replicates per location (Fig. A.1). Four trays were placed within a webbed mesh enclosure ( $\sim 0.5$  cm<sup>2</sup> openings) under the main viewing window, which allowed denticles and small shark teeth, but not animals, to enter the immediate area, and two were placed in a tank corner. The experimental sand substrate did not contain skeletal remains prior to deployment, so any denticles accumulating in the trays were shed by sharks in the tank.

Samples were weighed before and after each deployment to account for any changes to the experimental substrate. On average, the samples gained 171 g ( $SD = 118$  g), or 14% of their original weight, and there was no net loss. Around 30% of the weight gained was attributed to grains  $>3$  mm, which were not present in the clean CaribSea® sand substrate and could have entered the trays as a byproduct of feeding, thus contributing weight but not denticles to the samples. The remaining weight gained might have resulted from animal behavior (e.g. cownose rays displacing sediment) or sediment resuspension. We collected  $\sim 0.5$  kg bulk samples ( $n = 4$ ) of surface sediments outside the trays to quantify denticle abundance in the surrounding substrate and evaluate the extent to which this input of non-experimental sediment might have elevated our denticle shedding measurements.



All samples were processed to recover denticles. Samples were sieved, and the 63  $\mu\text{m}$  to 2 mm size fractions were quartered using a splitter (subsample  $M \pm SD$ ;  $264.0 \pm 31.2$  g). The subsamples were treated with 10% acetic acid to eliminate the calcium carbonate and then with 5% hydrogen peroxide to remove excess organic material (Dillon et al. 2017, Sibert et al. 2017). To facilitate denticle recovery, we dyed the samples with Alizarin Red S, a calcium-specific dye which preferentially stains skeletal remains such as ichthyoliths (Sibert et al. 2017). Denticles were separated from the residue under a stereo microscope and counted. Shark teeth were also manually removed from the 63  $\mu\text{m}$  to  $>2$  mm size fraction and counted to assess the magnitude of difference between teeth and denticle accumulation. Ray teeth and denticles were recognizable and were excluded from the analyses.

Denticles were visually identified to shark species using a dermal denticle reference collection (Dillon et al. 2017), as bonnethead and zebra shark denticles are morphologically distinguishable (Fig. 2.1 and Table A.1). Only 3% ( $n = 218$ ) of the denticles recovered could not be reliably identified (Fig. A.2) and were excluded from the species-level analyses. Denticle shedding rates were calculated as the number of denticles accumulating per day per  $\text{m}^2$  and were reported both in terms of total accumulation and the accumulation per individual of each species. In addition to reporting the raw estimates, denticle shedding rates were corrected by three factors that could bias the measured values: (1) non-experimental sediment input (described above); (2) body surface area (estimated as an ellipsoid) and published species-specific denticle densities (Raschi & Tabit 1992, Ferrón & Botella 2017, Creager & Porter 2018) (Table 2.1) because the two shark species differ in size and could possess different quantities of denticles on their bodies; and (3) shark space use, which could influence whether denticles settled in the trays after being shed (described in Section 2.3.5).

Denticle sinking trajectories are influenced by water flow patterns, which could potentially concentrate denticles in specific areas of the tank. To determine whether denticles accumulated in the replicate trays in a non-random manner, we used ANOVA to test for spatial differences across tray locations during each deployment as well as for the deployments in aggregate. In these analyses, systematic differences in denticle accumulation across tray locations might evidence preferential areas of accumulation on the tank bottom.

To explore differences in denticle shedding rates across shark species, we used generalized linear mixed models. Models were implemented using the R package *glmmTMB* with a Gamma error distribution (Brooks et al. 2017). Shark species was included as a fixed effect, and tray location and deployment month were included as random effects to account for spatial and temporal variability in denticle accumulation. Model diagnostics were assessed with the package *DHARMA* (Hartig 2021). Akaike's information criterion corrected for small sample size (AICc) was used for model selection, and likelihood ratio tests were used to calculate  $p$ -values. Models were re-run with each correction to examine their influence on the shedding rate estimations. All analyses were performed in R version 3.6.0 (R Core Team 2019, Wickham et al. 2019).

### 2.3.3 Denticle assemblage composition

Denticle morphology varies across the body of a shark, providing a means to examine whether denticles were preferentially lost from certain body regions. We categorized the denticles from a subset of 15 randomly selected samples ( $n = 3153$  denticles) into five previously recognized functional morphotypes: drag reduction, ridged abrasion strength, abrasion strength, generalized functions, and defense (Reif 1985, Ferrón et al. 2014, Dillon et al. 2017, Ferrón & Botella 2017). This classification approach produced functional morphotype and species-level information for each denticle.

We then compared the relative abundance of functional morphotypes in the trays to their proportional representation on the bodies of the two shark species in the tank. This comparison was used to test whether shedding rates differ across functional morphotypes within each species and provide insight into the process of denticle loss as inferred by these patterns. Proportional cover data were obtained from published morphometric studies of museum specimens (Dillon et al. 2017, 2020, Ferrón & Botella 2017). Similar methods were used in each study to quantify the body surface area covered by each functional morphotype. Zebra shark ( $n = 1$ ) and bonnethead shark ( $n = 2$ ) specimens were virtually divided into several regions (dorsolateral, ventral, and fins), and transects across each region were conducted on a coordinate system. Denticle morphology was visually inspected either using a binocular microscope or 40× magnification hand lens. When possible, these visual classifications were cross-checked with denticles in the reference collection. The relative proportions of each functional morphotype on the body and fins were scaled by the estimated surface area of each region to account for differences in body size (methods are described in detail by Ferrón & Botella 2017 and Dillon et al. 2020). Although denticle density can vary across the body of a shark, the proportional cover data were not overly sensitive to the amount of variation documented for bonnethead and zebra sharks (Raschi & Tabit 1992, Ferrón & Botella 2017, Creager & Porter 2018).

#### 2.3.4 Weathering analysis

Denticle weathering was assessed in 10 randomly selected denticles per species from each of the same subset of 15 samples ( $n = 300$  denticles). Each denticle was assigned a weathering score, which ranged from zero (pristine) to three (poor preservation) and was based on visual inspection of the crown, peaks, and base (Dillon et al. 2020) (Table A.2). The weathering scores were compared across shark species, denticle functional

morphotypes, tray locations, and deployments using ANOVA, Kruskal–Wallis, and Wilcoxon rank sum tests to describe denticle preservation within one month of being shed.

### 2.3.5 Shark behavioral surveys

To determine how behavior differed between the two shark species and, consequently, might have influenced denticle shedding rates, we conducted focal observations ( $n = 65$ ) from the main viewing window of the tank (Fig. A.1) on seven occasions between October 2019 and February 2020, spanning the months when denticle shedding rates were most variable. Observations took place during operating hours and were divided into morning (09:00–12:00 h;  $n = 22$ ), afternoon (12:00–15:00 h;  $n = 18$ ), and evening (15:00–18:00 h;  $n = 25$ ) sessions. Sessions were 3 h long, during which the behavioral surveys were conducted consecutively (~3 min gap between observation periods on average). Each survey lasted a maximum of 20 min or was terminated early if the shark left the field of view for longer than 2 min. Sharks were haphazardly selected (individuals could be identified using markings on their bodies), and each shark was observed 10 to 16 times. Thirteen of the 65 surveys were conducted during dive shows to investigate the effect of diver presence and feeding on shark behavior. These 13 surveys spanned morning ( $n = 3$ ), afternoon ( $n = 4$ ), and evening ( $n = 6$ ) dive shows and included observations of both bonnethead ( $n = 4$ ) and zebra sharks ( $n = 9$ ).

During each behavioral survey, we documented behaviors that were hypothesized to influence the rate and location of denticle shedding. Every 30 s, we recorded the sharks' activity state as well as vertical and horizontal position in the tank. The total number of interactions with the tank sides, tank bottom, and other large animals in the tank were also recorded, as they could artificially inflate shedding rates (Table 2.2). Because shark space use was not homogeneous throughout the tank, denticle shedding rate measurements were

corrected by the frequency of two behaviors during which any denticles lost would likely not be captured in the trays: (1) resting on the tank bottom (exclusive to zebra sharks) and (2) occupying space near the center of the tank away from the trays.

**Table 2.2** Shark behaviors documented during the behavioral surveys and their hypothesized effect on denticle shedding rates. **Bolded** categorical states were included as response variables in the generalized linear mixed models.

Behavior	Measurement frequency	Categorical state	Justification
Activity state	Every 30 s	<b>Traveling</b> Resting on tank bottom Other (describe)	Shark movement could induce denticle shedding and influence the location of denticle accumulation
Vertical position in the tank	Every 30 s	Top third Middle third <b>Bottom third</b>	Shark space use could influence the location of denticle accumulation
Horizontal position in the tank	Every 30 s	<b>Outer third</b> Inner two-thirds	Shark space use could influence the location of denticle accumulation
Contact with tank sides	Total count	–	Abrasion could dislodge denticles
Contact with tank bottom	Total count	–	Abrasion could dislodge denticles
Interactions with other large animals in the tank (approached within ~0.5 m)	Total count	–	Contact with other animals could induce denticle shedding

We used generalized linear mixed models to test whether behavior or position in the tank differed between species, time of day, or during dive shows. Models were implemented using the R package *glmmTMB* with a binomial error distribution (Brooks et al. 2017), and separate models were run with the following behavioral states as response variables: activity state (traveling), horizontal position (outer third, where the trays were located and contact with the tank sides was possible), and vertical position (bottom third, where contact with the tank bottom was possible) (Table 2.2). Additionally, a second set of models with a negative binomial error distribution were run with the interaction counts as the response variable and survey duration as an offset. In both sets of models, species, time of day, and dive show were included as fixed effects, and observation date was included as a random effect. Model diagnostics were assessed with the package *DHARMA* (Hartig 2021). AICc was used for

model selection (Table A.3), and likelihood ratio tests were used to calculate  $p$ -values. All analyses were performed in R (R Core Team 2019, Wickham et al. 2019).

### 2.3.6 Denticle shedding computer simulation

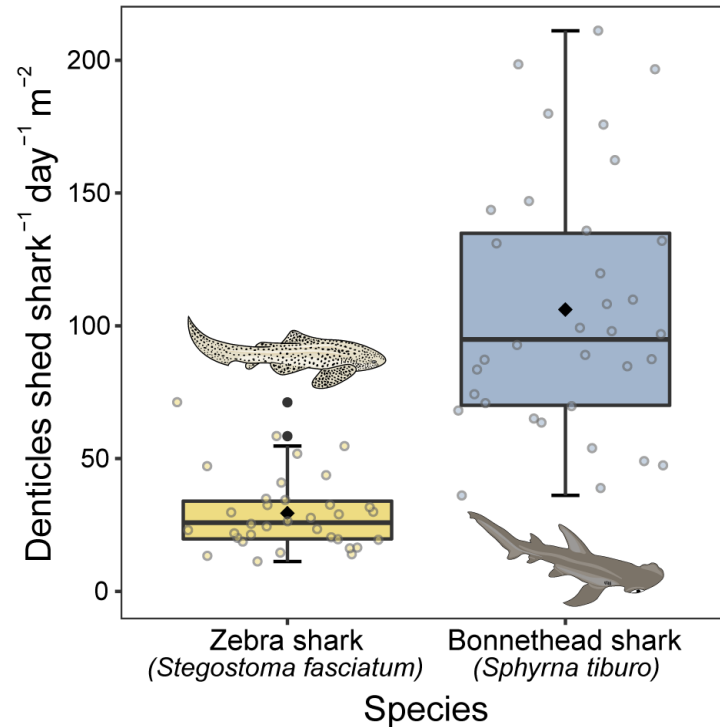
Using the aquarium-derived denticle shedding rates, we ran a computer simulation to explore how interspecific variation in denticle shedding could influence ecological interpretations of denticle accumulation and assemblage composition. In the simulation, bonnethead and zebra shark abundances were allowed to vary between 1 and 10 in a closed system (20 sharks maximum). All permutations of their abundances were computed to generate variation in both absolute shark abundance and relative species abundance. We then applied the shedding rates from our aquarium trials over a 90 d period to predict denticle accumulation in a 1 m<sup>2</sup> area. Denticle accumulations were determined for each species and were also disaggregated by functional morphotype using the ratios observed in the trays (see Section 2.3.3). The effect of denticle shedding on the relationship between shark count and denticle accumulation was plotted across the simulated scenarios to describe the limits of inference when characterizing shark communities using denticle assemblages.

## 2.4 Results

### 2.4.1 Denticle shedding rates

Denticles were frequently shed by sharks in the tank, with upwards of 33,000 denticles accumulating in the ~0.6 m<sup>2</sup> area sampled over the 190 d long study. Denticle shedding rates were similar across tray locations overall (ANOVA  $F_{2,29} = 0.35$ ,  $p = 0.71$ ; Fig. A.3A), although rates varied 1.1- to 2.3-fold across locations during each deployment (Fig. A.3B). Between 14 and 54 denticles accumulated per day in each tray, indicating that the five sharks in the tank shed multiple denticles every day that settled in the sampled area. In

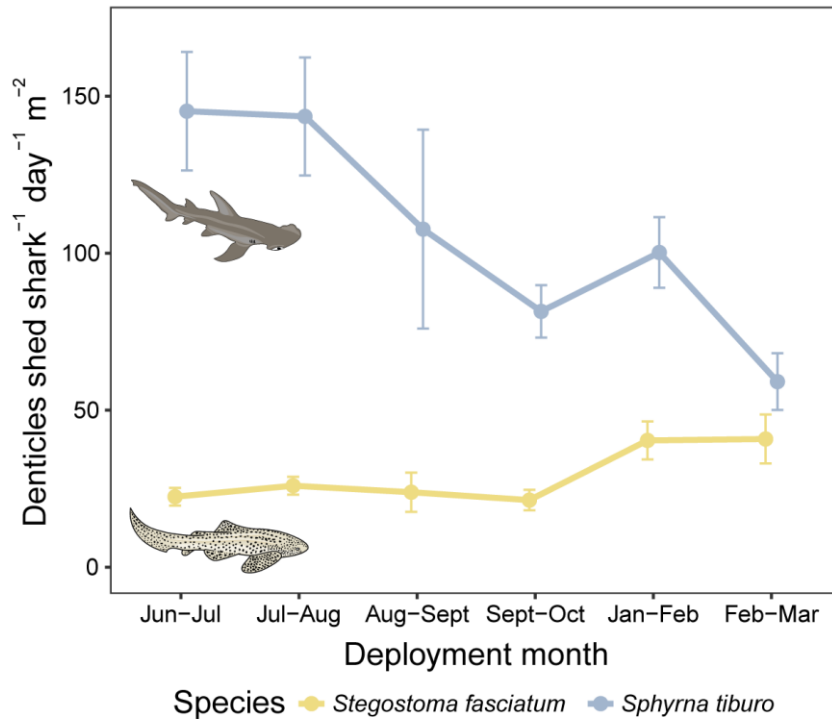
contrast, an estimated 146 shark teeth accumulated during the study, approaching the three to five order of magnitude difference between the number of denticles and teeth possessed by these species at any point in time.



**Figure 2.2** Denticle shedding rates for zebra sharks *Stegostoma fasciatum* (yellow) and bonnethead sharks *Sphyrna tiburo* (blue), aggregated across the 9 mo long study. Shedding rates were measured for each collection tray as the number of denticles accumulating  $\text{shark}^{-1} \text{d}^{-1} \text{m}^{-2}$  ( $n = 34$ ). Bonnethead sharks shed 3.6-fold more denticles on average than zebra sharks ( $p < 0.001$ ). Each boxplot shows the median (dark bar), mean (diamond), and interquartile range (box), and outliers are displayed as black points. The whiskers show the upper and lower extremes (calculated as  $Q3 + 1.5 \cdot \text{IQR}$  and  $Q1 - 1.5 \cdot \text{IQR}$ , respectively). Raw data are shown as jittered points.

Denticle shedding rates differed significantly between the two shark species in the tank. The mean shedding rate of bonnethead sharks ( $\text{denticles shed shark}^{-1} \text{d}^{-1} \text{m}^{-2}$ ) was 3.6 times higher than that of zebra sharks ( $\chi^2_1 = 73.80$ ,  $p < 0.001$ ; Fig. 2.2), although this difference ranged from 1.4 to 6.5 across deployments. When corrected by estimated non-experimental sediment input, denticle quantities, and shark space use, bonnethead sharks consistently maintained a higher shedding rate than zebra sharks (2.2- to 3.8-fold difference; Table 2.3).

Notably, correcting by denticle quantities did not have a large effect on the magnitude of difference in mean shedding rate between these two species, as their contrasting body sizes were offset by opposite patterns in denticle density (Table 2.1). As a result, the two bonnethead sharks in the tank produced more denticles than the three zebra sharks.



**Figure 2.3** Temporal variation in denticle shedding rates across the six deployments, which spanned a 9 mo long period from June to March. The mean shedding rate of bonnethead shark *Sphyrna tiburo* denticles (blue) decreased 2.5-fold during the study, whereas the mean shedding rate of zebra shark *Stegostoma fasciatum* denticles (yellow) remained relatively consistent across the first four deployments before increasing 1.7-fold in the final two deployments. The points and error bars show the mean denticle shedding rate and standard error for each deployment ( $n = 6$  trays per deployment, except the third deployment [ $n = 4$ ]).

Denticle shedding rates fluctuated across the monthly deployments. In aggregate, shedding rates varied 1.6-fold across deployments on average (Fig. A.4), although the pattern of temporal variation differed between the two shark species. The mean shedding rate of bonnethead shark denticles decreased from June to March by an overall factor of 2.5 (Fig. 2.3). In contrast, the mean shedding rate of zebra shark denticles was stable from June



to October before increasing by a factor of 1.7 in the final two deployments (Fig. 2.3). These patterns were consistent across tray locations, suggesting that they were not purely due to changes in shark space use over time in the tank (Fig. A.5). This temporal variation altered the ratio of bonnethead to zebra shark denticles recovered during each deployment despite shark density remaining constant.

The input of non-experimental sediment into the trays appeared to have a minimal effect on the observed patterns of denticle shedding. The bulk samples of surface sediments contained a mean  $\pm$  SD of  $554 \pm 242$  denticles  $\text{kg}^{-1}$ . Given the mean addition of  $135 \text{ g tray}^{-1}$  of grain sizes  $<3 \text{ mm}$ , we predict that  $\sim 75$  denticles could have been added by external processes, or approximately 8% of the 983 denticles that accumulated in each tray on average (falling within the SD of each deployment). Accordingly, the denticle shedding rates corrected by this non-experimental input were not substantially different from the raw measurements (Table 2.3). The tank was not hydro-vacuumed during the study, so these samples provide an upper estimate of denticles present in the substrate surrounding the trays.

**Table 2.3** Comparison of mean denticle shedding rates between zebra sharks *Stegostoma fasciatum* and bonnethead sharks *Sphyrna tiburo*. In addition to reporting the raw data, shedding rates were corrected by: (1) non-experimental sediment input, (2) denticle quantities (calculated as the product of body surface area and maximum observed denticle density; Table 2.1), (3) shark space use (based on the behavioral surveys), and (4) the corrections in aggregate. Shedding rates were calculated as denticles shed  $\text{shark}^{-1} \text{ d}^{-1} \text{ m}^{-2}$  with each correction applied. The magnitude of difference between the two species was determined both using the aggregated mean shedding rates as well as the rates during each deployment (provided here as a range). Test statistics are reported from the generalized linear mixed models.

Correction	Mean bonnethead shark shedding rate	Mean zebra shark shedding rate	Magnitude of difference (Range)	$\chi^2$	$p$
Raw data	106.1	29.5	3.6x (1.4-6.5)	73.80	<0.001
(1) Non-experimental input	103.0	27.3	3.8x (1.4-7.1)	70.70	<0.001
(2) Denticle quantity	$9.3 \times 10^6$	$2.9 \times 10^6$	3.2x (1.3-5.8)	68.82	<0.001
(3) Space use	89.3	37.7	2.4x (1.0-4.3)	45.35	<0.001
(4) All corrections	$7.6 \times 10^6$	$3.4 \times 10^6$	2.2x (0.9-4.2)	34.96	<0.001

#### 2.4.2 Denticle assemblage composition

The subset of denticles identified to functional morphotype broadly matched the expected proportions found on the body of each shark species in the tank (Fig. A.6). Most zebra shark denticles in the trays were classified as ridged abrasion strength ( $M \pm SE$ ;  $85.3 \pm 1.0\%$ ), with small numbers of generalized functions ( $7.4 \pm 0.7\%$ ) and abrasion strength ( $7.3 \pm 0.7\%$ ) denticles recovered. Likewise, the body and fins of zebra shark museum specimens were covered by ridged abrasion strength denticles (86.4% of body surface area), although generalized functions (8.3%) and abrasion strength (5.3%) denticles were also present. In contrast, most bonnethead shark denticles in the trays were classified as drag reduction ( $84.5 \pm 0.8\%$ ), with small numbers of abrasion strength ( $7.3 \pm 0.8\%$ ), ridged abrasion strength ( $4.8 \pm 0.5\%$ ), and generalized functions ( $3.4 \pm 0.5\%$ ) denticles recovered. Similarly, the body and fins of bonnethead shark museum specimens were characterized by drag reduction denticles (93.8% of body surface area), followed by abrasion strength (2.8%), ridged abrasion strength (2.4%), and generalized functions (1.0%) denticles. These corresponding rank abundances suggest that different functional morphotypes, which vary in their distribution on sharks' bodies, were shed at similar rates within each species.

#### 2.4.3 Denticle weathering

Denticles recovered from the trays were better preserved (median weathering score  $\pm$  median absolute deviation;  $1.0 \pm 1.2$ ) than denticles from the field. Although minor fragmentation of the crown or peaks was common (51% of denticles examined), both surface alteration and discoloration were rare, and denticles missing more than half of their crown comprised less than 3% of the assemblage. Around 23% of the denticles had an intact base, and base preservation, which could reflect differences in denticle morphology and whether they were lost through natural shedding or abrasion, was similar across functional

morphotypes (Kruskal–Wallis  $H_3 = 2.37$ ,  $p = 0.50$ ) and species (Wilcoxon  $W = 10671$ ,  $p = 0.37$ ). In general, weathering scores were consistent across deployments (Kruskal–Wallis  $H_5 = 5.82$ ,  $p = 0.32$ ) and areas of the tank (mesh enclosure vs. tank corner, Wilcoxon  $W = 10778$ ,  $p = 0.98$ ). However, drag reduction denticles ( $2.0 \pm 1$ ) were, on average, more weathered (Welch’s ANOVA  $F_{3,58.3} = 4.45$ ,  $p = 0.007$ ; Games–Howell post hoc test  $p < 0.05$ ) (Fig. A.7) and were more frequently observed with fragmented peaks (58%) than the other functional morphotypes (29%). Nevertheless, bonnethead shark denticles in aggregate ( $1.0 \pm 1.5$ ) had similar, although more variable, preservation relative to zebra shark denticles ( $1.0 \pm 0.7$ ).

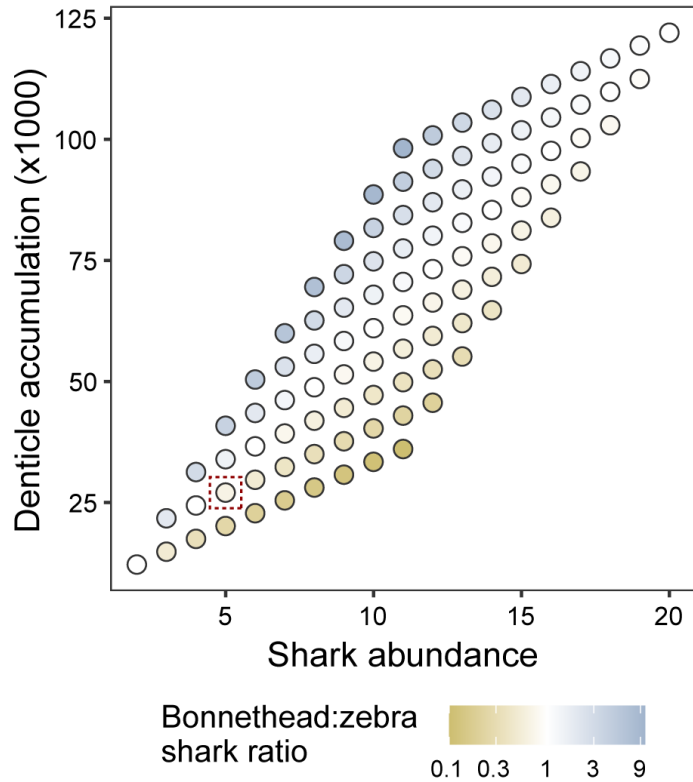
#### 2.4.4 Shark behavior

During the behavioral surveys, the bonnethead sharks in the tank displayed less behavioral variability than the zebra sharks, such that activity state differed significantly between species ( $\chi^2_1 = 616.38$ ,  $p < 0.001$ ) and time of day ( $\chi^2_1 = 77.26$ ,  $p < 0.001$ ). Bonnethead sharks were more active and consistently traveled around the tank, whereas zebra sharks alternated between traveling and resting, particularly in the evenings (Figs. A.8 and A.9). Both species were more active during dive shows ( $\chi^2_1 = 74.00$ ,  $p < 0.001$ ), with zebra sharks in particular spending less time resting on the tank bottom during shows. As a consequence of these activity patterns, bonnethead sharks spent more time in the upper two-thirds of the tank (78% of observations), whereas zebra sharks spent more time in the bottom third (51% of observations), where they would often rest on the tank bottom (38% of observations). Both species were more commonly observed in the outer third of the tank where the trays were located (59% of observations). Direct physical interactions with the tank sides, however, were moderately infrequent for both species ( $M \pm SD$ ;  $0.16 \pm 0.20$

$\text{min}^{-1}$ ). Taken together, both bonnethead and zebra sharks frequented the water column above the trays, enabling the capture of denticles, yet displayed distinct behaviors.

#### 2.4.5 Denticle shedding computer simulation

Using the empirical shedding rates to simulate theoretical denticle assemblages produced by different shark communities, we explored how shedding rates can influence the relationship between absolute shark abundance and denticle accumulation. For a shark community of a given size, the total number of denticles shed was driven by the relative proportions of species with fast and slow shedding rates (Fig. 2.4). For example, when fast-shedding bonnethead sharks were numerically dominant in the simulated community, denticle accumulation was substantially higher than when slow-shedding zebra sharks were dominant. Consequently, no single “true” shark density corresponded with each denticle accumulation value in the simulated scenarios, and the slope between these two metrics varied with community composition (Fig. 2.4). When disaggregated by functional morphotype, drag reduction denticles, which characterize bonnethead sharks, were overrepresented in the simulated assemblages relative to bonnethead shark abundances. Conversely, abrasion strength and ridged abrasion strength denticles, which characterize zebra sharks, outnumbered drag reduction denticles only when zebra sharks were several times more abundant than bonnethead sharks (Fig. A.10). As a result, the proportional representation of functional morphotypes in each simulated scenario did not inherently reflect the corresponding shark community composition given the contrasting shedding rates. This simulation underscores the value of considering shedding in denticle-based community reconstructions.



**Figure 2.4** Denticle accumulations simulated using the empirically measured denticle shedding rates over a 90 d period in a 1 m<sup>2</sup> area. The denticle accumulation produced by a given number of sharks varied with the ratio of fast-shedding (bonnethead sharks, in blue) to slow-shedding (zebra sharks, in yellow) species in the simulated community. The point reflecting the proportional abundance of sharks found in the Tropical Pacific Gallery is indicated by the red dashed box.

### 2.5 Discussion

Sharks lose their denticles, causing shark skin to be speckled with gaps where new denticles emerge to replace those lost (Reif 1985, Popp et al. 2020). This process of denticle replacement contributes to the accumulation of isolated denticles in sediments, yet the pattern and rate of denticle loss are unknown. Without understanding denticle shedding rates, it is challenging to generate reliable estimates of absolute shark abundance using denticle assemblages or assess their preservation potential. In this study, denticle shedding rates varied between the two shark species in the tank and, to a lesser extent, across months, a pattern which is broadly consistent with shark tooth replacement. Given the large

ecological and physiological differences between these two species, our estimates might span much of the natural spectrum of denticle shedding rates. Overall, we show that natural shedding contributes substantially to the prevalence of denticle assemblages in sediments, and we make suggestions for interpreting these assemblages in light of the variance in shedding among shark life modes.

Denticle accumulation into the trays over the 9 mo long study indicated that bonnethead sharks shed their denticles 3.6 times faster than zebra sharks on average. After correcting for denticle quantities, shark space use, and methodological factors, the mean magnitude of difference was 2.2-fold. This difference echoed the ~1- to 4.5-fold variation in tooth replacement rates reported across extant shark species (Ifft & Zinn 1948, Applegate 1967, Moss 1967, Märkel & Laubier 1969, Reif et al. 1978, Luer et al. 1990). Yet, our finding that the demersal species in the tank shed fewer denticles than the benthopelagic species was unexpected and suggests that abrasion alone did not dictate shedding rates. Rather, we propose that this interspecific variation might have resulted from differences in metabolism, ecological traits, or denticle characteristics. First, we hypothesize that higher activity levels, possibly mediated by metabolism (Killen et al. 2010, Bernal et al. 2012), can elevate denticle shedding. Bonnethead sharks are obligate ram-ventilators and are negatively buoyant, meaning that they must constantly move to breathe and maintain their position in the water column (Myrberg & Gruber 1974, Parsons 1990). Accordingly, captive bonnethead sharks patrol throughout the day (Myrberg & Gruber 1974), with activity states potentially tracking feeding schedules (Kelly et al. 2019), consistent with our findings in the behavioral surveys. In contrast, zebra sharks respire via buccal pumping and are observed, both in the wild (Dudgeon et al. 2008, 2013) and in the captive environment studied here, to remain sedentary for long periods each day and to exhibit nocturnal activity. In line with

these behavioral differences, bonnethead sharks likely have higher metabolic rates than zebra sharks (Parsons 1990, Carlson & Parsons 2003, Bernal et al. 2012, Payne et al. 2015), which could increase denticle shedding rates, as has been found for shark teeth (Breder 1942, Luer et al. 1990, Correia 1999).

In addition to physiology, denticle characteristics could influence shedding rates. Although bonnethead and zebra sharks possess similar numbers of denticles (~3–11 million), they could differ in their susceptibility to shedding, particularly upon contact with the tank. For example, zebra sharks' thick denticles are well suited for contact with rocky, sandy, or coral-rich substrates (Reif 1985, Raschi & Tabit 1992), concordant with this species' demersal lifestyle. In contrast, bonnethead sharks have thinner, smaller, and more hydrodynamic denticles (Fig. 2.1) that might be more prone to shedding because this benthopelagic species does not frequently encounter surfaces in the wild. Moreover, due to the tightly coupled relationship between bonnethead denticle ridge morphology and drag reduction properties (Raschi & Musick 1986, Lauder et al. 2016), physical wear of the denticle crown or ridges might rapidly decrease performance (e.g. Leidl et al. 2021), offsetting the energetic expense of continually shedding and replacing denticles. Thus, despite zebra sharks resting on the tank bottom and both species contacting the tank sides during the behavioral surveys, the effect of these behaviors on denticle shedding could vary between species. Overall, our findings show that, like shark teeth, denticle shedding rates vary between species with different life modes, physiologies, and denticle morphologies—a result which likely extends to other ecological groups of sharks. Although more experimental work is needed to disentangle the relative importance of these potential mechanisms in driving interspecific variation, these differences in denticle shedding rates imply that (1) raw denticle counts do not inherently mirror shark absolute abundances and

(2) shark community composition could influence total denticle accumulation if there is high variance in denticle shedding rates.

We observed temporal variation in denticle shedding despite shark density and tank conditions remaining stable throughout the study. The directionality and magnitude of these patterns differed between species, which could indicate distinct, interacting, or non-linear mechanisms. Neither photoperiod, water quality (e.g. temperature, alkalinity, or dissolved oxygen), water flow patterns, shark behavior, interactions with other large animals in the tank, aquarium attendance, nor hormonal changes associated with reproductive cycles could consistently explain these temporal patterns given the available data. However, we were unable to assess shark stress levels (i.e. in response to tank construction in November and December) or run controlled experiments to examine each hypothesized mechanism in isolation. Furthermore, a multi-year study would be needed to test whether these patterns follow seasonal cycles or, alternatively, whether they were caused by incidental changes to the tank environment. Although the proximate drivers remain inconclusive, temporal variation in denticle shedding might ultimately stem from metabolic rate, similar to shark tooth replacement (Luer et al. 1990, Correia 1999). At the same time, we expect environmental conditions and shark behavior to be more variable in a natural setting than in the controlled aquarium tank studied here, meaning that denticle shedding rates might be more dynamic in the wild. Given the ostensibly episodic nature of denticle shedding, we caution against using denticle accumulations over sub-annual time intervals to compare shark abundances. Yet, the denticle record in the field is typically time-averaged over years to millennia or longer, absorbing any short-term variability in denticle shedding rates. Variation in denticle accumulation over multi-year timescales is therefore more likely to



result from ecological factors such as changes in shark abundance and distribution or from differences in preservation across denticle morphotypes.

Denticles accumulating in the trays broadly reflected the rank abundance of functional morphotypes found on bonnethead and zebra sharks. Correspondence between the composition of denticle assemblages and shark communities has been found in shallow marine environments in both recent and deep time, demonstrating that the denticle record can measure shark functional diversity (Ferrón et al. 2014, Dillon et al. 2020). Here, because denticles recovered from the trays could be further identified to species, this correspondence also suggests that shedding rates were roughly consistent across the body of each shark and that specific functional morphotypes were not preferentially shed. The denticle record therefore most likely originates from natural shedding rather than the selective loss of denticles through abrasion or behavior. Although the effect of mortality was not addressed in this study, we expect pulses of denticle accumulation resulting from carcasses settling on the seafloor to be relatively rare yet recognizable. For example, work on fish scales has shown that scale deposition is primarily derived from shedding rather than predation or other mortality events (Shackleton 1988) and that outliers or anomalous samples can indicate instantaneous, mortality-driven deposition (O'Connell & Tunnicliffe 2001). However, unlike fish scales, denticles might also infrequently enter sediments via ingestion and defecation by other sharks and marine mammals due to their dissolution-resistant composition (Fertl 1996, Ford et al. 2011, Mourier et al. 2013, Engelbrecht et al. 2019), yielding a pattern of deposition similar to natural shedding. Nonetheless, given that frequent, indiscriminate shedding appears to be a major mechanism producing denticle accumulation in sediments, both shark abundance and diversity as well as the relative proportions of

functional morphotypes across sharks' bodies should contribute to denticle assemblage composition in the field.

Denticles recovered from the trays were well-preserved, demonstrating that denticles are largely intact when shed and are later exposed to taphonomic processes as they accumulate and are buried in sediments. On the whole, the median weathering score in this study (1.0) was lower (better preserved) than that of denticles recovered from modern (1.3–2.0) and mid-Holocene (1.3) reef sediments using the same scoring criteria, and variation in preservation across denticles was similar (Dillon et al. 2020, 2021). Likewise, the frequency of denticles with intact bases was over twice as high in the trays (23%) as in reef sediments (~8–11%), suggesting that although some denticles are shed with their base intact, the bases are often lost during or shortly after shedding. Consistent with previous work (Dillon et al. 2020, 2021), drag reduction denticles were more weathered than the other functional morphotypes, as their peaks are prone to fragmentation. These weathering scores represent a baseline of denticle preservation around the time of deposition, providing context for interpreting denticle weathering in modern and fossil assemblages that have incurred additional taphonomic damage. Such comparisons should, nonetheless, be interpreted with care as they do not account for denticles lost through complete fragmentation or dissolution.

Building from our empirical measurements, the computer simulation examined the implications of interspecific variation in denticle shedding rates when interpreting denticle accumulation in the field. We share this simple, two-species exercise to illustrate the interpretive boundaries of the denticle record, rather than to offer a means to predict shark abundances using field data, given that wild systems are inevitably more complex. In the simulation, shedding rates governed the linkage between absolute shark abundance and denticle accumulation, causing sharks with fast shedding rates to be overrepresented in the

simulated denticle record. Simulated shark communities dominated by fast-shedding species therefore produced larger denticle assemblages that were compositionally skewed toward those species. Accordingly, using denticle abundances alone to reconstruct shark densities from an isolated sample might be intractable, yet such abundances could nonetheless provide insight into relative changes across samples. Denticle shedding rate measurements collected at the level of shark species or life mode, as well as information about shark body size, denticle densities, and taphonomic alteration (Dillon et al. 2021), would be needed to construct more reliable shark density estimates from the denticle record. In the absence of this information, we suggest analyzing complementary absolute and relative abundance metrics across time points or between sampling locations to conservatively characterize community change. These metrics include: (1) absolute denticle accumulation rates, corrected by an age-depth model; (2) the absolute accumulation rate of each functional morphotype; (3) the relative proportion of each functional morphotype; and (4) beta diversity. Calculating both absolute and relative denticle abundances can help distinguish between a meaningful ecological change and an artifact of proportional math (Jackson 1997). These calculations can also be used to reveal any potential feedback between absolute denticle accumulation and assemblage composition (e.g. identifying whether patterns in overall denticle accumulation are driven by shifts in a numerically dominant species with an especially fast or slow shedding rate). Nevertheless, the two species included in this simulation likely represent opposite ends of the shedding rate spectrum. If so, the proportions of fast- and slow-shedding species and their respective shedding rates might be more balanced in a wild shark community, where denticle accumulation rates have been found to correlate with shark abundances (Dillon et al. 2020). Regardless, this simulation helps constrain the inferences that can be made using denticle assemblages. Similar

exercises could be instructive when interpreting other multi-element fossil assemblages, such as fish teeth, otoliths, or sea urchin spines.

Multiple logistical challenges hinder the collection of denticle shedding rate measurements from individual sharks. Conducting this study in an aquarium provided a controlled setting with a known and constant shark density, allowing repeated measurements of denticle shedding. At the same time, captivity could have artificially altered shedding rates in five primary ways. First, shark behavior and movement were limited by the tank environment. Physical contact with the enclosure, a scenario not encountered in the wild, could induce denticle loss. Furthermore, the artificial light, high fish density, dive shows, tank maintenance, feeding regimen, and visitor attendance could have affected shark behavior or stress levels, indirectly influencing shedding rates. Although we explored some of these potential confounding variables, our behavioral surveys might have lacked the resolution to infer an effect on denticle shedding. On the other hand, we were not able to test how diet, ontogeny, migration, water temperature, or other seasonal behaviors or metabolic shifts affect shedding—particularly over annual timescales in a biodiverse shark community. For example, natural oscillations in environmental conditions in the wild could augment the temporal variability of denticle shedding rates. As such, caution should be exercised when extrapolating measurements from captive sharks to wild contexts. Second, all patterns observed in this study precede any taphonomic alteration that would occur as denticle assemblages are buried, reworked, or exhumed after initial deposition. Third, water flow can influence denticle sinking trajectories, although flow in the tank was relatively low and localized, particularly when compared to a natural marine setting. These flow patterns did not appear to create non-random concentrations of denticles across the sampled tray locations (Fig. A.3). Given that denticles are 2–3 times denser than seawater, they likely

sank quickly relative to any currents in the tank. Nonetheless, future empirical or theoretical work to model denticle sinking rates and pathways could illuminate how far denticles are displaced horizontally as they settle under different flow regimes. Fourth, the input of non-experimental sediment into the trays could have elevated the observed shedding rates, although this effect appeared to be minor. Fifth, the large tank size prevented the measurement of absolute shedding rates given that every denticle accumulating in the tank substrate could not be counted. Rather, we sub-sampled the process of denticle accumulation as a proxy for denticle shedding. Despite these limitations, our results provide first insight into the range of variation in denticle shedding rates between sharks with different life modes as well as a framework to collect these measurements for additional species.

Collectively, we demonstrate that the two shark species in this study contribute unevenly to the denticle record, highlighting denticle shedding rates as an important and dynamic biological lens through which shark abundance can be determined from denticle accumulation. Our denticle shedding rate measurements build on previous advances in the identification, quantification, and recovery of denticles from sediments (Helms & Riedel 1971, Doyle & Riedel 1979, Tway 1979, Reif 1985, Ferrón et al. 2014, Dillon et al. 2017, 2020, Ferrón & Botella 2017, Sibert et al. 2017), which have illuminated the prevalence and ecological value of these understudied microfossils. Our findings provide a first step in calibrating the relationship between shark density and denticle accumulation to improve our understanding of how sharks are represented as disarticulated denticle assemblages in the fossil record. They also offer guidance when evaluating denticle assemblages as a proxy for shark abundance in the field and call attention to the natural complexities when interpreting these abundance data.

**Data Availability.** Data are archived in the Dryad Digital Repository:

<https://doi.org/10.25349/D9K32M>. The R code used in this study is available at

<https://github.com/erinmdillon/denticle-shedding-meps2021> and archived on Zenodo:

<https://doi.org/10.5281/zenodo.5637265>.

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**Author Contributions.** E.M.D., A.O., and D.J.M. designed the study and methodology; E.M.D., A.B., and K.D.P. collected and processed the sediment samples; K.D.P. collected the behavioral data; E.M.D. analyzed the data with input from K.D.L.; E.M.D. wrote the manuscript. All authors contributed to drafts and gave final approval for publication.

# **CHAPTER 3: DERMAL DENTICLE ASSEMBLAGES IN CORAL REEF SEDIMENTS CORRELATE WITH CONVENTIONAL SHARK SURVEYS**

## ***3.1 Abstract***

It is challenging to assess long-term trends in mobile, long-lived and relatively rare species such as sharks. Despite ongoing declines in many coastal shark populations, conventional surveys might be too fleeting and too recent to describe population trends over decades to millennia. Placing recent shark declines into historical context should improve management efforts as well as our understanding of past ecosystem dynamics. A new palaeoecological approach for surveying shark abundance on coral reefs is to quantify dermal denticle assemblages preserved in sediments. This approach assumes that denticle accumulation rates correlate with shark abundances. Here, we test this assumption by comparing the denticle record in surface sediments to three conventional shark survey methods at Palmyra Atoll, Line Islands, central Pacific Ocean, where shark density is high and spatially heterogeneous. We generally found a significant positive correlation between denticle accumulation rates and shark abundances derived from underwater visual census, baited remote underwater video and hook and line surveys. Denticle accumulation rates reflected shark abundances, suggesting that denticle assemblages can preserve a signal of time-averaged shark abundance in low-energy coral reef environments. We offer suggestions for applying this tool to measure shark abundance over long timescales in other contexts.

### ***3.2 Introduction***

Long-term shark abundance data can help evaluate the extent of shark declines, set appropriate management targets and provide insight into how sharks influence food web ecology (Myers & Worm 2003, Lotze & Worm 2009, Ferretti et al. 2010, Roff et al. 2016). However, conventional surveys and fisheries catch data typically have short survey periods that only span the last few decades, cover small areas and can miss rare and mobile species like sharks (Burgess et al. 2005, McClanahan et al. 2007, Lotze & Worm 2009). These limitations might explain, in part, why surveys conducted at the same sites can return shark abundance estimates that vary by orders of magnitude (Ward-Paige et al. 2010, Bradley et al. 2017a), confounding our understanding of shark community dynamics over time and space. This is a particularly pervasive issue in coral reef ecosystems, where sharks are important predators (Roff et al. 2016), cultural symbols (e.g. Riesenfeld 1950) and ecotourism attractions (Cisneros-Montemayor et al. 2013), yet are vulnerable to exploitation and have declined in many regions (Ferretti et al. 2010). Here, we examine the use of an alternate method for surveying sharks—dermal denticle counts—to improve contemporary and historical reference points of shark abundance on coral reefs.

Dermal denticles are small (<2 mm), tooth-like scales that cover the bodies of elasmobranchs (Fig. 3.1). After being shed, denticles sink and become incorporated into marine sediments (Helms & Riedel 1971, Dillon et al. 2017, Sibert et al. 2017). This accumulation of denticles in sediments is time-averaged, meaning that denticles shed by non-contemporaneous individuals appear together in a single temporally mixed assemblage. Denticle assemblages can preserve evidence of shark occurrences (Fig. 3.2), even where sharks are rare or are not easily observed in conventional surveys (Dillon et al. 2017, Sibert et al. 2017). Denticles also preserve well in fossil sediments since they are composed of



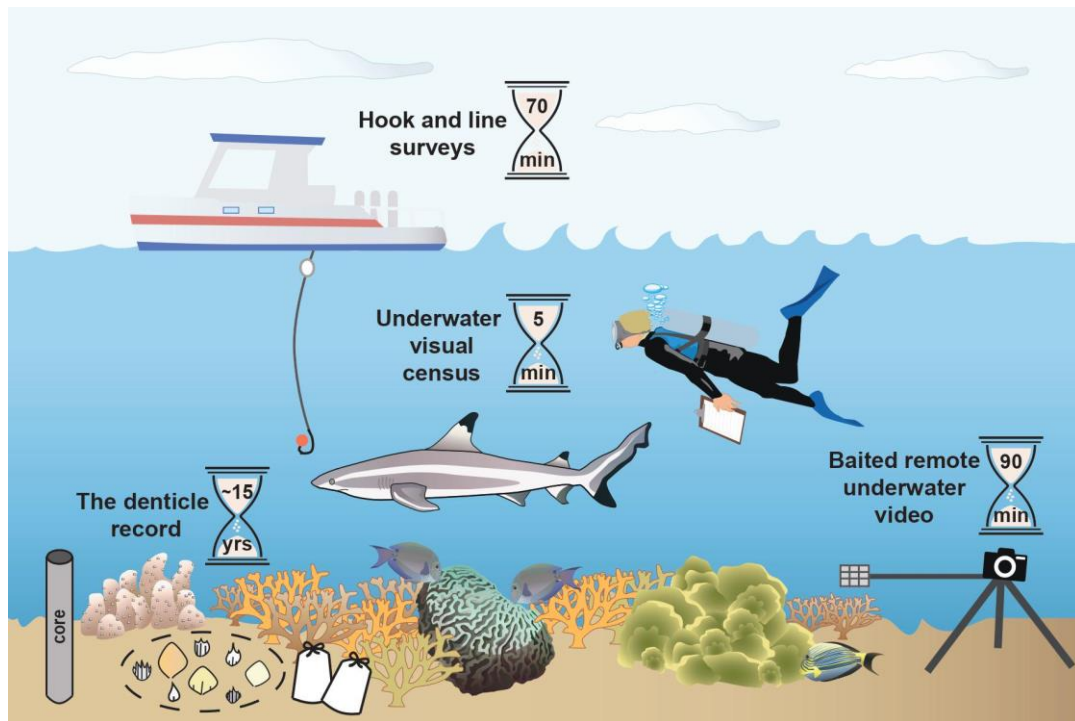
calcium phosphate (Helms & Riedel 1971, Sibert & Norris 2015), and they differ morphologically across taxa with different ecological life modes (Reif 1985, Ferrón et al. 2014, Dillon et al. 2017, Ferrón & Botella 2017), offering a window into shark community dynamics over deeper ecological and geological time. However, it is unclear if denticle accumulations reflect shark abundances or how taphonomic processes—which affect how organic remains and their ecological attributes become preserved in the fossil record (Behrensmeier et al. 2000)—selectively alter the preservation, transport and temporal context of the denticle record (Kidwell & Flessa 1995, Kidwell 2013).



**Figure 3.1** Light microscope image of a blacktip reef shark *Carcharhinus melanopterus* denticle. Scale bar = 200  $\mu\text{m}$ . Image credit: V. Chan.

We explored the relationship between denticles preserved in surface sediments and contemporary shark abundances measured by three conventional shark survey methods to test how well shark abundance can be estimated from the denticle record. Such live–dead comparisons are used to test the fidelity of death assemblages for other taxa (e.g. molluscs) in surface sediments (Kidwell 2013), although they can also help indicate the reliability of buried historical layers, which are otherwise challenging to assess directly (Kidwell 2009).

In doing so, this approach assumes that denticle assemblages in surface sediments are reasonable analogues of the fully buried record (Kidwell 2013). We conducted this study at Palmyra Atoll, a protected, remote island in the central Pacific Ocean where shark populations have been monitored for over a decade and are likely at or near their site-specific carrying capacity (Bradley et al. 2017a). Consequently, mismatch between contemporary surveys and denticle assemblages is less likely to be caused by anthropogenic-driven shark declines than in fished regions and, instead, could indicate taphonomic biases, time-averaging or contemporary survey error. We found that denticle accumulation rates corresponded with shark abundances, supporting the denticle record's ability to record relative shark abundance in low-energy coral reef environments.



**Figure 3.2** Sample durations for the denticle record and conventional survey methods used in this study. The hourglasses show the average duration of a single replicate. Underwater visual census and baited remote underwater video surveys had standardized durations, hook and line surveys varied from 7–274 min and the denticle record was averaged over years to decades. Several graphics were obtained from the Integration and Application Network image library: J. Hawkey, D. Kleine, T. Saxby, D. Tracey, and J. Woerner, Integration and Application Network, University of Maryland Center for Environmental Science ([ian.umces.edu/image-library/](http://ian.umces.edu/image-library/)).

### 3.3 Methods

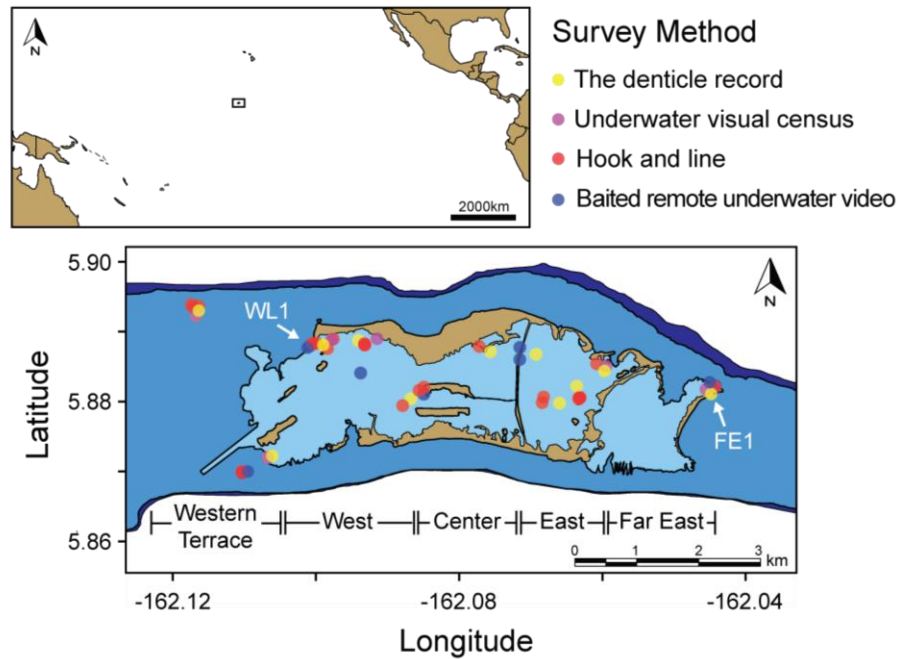
#### 3.3.1 Study area

Palmyra Atoll Fish and Wildlife Refuge, central Pacific Ocean (5°54' N, 162°05' W) is remote, unfished and uninhabited, apart from a limited research presence. Shark density at the atoll is high, spatially heterogeneous and has remained relatively stable over the past decade (Bradley et al. 2017a). Blacktip reef sharks *Carcharhinus melanopterus* are the most common shark species in the lagoon and backreef habitats (Papastamatiou et al. 2009a, 2017), followed by grey reef sharks *Carcharhinus amblyrhynchos* and whitetip reef sharks *Triaenodon obesus*. However, at least 15 other shark species have been documented by deep reef cameras or shallow surveys (Mundy et al. 2010, Papastamatiou et al. 2014, Y. P. Papastamatiou & J. G. Eurich, pers. comm.).

#### 3.3.2 Collection and recovery of denticle assemblages

We collected surface sediments from 11 sites at Palmyra Atoll in the lagoon ( $n = 8$ ) and backreef ( $n = 3$ ) habitats (Fig. 3.3). These sites were spread across five broadly defined areas: Western Terrace, West Lagoon, Center Lagoon, East Lagoon and Far East (following Gardner et al. 2014a). Sampling was restricted to low-energy habitats to reduce the likelihood that the denticle assemblages had been transported, sorted, reworked or exposed to physical taphonomic biases (Kidwell & Flessa 1995, Kidwell 2013). We expect the sediment record to be relatively undisturbed by storms because Palmyra Atoll lies outside the path of nearly all tropical cyclones (Clark 2008), although it does occasionally experience high wind and waves from distant storms (Gardner et al. 2014b). Sediment collection sites were selected to overlap with available shark abundance data from underwater visual census (UVC), baited remote underwater video (BRUV) and hook and

line surveys, which varied by nearly an order of magnitude across sites (Fig. B.1 and Table 3.1). In particular, sediment collection sites were matched with survey locations in the same habitat within a 0.5 km radius ( $M = 0.23$  km) and were spaced 0.5–2.5 km apart.



**Figure 3.3** Survey locations at Palmyra Atoll, central Pacific Ocean. Conventional surveys were matched with the denticle record at 11 sites spread across five broadly defined areas of the atoll: Western Terrace, West Lagoon, Center Lagoon, East Lagoon and Far East. The arrows indicate the sites at Far East 1 (FE1) and West Lagoon 1 (WL1). The land is tan, the lagoon is light blue, the reef terrace and backreef are blue and the forereef is dark blue.

At each site, we collected 10 replicate ~0.8 kg bulk samples of sediments from the top <10 cm via SCUBA in patches of mud, silt and sand in areas with scattered branching coral matrix or adjacent to coral heads. Water depths varied between 1.8 and 6.1 m. Replicate samples within a site were spaced 1–5 m apart. The substrate was moderately bioturbated by crustaceans, worms and holothurians, although the density of burrow holes and tracks was visibly similar across sites. Areas with high bioturbation by callianassid and alpheid shrimp were avoided. Sediment sampling was conducted in 2015 and 2016, with different sites sampled in each year.

**Table 3.1** Sediment characteristics, denticle accumulation rates and abundances and shark abundances measured by underwater visual census (UVC), baited remote underwater video (BRUV) and hook and line surveys. Sites are ordered west to east across the atoll. Where applicable, the mean and standard deviation are reported ( $M \pm SD$ ).

Site name	Habitat	Area	Sorting	Mean grain size & sorting description	Days of sediment trap data	Sedimentation rate ( $g/m^2/day$ ; $M \pm SD$ )	Vertical accumulation rate ( $cm/yr$ ; $M \pm SD$ )	Average estimated years per sample	Denticle accumulation rate (denticles/kg sediment/year; $M \pm SD$ )	Denticle abundance (denticles/kg sediment; $M \pm SD$ )	UVC surveys (sharks/ $m^2$ )	Hook and line surveys (shark catch/hr)	BRUV surveys (MaxN)
Western Terrace 1	Backreef	Western Terrace	1.61	Fine sand, poorly sorted	423	12.7 $\pm$ 6.6	0.46 $\pm$ 0.24	15	1.97 $\pm$ 1.4	29.7 $\pm$ 21.6	0.00053	0.84	-
Western Terrace 2	Backreef	Western Terrace	1.46	Fine sand, poorly sorted	377	41.8 $\pm$ 19.8	1.39 $\pm$ 0.66	5	1.98 $\pm$ 1.2	10 $\pm$ 5.9	0.00053	0.62	3
West Lagoon 1	Lagoon	West Lagoon	2.60	Medium sand, very poorly sorted	233	17.2 $\pm$ 7.0	0.57 $\pm$ 0.23	12	4.48 $\pm$ 1.7	54.8 $\pm$ 21.3	0.00159	0.90	1
West Lagoon 2	Lagoon	West Lagoon	2.32	Medium sand, very poorly sorted	118	13.7 $\pm$ 1.4	0.55 $\pm$ 0.06	13	2.71 $\pm$ 0.8	34.2 $\pm$ 10.4	0.00273	1.27	4
West Lagoon 3	Lagoon	West Lagoon	1.86	Coarse sand, poorly sorted	120	17.1 $\pm$ 7.2	0.52 $\pm$ 0.22	13	4.68 $\pm$ 1.9	62.9 $\pm$ 25.5	-	1.42	5
Central Lagoon 1	Lagoon	Central Lagoon	1.51	Fine sand, poorly sorted	209	25.3 $\pm$ 8.3	0.71 $\pm$ 0.23	10	0.85 $\pm$ 0.8	8.3 $\pm$ 7.7	-	0.00	2
East Lagoon 1	Lagoon	East Lagoon	1.74	Fine sand, poorly sorted	191	7.2 $\pm$ 2.0	0.20 $\pm$ 0.06	35	0.32 $\pm$ 0.3	10.9 $\pm$ 9.2	-	-	2
East Lagoon 2	Lagoon	East Lagoon	1.42	Medium sand, poorly sorted	191	7.2 $\pm$ 2.0	0.20 $\pm$ 0.06	35	1.19 $\pm$ 0.4	41.1 $\pm$ 13.7	-	0.00	-
East Lagoon 3	Lagoon	East Lagoon	1.23	Medium sand, poorly sorted	147	38.7 $\pm$ 24.9	1.09 $\pm$ 0.70	6	6.05 $\pm$ 1.9	39 $\pm$ 12.1	-	2.41	-
East Lagoon 4	Lagoon	East Lagoon	1.87	Fine sand, poorly sorted	115	26.2 $\pm$ 8.1	0.96 $\pm$ 0.30	7	8.85 $\pm$ 3.9	64.6 $\pm$ 28.7	0.00510	1.36	-
Far East 1	Backreef	Far East	2.10	Medium sand, poorly sorted	247	16.3 $\pm$ 9.7	0.66 $\pm$ 0.39	11	1.86 $\pm$ 1.3	19.7 $\pm$ 13.6	0.00053	4.57	3

Sediment samples were processed to extract denticles and describe sediment characteristics. First, we dried, weighed and sieved the sediments, from which grain size distributions, mean grain size and sorting were calculated (Folk & Ward 1957). The 106–250  $\mu\text{m}$ , 250–500  $\mu\text{m}$  and 500  $\mu\text{m}$ –2 mm size fractions were digested with 10% glacial acetic acid to eliminate the calcium carbonate components, and the remaining particles were treated with 100–200 ml of 5% hydrogen peroxide and heated for no more than 15 min to remove organic material (see Dillon et al. 2017 and Sibert et al. 2017 for the full protocol). Denticles were picked from the residue under a dissecting microscope and counted. Denticles missing more than half of their crown were excluded to avoid double counting; such fragments composed <7% of the total denticle assemblage. Denticle abundance was calculated as the total denticle count per sample divided by the dry weight of the sediment fractions, yielding denticles per kg sediment.

Denticle abundances were corrected by sedimentation rates at each site to produce denticle accumulation rates, measured as total denticle count per kg sediment per year (following Sibert et al. 2017). We used sedimentation data obtained from polyvinyl chloride (PVC) sediment traps over replicate 5–139 d periods between 2006 and 2009 from two sources, our own field collections and published data (Williams et al. 2011, Knapp et al. 2013; Supplementary Methods in Appendix B.1). Sedimentation rates were converted into vertical accumulation rates per year using mean sediment densities (Table 3.1).

Differences in denticle accumulation rates between sites ( $n = 11$ ), areas of the atoll, ( $n = 5$ ) and habitats ( $n = 2$ ) were analyzed using Welch's ANOVA (R Core Team 2019). To assess the sampling effort needed to detect differences in denticle accumulation rates between sites, we performed a power analysis with a Type I error level of 0.05 for Welch's ANOVA (Levy 1978, Shieh & Jan 2013). To inform how best to invest field and laboratory

sampling effort, the number of samples needed to capture the variation in denticle abundance between replicates at each site was explored via randomized resampling. Denticle abundances from the 10 replicate samples collected at each site were randomly resampled 10,000 times without replacement, and the cumulative mean denticle count per kg sediment was calculated. We then determined the difference between the cumulative mean as each replicate sample was added to the set and the final cumulative mean. The closer this difference was to zero, the more appropriate the number of replicates was for capturing within-site variation in denticle abundance, with the assumption that the 10 replicates did adequately capture this variation. This exercise was carried out for each sediment collection site, and the differences were plotted to visually examine the trade-offs between sampling effort and variance (Fig. B.2).

### 3.3.3 Denticle classification

Denticle assemblage composition was determined by measuring and classifying the denticles from three randomly selected replicates per site ( $n = 574$  denticles). We described the crown, peaks and ridges using a dissecting microscope (following Dillon et al. 2017; Table B.1). We then visually categorized the denticles into previously recognized functional morphotypes: drag reduction, ridged abrasion strength, abrasion strength, generalized functions and defense (Reif 1985, Ferrón et al. 2014, Dillon et al. 2017). To verify these classifications, a multinomial logistic regression was trained with our denticle reference collection (Dillon et al. 2017) and used to predict the functional morphotype of each denticle based on the measurements taken (Tables B.1–B.4 and Supplementary Methods in Appendix B.1).

### 3.3.4 Assessing denticle preservation

To assess preservation, the denticles in each randomly selected replicate were assigned a weathering score ( $n = 574$ ). Weathering scores ranged from zero (pristine) to three (poor preservation) and were based on visual inspections of the crown, peaks and base (Fig. B.3 and Table B.5). Weathering scores were compared across functional morphotypes, denticle characters, habitats and areas of the atoll as well as with sediment characteristics and denticle abundances to explore how preservation could affect our interpretation of the denticle record.

### 3.3.5 Testing for concordance between the denticle record and conventional shark surveys

#### **3.3.5.1 Underwater visual census surveys**

Stationary point counts were used to obtain diver-based visual measurements of relative shark abundance. During these surveys, a diver was positioned in the center of a circle with a 10 m radius for 5 min (McCauley et al. 2012b). Divers endeavored to count individual sharks only once. Six sites corresponding to the sediment collection sites (Fig. 3.3) were surveyed, with four to seven replicate surveys conducted at each site during daylight hours over a two-month period in 2006. Shark density was expressed as sharks  $m^{-2}$ .

#### **3.3.5.2 Baited remote underwater video surveys**

Baited remote underwater video (BRUV) surveys were used to obtain video-based measurements of relative shark abundance. GoPro™ Hero4 cameras mounted on PVC or metal frames were deployed for 120 min at depths between 1 and 7 m at seven sites corresponding with the sediment collection sites (Fig. 3.3), with one to three replicate surveys conducted at each site (Bradley et al. 2017b). Each BRUV system was baited with



0.5 kg mackerel. All BRUVs were deployed during daylight hours between 2014 and 2016. We used the SeaGIS<sup>®</sup> software EventMeasure (version 4.4; Bacchus Marsh, Australia) to analyze the first 90 min of video footage. For each survey, we recorded the maximum number of individuals in a single video frame (MaxN)—a conservative metric of relative abundance in BRUV surveys—for each shark species.

### **3.3.5.3 Hook and line fishing surveys**

Hook and line surveys were conducted on six occasions between 2013 and 2014 during daylight hours (Bradley et al. 2017a). Sampling trips lasted 7–10 d, and trips were spaced at least 58 d apart to decrease the likelihood of behavioral effects. Sampling was unstructured, and fishing locations were selected opportunistically to cover the lagoon and backreef habitats. Data were available for ten of the eleven sediment collection sites (Fig. 3.3), with one to five replicate surveys conducted at each site. Chum was used to attract sharks to the boat, where they were caught using hand lines baited with a single barbless circle hook. Fishing effort (hours spent scientific fishing) varied across sampling trips and sites, and shark abundance was expressed as shark catch per hour. Recaptured individuals were not included in the abundance estimates.

### **3.3.5.4 Between-methods comparisons**

Data from the three conventional shark survey methods were not available at every sediment collection site, although there was overlap between at least two conventional methods and the denticle record at eight of the eleven sites (Table 3.1). Spatial autocorrelation between sediment collection sites was assessed using Moran's I but was non-significant. The three conventional survey methods primarily detected *C. melanopterus*, with *C. amblyrhynchos* rarely observed. The abundances of these two species were summed to

yield total shark abundance with which the denticle record was compared. Pairwise comparisons between denticle accumulation rates and shark abundances, as detected by each of the conventional survey methods, were made using a series of one-sided Spearman's rank correlations with sequential Bonferroni corrections. This was done with denticle accumulation rates calculated using the mean (including  $\pm 1\sigma$ ), minimum and maximum sedimentation rates at each site. Pairwise comparisons were also made between denticle abundances and shark abundances. Shark abundances from the conventional survey methods were compared using one-sided Spearman's rank correlations (R Core Team 2019).

### **3.3.5.5 *N*-mixture model**

To better understand linkages between the denticle record and shark abundance given the challenges inherent in detecting rare, mobile taxa such as sharks, the temporally replicated data from the conventional survey methods were integrated using an *N*-mixture model to predict relative shark abundance at each site while accounting for differences in detection probability (Royle 2004). We assumed a closed population with respect to mortality, recruitment and movement and that the counts were independent. Survey duration (effort) and method were included as covariates for detection, and habitat type (lagoon or backreef), substrate angle (along a sand-flat ledge or horizontal) and year were included as covariates for abundance. The data were fitted using a negative binomial mixing distribution with an upper index of integration (*K*) of 600 using the R package *unmarked* (Fiske & Chandler 2011). Model support was assessed using a maximum likelihood-based approach (Table B.6), and goodness-of-fit was examined using the R package *nmixgof* (Knape et al. 2018; Supplementary Methods in Appendix B.1). No model received unequivocal support, so we model-averaged the predicted abundance values for each site across the top three models (Tables B.7 and B.8). Due to the potential limitations of this modelling approach

given the use of unmarked animals and its sensitivity to statistical assumptions (Barker et al. 2018, Link et al. 2018, but see Kéry 2018), these predicted values were interpreted as relative abundances, rather than absolute abundances. Detection probabilities for each survey method were also estimated using model-averaging.

Multiple regression was used to determine the relationship between relative shark abundance (integrated across survey methods), environment and the denticle record. Shark abundance predicted from the  $N$ -mixture model (Table B.8) and habitat type were included in the model as predictors, and the model was run twice—once with denticle accumulation rates and once with denticle abundances as the response variable (R Core Team 2019).

### **3.3.5.6 Denticle assemblage composition**

To test how well the denticle record reflects shark diversity, we examined denticle assemblage composition in relation to the two species detected by the conventional survey methods. Using our reference collection, we determined the rank abundance of functional morphotypes that characterize *C. melanopterus* and *C. amblyrhynchos* (Supplementary Methods in Appendix B.1) and compared this with the rank abundance of morphotypes found in the sediments. We also compared denticle assemblages between sites using Kruskal–Wallis tests (R Core Team 2019).

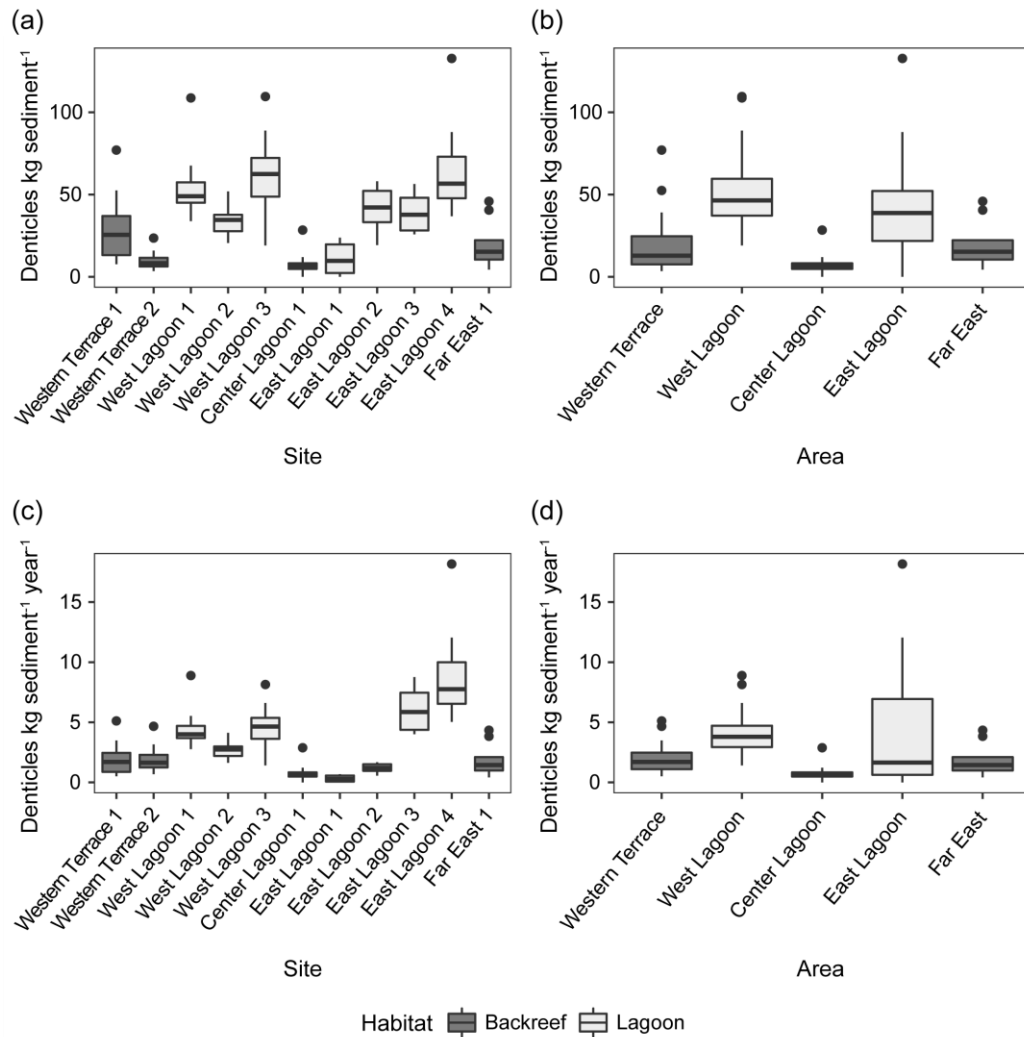
## **3.4 Results**

### **3.4.1 Spatial variation in the denticle record**

Denticle abundances ranged from 0 to 133 ( $M \pm SD$ ;  $34 \pm 26$ ) denticles  $\text{kg}^{-1}$  sediment across sites (Fig. 3.4 and Fig. B.1). Sedimentation rates differed across sites and areas of the atoll (Welch's ANOVA  $F_{9,20.9} = 11.74$ ,  $p < 0.0001$  and Welch's ANOVA  $F_{4,34.8} = 3.67$ ,  $p = 0.013$  respectively; Fig. B.4) and, without accounting for mixing or reworking of older

buried sediments, indicated that the samples spanned at least 15 y on average (Table 3.1). When denticle abundances were corrected by these sedimentation rates, denticle accumulation rates ranged from 0 to 18 ( $3.1 \pm 3.0$ ) denticles  $\text{kg}^{-1}$  sediment  $\text{year}^{-1}$ . Denticle accumulation rates were generally higher in the lagoon ( $3.6 \pm 3.3$ ) than on the backreef ( $1.9 \pm 1.3$ ), with the highest rates found in the East and West Lagoons (Welch's ANOVA  $F_{4,38.9} = 16.12$ ,  $p < 0.0001$ ; Fig. 3.4). Denticle accumulation rates varied little across sites in the West Lagoon ( $4.0 \pm 1.8$ ) but were more variable across sites in the East Lagoon ( $4.0 \pm 4.2$ ). The Center Lagoon ( $0.8 \pm 0.8$ ) had the lowest denticle accumulation rates (Fig. 3.4). Neither denticle abundances nor denticle accumulation rates were correlated with sorting (Spearman  $r = 0.32$ ,  $p = 0.3$  and Spearman  $r = 0.24$ ,  $p = 0.5$  respectively; Fig. B.5) or mean grain size (Kruskal–Wallis  $H_2 = 2.91$ ,  $p = 0.2$  and Kruskal–Wallis  $H_2 = 1.49$ ,  $p = 0.5$  respectively), which served as proxies for depositional environment.

In the randomized resampling exercise, the difference between the total cumulative site mean and the cumulative mean after adding each replicate in a randomized order leveled off at five to seven replicates, depending on the variance in denticle abundance (Fig. B.2). With five to seven replicates, there was a 5–9 denticles  $\text{kg}^{-1}$  sediment maximum difference from the global cumulative mean at each site, which fell around each site's standard deviation (6–29 denticles  $\text{kg}^{-1}$  sediment). When comparing denticle accumulation rates across sites, analyzing all 10 replicates per site yielded a power of 0.7, whereas analyzing five to seven replicates per site yielded a power of 0.3–0.5. Thus, at least seven replicates should be collected per site to capture the spatial variability in the denticle record at Palmyra Atoll, and at least 10 replicates should be collected to detect differences across sites.



**Figure 3.4** Boxplot of denticle abundances across (a) sites and (b) areas of the atoll and denticle accumulation rates across (c) sites and (d) areas of the atoll, colored by habitat. Each boxplot shows the median (dark bar) and interquartile range (box), and outliers are displayed as points. In each plot, at least one group differed from the others ( $p < 0.0001$ ).

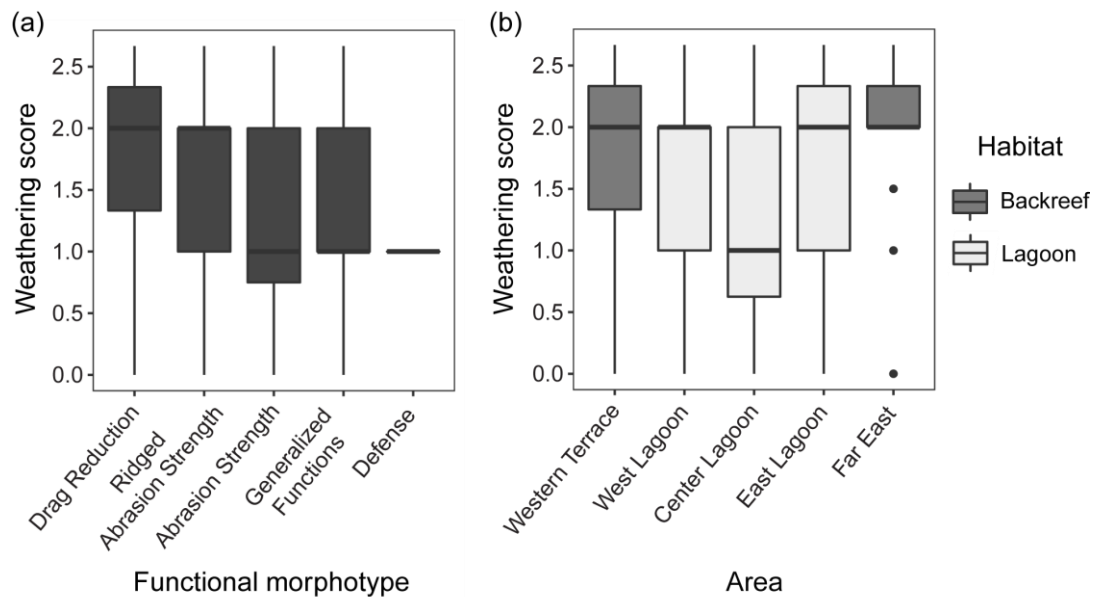
### 3.4.2 Denticle preservation

Denticles were preserved at all sites and were only moderately weathered (median weathering score  $\pm$  median absolute deviation:  $2 \pm 0.7$ ). Although denticle bases were often missing, the crowns were usually intact, permitting measurement and classification.

Weathering scores varied across functional morphotypes and denticle characters, although these differences were often inconsequential for classification. Drag reduction denticles

(which characterize *C. melanopterus* and *C. amblyrhynchos*) were more weathered than the other functional morphotypes (Kruskal–Wallis  $H_3 = 41.84$ ,  $p < 0.0001$ ; Dunn's test  $p < 0.01$ ; Fig. 3.5A). Similarly, thin denticles (Mann–Whitney  $U = 21574$ ,  $p < 0.0001$ ) and those with ridges (Mann–Whitney  $U = 8882.5$ ,  $p < 0.0001$ ) were more weathered than thicker denticles without ridges. Weathering scores were not affected by other denticle characters, such as crown size (Kruskal–Wallis  $H_8 = 10.52$ ,  $p = 0.2$ ) or the number of peaks (Mann–Whitney  $U = 27176$ ,  $p = 0.1$ ).

Denticles were better preserved in the lagoon than on the backreef (Mann–Whitney  $U = 22881$ ,  $p = 0.008$ ; Fig. 3.5B). Weathering scores were not correlated with sorting (Spearman  $r = -0.05$ ,  $p = 0.9$ ), mean grain size (Kruskal–Wallis  $H_2 = 2.39$ ,  $p = 0.3$ ) or denticle abundance (Spearman  $r = 0.1$ ,  $p = 0.7$ ), suggesting that low denticle abundances were not purely due to poor preservation.



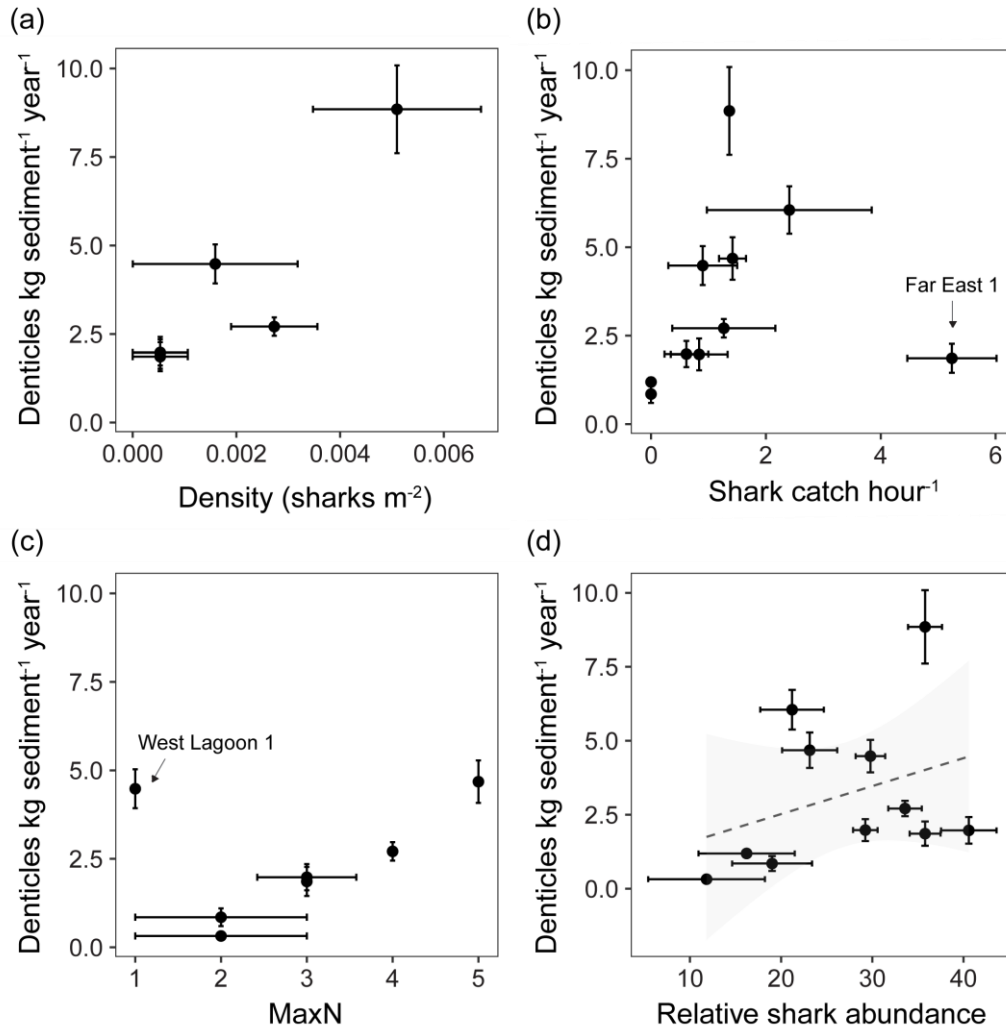
**Figure 3.5** Denticle weathering scores by (a) functional morphotype and (b) area of the atoll, colored by habitat. Weathering scores ranged from zero (pristine) to three (poorly preserved). Each boxplot shows the median (dark bar) and interquartile range (box), and outliers are displayed as points. Preservation was lowest in drag reduction denticles and denticles recovered from the backreef.

### 3.4.3 Concordance between the denticle record and conventional shark surveys

The denticle record was well-aligned with the three conventional survey methods at all sites except two (Fig. 3.6 and Fig. B.6). There was a significant positive correlation between denticle accumulation rates and shark abundances measured by UVC surveys (Spearman  $r = 0.88$ ,  $p = 0.031$  adjusted; Fig. 3.6A). Denticle accumulation rates and hook and line surveys were positively correlated at all sites except Far East 1 (Fig. 3.3), which had a low denticle accumulation rate but high shark catch per hour (Spearman  $r = 0.91$ ,  $p = 0.0009$  adjusted; Fig. 3.6B). Denticle accumulation rates and BRUV surveys were positively correlated at all sites except West Lagoon 1 (Fig. 3.3), which had a high denticle accumulation rate but low MaxN value (Spearman  $r = 0.97$ ,  $p = 0.002$  adjusted; Fig. 3.6C). These correlations were largely robust to variation in sedimentation rate, as over half the correlations remained statistically significant in the sensitivity analysis (Table B.9). Furthermore, denticle abundances were positively correlated with UVC and BRUV surveys (Spearman  $r = 0.88$ ,  $p = 0.031$  adjusted and Spearman  $r = 0.85$ ,  $p = 0.046$  adjusted respectively; Fig. B.7A,C) but not with hook and line surveys (Spearman  $r = 0.58$ ,  $p = 0.16$  adjusted; Fig. B.7B). Finally, there were non-significant correlations between the three conventional survey methods ( $p > 0.05$  for each pairwise comparison; Fig. B.6).

The  $N$ -mixture model demonstrated that the detection probability of each survey method increased with survey effort. When effort was held constant at one hour, detection probability was highest for UVC (0.56), followed by hook and line (0.42) and BRUV surveys (0.37). However, because survey effort varied across methods, hook and line and BRUV surveys had higher actual detection probabilities due to their longer durations. Survey year was associated with shark abundance in one of the top-ranked models, which likely indicated temporal variability, although a limited number of environmental parameters

were included in the model due to data availability (Table B.7). After accounting for these differences in detection, both relative shark abundance and habitat were decent predictors of denticle accumulation rate ( $p = 0.037$  and  $p = 0.041$  respectively;  $R^2 = 0.36$ ; Fig. 3.6D) and denticle abundance ( $p = 0.04$  and  $p = 0.019$  respectively;  $R^2 = 0.43$ ) in the multiple regression models (Table B.10).



**Figure 3.6** Correlation between denticle accumulation rates (denticles kg<sup>-1</sup> sediment year<sup>-1</sup>) and (a) shark density derived from UVC surveys, (b) shark catch per hour derived from hook and line surveys, (c) MaxN derived from BRUV surveys, and (d) relative shark abundance integrated across methods (dashed regression line with 95% confidence intervals). Points represent  $M \pm SE$  at each site with corresponding data.



#### 3.4.4 Denticle assemblage composition

The subset of denticles that was identified to functional morphotype was comprised primarily of drag reduction denticles (69.0%), followed by ridged abrasion strength (12.8%), abrasion strength (8.1%), generalized functions (7.8%) and defense denticles (0.2%). Around 2% remained unclassified. One defense denticle closely resembled a diagnostic tiger shark *Galeocerdo cuvier* denticle (Muñoz-Chápuli 1985, Dillon et al. 2017) from our reference collection (Fig. B.8). The relative abundance of each functional morphotype did not differ across sites ( $p > 0.05$  for all morphotypes). Notably, denticle assemblage composition overlapped with the functional morphotypes possessed by the two species detected by the conventional surveys. Most of the body and fins of *C. melanopterus* and *C. amblyrhynchos* in our reference collection were characterized by drag reduction denticles (75.0% of denticles examined; Fig. 3.1), although ridged abrasion strength (14.3%), abrasion strength (9.7%) and generalized functions (1.0%) denticles were also present. Overall, the rank abundance of functional morphotypes recovered from the sediments resembled that of the commonly observed species at the atoll.

### 3.5 Discussion

#### 3.5.1 Denticle accumulation rates reflect relative shark abundances at Palmyra Atoll

Denticle accumulation rates were, in almost all instances, highly correlated with shark abundances detected by UVC, BRUV and hook and line surveys at Palmyra Atoll. This is likely because sites with more sharks accumulated more denticles. Moreover, patterns of residency and movement shape the distribution of shark abundance and could influence denticle accumulation. For example, denticle accumulation rates were highest in the East and West Lagoons, where *C. melanopterus* shows strong site fidelity and small home ranges

(Papastamatiou et al. 2009b, 2010). In contrast, *C. melanopterus* occasionally transits through the backreef and Center Lagoon (Papastamatiou et al. 2009a, 2010, 2017), where shark abundances and denticle accumulation rates were lower. The denticle record therefore could be recording long-term spatial variation in the abundance and habitat use of this species.

The denticle record did not align with all the individual surveys—namely the hook and line survey at Far East 1 and the BRUV survey at West Lagoon 1. Far East 1 is a shallow backreef site that can become isolated from the adjacent forereef at low tide (Rogers et al. 2017), so shark catch per hour could have been abnormally high due to the presence of sharks from the neighboring forereef that were attracted by the bait plume at high tide or, alternatively, sharks that were trapped at low tide. In the lagoon, *C. melanopterus* uses the sand-flats in a tidally dependent manner, and larger individuals patrol the sand-flat ledges (Papastamatiou et al. 2009b). The BRUV deployment at West Lagoon 1 faced the sand-flat rather than the ledge, so it might not have been well-positioned to detect sharks in the vicinity. Although bait was used, the small quantity of bait and relatively low water movement in the lagoon (Rogers et al. 2017) could have limited the area of attraction. Therefore, whereas these short surveys might have captured ephemeral, behaviorally driven fluctuations in shark abundance, the denticle record likely recorded longer-term averages.

There was no significant correlation between the three conventional survey methods, matching previous observations of imperfect correspondence (Ward-Paige et al. 2010, McCauley et al. 2012b, Bradley et al. 2017a). They took different approaches to measuring shark abundance, had varying detection probabilities and sampling effort, and were conducted in different years, all of which likely contributed to their lack of agreement (Fig. 3.2). Regardless, relative shark abundance was positively correlated with denticle

accumulation rate, even when we accounted for imperfect shark detection probability and estimated population size using an  $N$ -mixture model. This suggests that the shark abundance gradient across sites, which was reflected in the denticle record, did not purely stem from each method's observational biases.

### 3.5.2 Denticle assemblage composition captures a signal of shark functional diversity

Most isolated denticles cannot be identified to species, in contrast to the high taxonomic resolution of conventional shark surveys. Regardless, we found that the relative proportions of functional morphotypes recovered from sediments were similar to those characterizing the two species detected by the conventional surveys. In contrast, the tiger shark denticle was an unexpected find, considering that this species has only been anecdotally reported at Palmyra Atoll (J. D. Collen, pers. comm.). The denticle record might therefore be capturing a larger proportion of shark functional diversity than the conventional surveys used in this study, perhaps due to the longer timescales over which denticles accumulate (Fig. 3.2).

### 3.5.3 Applying the denticle record to survey sharks

In low-energy coral reef environments, the denticle record can preserve a time-averaged signal of relative shark abundance. Here, we provide several suggestions for using the denticle record to explore shark communities in other regions.

#### **3.5.3.1 Site selection**

To improve the fidelity of denticle assemblages, sampling should be constrained to low-energy habitats such as lagoons, bays or backreefs. Under these conditions, denticles are likely to sink quickly after being shed, given that their primary constituent (hydroxyapatite) is ~3.1 times denser than seawater (Helms & Riedel 1971), and are less likely to be size-sorted. Under high-energy conditions, denticles are more likely to be sorted, selectively lost

or damaged, resulting in mismatch between denticle accumulation and shark abundance or skewing denticle assemblage composition toward thicker, more durable morphotypes. In Palmyra Atoll's lagoon, denticles were well-preserved, consistent with results from mid-Holocene lagoonal reefs (Dillon et al. 2017) and deep-sea cores (Sibert & Norris 2015). Denticles found in the backreef tended to be more weathered and might have experienced selective loss or sorting. Two samples from the forereef not used in this study contained few denticles despite sharks being abundant in this habitat, and those denticles were in poor condition, strongly suggesting the avoidance of such high-energy sites.

### **3.5.3.2 Establishing temporal context**

Temporal context is needed to infer shark abundance from denticle abundance. While sedimentation and reef accretion rates determine the minimum amount of time encompassed by a sample, biological (e.g. bioturbation, deposit feeding) and physical (e.g. wave energy) processes can mix sediments (Flessa et al. 1993, Olszewski 2004, Kosnik et al. 2015). This mixing is a key process affecting death assemblage composition, as it blends new inputs from the living community with older buried cohorts (Tomašových & Kidwell 2011, Kidwell 2013, Tomašových et al. 2019a). For this reason, the amount of time-averaging should be resolved and, depending on the study aims, constrained.

Examining changes in the denticle record over human timescales requires high-resolution samples (e.g. sediment cores). One way to constrain time-averaging is to collect samples from branching coral framework, which forms an interlocking matrix that restricts the vertical movement of sediment particles trapped within. Reef accretion rates can be measured by dating pieces of coral (Cramer et al. 2017) or smaller particles like otoliths (Lin et al. 2019) and used to calculate denticle accumulation rates and estimate sample age.

Although denticles accumulating in moderately bioturbated sediments cannot reveal fine-scale historical trends, they can help describe spatial patterns of abundance and indicate recent change. The bulk samples used in this study were collected from well-oxygenated sediments with little to no branching coral framework, which can increase time-averaging (Kowalewski 1996, Kosnik et al. 2015), leaving us unable to interpret changes in shark abundance over time. However, shark populations at Palmyra Atoll have remained relatively stable over at least the last decade (Bradley et al. 2017a), and, with the exception of a brief period of human occupation during World War II, there has not been a strong human presence at the atoll (Collen et al. 2009). Given this low anthropogenic pressure, Palmyra Atoll is an ideal location to reveal taphonomic patterns in the denticle record. In contrast, areas that have experienced rapid and recent shark declines are likely to show mismatch between time-averaged denticle assemblages and contemporary surveys (c.f. Roff et al. 2013, Albano et al. 2016, Tomašových & Kidwell 2017).

Quantifying the amount of time encompassed by bioturbated sediments can be challenging and time intensive. Here, we used sedimentation rates to calculate denticle accumulation rates, as methods to date denticles and build denticle-specific time-averaging models have not yet been developed. However, using sedimentation rates has several drawbacks. First, we expect the samples to be more time-averaged than indicated by sedimentation rates alone due to the presence of bioturbating fauna (Olszewski 2004, Kosnik et al. 2015, Tomašových et al. 2019b). Moreover, bioturbation depths were not known, so time-averaging could have differed across sites. Second, sedimentation was measured over three years, which might not scale linearly over decades (Sadler 1981). However, sedimentation rates measured from a gravity core collected in the West Lagoon, spanning a period from 1840 to 2003 (Collen et al. 2011), corresponded with the rates used in this

study. Third, resuspension could have artificially inflated sedimentation rates. To reduce resuspension, we used sediment traps with a diameter-to-length ratio of 10, well in excess of the recommended ratio of three to five for low-energy habitats (Bloesch & Burns 1980). Finally, given that denticle accumulation rates can be sensitive to the accuracy of the timescale used to calculate them (Sibert et al. 2017), we ran sensitivity analyses to ensure that correlations between denticle accumulation rates and shark abundances were robust to variation in sedimentation.

### **3.5.3.3 Sample volume and replication**

Denticles comprise a small fraction of coral reef sediments, so large volumes are needed to obtain enough denticles for robust statistical analyses. The minimum per-sample volume should aim to recover at least ~30 denticles when estimating total abundance (Sibert et al. 2017) and likely more when examining community composition (Heck et al. 1975), depending on the standing diversity and taxonomic identification attainable. Sample volumes can be determined with preliminary sampling or estimated using information about reef accretion rates and contemporary shark abundance. For example, if shark abundance is high and/or reef accretion is low, <1–3 kg replicates should be sufficient, whereas if shark abundance is low and/or reef accretion is high, 10 kg replicates might be needed.

### **3.5.3.4 Implications for fully buried denticle assemblages**

Reconstructing shark abundances over millennia requires sampling the fossil record using sediment cores or fossil reefs. Although the differential loss of taxa or diagnostic characters during burial can hinder interpretations of the fossil record (Kidwell 2013), ichthyoliths have relatively little preservation bias (Helms & Riedel 1971, Doyle & Riedel 1979; see Section 3.5.3.1), supporting the ability of denticle assemblages in surface

sediments to be reasonable proxies for permanently buried assemblages. Nonetheless, quantifying down-core trends in denticle weathering and radiometrically dating denticles might help verify whether the preservation and age-frequency distribution (temporal resolution) of denticle assemblages changes during burial.

### **3.5.3.5 Future work and limitations**

Several uncertainties remain about the relationship between denticle assemblages and shark communities. First, little is known about denticle shedding rates and whether they differ between demersal and pelagic species or as a function of shark age, body size, behavior or denticle morphology, which could cause some species to be over- or under-represented in the denticle record. Second, models should be built to test how water velocity and denticle morphology affect denticle sinking rates and horizontal transport. Third, variability in denticle morphology across sharks' bodies and between taxa currently limits identification to functional group and shark family (Reif, 1985, Dillon et al. 2017). Machine learning techniques could help identify denticles to genus or species, improving comparisons between denticle assemblage composition and diversity metrics derived from conventional shark surveys. Finally, although we found correlations between denticle accumulation rates and shark abundances at the scale of several kilometers at Palmyra Atoll, additional work is needed to resolve whether this association holds across islands with different shark densities and community compositions.

### **3.6 Conclusions**

As coral reefs accrete, they preserve a record of the abundances and composition of a variety of taxa, including corals, urchins and fish (Pandolfi & Jackson 2006, Cramer et al. 2017, Lin et al. 2019). Our results add sharks to this list. The correspondence between

denticle accumulation rates and shark abundances supports the use of the denticle record as a proxy for estimating relative shark abundance in low-energy coral reef environments. For example, mismatch between denticle assemblages in surface sediments and contemporary surveys can be used to demonstrate recent change. Furthermore, although these surface assemblages receive input from the contemporary shark community and might undergo additional taphonomic processes during burial (Behrensmeyer et al. 2000), testing their fidelity can aid the interpretation of deeper palaeoecological records (Kidwell 2009). Denticles recovered from fossil reefs present a valuable opportunity to define historical ranges of variation (O'Dea et al. 2017) and reconstruct patterns of change over millennia. Such long-term data can help determine baseline shark abundance, understand natural variation, interpret sharks' roles in natural and human-impacted systems and set management targets informed by local historical conditions.

**Data Availability.** Data are available at the Dryad Data Repository:  
<https://doi.org/10.25349/D9CP4C> (Dillon et al. 2020).

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Raredon and collection manager J. Williams, for facilitating access to their ichthyology collections. Finally, we thank Lee Hsiang Liow and four anonymous reviewers for their insightful comments. This research was conducted under U.S. Fish and Wildlife special use permits (#12533-14011, #12533-13011, #12533-12011, #12533-11007, #12533-10011, #12533-09010, #12533-08011 and #12533-07006), and the methods were carried out in accordance with relevant guidelines and regulations stated in our Institutional Animal Care and Use Committee, University of California, Santa Barbara, Protocol no. 856. Study collaboration and funding were provided by the U.S. Department of the Interior, Bureau of Ocean Energy Management, Environmental Studies Program, Washington, DC under Agreement Number MP1400039 (K.D.L.). Any use of trade, product or firm names in this publication is for descriptive purposes only and does not imply endorsement by the U.S. Government. Additional funding was obtained from the Alfred P. Sloan Foundation (D.J.M.), the Swerdlow Family (D.J.M.), the Worster Family (E.M.D.), the Sistema Nacional de Investigadores of SENACYT (A.O.), the Marisla Foundation (J.E.C.) and the Victoria University of Wellington, New Zealand (J.P.A.G.). E.M.D. is supported by a UC Santa Barbara Chancellor's Fellowship. This is contribution PARC-155 of the Palmyra Atoll Research Consortium. The authors declare no competing financial interests.

**Author Contributions.** E.M.D., A.O., K.D.L., D.J.M. and R.D.N. designed the study and methodology; E.M.D. and K.D.L. collected the sediment samples; J.E.C., D.J.M., D.B. and J.P.A.G. contributed data; E.M.D. processed the samples and analyzed the data; G.V.D. assisted with the analysis; E.M.D. and A.O. wrote the manuscript. All authors contributed to drafts and gave final approval for publication.

# CHAPTER 4: FOSSIL DERMAL DENTICLES REVEAL THE PRE-EXPLOITATION BASELINE OF A CARIBBEAN CORAL REEF SHARK COMMUNITY

## *4.1 Abstract*

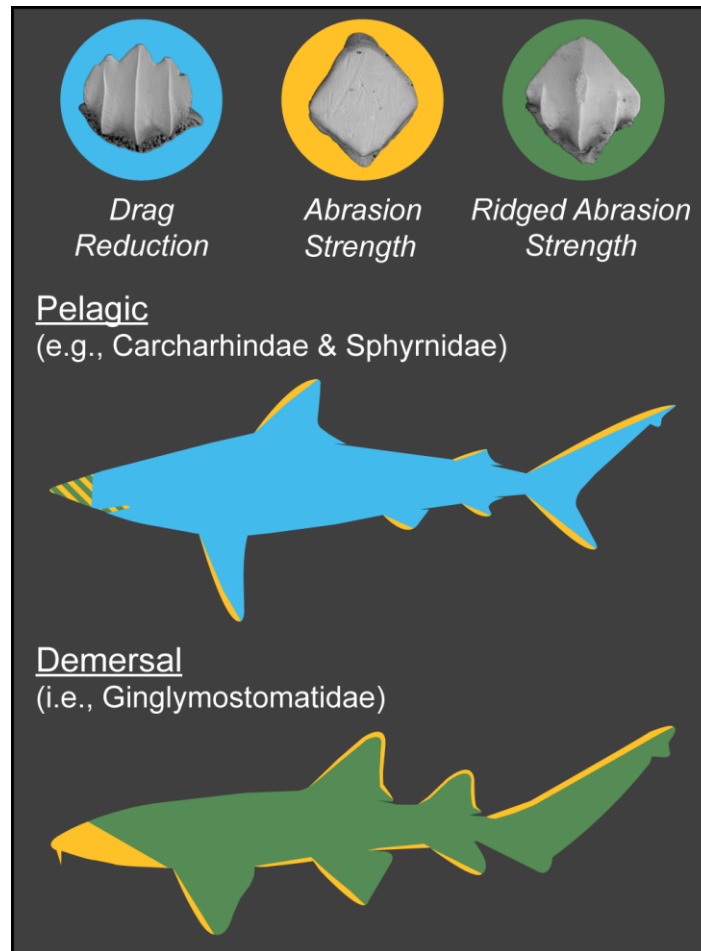
Pre-exploitation shark baselines and the history of human impact on coral reef-associated shark communities in the Caribbean are poorly understood. We recovered shark dermal denticles from mid-Holocene (~7 ky ago) and modern reef sediments in Bocas del Toro, Caribbean Panama, to reconstruct an empirical shark baseline before major human impact and to quantify how much the modern shark community in the region had shifted from this historical reference point. We found that denticle accumulation rates, a proxy for shark abundance, declined by 71% since the mid-Holocene. All denticle morphotypes, which reflect shark community composition, experienced significant losses, but those morphotypes found on fast-swimming, pelagic sharks (e.g. families Carcharhinidae and Sphyrnidae) declined the most. An analysis of historical records suggested that the steepest decline in shark abundance occurred in the late 20th century, coinciding with the advent of a targeted shark fishery in Panama. Although the disproportionate loss of denticles characterizing pelagic sharks was consistent with overfishing, the large reduction in denticles characterizing demersal species with low commercial value (i.e. the nurse shark *Ginglymostoma cirratum*) indicated that other stressors could have exacerbated these declines. We demonstrate that the denticle record can reveal changes in shark communities over long ecological timescales, helping to contextualize contemporary abundances and inform shark management and ecology.

## ***4.2 Introduction***

Historical accounts often depict remarkable numbers of sharks on Caribbean coral reefs (Jackson 1997, Jackson 2001, Ward-Paige et al. 2010), yet empirical evidence of past shark abundances is limited. Although declines in oceanic shark populations over the last century have been well documented (Baum et al. 2003, Baum & Myers 2004, Ferretti et al. 2010), much less is known about how humans have shaped reef-associated, coastal shark communities, especially over long time periods. Sharks on many Caribbean coral reefs could have experienced earlier and more intense exposure to human stressors than their offshore counterparts, owing to their greater proximity to human populations (Lotze et al. 2006, Ward-Paige et al. 2010), the antiquity of fishing (Jackson et al. 2001, Wing & Wing 2001, Hawkins et al. 2004), and the widespread degradation of reef ecosystems, which preceded systematic monitoring (Hughes 1994, Jackson 1997, Pandolfi et al. 2003, Cramer et al. 2020a). Without baseline data to document what has been lost, it is challenging to implement effective management practices and to understand sharks' natural functions as mobile predators on reefs (McClenachan et al. 2012, Roff et al. 2016).

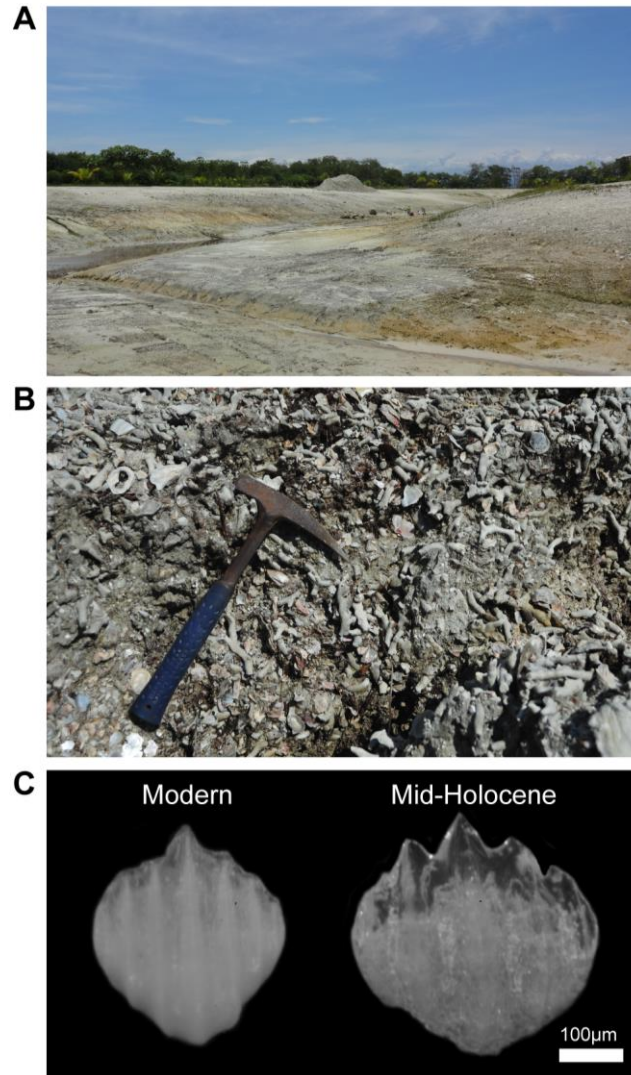
To examine how shark abundances have changed over long ecological timescales, we used dermal denticles—the microscopic (<2 mm) tooth-like scales that cover elasmobranchs' bodies—to reconstruct shark communities on a Caribbean coral reef before major human impact. Denticles are shed naturally and accumulate in marine sediments, where they preserve as fossils (Helms & Riedel 1971). Denticle accumulations reflect shark abundances in low-energy reef habitats (Dillon et al. 2020), and denticle morphology varies across sharks with different ecological modes, as it is coupled to denticle function (Reif 1985, Raschi & Tabit 1992, Dillon et al. 2017, Ferrón & Botella 2017) (Fig. 4.1). Because sharks have several orders of magnitude more denticles than teeth, denticles are far more

abundant in reef sediments, facilitating statistical analyses (Dillon et al. 2017, Ferrón & Botella 2017, Dillon et al. 2020). As such, denticle assemblages can yield rigorous ecological information about past shark communities.



**Figure 4.1** Drag reduction, abrasion strength, and ridged abrasion strength denticles (Inset, scanning electron microscope images) are the three most common functional morphotypes found on reef-associated sharks. Generalized functions and defense denticles (not shown) are less common. Fast-swimming, pelagic taxa, such as the families Carcharhinidae and Sphyrnidae (defined here as including both near-shore and oceanic species, following Ferrón & Botella 2017), are characterized by hydrodynamic drag reduction denticles with riblets that improve swimming performance (often covering >80 to 90% of their bodies), although abrasion strength and ridged abrasion strength denticles are found along the leading edges of the fins and around the snout. The cross-hatching denotes a combination of morphotypes. Demersal taxa, such as the family Ginglymostomatidae, possess both ridged abrasion strength and abrasion strength denticles (often covering ~60 and 40% of their bodies, respectively), which provide protection during contact with sandy, hard, or coral-rich substrates. The dominance of different functional morphotypes on pelagic and demersal sharks enables changes in their relative abundances to be ascertained from the denticle record. Squamation patterns (lateral view) are portrayed from museum specimens (Dillon et al. 2017, Ferrón & Botella 2017, Dillon et al. 2020), with a focus on species documented in Caribbean Panama.

We extracted denticles from a mid-Holocene fringing reef in Bocas del Toro, Panama, that formed  $\sim 7$  ka (Fredston-Hermann et al. 2013) (Fig. 4.2 and Fig. C.1), which predates the earliest evidence of human settlement in this region by several thousand years (Linares 1977, Ranere & Cooke 1991, Baldi 2011, Wake et al. 2013) yet represents a time when environmental conditions were similar to the modern day (O’Dea et al. 2020). We then compared this empirical baseline with denticle assemblages recovered from nearby modern reefs to quantify the magnitude of change between the mid-Holocene and modern time periods. To shed light on the timing and mechanisms driving shifts in shark abundance, we compiled and analyzed published archaeological, historical, ecological, and fisheries records, which offer insight into human interactions with sharks in the region during different cultural periods in Panama’s history. Taken together, our findings revealed that shark communities on these reefs in western Caribbean Panama not only experienced severe declines but had been functionally restructured since the mid-Holocene.

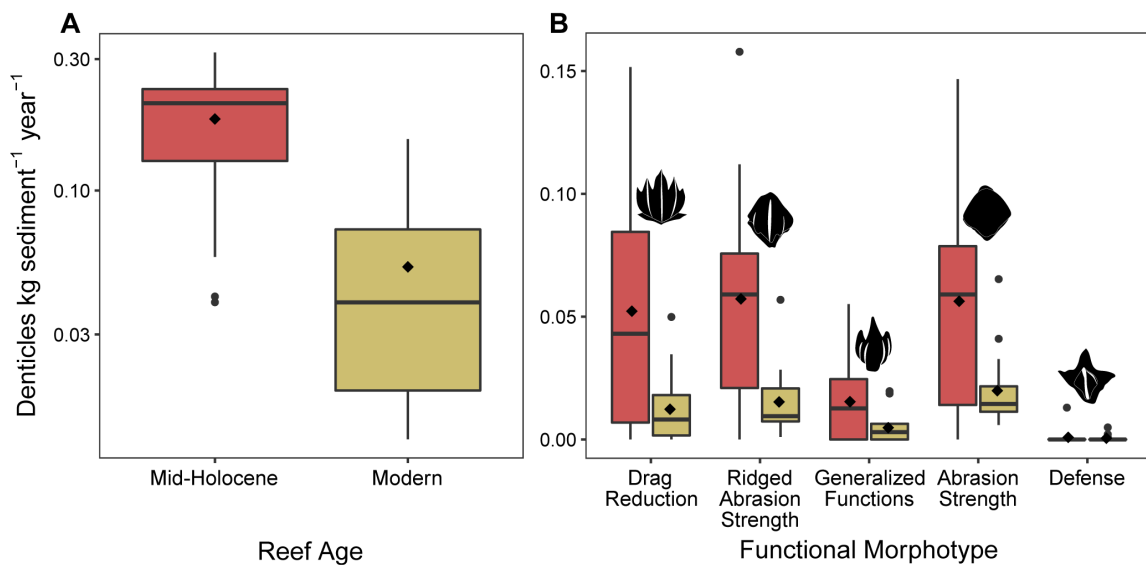


**Figure 4.2** Mid-Holocene coral reef in Almirante Bay, Bocas del Toro, Panama. (A) Samples were collected from a ~50 ha exposed area of this reef. (B) In situ and in life position branching coral framework and sediments were bulk sampled to access the denticle record. (C) Denticles recovered from the mid-Holocene and modern reefs were well preserved.

### 4.3 Results

Denticle accumulation rates (denticles accumulating per kilogram sediment per year)—a proxy for shark abundance—were 3.4 times higher on the mid-Holocene reef ( $n = 15$  sediment samples,  $n = 183$  denticles, and  $0.18 \pm 0.090$  [ $M \pm SD$ ]) than on the modern reefs ( $n = 16$  sediment samples,  $n = 389$  denticles, and  $0.053 \pm 0.042$ ), representing a 71% decline

(59 to 79% decline with jackknife sampling) in mean denticle accumulation between the two time periods ( $\chi^2 = 4.68$ ,  $p = 0.030$ ; Fig. 4.3A). This significant difference largely persisted when we accounted for uncertainty in the Uranium–Thorium dates used to establish the sample ages and calculate denticle accumulation rates (Tables C.1 and C.2). Denticle accumulation also varied between sites within each time period, with a 5.4-fold difference observed across the modern reef means and a 5.6-fold difference observed across the mid-Holocene reef means ( $\chi^2 = 33.88$ ,  $p < 0.001$ ; Figs. C.2–C.4).



**Figure 4.3** Change in denticle accumulation rates between the mid-Holocene (red) and modern (gold) time periods. (A) The mean denticle accumulation rate declined by 71% between the two time periods ( $p = 0.030$ ). (B) The mean accumulation rate of each morphotype also declined over time, ranging from a 76% decline in drag reduction denticles to a 42% decline in defense denticles ( $p < 0.05$ ). Morphotypes are ordered from left to right by the amount of decline. Mid-Holocene and modern accumulation rates were calculated from 15 sediment samples ( $n = 183$  denticles) and 16 sediment samples ( $n = 389$  denticles), respectively. Each boxplot shows the median (dark bar), mean (diamond), and interquartile range (box), and outliers are displayed as points. The vertical axis is  $\log_{10}$  transformed in A but not in B because of the presence of zeros. Denticle illustrations credit: Ashley Diedenhofen (artist).

To evaluate shifts in shark community composition over time, the denticles were classified into five previously recognized morphotypes (Reif 1985, Raschi & Tabit 1992, Dillon et al. 2017, Ferrón & Botella 2017), which have different functions and are associated with different ecological groups of sharks (Fig. 4.1). The accumulation rate of all five

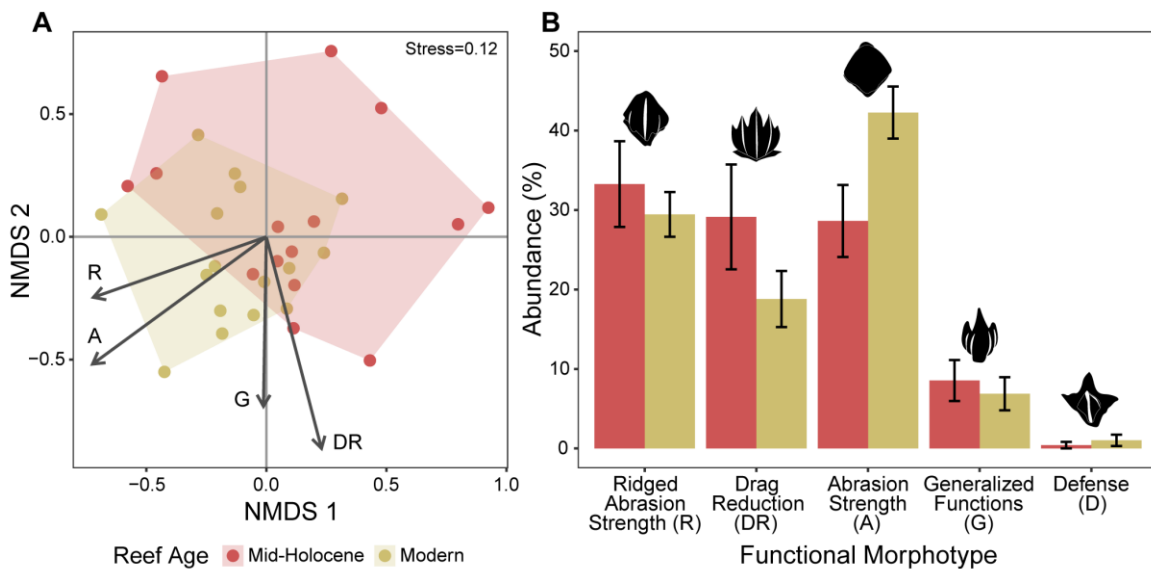
denticle functional morphotypes declined over time (Fig. 4.3B), mirroring the pattern in total denticle accumulation. Drag reduction denticles underwent the largest decline (76%;  $\chi^2 = 7.83$ ,  $p = 0.0051$ ), followed by ridged abrasion strength (73%;  $\chi^2 = 6.60$ ,  $p = 0.010$ ), generalized functions (69%;  $\chi^2 = 7.23$ ,  $p = 0.0072$ ), abrasion strength (65%;  $\chi^2 = 7.36$ ,  $p = 0.0067$ ), and defense denticles (42%;  $\chi^2 = 4.15$ ,  $p = 0.042$ ). Although the declines in drag reduction, abrasion strength, and ridged abrasion strength denticles differed by only 11%, together these shifts yielded a 45% decrease in the ratio of pelagic to demersal denticle accumulation rates between the mid-Holocene and modern time periods. Thus, despite these sweeping declines, the marginally greater decrease in the accumulation of drag reduction denticles relative to abrasion strength and ridged abrasion strength denticles suggested a proportionally larger reduction in denticles characteristic of fast-swimming, pelagic sharks (75% decline)—defined here as including both near-shore and oceanic species—as compared to denticles characteristic of demersal sharks (69% decline).

Differences in the absolute magnitude of decline across each denticle morphotype, although ostensibly subtle, were enough to alter the functional composition of denticle assemblages over time. Drag reduction, abrasion strength, and ridged abrasion strength morphotypes dominated both the mid-Holocene and modern denticle assemblages (>90%), yet the assemblage composition shifted significantly between the two time periods (permutational multivariate analysis of variance [PERMANOVA]  $F = 3.24$ ,  $p = 0.024$ ; Fig. 4.4A), even after accounting for differences across sites (PERMANOVA  $F = 2.34$ ,  $p = 0.006$ ; Fig. C.5).

Abrasion strength denticles were proportionally more abundant and drag reduction denticles were proportionally less abundant in the modern samples relative to the mid-Holocene samples (Fig. 4.4B). In contrast, the proportion of ridged abrasion strength



denticles remained similar over time (Fig. 4.4B). Furthermore, the relative abundances of abrasion strength and ridged abrasion strength denticles shed from pelagic sharks, which cover only a small proportion of their bodies (Fig. 4.1), were consistently low in both time periods (Fig. C.6). Consequently, although there was overlap in the functional morphospace that encompassed the modern and historical ranges of variability (Fig. 4.4A), the modern denticle assemblage reflected the persistence of demersal sharks.



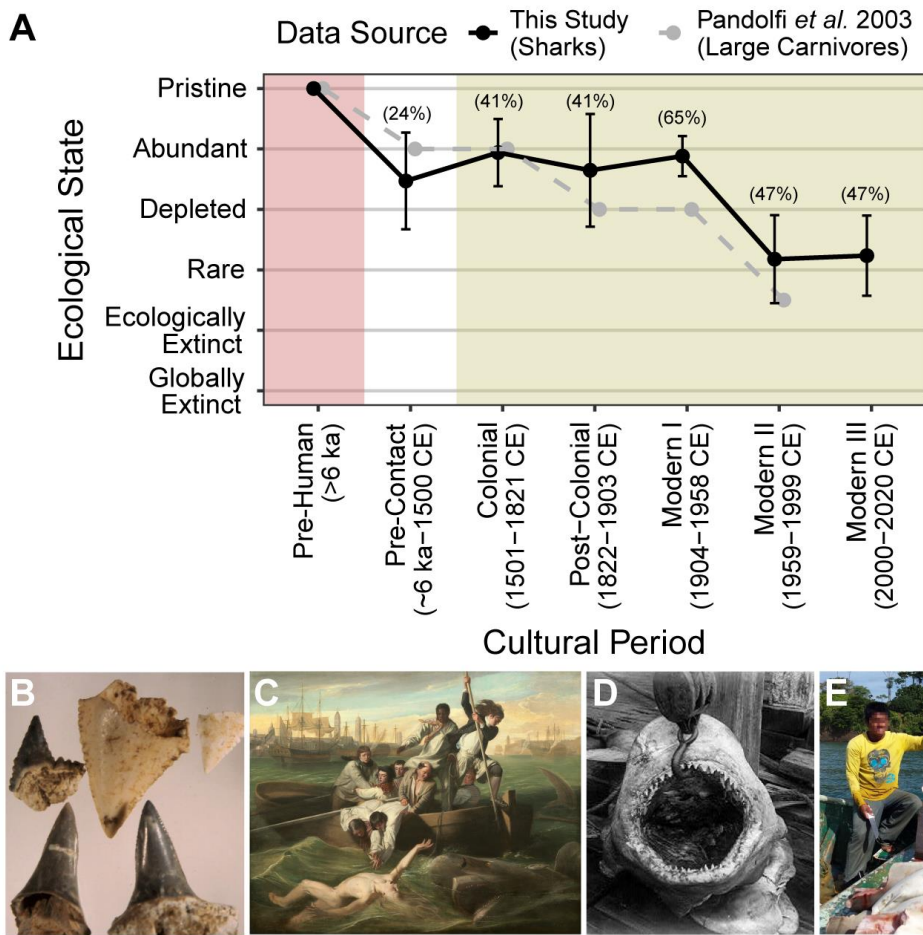
**Figure 4.4** The mid-Holocene (red) denticle assemblage ( $n = 183$ ) was compositionally different from the modern (gold) assemblage ( $n = 389$ ). (A) Nonmetric multidimensional scaling ordination depicts the assemblages in functional morphospace, with each point representing a sediment sample within the shaded convex hull. Although there was overlap between the mid-Holocene and modern assemblages, the centroid of the modern assemblage shifted ( $p = 0.024$ ), corresponding with a higher proportion of denticles characteristic of demersal sharks. (B) The relative abundances of ridged abrasion strength, drag reduction, and abrasion strength denticles were similar in the mid-Holocene assemblage. In contrast, drag reduction denticles were proportionally less common, and abrasion strength denticles were proportionally more common in the modern assemblage. Error bars indicate the SE around each mean.

Because denticle accumulation is driven by shark abundance (Dillon et al. 2020), the higher denticle abundances discovered on the mid-Holocene reef indicated that sharks might have been over three times more numerous in the region historically. Alternatively, this pattern could have resulted from the presence of larger sharks, which possess more denticles

and, accordingly, might contribute more to the denticle record. To investigate this counter hypothesis, we compared patterns of denticle size over time, as denticle crowns scale allometrically with shark length within species (Raschi & Musick 1986). The size–frequency distribution of all denticles combined was not significantly different between the mid-Holocene and modern time periods (Kolmogorov–Smirnov  $D = 0.11$ ,  $p = 0.13$ ), although the median denticle crown size was marginally larger in the modern samples (Wilcoxon  $W = 29500$ ,  $p = 0.021$ ). This was likely due to the higher representation of abrasion strength and ridged abrasion strength denticles, which are typically larger than the other morphotypes. When disaggregated by ecological mode, median denticle crown size and size–frequency distributions were not significantly different over time for both demersal (Wilcoxon  $W = 11889$ ,  $p = 0.44$ ; Kolmogorov–Smirnov  $D = 0.12$ ,  $p = 0.25$ ) and pelagic (Wilcoxon  $W = 2481$ ,  $p = 0.17$ ; Kolmogorov–Smirnov  $D = 0.15$ ,  $p = 0.41$ ) sharks (Fig. C.7 and Table C.3), suggesting that, in aggregate, shark sizes remained similar between the two time periods. However, because the allometric relationship between denticle size and shark length varies among species (Raschi & Musick 1986), changes in individual species’ size structures could have been obscured by the functional-level resolution of the denticle classifications used here.

To determine whether taphonomic processes might have affected our interpretation of the denticle record, we examined patterns of denticle weathering and relationships between denticle abundance and sediment characteristics. The denticles recovered were, on the whole, well preserved (Fig. 4.2C), and the assemblages reinforced previous findings that macro- and microskeletal remains on these reefs represent time-averaged, autochthonous accumulations of foraminifera (Gudnitz et al. 2021), fish (Lin et al. 2019), molluscs (Fredston-Hermann et al. 2013, O’Dea et al. 2014), and corals (O’Dea et al. 2020) in three

notable ways. First, denticle abundances were not correlated with reef accretion rates (Spearman  $r = 0.52$ ,  $p = 0.19$ ), the weight of coral in each sample (Spearman  $r = -0.27$ ,  $p = 0.14$ ), or sorting estimates (Spearman  $r = -0.05$ ,  $p = 0.79$ ), suggesting that denticle deposition was independent from reef growth and sediment production and that denticles had not been preferentially swept away by water movement. Second, the denticle assemblages were dominated by functional morphotypes characteristic of species in the families Ginglymostomatidae, Carcharhinidae, and Sphyrnidae (Dillon et al. 2017), consistent with the shark communities typically observed on Caribbean reefs (Robertson & Van Tassell 2019). Third, median denticle weathering scores were similar across time periods (Wilcoxon  $W = 38391$ ,  $p = 0.59$ ; Fig. C.8A) and sites (Kruskal–Wallis  $H_7 = 3.88$ ,  $p = 0.79$ ; Figs. C.8B and C.9), suggesting that the mid-Holocene assemblage had not undergone greater taphonomic alteration than its modern counterpart. There was also no correlation between weathering scores and denticle abundances (Spearman  $r = 0.08$ ,  $p = 0.65$ ; Fig. C.10), implying no selective dissolution or winnowing. Drag reduction denticles were more weathered than the other morphotypes (Kruskal–Wallis  $H_5 = 95.28$ ,  $p < 0.001$ , Dunn’s test  $p < 0.05$ ; Fig. C.8C), although this difference was consistent between time periods (Fig. C.8D). Therefore, selective preservation or sorting likely did not affect the observed patterns of denticle abundance or assemblage composition.



**Figure 4.5** Historical ecology of sharks in Caribbean Panama. (A) The ecological state of sharks was inferred from 91 published records and revealed an apparent decline in shark abundance since humans arrived in the region. The steepest decline occurred after the mid-20th century when sharks became described as rare. This pattern closely tracks an assessment of all large marine carnivores in the region using the same evaluation criteria (gray dotted line) (Pandolfi et al. 2003). The points and error bars show the mean and SD of ecological state scores across respondents ( $n = 17$ ), and the percentages indicate the prevalence of self-reported “high confidence” responses (44% across all cultural periods). The shading indicates the maximum time span of the modern (gold) and mid-Holocene (red) denticle record (the mid-Holocene record extends beyond the lower limit of the horizontal axis). Cultural periods are defined in Table C.6, and the ecological state of sharks in the prehuman cultural period was assigned to be pristine (following Pandolfi et al. 2003). Human interactions with sharks are also depicted in imagery from across the Caribbean: shark teeth recovered from a midden in the settlement at Black Creek, Costa Rica (4000 to 2500 B.P.) (B); shark attack rescue in Havana, Cuba (1778 CE) (C); shark caught in the Panama Canal Zone (1910 CE) (D); and shark fishing in Bocas del Toro, Panama (2015 CE) (E). Images credits: Norberto Francisco Baldi Salas (photographer)/National Gallery of Art, Washington/John Singleton Copley/Library of Congress, Prints & Photographs Division, LC-USZ62-98280.

To independently reconstruct the ecological history of sharks in the region and constrain when shark abundance declined between the mid-Holocene and modern time periods represented by the time-averaged denticle assemblages, we analyzed published archaeological, historical, ecological, and fisheries data (Tables C.4 and C.5). These records ( $n = 91$ ) were divided into seven cultural periods in Caribbean Panama's history (Table C.6) and were blindly reviewed ( $n = 17$  reviewers) to assign a semiquantitative ecological state to each cultural period based on perceived shark abundance (Table C.7). Shifts in the ecological state of sharks in Caribbean Panama over the last several millennia corroborated the declines in denticle accumulation rates that we report here. Furthermore, these records suggested that the most precipitous decline occurred in the late 20th century, when perceived shark abundance dropped from abundant to rare (Fig. 4.5 and Fig. C.11). The ecological state of sharks subsequently remained rare throughout the most recent cultural period.

#### ***4.4 Discussion***

Caribbean coral reefs and their associated shark communities suffer from the shifting baseline syndrome, as substantial human impacts were manifest long before monitoring began (Jackson 1997, Jackson et al. 2001, Aronson et al. 2004, McClenachan et al. 2006, McClenachan & Cooper 2008, Fredston-Hermann et al. 2013, O'Dea et al. 2014, Cramer et al. 2017, Łukowiak et al. 2018, Cramer et al. 2020a, Cramer et al. 2020b). Reef sharks have been depleted in many regions (Ferretti et al. 2010, MacNeil et al. 2020) including the Caribbean (Ward-Paige et al. 2010), yet we do not know what shark carrying capacities were on Caribbean reefs before people began fishing and altering the landscape, thus hindering efforts to set management targets informed by local expected conditions. It is also unclear whether shark communities were compositionally different in the past and how any

structural changes might have affected their ecological functions on reefs (Roff et al. 2016). Our approach, which leverages shark dermal denticles preserved in mid-Holocene and modern reef sediments, helps resolve this issue. As a first application, we used the denticle record here to gain insight into the state of shark communities before harvesting in one area of western Caribbean Panama.

We found that the overall denticle accumulation rate decreased by 71% since the mid-Holocene on reefs in Bocas del Toro, suggesting that sharks were over three times more abundant before humans began using marine resources in the region. All denticle functional morphotypes declined over time, indicating a loss of sharks with different ecological modes. However, the accumulation rate of denticles found on fast-swimming, pelagic taxa, such as near-shore and oceanic species in the families Carcharhinidae and Sphyrnidae (i.e. drag reduction denticles; Fig. 4.1), declined more than those characterizing demersal taxa, such as the nurse shark *Ginglymostoma cirratum* (i.e. ridged abrasion strength and abrasion strength denticles). The high representation of demersal sharks in the modern denticle assemblage mirrors survey data, which show that the shark community today in Bocas del Toro is dominated by nurse sharks (Chevis et al. 2020)—a finding which extends to many Caribbean reefs (Ward-Paige et al. 2010). Yet, by revealing that nurse sharks were relatively less common in the past, the denticle record suggests that their current dominance likely does not reflect the historical state of shark communities in the area.

The 71% decline in denticle accumulation rates between the mid-Holocene and modern time periods echoes postindustrial shark declines estimated using fishery-dependent and -independent data (e.g. Baum et al. 2003, Baum & Myers 2004, Ward & Myers 2005, Myers et al. 2007, Ferretti et al. 2010, Pacoureau et al. 2021) and space-for-time substitutions, which rely on surveys in protected areas to infer unfished shark biomass (e.g. Friedlander et

al. 2002, Robbins et al. 2006, Nadon et al. 2012, Valdivia et al. 2017). For example, longline data from the Gulf of Mexico showed declines of 45 to 99% in oceanic sharks between the 1950s and 1990s (Baum & Myers 2004 although see Burgess et al. 2005 for criticism), and reef shark densities across the central-western Pacific Ocean have declined by more than 90% from simulated baselines (Nadon et al. 2012). Likewise, a 71% decline in abundance since 1970 was reported across 18 oceanic shark and ray species globally using the Living Planet Index, including a 46% decline in the Atlantic Ocean (Pacoureau et al. 2021).

Although this congruence between methods builds confidence in the denticle record, time series data for sharks—particularly those inhabiting coastal habitats such as coral reefs—are temporally and geographically limited, and prehuman baselines are largely unknown (Lotze et al. 2006, Ferretti et al. 2010). In some instances, recent estimates of decline could easily underestimate the full magnitude of change from pre-exploitation baselines because significant losses could have occurred prior to the reference points used (e.g. Heithaus et al. 2007), given some species' vulnerability to even mild artisanal fishing pressure (Stevens et al. 2000, Stallings 2009, Ward-Paige et al. 2010). In other instances, such as in Bocas del Toro where this study was conducted, no long-term data exist as shark surveys have only recently been implemented (Chevis et al. 2020). Denticle-based reconstructions of reef shark communities can complement these ecological and fishery-based approaches by (1) documenting historical changes in shark abundance where data are sparse and (2) producing empirical baselines that can predate human impact and characterize natural variability. Our study illustrates this method's potential to access millennial-scale records of shark communities in other regions with different human histories, contemporary human impact, and oceanographic settings by sampling exposed fossil reefs and reef cores.

Our analysis of the perceived ecological state of sharks in Caribbean Panama based on historical records mirrored the decline in denticle accumulation and indicated that it might have occurred relatively recently. Archaeological evidence shows that shark harvesting began as early as 4000 to 2500 B.P. (Baldi 2011). Despite this prehistorical fishing, European explorers described sharks as numerous, and anecdotes of seas teeming with sharks continued into the early 20th century, contradicting their rarity in the region today (Table C.4). The absence of sharks was most apparent in these historical sources after the mid-20th century, corroborating trajectories of change reconstructed for all large marine carnivores in the region, including sharks, using a similar approach (Pandolfi et al. 2003) (Fig. 4.5). Marine carnivores in aggregate, however, experienced earlier and larger declines, which was anticipated given that this group included the heavily harvested loggerhead and hawksbill sea turtles (McClenachan et al. 2008) and the now extinct Caribbean monk seal (McClenachan & Cooper 2008). If taken at face value, these findings suggest that although sharks have been harvested in the region for millennia, intensive harvesting did not occur or did not have a significant impact on sharks until after more valuable resources had been depleted.

The pattern, timing, and rate of these declines implicate human activities. Overfishing, which is recognized as the primary threat to shark populations globally (Jackson et al. 2001, Ferretti et al. 2010, Dulvy et al. 2014), appears to be the most parsimonious explanation for the pronounced loss of pelagic sharks observed in this study. In Caribbean Panama, sharks first became described as rare in the cultural period spanning 1959 to 1999, coinciding with, although potentially preceding, the advent of a targeted shark fishery in the 1980s (Harper et al. 2014). Today, sharks continue to be caught and sold along Panama's Caribbean coast (Monzini 2004). Over a 13-mo-long fisheries survey in the city of Colón, all but one shark



landed were species with drag reduction denticles (Fig. C.12), indicating that modern day Panamanian fisheries selectively catch pelagic sharks. Similar selectivity is apparent in historical accounts (Meek & Hildebrand 1923) and prehistorical records from Caribbean Panama, with teeth and vertebrae belonging to sharks in the families Carcharhinidae and Sphyrnidae, but not Ginglymostomatidae, found in middens (Baldi 2011, Wake et al. 2013). Fishing mortality can therefore help explain the large selective loss of sharks with drag reduction denticles since the mid-Holocene in Bocas del Toro. However, we also observed a substantial reduction in the accumulation of abrasion strength and ridged abrasion strength denticles, which are primarily found on the nurse shark *G. cirratum* in our study region. Nurse sharks are rarely landed (Baldi 2011, Wake et al. 2013, Harper et al. 2014) (Fig. C.12), exhibit low at-vessel mortality (Ellis et al. 2017), and have little commercial value (Anglo-American Caribbean Commission 1945, Castro 2000), suggesting that fishing alone cannot explain their decline over time.

The large reduction in denticles belonging to shark taxa that are infrequently harvested highlights the additional contribution of indirect human pressures to shark declines in Caribbean Panama. The Bocas del Toro region has experienced major environmental and ecological changes since the beginning of the 20th century, stemming from agriculture, land clearing, and coastal development (Cramer 2013). Benthic habitats across the archipelago have undergone dramatic transformations, including shifts in the dominant reef builders as well as decreased coral cover, due to deteriorating water quality, disease, bleaching, deep water hypoxia, and hydrological change (Aronson et al. 2004, Fredston-Hermann et al. 2013, Cramer et al. 2020b, O’Dea et al. 2020, Figuerola et al. 2021)—a pattern of degradation documented across the greater Caribbean (Cramer et al. 2020a, Jackson et al. 2014). These anthropogenic disturbances, in turn, could have degraded habitat for both

sharks and their prey, in addition to lowering prey nutritional quality (de Sousa Rangel et al. 2021). This habitat loss was compounded by the intensification of fish and invertebrate harvesting in the 1970s (Harper et al. 2014), which likely reduced available prey for all sharks. In addition to human impacts, oceanographic variability (Nadon et al. 2012) or natural population fluctuations (Kessel et al. 2016) could have contributed to the changes observed in the denticle record. Continuous time series of denticle accumulation rates could refine the timing and pattern of shark decline between the two end members presented in this study and, when combined with coeval abiotic and biotic proxies, could help further disentangle the relative importance of these human and nonhuman drivers.

The size–frequency distribution of denticle crowns, which scale allometrically with shark length within species, was similar between the mid-Holocene and modern assemblages, suggesting that the observed decline in denticle accumulation did not result purely from changes in shark body size. This lack of change in denticle size structure deviates from well-substantiated declines in the mean length of exploited shark populations in the Caribbean and other regions (Stevens et al. 2000, Baum & Myers 2004, Heithaus et al. 2007, Myers et al. 2007) but should not be construed here as evidence that size shifts did not occur in Bocas del Toro. There are at least two reasons why changes in shark population size structure might not have been detected in the denticle assemblages sampled in this study. First, lagoonal areas within Bocas del Toro provide habitat for multiple species of juvenile sharks, as documented by the denticle record (Fig. C.7A) and modern observations (Gonzalez et al. 2019). As such, this record might provide a truncated view of the shark community size structure. Such a view is sufficient to measure shifts in relative abundance over time, but it would not provide adequate insight into shifts in the size structure of the whole shark community (e.g. if larger pelagic sharks that spent more time offshore than in

these shallow reef habitats were preferentially culled, their loss would not be recorded at these sites). Second, at present, we were unable to resolve these patterns at the species level, which would be needed to measure shifts in population size structure. Although we cannot conclusively reject the possibility that some species became smaller over time, a strong and systematic shift in shark length would likely be reflected in denticle size at the functional level due to the low species selectivity of fishing in the region. The absence of such a pattern within denticle morphotypes suggests that demographic changes were not a dominant mechanism driving their decline over time. Further work to increase the taxonomic resolution of denticle classifications and establish the allometric scaling relationship for each shark species in the region, in addition to conducting work in locations with only adult shark habitat, could help unlock the potential to use denticle assemblages to study the size structure of shark populations.

The decline in reef shark abundance, reflected by denticle assemblages in Bocas del Toro, parallels global losses across marine megafauna (Pimiento et al. 2020) and apex consumers (Estes et al. 2011), yet the ecological consequences are still being unraveled. The denticle record can contribute historical perspective to how shark declines might have affected ecosystem processes and can help test predictions rooted in ecological theory. Theory predicts that the threefold loss of meso and apex predators likely altered food web structure and stability through a decrease in predation and scavenging and a possible loss of functional redundancy (Bascompte et al. 2005, Rezende et al 2009, Frisch et al. 2016, Roff et al. 2016). The removal of predators might have also diminished nonconsumptive effects on prey behavior and foraging (Heithaus et al. 2008), nutrient cycling (Estes et al. 2011, Roff et al. 2016), and cross-ecosystem linkages (McCauley et al. 2012a). However, it is less clear if these declines drove cascading effects or if such effects were dampened by harvest

pressure exerted on both shark and teleost predators. Likewise, if we assume consistency in sharks' ecological functions through time, theory predicts that the shifts we documented in shark community composition likely altered predation pressure, given the trophic differences between pelagic and demersal sharks. Nurse sharks are sedentary, have one of the slowest reported metabolisms of any shark species, and primarily consume benthic invertebrates and small teleost fish (Castro 2000, Whitney et al. 2016). They, therefore, occupy a lower trophic position than many adults in the families Carcharhinidae and Sphyrnidae (Rezende et al. 2009, Roff et al. 2016) and likely would have a smaller impact on reef food webs because of their reduced energy requirements (Whitney et al. 2016). Nonetheless, additional evidence would be needed to demonstrate how lower trophic guilds responded to the heightened dominance of this demersal mesopredator over time. These predictions could be tested by analyzing the skeletal remains of shark prey items. Fish teeth, otoliths, and mollusc shells are preserved alongside denticles in the fossil record (Fredston-Hermann et al. 2013, Cramer et al. 2017, Lin et al. 2019, O'Dea et al. 2020) and could reconstruct components of sharks' trophic interactions through time. If paired with contemporaneous oceanographic (e.g.  $\delta^{18}\text{O}$ ) and habitat (e.g. coral abundance) proxies, these data could help reveal whether historical shark declines precipitated a trophic cascade or, alternatively, whether lower trophic guilds were shaped by bottom-up forces (see Jackson et al. 2001, Roff et al. 2016, and Desbiens et al. 2021). Additionally, nitrogen isotopes (Kast et al. 2016) could document temporal changes in predator and prey trophic levels and quantify trophic overlap between mesopredatory sharks and teleost predators, which is known to buffer against trophic cascades (Roff et al. 2016). The historical context derived from these analyses could augment the contemporary evidence used to assess sharks' trophic roles on reefs.

Denticle-informed shark baselines can help guide ecosystem management by reshaping perceptions of what a natural shark community in Caribbean Panama, or elsewhere, looked like before human disturbance. Shark abundances on remote, protected islands have often been used to estimate pre-exploitation baselines, although these spatial reference points are only available in a limited number of regions and arguably not in the Caribbean (Jackson 2001, Jackson et al. 2001). The denticle accumulation rates on the mid-Holocene reef in Panama were around an order of magnitude lower than modern accumulation rates found on a remote, un-fished Pacific atoll (Dillon et al. 2020). This disparity suggests that pre-exploitation shark abundance on this inshore Caribbean reef was much lower than the high abundances observed on many uninhabited, oceanic islands today and supports the prediction that there could be important bottom-up forcing that regulates shark populations and contributes to natural geographic heterogeneity in shark carrying capacities (Nadon et al. 2012, Valdivia et al. 2017). Therefore, making generalizations using baselines derived from regions or time periods with dissimilar environments or ecological histories could yield impractical restoration targets for sites that historically supported different shark densities. Rather, comparing prehuman and modern shark communities can be insightful for evaluating site-specific management strategies and goals, even if these historical abundances cannot be restored.

The denticle record has several limitations that must be considered when using it to reconstruct shark communities. First, variation in denticle morphology across a shark's body currently precludes the identification of isolated denticles beyond the family level (Reif 1985, Dillon et al. 2017, Ferrón & Botella 2017). Nonetheless, interpreting the denticle record at the level of functional morphotype and broadly relating those morphotypes to shark taxa, as we have done here, can provide an ecologically meaningful assessment of

higher-level taxonomic and functional shifts in shark communities. Second, because the bulk samples in this study represent time-averaged accumulations, the modern denticle assemblages were estimated to encompass the last ~50 to 360 y, which could dampen the extent of decline observed in denticle accumulation over time.

One advantage of this time averaging, however, is that the denticle assemblages capture some of the natural fine-scale temporal variability across the modern and mid-Holocene sites, representing an average shark community by integrating over many points in time. These samples also incorporate natural spatial variability in shark abundance, as habitats interdigitate over decades to centuries. Third, we sampled five localities across a single mid-Holocene reef tract as it is, at present, the only known exposed reef of this age in Caribbean Panama. This fossil reef offers unique insight into pre-exploitation shark baselines in the local region, although the rarity of such sites hinders large-scale spatial replication. Nonetheless, variation in denticle accumulation across the sites we sampled was similar between time periods and comparable to contemporary shark surveys in Bocas del Toro (Chevis et al. 2020). As additional Holocene sites are detected, our study provides a framework for comparing denticle assemblages over time and across locations to describe geographic patterns of shark decline. Lastly, variation in denticle densities (Raschi & Tabit 1992), shedding rates, and taphonomic biases could confound estimates of absolute shark abundance derived from denticle accumulation rates (Supplementary Methods in Appendix C.1). In this study, we found no evidence to suggest that the trends in denticle accumulation were caused by taphonomic processes, such as size sorting, selective dissolution, or preservation, or by reef accretion rates. However, denticle shedding rates likely vary across sharks with different ecological modes and denticle quantities, decoupling true abundances

from denticle accumulation. Instead, reconstructing relative shark abundances, as we have done here, provides a conservative metric of shark community shifts.

#### ***4.5 Conclusions***

Our evidence adds to the growing body of paleoecological research investigating the effects of overharvesting (Cramer et al. 2017, Łukowiak et al. 2018, O’Dea et al. 2020) and habitat change (Aronson et al. 2004, Fredston-Hermann et al. 2013, Cramer et al. 2020b, O’Dea et al. 2020) on reef-associated coral, mollusc, sponge, and teleost fish communities in Bocas del Toro, Panama. In this study, we quantify shark abundance before major human impact using the fossil record. Our data show that reef-associated sharks in this area of the Caribbean have been severely depleted by both long-term harvesting, which accelerated in the second half of the 20th century, as well as by habitat degradation, which began even earlier. We also demonstrate that denticles are abundant and well preserved in reef sediments, providing a record of reef shark abundance and functional diversity over millennia. This first application establishes denticle assemblages as a promising approach for answering long-standing questions about the baseline conditions of shark communities, the drivers of shark declines over long ecological timescales, and their ultimate ecological and conservation implications.

#### ***4.6 Methods***

**General Setting.** Sampling was conducted in Almirante Bay, Bocas del Toro, Caribbean Panama (centered at 9.2993° N and 82.2312° W), a sheltered, semi enclosed lagoonal system that sits outside the hurricane belt. Over 30 shark species have been observed in, or their ranges cover, the Bocas del Toro archipelago (Robertson & Van Tassell 2019), and Almirante Bay could provide a nursery habitat for small coastal shark species (Gonzalez et

al. 2019). Surveys conducted in the bay since 2016 have reported just seven shark species, with the nurse shark *G. cirratum* accounting for most of the sightings (Chevis et al. 2020). Although contemporary shark abundance varies spatially across the archipelago, reported abundances were similarly low near all reefs sampled in this study, including those adjacent to the fossil site (Chevis et al. 2020).

**Mid-Holocene Reef.** The mid-Holocene reef tract (Fig. 4.2) occupies a ~50-ha area on the leeward side of Isla Colón, buffered from waves and currents by the Plio-Pleistocene sediments that comprise the island (Coates et al. 2005). The reef is located alongside the modern coastline and accreted ~7 ka under similar oceanographic and climatic conditions to the reefs in Almirante Bay today (Giry et al. 2012, Fredston-Hermann et al. 2013, O’Dea et al. 2020, Gudnitz et al. 2021). Foraminiferal assemblages characterize this mid-Holocene site as a patch reef with seagrass facies and molluscan muds, similar to modern habitats within the bay (Gudnitz et al. 2021). This congruence suggests that the mid-Holocene reef should be reasonably representative of shark communities found in similar habitats today. The reef matrix was found to be well preserved, consisting of corals in life position without indication of physical disturbance and unsorted carbonate muds and silts containing autochthonous biogenic material accumulating within this branching coral framework (Fredston-Hermann et al. 2013, Lin et al. 2019, O’Dea et al. 2020). Human settlements are not recorded on Panama’s Caribbean coast until after ~6 ka (Linares 1977, Ranere & Cooke 1991), and the earliest evidence in the Bocas del Toro region dates to ~4 ka (Baldi 2011, Wake et al. 2013). Being the only known exposed mid-Holocene reef in this region, this site provides a unique, albeit rare, window into the shark community inhabiting a Caribbean reef before major human impact at a time when the environment was similar to the modern day.



**Sample Collection.** To compare the denticle-defined shark baseline with the modern assemblage, we collected replicate ~9 kg bulk samples of fine surface sediments and reef framework from five localities on the mid-Holocene reef ( $n = 15$  samples;  $n = 3$  replicates per locality) and three modern reefs in Almirante Bay ( $n = 16$  samples;  $n = 4$  to 6 replicates per site) (Fig. C.1) in 2014. At the mid-Holocene reef site, samples were collected from the uppermost section of the reef facies, covering a stratigraphic depth of ~10 cm. On the modern reefs, samples were excavated from the uppermost <10 cm at water depths of 2 to 4 m, which overlap with the estimated paleodepths at the mid-Holocene site (O’Dea et al. 2020) and constitute a similar environmental setting (Fredston-Hermann et al. 2013, Gudnitz et al. 2021). Sampling was constrained to low-energy habitats with branching coral framework, which restricts vertical mixing and reworking (Cramer et al. 2017, Lin et al. 2019, O’Dea et al. 2020), to reduce the influence of taphonomic processes and facilitate comparisons over time.

**Quantifying the Denticle Assemblages.** Sediment samples were sieved and processed to isolate the denticles. The 106 to 250  $\mu\text{m}$ , 250 to 500  $\mu\text{m}$ , and 500  $\mu\text{m}$  to 2 mm size fractions were treated with 10% acetic acid to eliminate the calcium carbonate components and then with 5% hydrogen peroxide to remove excess organic material (Dillon et al. 2017, Sibert et al. 2017). Denticles were picked from the residue and counted, and denticles missing more than half of their crown were excluded to avoid double counting. The total denticle count per sample was divided by the dry weight of the sediment fractions to calculate denticle abundance.

To determine assemblage composition, denticles were measured and visually classified using a reference collection into five recognized functional morphotypes: drag reduction, ridged abrasion strength, abrasion strength, generalized functions, and defense (Reif 1985,

Raschi & Tabit 1992, Dillon et al. 2017, Ferrón & Botella 2017). These classifications were verified with a multinomial logistic regression model trained on the reference collection (Dillon et al. 2017), which was used to predict the morphotype of each denticle (Tables C.8–C.10 and Supplementary Methods in Appendix C.1).

**Temporal Context and Accumulation Rates.** Uranium–Thorium and calibrated radiocarbon dating of coral pieces were used to estimate the age and amount of time encompassed by the sediment samples and to calculate reef accretion rates (Table C.1 and Supplementary Methods in Appendix C.1). Dates from the mid-Holocene site corroborated previous work, demonstrating that this reef accreted over a period of at least 1,500 y from 7.2 to 5.7 ka (Fredston-Hermann et al. 2013, O’Dea et al. 2020), whereas the modern samples spanned the last ~50 to 360 y (mean = 159 y; expressed relative to the collection year). Reef accretion rates were estimated using linear interpolation between dates after removing age reversals (Fig. C.13). Denticle abundances were corrected by reef accretion rates at each site to calculate absolute denticle accumulation rates. The  $2\sigma$  errors on each date were incorporated into a sensitivity analysis to determine how much this analytical uncertainty affected our interpretation of change in denticle accumulation rates (Table C.2).

**Analyzing Change over Time in Denticle Assemblages.** Generalized linear mixed models using a Gamma error distribution and site as a random effect were used to test for differences in denticle accumulation rates over time, while accounting for variation across sites. A negative binomial error distribution was used to test for differences in the counts of each functional morphotype, offset by kilograms sediment per year, over time. Models were compared using small-sample corrected Akaike information criterion, and nested likelihood ratio tests were used to obtain  $p$  values. Models were implemented using the R package *glmmTMB*, and the assumptions were checked using the package *DHARMA*. Jackknife

sampling was used to estimate bias, given natural spatial variation. Spearman rank correlations were used to explore relationships between denticle abundance, weathering, and sediment characteristics (sorting was calculated using the Folk and Ward method in the package *G2Sd*). Differences in the shapes and medians of the denticle size–frequency distributions were evaluated with Kolmogorov–Smirnov and Wilcoxon tests.

To assess changes in denticle assemblage composition, denticle counts were square root transformed, and Bray–Curtis dissimilarities were ordinated using nonmetric multidimensional scaling. The function *envfit* was applied to overlay biplot vectors and identify the morphotypes that contributed to the ordination patterns. We used PERMANOVA to test for differences in dissimilarities over time, while controlling for site differences using the function *adonis2* in the package *vegan*. Unidentified denticles were removed from the analyses. Changes were reported in terms of the relative abundance of each morphotype, which was positively correlated with the absolute count (Fig. C.14). All analyses were performed in R (R Core Team 2019).

**Weathering Analysis.** Each denticle was assigned a weathering score, which ranged from zero (pristine) to three (poor preservation) and was based on visual inspection of the crown, peaks, and base (criteria are described in Dillon et al. 2020). Scores were compared across time periods, sites, and functional morphotypes to assess denticle preservation.

**Exploring Changes in the Perceived Ecological State of Sharks.** Published archaeological studies ( $n = 15$ ), anecdotes and ethnographic accounts ( $n = 47$ ), ecological surveys ( $n = 12$ ), and fisheries reports ( $n = 17$ ) from Caribbean Panama were compiled to evaluate human perceptions and harvesting of sharks over the last ~4 ka (Table C.5). These records were separated into seven cultural periods in Caribbean Panama’s history, which were described in terms of human resource use (Table C.6). To evaluate these disparate data

types, the records (e.g. Table C.4) were interpreted using established criteria (Pandolfi et al. 2003, Kittinger et al. 2011) to assign a semiquantitative ecological state to each cultural period based on perceived shark abundance (Table C.7). Ecological states were determined using the data in aggregate for each cultural period, and they were based on the most frequent state given the potential for variation in perceptions of shark abundance. To constrain personal biases, the accounts were reviewed by 17 individuals. The study protocol was approved and designated as exempt by the Human Subjects Committee, which serves as the Institutional Review Board of the University of California, Santa Barbara (IRB Protocol #3–20–0211). Written consent was obtained through completion of the questionnaire. Identifying information was removed from the metadata before evaluation, and the cultural periods were blinded (following Al-Abdulrazzak et al. 2012). Respondents were also asked to report how confident they were in each of their responses (high, neutral, or low) and to provide a short justification for each response in the questionnaire (Supplementary Methods in Appendix C.1).

**Data Availability.** Data are archived in the Dryad Digital Repository: <https://doi.org/10.25349/D9WP5D>. All other data are included in the manuscript and Appendix C.

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## CHAPTER 5: CONCLUSIONS

In this dissertation, I refine a method to recover, classify, and interpret shark dermal denticles accumulating in coral reef sediments over millennia. My work builds on previous advances, which first illuminated the prevalence and diagnostic features of these understudied microfossils (Helms & Riedel 1971, Doyle & Riedel 1979, Tway 1979, Reif 1985, Ferrón et al. 2014, Ferrón & Botella 2017, Sibert et al. 2017). I measure denticle shedding rates, morphological variation, preservation, and ecological fidelity with modern shark communities—factors which influence how sharks are represented as denticles in the fossil record. By calibrating the relationship between shark abundance and denticle accumulation, this research offers guidance for analyzing denticle assemblages as a proxy for relative shark abundance through time.

By testing the utility of the denticle record, I also reveal interpretational boundaries that require caution and further exploration. Importantly, more work is needed to generate reliable estimates of absolute shark abundance from denticle accumulations. Only a small number of the denticles shed by sharks are preserved in the fossil record. This accumulation is governed by the number of denticles on a shark's body, the rate at which they are shed, and their preservation and mixing after being deposited in sediments. Better constraints on each of these variables would improve our ability to reconstruct absolute shark densities using the denticle record. Such estimates could be further validated by comparing denticle accumulation across regions or islands with different shark densities. Nonetheless, denticle assemblages provide valuable information about relative differences in shark abundance and community composition between time periods and locations.

As this method continues to advance, future research could work toward extracting denticles more efficiently from sediments, improving denticle classifications, and better

understanding the temporal and spatial resolution of denticle assemblages. Here, I outline four potentially promising avenues of method refinement.

First, machine learning techniques (e.g. convolutional neural networks) or high-throughput morphological data collection (e.g. using AutoMorph; Hsiang et al. 2017, Sibert & Rubin 2021) could be applied to increase the taxonomic resolution of denticle classifications. Denticle morphology varies across the body of a shark as well as between species and ontogenetic stages, limiting denticle classifications to functional groups and shark families. Analyses of this morphological variation using high-resolution images and automated measurements could increase the reliability of classifications beyond the family level. Achieving higher taxonomic resolution could improve estimates of community composition, reveal population size structure (using allometric relationships between denticle crown size and shark length), inform species management, and assign denticles to meso and apex predator trophic groups to test hypotheses about trophic cascades.

Second, alternative extraction techniques such as density separation (Sibert et al. 2017) could be tested to increase sample processing efficiency, particularly given the large sample sizes that are needed to obtain enough denticles for statistical analysis in some regions. Improved denticle recovery methods would increase the feasibility of large-scale temporal or spatial comparisons of shark communities using the denticle record.

Third, direct dates of fossil dermal denticles have yet to be published. In this dissertation, I use Uranium–Thorium dates of coral pieces to estimate the age and amount of time encompassed by sediment samples from which denticles were recovered. However, denticles assemblages in these samples are time-averaged (age mixed) and might filter through the coral framework, producing age offsets between coral pieces and denticles in the

same samples. Dates of denticles could be used to measure time-averaging in denticle assemblages and improve calculations of denticle accumulation rates.

Finally, denticle sinking rates and pathways should be modeled and empirically verified to determine how far a denticle is transported horizontally as it sinks under different flow regimes after being shed. Such outputs could be used to describe the spatial resolution of denticle assemblages.

Fossil denticle assemblages have created new opportunities to assess how abundant sharks were before human disturbance and the extent to which they have shifted from pre-exploitation baselines. This method is now ripe to be applied to questions of broad ecological and conservation significance, such as: To what extent do shark baselines vary across regions, and do they oscillate through time? What drives natural spatial and temporal variability in reef shark communities before human exploitation? How have shark declines altered reef trophic structure and ecosystem functioning? Did trophic cascades occur before contemporary monitoring? Do oceanographic conditions shape shark resilience or recoveries? And how can denticle-defined shark baselines best inform management targets and address conservation priorities?

Some of this work is already in progress. For example, I aim to quantify variation in pre-exploitation shark abundance and resilience across Pacific and Caribbean coral reefs with different human and environmental histories. I am also integrating denticles with complementary records, including coral skeletons, fish otoliths, and paleoenvironmental proxies, to test hypotheses about top-down and bottom-up forcing on historical reefs. As we work to curb shark declines and restore sharks and their ecological functions to marine ecosystems, this dissertation adds denticle assemblages to the toolbox by contributing previously inaccessible information about coastal shark communities over millennia.



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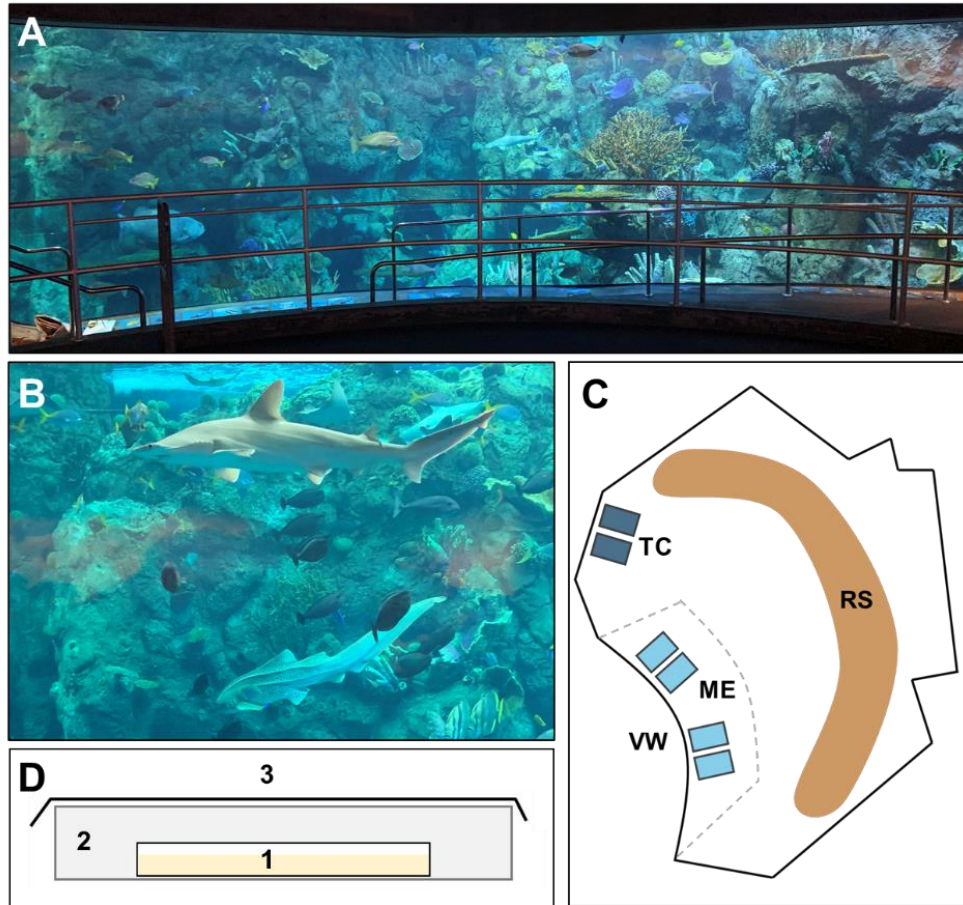
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## APPENDICES

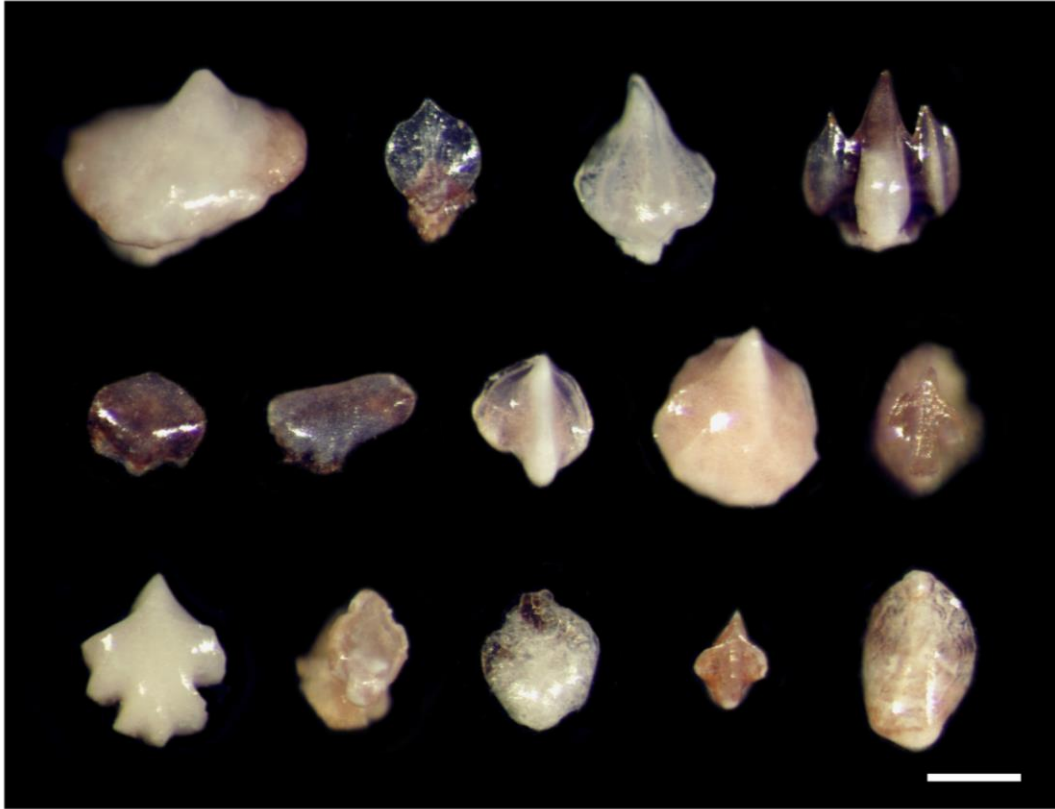
### Appendix A: Chapter 2

#### Appendix A.1 Supplementary Figures

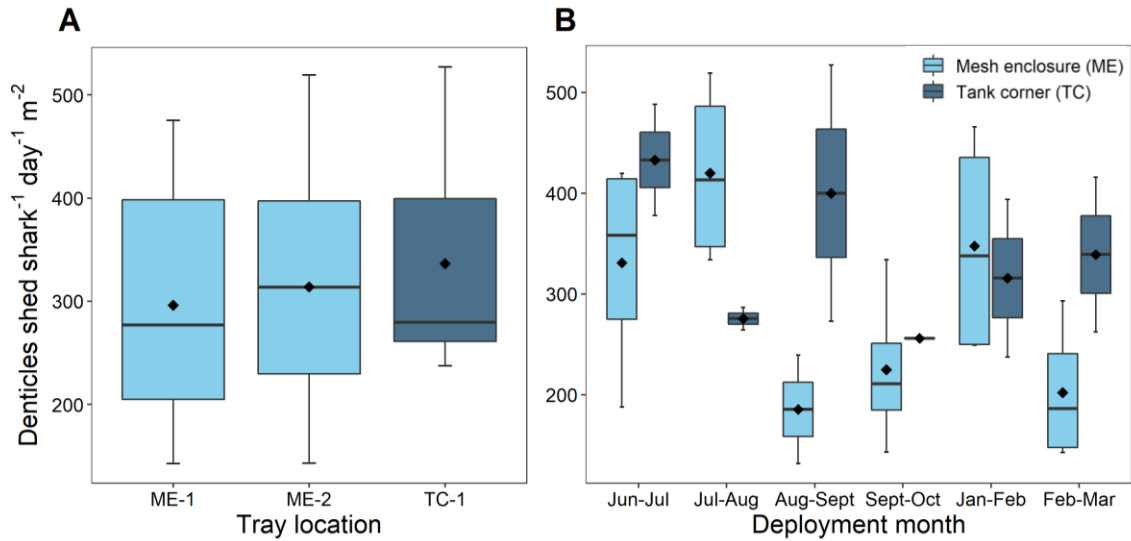


**Figure A.1** Tropical Pacific Gallery at the Aquarium of the Pacific. (A) Main viewing window where the behavioral surveys were conducted, which provided a panorama view of the tank. (B) The tank housed two bonnethead sharks *Sphyrna tiburo* (top) and three zebra sharks *Stegostoma fasciatum* (bottom). (C) Tank schematic, showing the approximate locations of the trays (light and dark blue rectangles;  $n = 6$ ) in the webbed mesh enclosure (ME; bounded by dashed gray line) and tank corner (TC). The artificial reef structure (RS; brown shading) is visible from the main viewing window (VW) in panel A. Tank

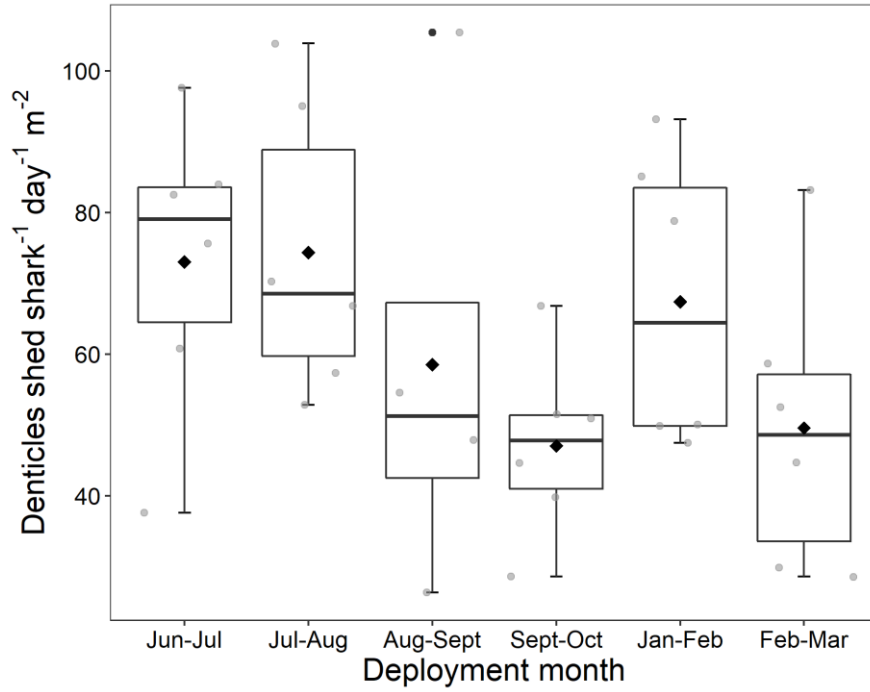
dimensions are not to scale. (D) Experimental tray design, comprising a 4.5 l Pyrex® tray containing 1.2 kg of CaribSea® aragonite sand substrate [1] placed within a 26 l Sterilite® container [2] covered with a mesh baffle (~2 cm<sup>2</sup> openings) [3]. Trays were used to measure net denticle deposition, allowing for resuspension but not capturing entrained denticles (see Browne et al. 2012). Basin-like geometries also have high trapping efficiency, collecting near 100% of the calculated particle flux in flume experiments (Gardner 1980), while remaining out of sight to aquarium visitors.



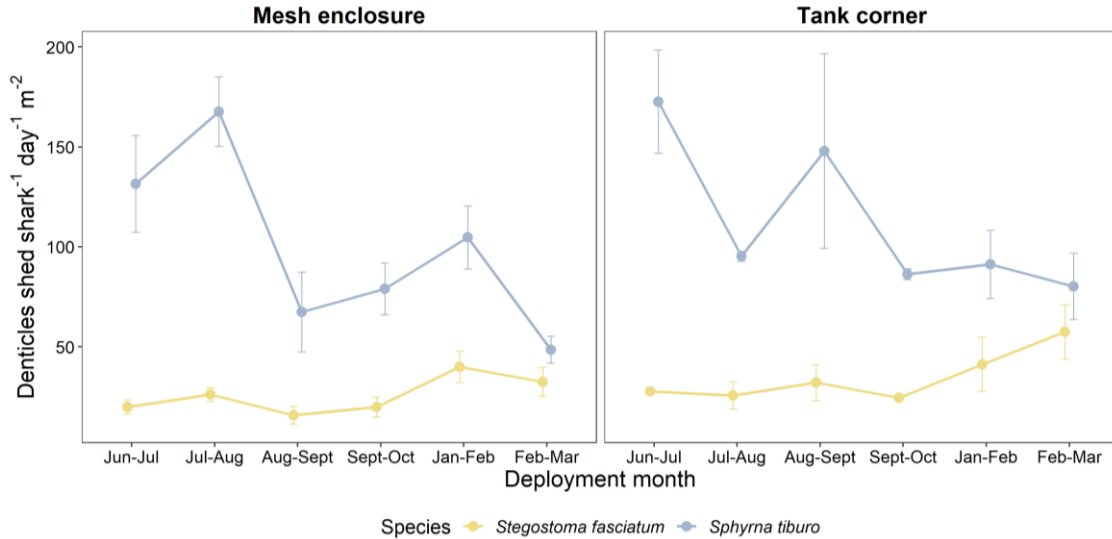
**Figure A.2** Examples of denticles that could not be reliably identified. These morphotypes represented only 3% ( $n = 218$ ) of the denticles recovered from the trays. Scale bar = 200  $\mu\text{m}$ .



**Figure A.3** Denticle shedding rates across tray locations in the tank. (A) Shedding rate measurements were similar among trays located inside the mesh enclosure (ME) and in the tank corner (TC) when aggregated across deployments ( $p = 0.71$ ). Colors correspond to the diagram in Fig. A.1C. (B) Shedding rate measurements varied by a factor of 1.1 to 2.3 across tray locations during each deployment but were not systematically offset. Each boxplot shows the median (dark bar), mean (diamond), and interquartile range (box), and outliers are displayed as points.

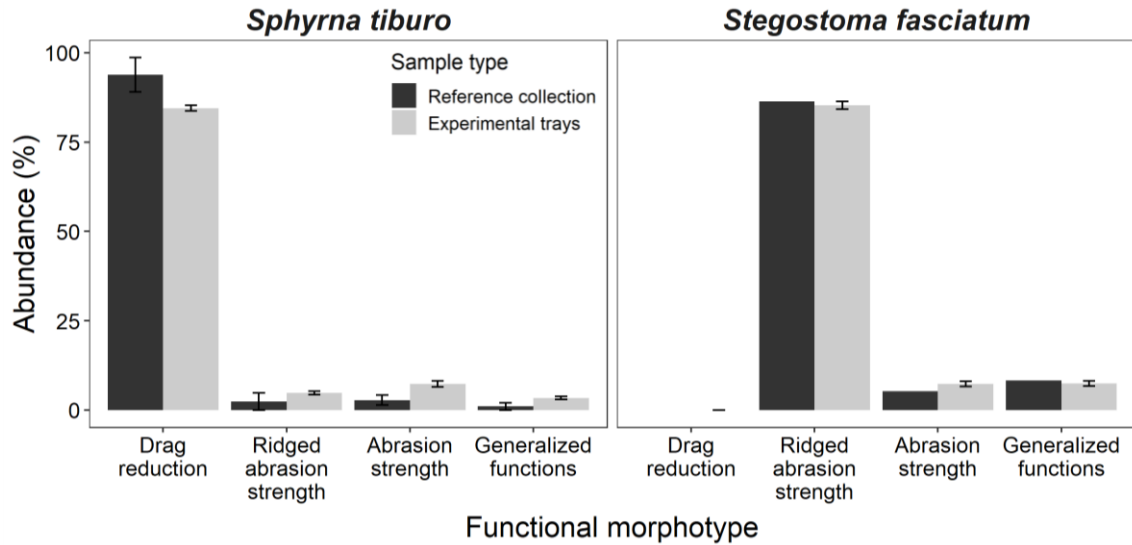


**Figure A.4** Total denticle shedding rate in each deployment aggregated across species. Each boxplot shows the median (dark bar), mean (diamond), and interquartile range (box), and outliers are displayed as black points. Raw data are represented by jittered gray points.

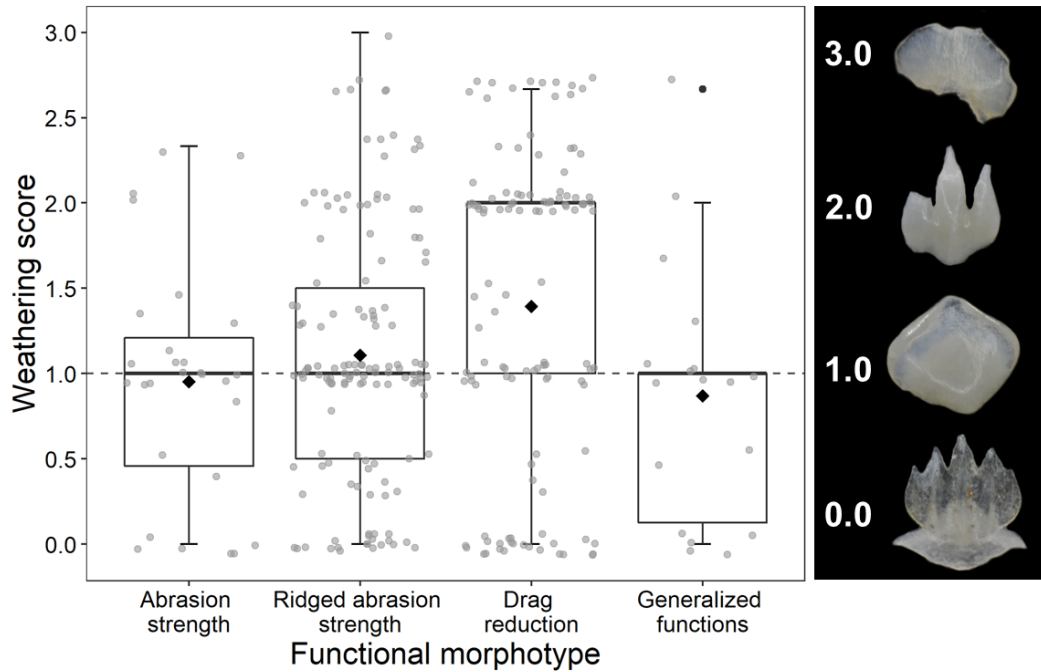


**Figure A.5** Variation in denticle shedding rates across the six deployments, disaggregated by species and tray location. Trays in both locations displayed a similar temporal pattern, with bonnethead shark *Sphyrna tiburo* shedding rates (blue) decreasing over time and zebra shark *Stegostoma fasciatum* shedding rates (yellow) remaining consistent across the first four deployments before increasing slightly. The points and error bars show the mean denticle shedding rate and standard error for each deployment ( $n = 6$  trays per deployment, except the third deployment [ $n = 4$ ]).

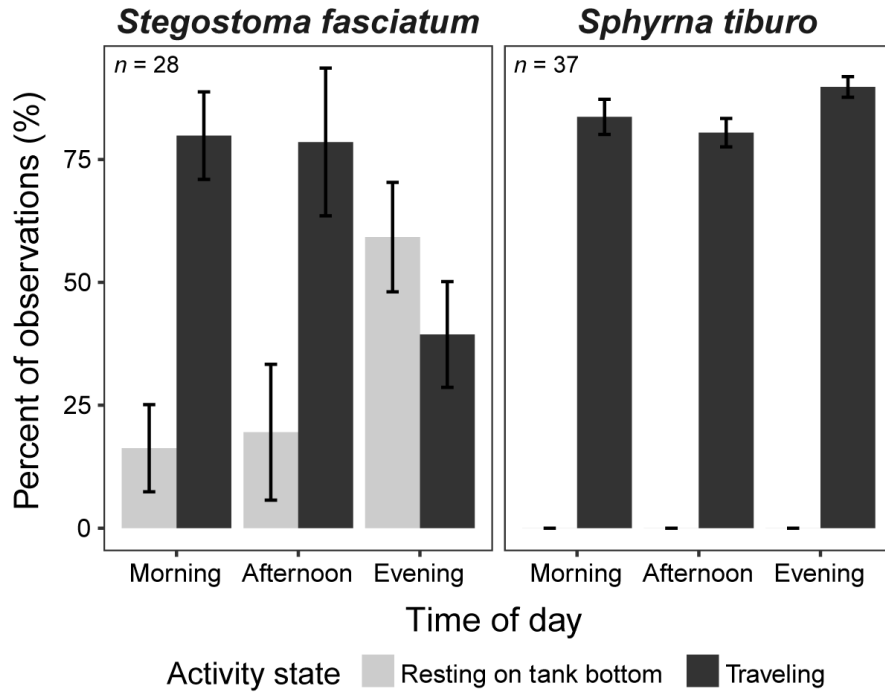




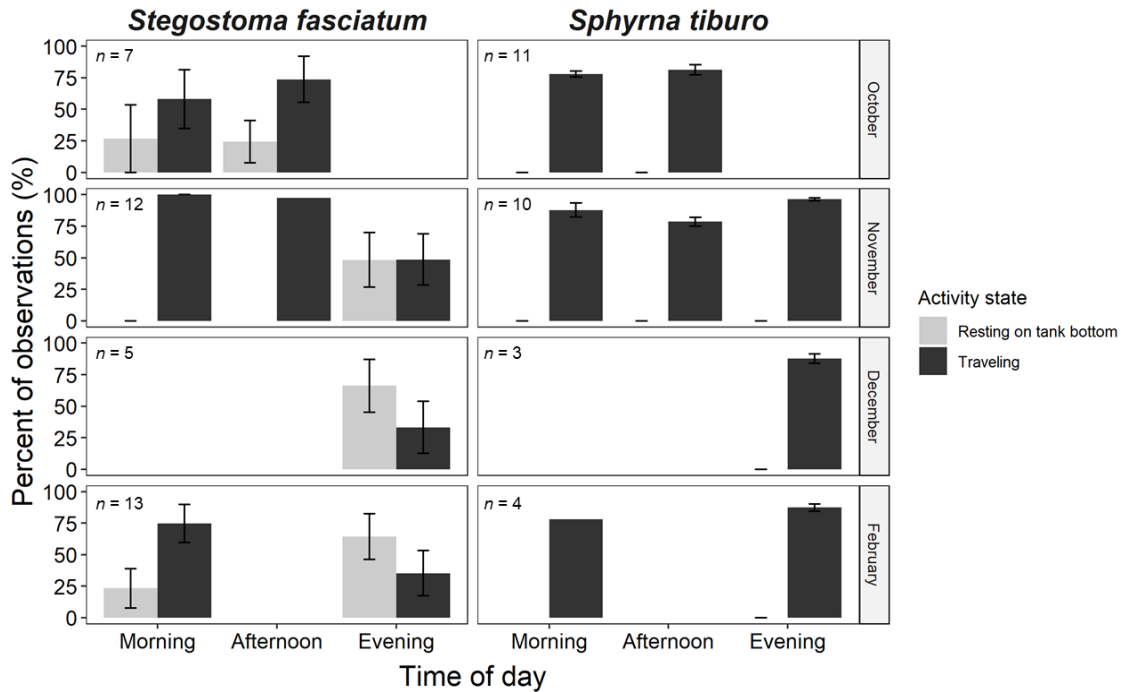
**Figure A.6** Expected proportional abundance of denticle functional morphotypes on the body of each species (black) based on bonnethead shark (left;  $n = 2$ ) and zebra shark (right;  $n = 1$ ) museum specimens (Dillon et al. 2017, 2020, Ferrón & Botella 2017), compared with the proportional abundances found in the trays (gray;  $n = 3153$  denticles). The rank abundances of functional morphotypes found on each species roughly corresponded with the abundances recovered from the trays. The bars show the mean abundance and standard error for each functional morphotype.



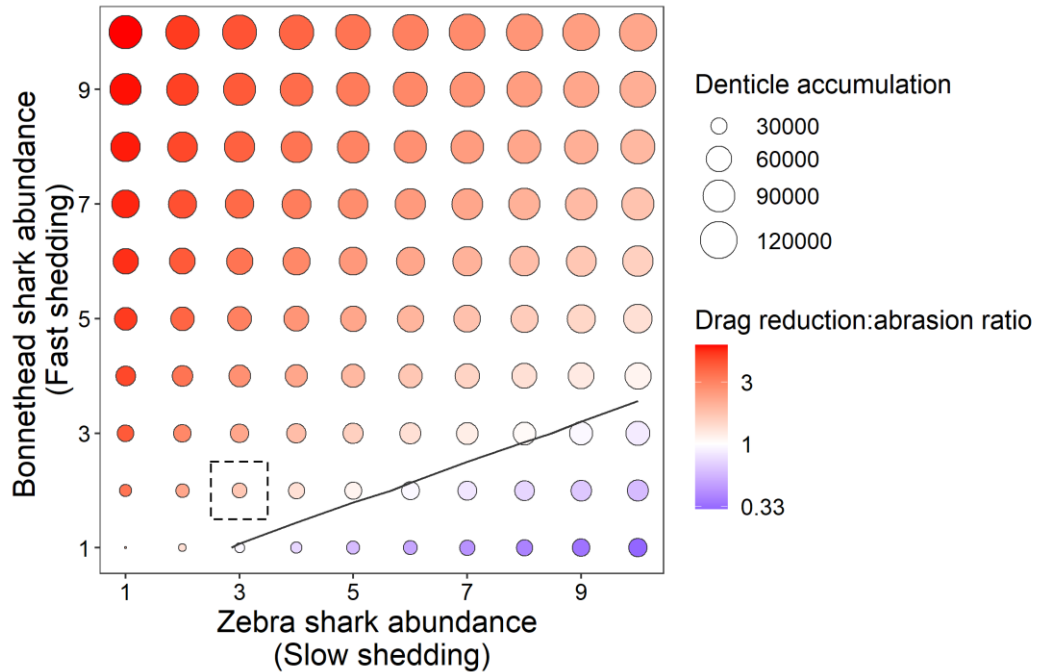
**Figure A.7** Weathering scores across denticle functional morphotypes. Lower scores indicate better preservation. Drag reduction denticles were more weathered on average than the other morphotypes ( $p = 0.007$ ; Games–Howell test  $p < 0.05$ ). Each boxplot shows the median (dark bar), mean (diamond), and interquartile range (box), and outliers are displayed as black points. Raw data are represented by jittered gray points. The dashed line indicates the median score across all denticles. Examples of each weathering score are provided for context.



**Figure A.8** Zebra shark *Stegostoma fasciatum* and bonnethead shark *Sphyrna tiburo* activity states during the behavioral surveys ( $n = 65$ ). Bonnethead sharks were always traveling when visible, whereas zebra sharks alternated between traveling and resting. The bar plot and error bars show the mean percent of observations during which each activity state was recorded (i.e. when the shark was visible) and standard error at each time of day (morning, 09:00–12:00 h; afternoon, 12:00–15:00 h; and evening, 15:00–18:00 h).



**Figure A.9** Shark activity remained relatively consistent across months during the behavioral surveys. Surveys were conducted during the months when denticle shedding rates were most variable. Between three and thirteen surveys were conducted per species each month ( $n = 65$  surveys over seven sampling occasions). Observations were not available at every time of day each month (blank areas indicate no data). The bars show the mean percent of observations during which each activity state was recorded (i.e. when the shark was visible) and standard error at each time of day (morning, 09:00–12:00 h; afternoon, 12:00–15:00 h; and evening, 15:00–18:00 h).



**Figure A.10** Denticle accumulations simulated using the empirically measured shedding rates over a 90 d period in a 1 m<sup>2</sup> area. Fast-shedding bonnethead sharks contributed more to the denticle assemblages than slow-shedding zebra sharks. Drag reduction denticles, which characterize bonnethead sharks, were therefore overrepresented in the simulated denticle assemblages relative to bonnethead shark abundances. In contrast, the functional morphotypes found on zebra sharks (ridged abrasion strength and abrasion strength denticles) outnumbered drag reduction denticles only in scenarios in which zebra sharks were several times more abundant than bonnethead sharks. Accordingly, the 1:1 ratio (black line) between drag reduction denticles and the summation of ridged abrasion strength and abrasion strength denticles (defined here as abrasion) was offset from the 1:1 ratio between bonnethead and zebra shark abundances. The shark community in the Tropical Pacific Gallery, from which the shedding rates were calculated, is indicated by the dashed box.

## Appendix A.2 Supplementary Tables

**Table A.1** Description of denticle characteristics used to classify bonnethead shark *Sphyrna tiburo* and zebra shark *Stegostoma fasciatum* denticles. Denticle images for each species are displayed in Fig. 2.1. Criteria follow Dillon et al. 2017.

Criteria	<i>Sphyrna tiburo</i>	<i>Stegostoma fasciatum</i>
Crown size (relative)	Smaller	Larger and thicker
Crown shape	Elliptical, circular, or diamond-shaped	Lanceolate or diamond-shaped
Crown microstructures	Microstructures	No microstructures
Ridge number and spacing	>3 (often 5 or 7) thinly-spaced ridges; in the absence of ridges, the crown is lobed	1, 3, or 5 widely-spaced ridges (or no ridges)
Number of peaks	Often multiple peaks	Single peak

**Table A.2** Weathering score criteria. Weathering was determined based on the state of the denticle base, crown, and peaks, which were each assigned a point value (Dillon et al. 2020). Each value was multiplied by a scaling factor to give equal weight to the base, crown, and peaks. Scaled point values were then summed to yield the final score, which ranged from zero (pristine) to three (poorly preserved). Examples of each whole weathering score are provided in Fig. A.7. Discoloration (loss of original coloration) and surface alteration (abrasion or wear on the crown surface) were independently assessed but rarely observed.

Character	Description	Points	Scaling factor
Base	Present	0	1/2
	Partially present	1	1/2
	Absent	2	1/2
Crown	Fully intact	0	1/3
	Light damage (90-99% intact)	1	1/3
	Damage (51-89% intact)	2	1/3
	Fragment ( $\leq$ 50% intact)	3	1/3
Peaks	Intact	0	1
	Fractured	1	1

**Table A.3** Generalized linear mixed model selection results, which explored the influence of species, time of day, and dive shows on shark behavior. Species, time of day, and dive show were included as fixed effects, and observation date was included as a random effect. An asterisk denotes the interaction between factors. The three-way interaction with species, time of day, and dive show is not reported due to the low number of observations conducted during dive shows ( $n = 13$ ). The top-ranked models are bolded ( $\Delta\text{AICc} < 2$  threshold) and discussed in the main text. These parameters were possibly poor predictors of total contact with the tank and interactions with other large animals, producing many models with similar support.

Response	Model	Error distribution	K	AICc	$\Delta\text{AICc}$	Cum. wt	
Activity state (traveling)	<b>species + time of day + dive show + (1 date)</b>	<b>Binomial</b>	<b>6</b>	<b>1125.2</b>	<b>0.0</b>	<b>1</b>	
	species + time of day + (1 date)	Binomial	5	1197.2	72.0	1	
	species + dive show + (1 date)	Binomial	4	1198.4	73.2	1	
	species * dive show + (1 date)	Binomial	5	1200.5	75.3	1	
	species * time of day + (1 date)	Binomial	7	1201.2	76.0	1	
	species + (1 date)	Binomial	3	1252.4	127.2	1	
	time of day * dive show + (1 date)	Binomial	7	1718.0	592.8	1	
	time of day + dive show + (1 date)	Binomial	5	1739.6	614.4	1	
	time of day + (1 date)	Binomial	4	1742.4	617.2	1	
	dive show + (1 date)	Binomial	3	1815.8	690.5	1	
	null	Binomial	2	1816.3	691.1	1	
	Horizontal position in the tank (outer third)	<b>species * dive show + (1 date)</b>	<b>Binomial</b>	<b>5</b>	<b>2292.8</b>	<b>0.0</b>	<b>1</b>
		time of day * dive show + (1 date)	Binomial	7	2319.4	26.6	1
species + time of day + dive show + (1 date)		Binomial	6	2324.7	31.9	1	
time of day + dive show + (1 date)		Binomial	5	2326.6	33.8	1	
species + dive show + (1 date)		Binomial	4	2329.0	36.2	1	
dive show + (1 date)		Binomial	3	2332.5	39.8	1	
species * time of day + (1 date)		Binomial	7	2335.4	42.6	1	
time of day + (1 date)		Binomial	4	2346.6	53.8	1	
species + time of day + (1 date)		Binomial	5	2347.0	54.3	1	
species + (1 date)		Binomial	3	2351.2	58.5	1	
null		Binomial	2	2352.6	59.8	1	
Vertical position in the tank (bottom third)		<b>species + time of day + dive show + (1 date)</b>	<b>Binomial</b>	<b>6</b>	<b>1540.2</b>	<b>0.0</b>	<b>0.95</b>
		species * time of day + (1 date)	Binomial	7	1546.2	6.0	1
	species + time of day + (1 date)	Binomial	5	1618.4	78.2	1	
	species * dive show + (1 date)	Binomial	5	1626.3	86.0	1	
	species + dive show + (1 date)	Binomial	4	1628.5	88.3	1	
	species + (1 date)	Binomial	3	1681.6	141.4	1	
	time of day * dive show + (1 date)	Binomial	7	2089.1	548.9	1	
	time of day + dive show + (1 date)	Binomial	5	2113.2	573.0	1	
	time of day + (1 date)	Binomial	4	2118.2	578.0	1	
	dive show + (1 date)	Binomial	3	2197.7	657.5	1	
	null	Binomial	2	2200.7	660.4	1	
	Total contact with the tank (count)	<b>species + time of day + (1 date) + offset(log(survey duration))</b>	<b>Negative binomial</b>	<b>6</b>	<b>352.7</b>	<b>0.0</b>	<b>0.19</b>
		<b>species + (1 date) + offset(log(survey duration))</b>	<b>Negative binomial</b>	<b>4</b>	<b>353.0</b>	<b>0.3</b>	<b>0.36</b>
<b>time of day + (1 date) + offset(log(survey duration))</b>		<b>Negative binomial</b>	<b>5</b>	<b>353.3</b>	<b>0.5</b>	<b>0.5</b>	
<b>species + time of day + dive show + (1 date) + offset(log(survey duration))</b>		<b>Negative binomial</b>	<b>7</b>	<b>353.6</b>	<b>0.8</b>	<b>0.63</b>	
<b>time of day + dive show + (1 date) + offset(log(survey duration))</b>		<b>Negative binomial</b>	<b>6</b>	<b>353.9</b>	<b>1.2</b>	<b>0.73</b>	
<b>null</b>		<b>Negative binomial</b>	<b>3</b>	<b>354.1</b>	<b>1.4</b>	<b>0.83</b>	
<b>species + dive show + (1 date) + offset(log(survey duration))</b>		<b>Negative binomial</b>	<b>5</b>	<b>354.7</b>	<b>1.9</b>	<b>0.9</b>	
dive show + (1 date) + offset(log(survey duration))		Negative binomial	4	355.7	3.0	0.94	
species * dive show + (1 date) + offset(log(survey duration))		Negative binomial	6	356.2	3.4	0.98	
species * time of day + (1 date) + offset(log(survey duration))		Negative binomial	8	357.8	5.1	0.99	
time of day * dive show + (1 date) + offset(log(survey duration))	Negative binomial	8	358.6	5.9	1		
Interactions with other large animals (count)	<b>time of day + (1 date) + offset(log(survey duration))</b>	<b>Negative binomial</b>	<b>5</b>	<b>227.3</b>	<b>0.0</b>	<b>0.25</b>	
	<b>species + time of day + (1 date) + offset(log(survey duration))</b>	<b>Negative binomial</b>	<b>6</b>	<b>227.6</b>	<b>0.3</b>	<b>0.46</b>	
	<b>species * time of day + (1 date) + offset(log(survey duration))</b>	<b>Negative binomial</b>	<b>8</b>	<b>228.0</b>	<b>0.7</b>	<b>0.64</b>	
	<b>species + time of day + dive show + (1 date) + offset(log(survey duration))</b>	<b>Negative binomial</b>	<b>7</b>	<b>228.6</b>	<b>1.3</b>	<b>0.77</b>	
	<b>time of day + dive show + (1 date) + offset(log(survey duration))</b>	<b>Negative binomial</b>	<b>6</b>	<b>228.8</b>	<b>1.5</b>	<b>0.88</b>	
	species + (1 date) + offset(log(survey duration))	Negative binomial	4	231.3	4.1	0.92	
	null	Negative binomial	3	231.9	4.6	0.94	
	species + dive show + (1 date) + offset(log(survey duration))	Negative binomial	5	232.2	5.0	0.96	
	time of day * dive show + (1 date) + offset(log(survey duration))	Negative binomial	8	232.5	5.2	0.98	
	dive show + (1 date) + offset(log(survey duration))	Negative binomial	4	233.3	6.0	0.99	
	species * dive show + (1 date) + offset(log(survey duration))	Negative binomial	6	234.0	6.8	1	

### Appendix A.3 Supplementary References

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## ***Appendix B: Chapter 3***

### Appendix B.1 Supplementary Methods

**Multinomial Logistic Regression.** Multinomial logistic regression was used to help verify the visual denticle classifications given that denticle functional morphotypes fall along a morphological continuum (Dillon et al. 2017, Reif 1985). First, we characterized the denticles in our reference collection, which comprises denticles from 37 shark species with a focus on reef-associated species (Dillon et al. 2017), as well as the randomly selected subset of denticles recovered from the sediments ( $n = 580$ ). The crown, peaks, and ridges were measured using a dissecting microscope (Table B.1). This set of denticle characters was refined from Dillon et al. 2017 to include characters that were important for distinguishing between functional morphotypes.

The multinomial logistic regression model, built using the R package *nnet* (Venables & Ripley 2002), was trained and validated on the reference collection denticles ( $n = 213$ ) to determine its accuracy. The denticles were split into  $N$  groups ( $N = 2, 3, 4, 5, 6, 7, \text{ or } 8$ ), and either  $N$  or  $N-1/N$  randomly selected specimens were used to train the classification of the remaining denticles (following Mitra et al. 2019). Regardless of the training set size, the overall accuracy of the model was 84%, although it varied across functional morphotypes (Table B.2).

The multinomial logistic regression model was then trained with all the reference collection denticles and used to classify the denticles recovered from the sediments (Table B.3). There was a 75% correspondence between the visual classifications and the model predictions (Table B.4). Many of the conflicting classifications were visually identified as generalized functions denticles, which can vary in their morphology. The visual classifications were used in all reported analyses.

**Sediment Trap Deployments.** Sedimentation data were obtained from two sources – our own field collections and published data (Williams et al. 2011, Knapp et al. 2013). Sedimentation rates were measured using PVC sediment traps (50–60 cm in length and 51 mm in internal diameter) placed at or near each sediment collection site. Traps were deployed in aluminum supports to keep them vertical and flush with the substrate surface. To reduce resuspension, the traps had a diameter-to-length ratio of 10 (Bloesch & Burns 1980, Gardner 1980, Butman et al. 1986). Resuspension was suspected in five deployments,

and these values were excluded from the analyses. Overall, between 115 and 423 days of sediment trap data were available for each site (Table 3.1) within a 0.6 km radius (mean 0.26 km) from a similar depth and habitat.

After collection, sediment samples were preserved in small volumes of seawater (50–100 ml) with the addition of 5% Lugol's iodine. In the laboratory, sediment samples were concentrated onto pre-ashed and pre-weighed Whatman GF/C filters. The filters and sediment were dried at 60° C to a constant weight. Total particulate matter ( $\text{g m}^{-2} \text{ day}^{-1}$ ) was calculated from the dry weight, number of days the sediment trap was deployed, and the area of the trap opening. Sediment trap dimensions and methods were comparable across data sources.

***N*-mixture Model.** We used an *N*-mixture model to estimate shark abundance across the three conventional survey methods at each site while accounting for imperfect detection. The ecological process describing shark abundance was modelled as a negative binomial distribution with habitat type, substrate angle, and year as covariates. The observational process was modelled as a binomial distribution with survey duration and method as covariates. Ecological covariates were measured at each site, although site itself was not included in the model as a fixed effect. Covariates were checked for collinearity and then scaled and normalized as appropriate prior to inclusion in the model.

Model support was assessed in two ways using a maximum likelihood-based approach. First, we used the small sample size corrected version of Akaike information criterion (AICc), for which the number of sites was treated as the sample size. Second, we followed a two-step process (described in Doherty et al. 2012) in which the global covariate structure for abundance was fixed and the different combinations of detectability covariates were modelled (best model selected using AICc). Next, the detectability covariates were fixed based on this top-ranked model and the different combinations of abundance covariates were modelled. The two top-ranked models derived from this process were consistent with the three best models ( $\Delta\text{AICc} < 2$ ) obtained using our first approach (Table B.6).

Model goodness-of-fit was assessed in several ways. Negative binomial and Poisson abundance mixture models were compared using AICc, and goodness-of-fit of the top three models was examined using the R package *nmixgof* (Knape et al. 2018). Although quantile-quantile plots of the site-sum randomized quantile residuals and residual plots against fitted values for both the negative binomial and Poisson mixtures showed good fit, the negative

binomial abundance mixtures had a lower AICc value and were less overdispersed than the Poisson abundance mixtures. Using a negative binomial abundance mixture model, we then tested the identifiability of the models to determine whether abundance estimates stabilized with increasing K (Dennis et al. 2015, Kéry 2018). The top three models were each fitted with five different values of K (150, 250, 400, 600, and 1000). Abundance estimates stabilized with a K of 400 and above, whereby the abundance intercept between the two K values differed by less than 0.01 (Knape et al. 2018).

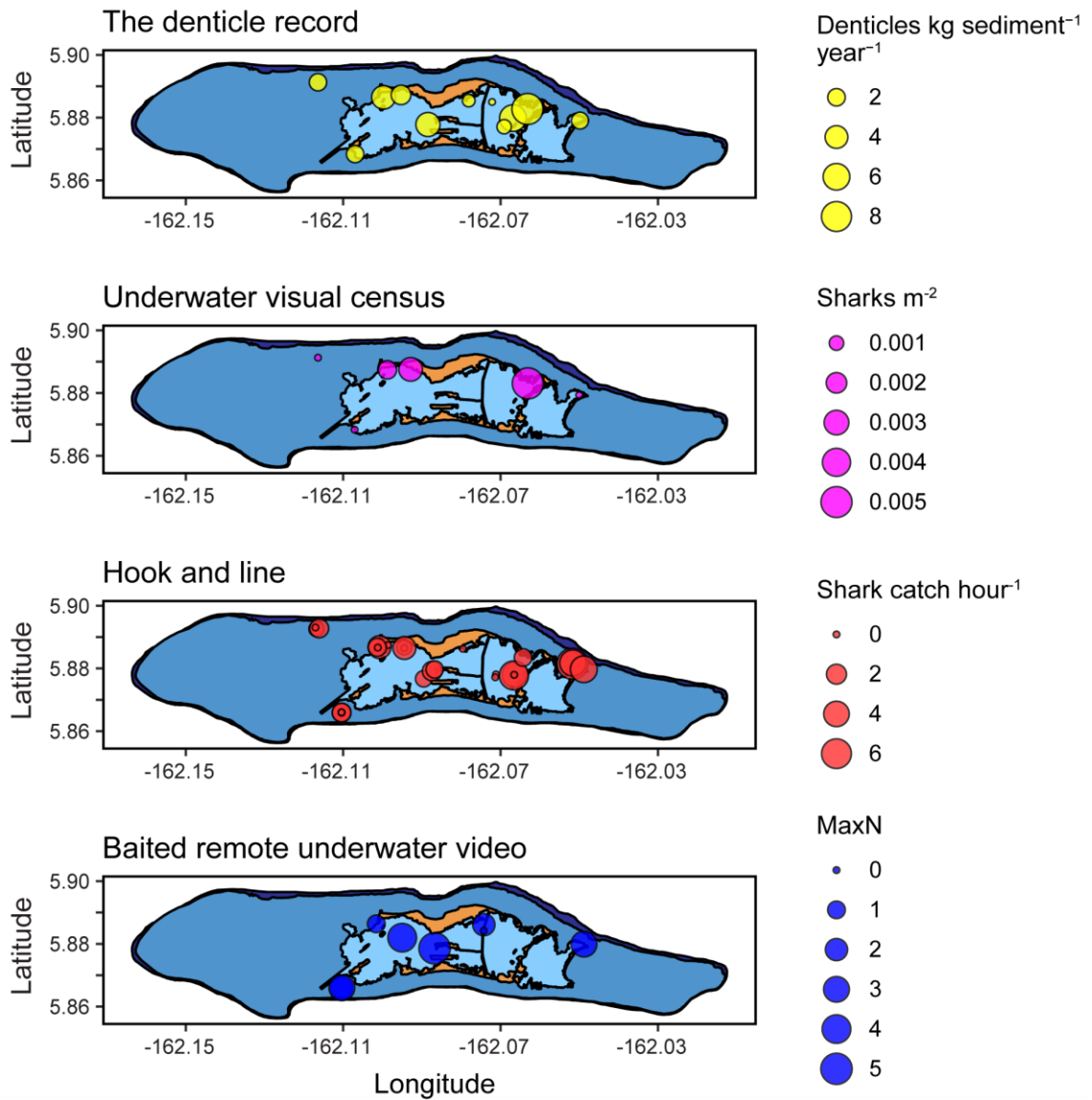
### **Characterizing *Carcharhinus melanopterus* and *Carcharhinus amblyrhynchos***

**Denticles.** We determined the relative abundances of denticle functional morphotypes on the bodies of *Carcharhinus melanopterus* and *Carcharhinus amblyrhynchos* using our denticle reference collection, paired with an observational transect across the body of one museum specimen per species. Our reference collection consists of denticles isolated from ~1 cm<sup>2</sup> pieces of skin excised from the bodies of 37 different shark species (see Dillon et al. 2017 for details). It includes denticles from 12 standardized locations across the body and fins of *C. melanopterus* and *C. amblyrhynchos*. Denticles at each sampling location were measured and classified to functional morphotype.

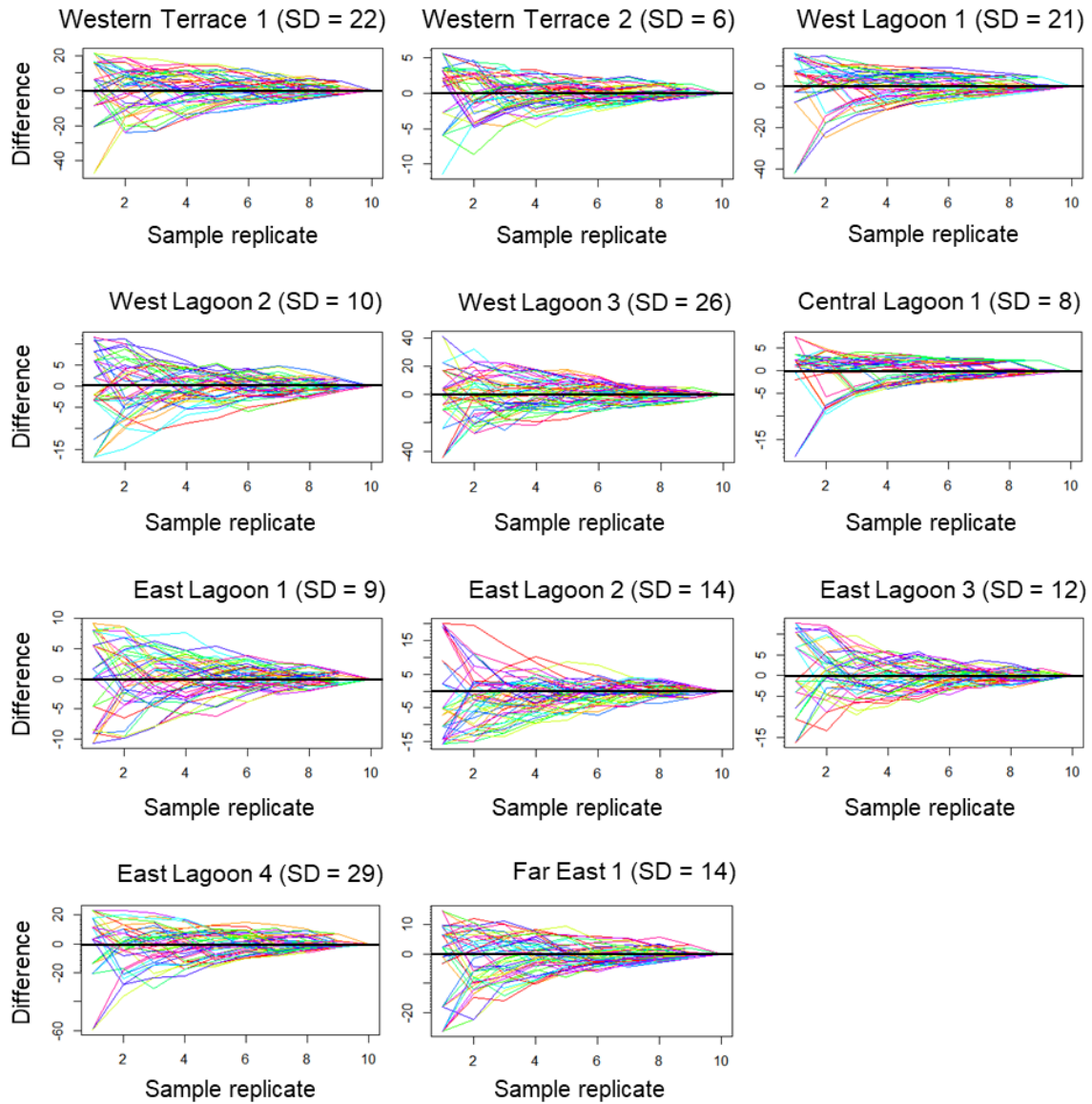
Because denticle morphology varies across a shark's body, we supplemented our observations from the reference collection with non-invasive transects to better understand how these reference denticles are represented on the bodies of *C. melanopterus* and *C. amblyrhynchos*. Two specimens from the ichthyology collection at the Smithsonian National Museum of Natural History were characterized. Similar to Ferrón & Botella 2017, the total length of each specimen was measured and used to virtually divide the shark into eleven equally sized regions (i.e. 10 dividing marks). At each dividing mark, a flexible measuring tape was laid along the dorsoventral axis of the shark. Moving from the dorsal side to the ventral side, the dominant denticle functional morphotype in a 1 cm<sup>2</sup> area was visually classified using a 40× magnification hand lens every 10 cm along the measuring tape. The dorsal, caudal, and right pectoral fin were characterized using a similar approach. Four transects (two along the fin edges and two through the center) were laid across each fin, starting at the tip of the fin. At three equally spaced points along each measuring tape, the dominant denticle functional morphotype in a 1 cm<sup>2</sup> area was visually classified. In total, this approach yielded around 60 data points per specimen. The visual classifications were cross-checked with denticles in our reference collection. The relative proportions of each

functional morphotype on the body and fins were calculated and weighted by the estimated surface area (i.e. because the fins represent a small proportion of each specimen's total surface area, yet sampling effort was nearly equal between the body and fins). The surface area of the body was treated as an ellipsoid, and the surface area of each fin was treated as a double-sided triangle. These weighted proportions were used to determine the rank abundance of each functional morphotype on the bodies of these two species.

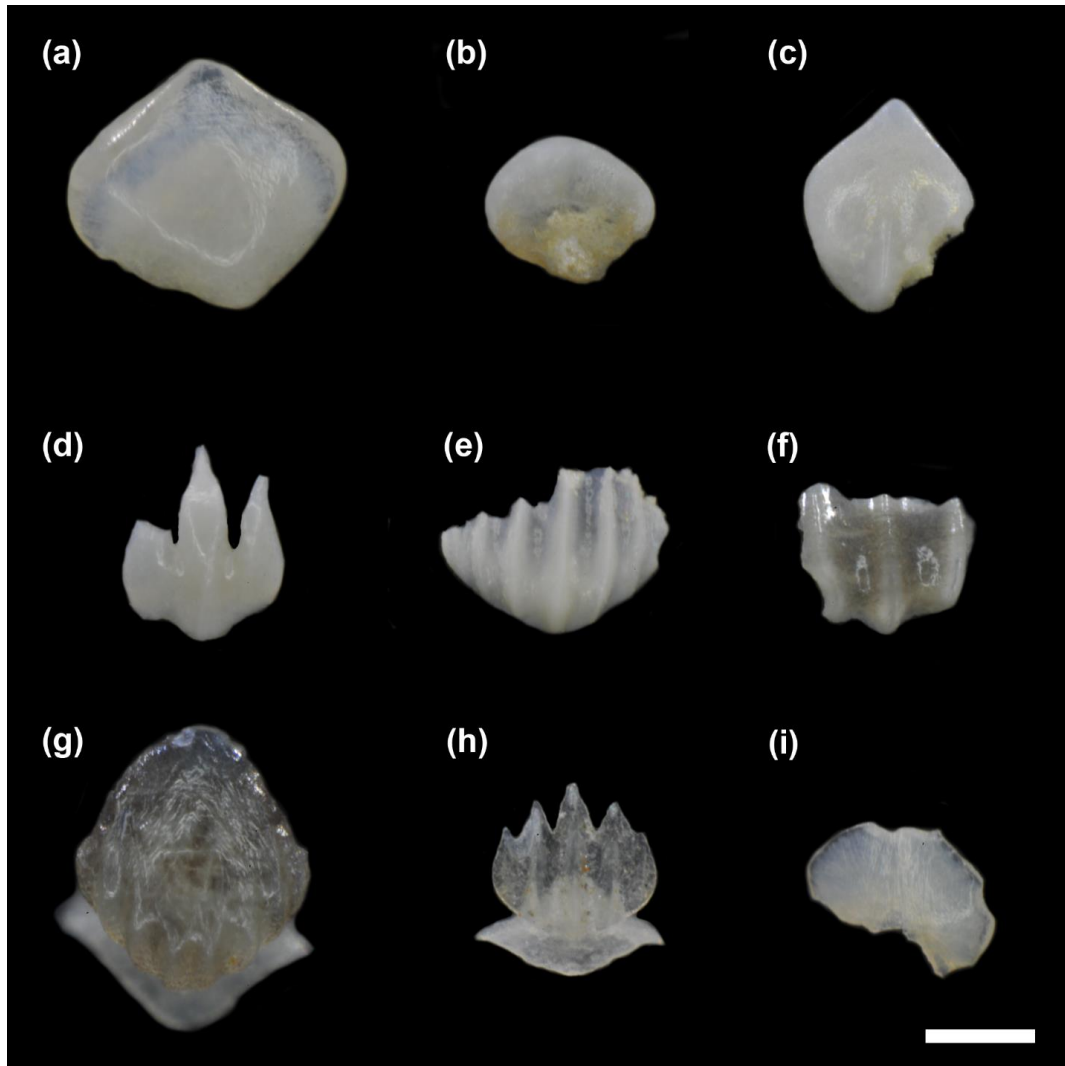
Appendix B.2 Supplementary Figures



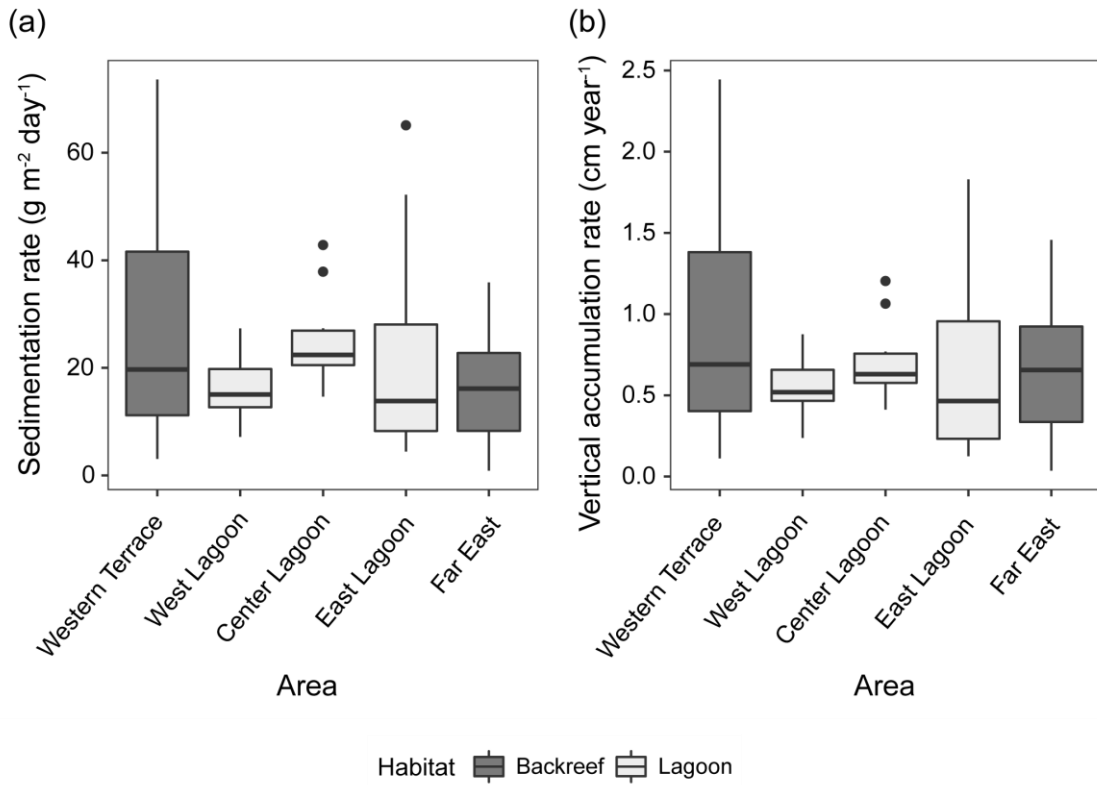
**Figure B.1** Relative shark abundances measured by the denticle record, underwater visual census, hook and line, and baited remote underwater video surveys. Circle diameters are scaled to mean shark abundance and are centered on the survey coordinates. The land is colored tan, the lagoon is colored light blue, the reef terrace and backreef are colored blue, and the forereef is colored dark blue.



**Figure B.2** Random resampling exercise plots for each sediment collection site, which show the difference between the cumulative mean denticle abundance in all samples at that site and the cumulative mean as each replicate was added to the set. After each sample replicate was added, the difference was calculated to explore how it changed with increased replication. The closer this difference was to zero (black line), the more appropriate the number of replicates was for capturing within-site variation (SD, standard deviation) in denticle abundance. Each colored line represents a single resampling run.

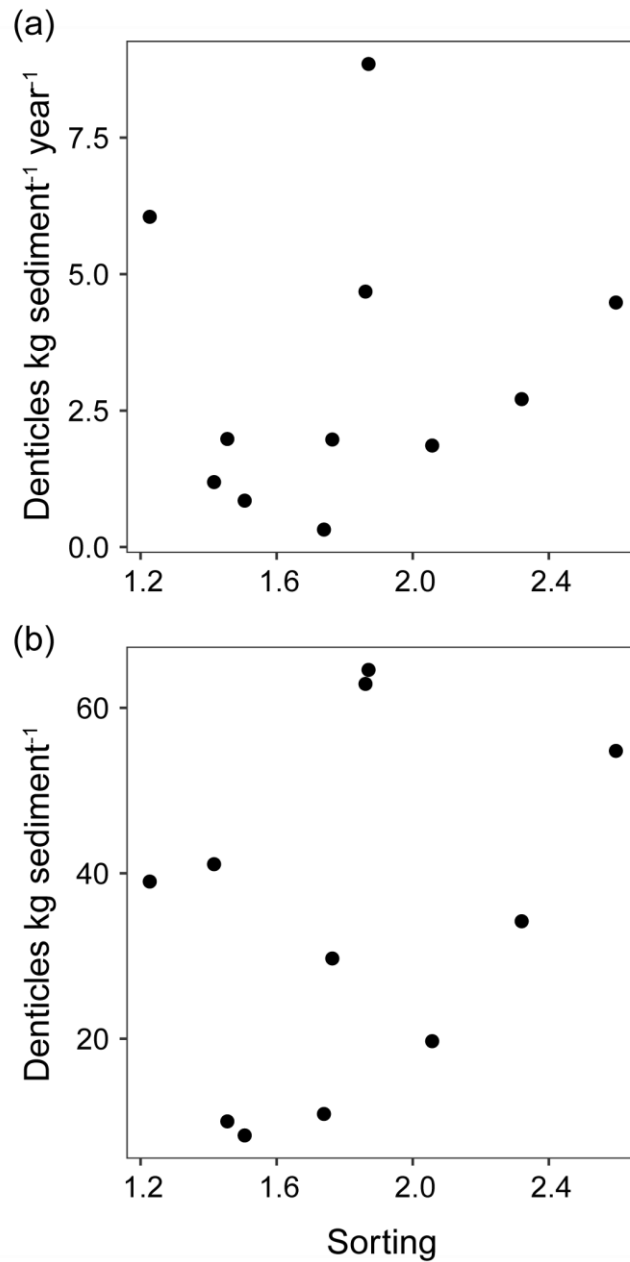


**Figure B.3** Examples of the weathering score criteria and denticle functional morphotypes. Matching descriptions of the base, crown, and peaks are in Table B.5. Denticle (h) has pristine preservation (weathering score of 0). Denticles (a), (b), and (g) are slightly weathered (weathering score from  $>0$  to 1). Denticles (c) and (d) are moderately weathered (weathering score from  $>1$  to 2). Denticles (e) and (f) are weathered (weathering score from  $>2$  to 2.9). Denticle (i) has poor preservation (weathering score of 3). Denticles (e), (f), and (h) are drag reduction, denticle (g) is ridged abrasion strength, denticles (a) and (c) are abrasion strength, and denticles (b) and (d) are generalized functions. Denticle (i) is too weathered to be identified to functional morphotype. All denticles are oriented with the crown facing upward except (b), which is inverted to display the partially present base. Scale bar = 200 $\mu$ m.

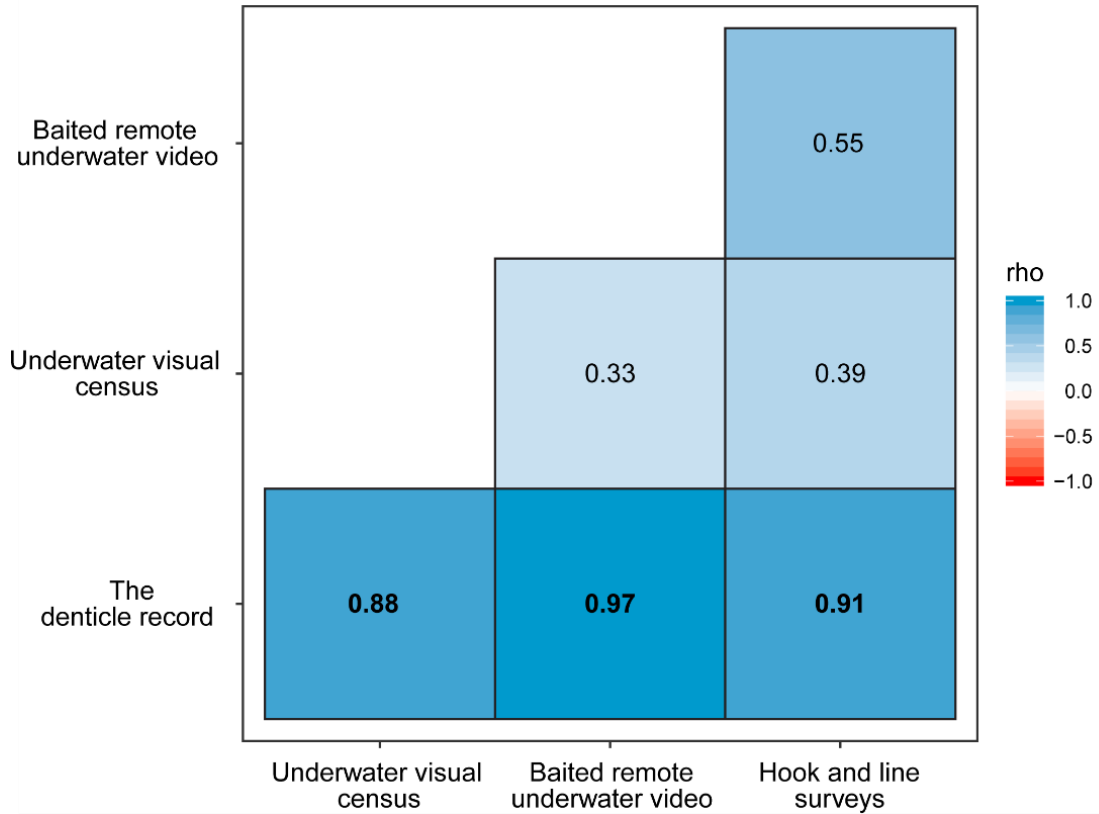


**Figure B.4** Boxplot of (a) measured sedimentation rates and (b) calculated vertical accumulation rates by area of the atoll, colored by habitat. Each boxplot shows the median (dark bar) and the interquartile range (box), and outliers are displayed as points. Sedimentation rates differed across sites ( $p < 0.0001$ ) and areas ( $p = 0.013$ ) but not habitats ( $p > 0.05$ ). Vertical accumulation rates differed across sites ( $p < 0.0001$ ) but not areas or habitats ( $p > 0.05$  for both).

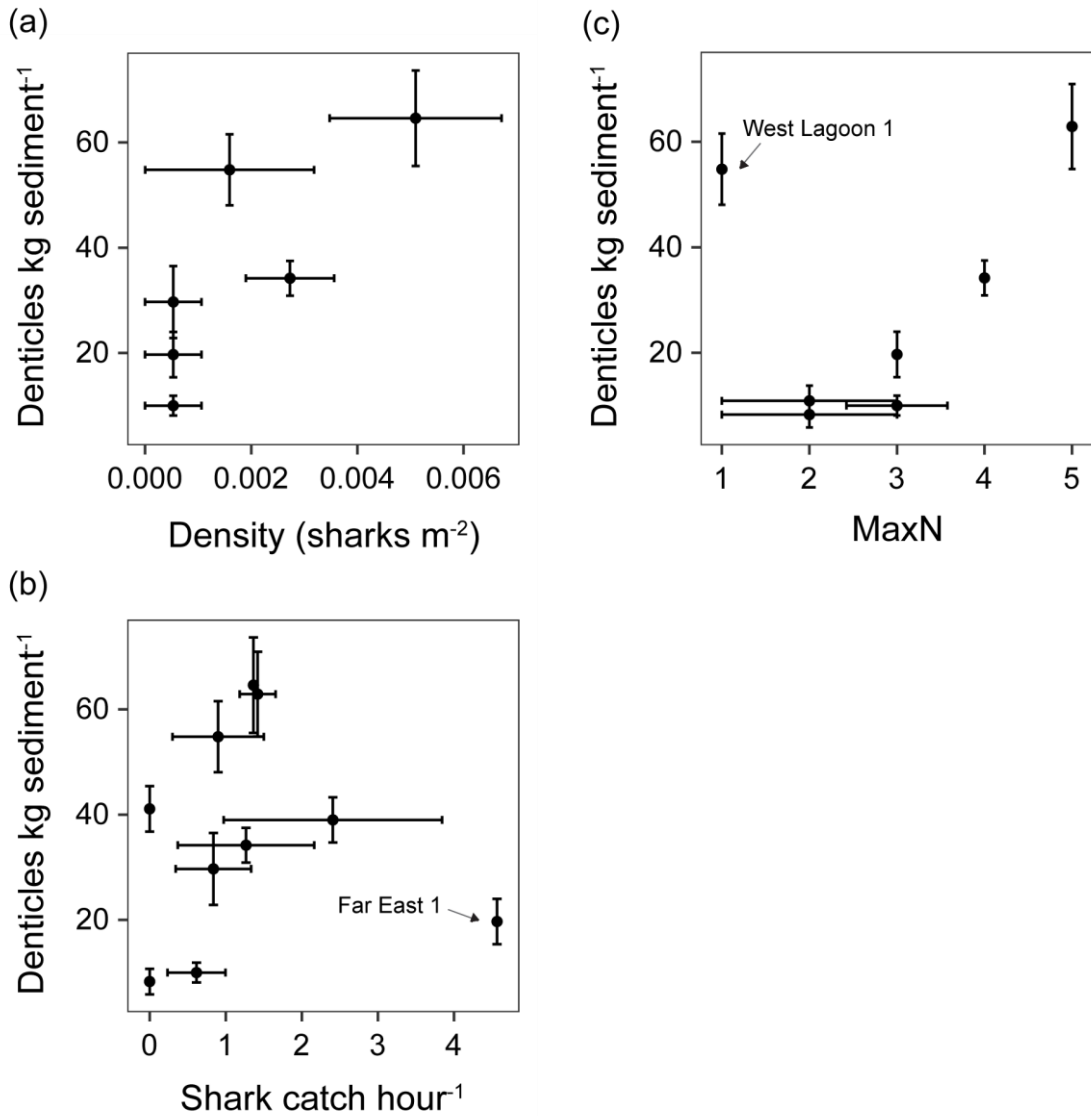




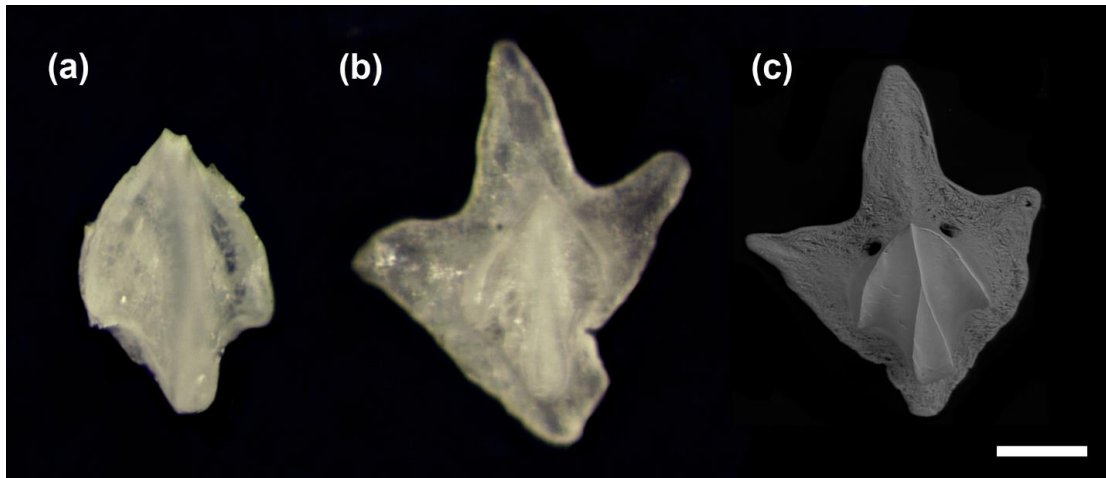
**Figure B.5** Correlation between (a) denticle accumulation rates and sorting and (b) denticle abundances and sorting. Sorting, which was calculated from the grain size distributions, was used as a proxy for depositional environment. Neither of these correlations were significant ( $p > 0.05$  for both), suggesting that in these low-energy habitats, sediments that are more sorted do not necessarily have fewer denticles.



**Figure B.6** Correlation matrix of shark abundance derived from underwater visual census surveys, baited remote underwater video surveys, hook and line surveys, and the denticle record. Spearman's rank correlation coefficients ( $\rho$ ) are shown for each pairwise comparison, with color indicating the correlation strength. Statistically significant correlations are bolded ( $p < 0.05$ ). The denticle record (denticle accumulation rate) was strongly correlated with underwater visual census (sharks  $\text{m}^{-2}$ ), hook and line (shark catch  $\text{hour}^{-1}$ ), and baited remote underwater video surveys (MaxN) for all but two individual surveys (excluded here).



**Figure B.7** Correlation between denticle abundances (denticles kg sediment<sup>-1</sup>) and (a) shark density derived from underwater visual census surveys, (b) shark catch hour<sup>-1</sup> derived from hook and line surveys, and (c) MaxN derived from baited remote underwater video surveys. Points represent mean  $\pm$  SE at each site with corresponding data.



**Figure B.8** Tiger shark (*Galeocerdo cuvier*) denticles. (a) Light microscope image of a tiger shark denticle found in the Center Lagoon compared with (b) a light microscope image of a denticle taken from the pectoral fin of a juvenile tiger shark in our reference collection and (c) a scanning electron microscope image of a denticle taken from the dorsal fin of a juvenile tiger shark in our reference collection. Note that the base is missing in (a). Scale bar = 100µm.

### Appendix B.3 Supplementary Tables

**Table B.1** Denticle characters measured for the weathering and morphometric analyses. This selection of denticle characters was refined from Dillon et al. 2017 to include characters that could be affected by weathering and characters that are important for distinguishing between functional morphotypes.

Character	Description
Crown shape	1 Circular or elliptical
	2 Lanceolate or teardrop-shaped
	3 Diamond-shaped, square, or triangular
	4 Cruciform or arrow-shaped
	5 Lobed on all sides
Crown size	$\sqrt{(\text{length} \times \text{width})}$ ; grouped into 100 $\mu\text{m}$ size classes
Crown thickness ratio	Thin: $\sqrt{(\text{length} \times \text{width})}/\text{thickness} \geq 4$
	Thick: $\sqrt{(\text{length} \times \text{width})}/\text{thickness} < 4$
Number of peaks	0 Single peak
	1 >1 peak
Presence of ridges	0 No ridges
	1 $\geq 1$ ridge
Ridge length	1 Incomplete, medially-reduced ridges
	2 Complete ridges

**Table B.2** Confusion matrix from the multinomial logistic regression model trained and validated with our denticle reference collection ( $n = 186$  training,  $n = 27$  testing). The numbers indicate the proportions of cross-validated denticles that were classified into each predicted group. Each row sums to one, and values of one across the diagonal would indicate perfect performance. The largest proportion in each row is bolded. Overall model accuracy was 84%, although accuracy varied across functional morphotypes.

Actual Group	Predicted Group				
	Abrasion Strength	Defense	Drag Reduction	Ridged Abrasion Strength	Generalized Functions
Abrasion Strength	<b>0.875</b>	0	0	0	0.125
Defense	0	<b>0.667</b>	0.333	0	0
Drag Reduction	0	0	<b>0.952</b>	0.024	0.024
Ridged Abrasion Strength	0	0.25	0.083	<b>0.667</b>	0
Generalized Functions	0	0	0.4	0	<b>0.6</b>

**Table B.3** Estimates of coefficient values (Coeff) and standard errors (SE) for covariates influencing the assignment of functional morphotypes using the multinomial logistic regression model. Coefficients were estimated using a maximum likelihood estimation approach and are expressed here as log odds. The abrasion strength functional morphotype is the reference level and likelihoods are expressed with respect to that outcome.

Predictor	Defense		Drag Reduction		Ridged Abrasion Strength		Generalized Functions	
	Coeff	SE	Coeff	SE	Coeff	SE	Coeff	SE
(Intercept)	-107.60	0.53	-135.41	0.64	-109.04	0.38	-81.27	1.10
Number of peaks	-69.43	0.00	13.22	0.39	-71.20	0.00	12.94	0.39
Presence of ridges	155.00	0.53	150.74	0.64	200.51	0.38	81.57	0.70
Ridge length	47.87	1.20	49.15	1.10	46.56	0.87	43.04	1.01
Crown size 100-200 $\mu$ m	-4.27	0.68	24.95	0.66	-48.93	0.92	40.07	0.96
Crown size 201-300 $\mu$ m	66.69	0.60	45.07	0.55	23.91	0.47	60.31	0.66
Crown size 301-400 $\mu$ m	28.84	0.88	7.46	0.58	-12.91	0.70	24.36	0.58
Crown size 401-500 $\mu$ m	-13.45	0.79	-35.11	0.64	-55.54	0.64	-16.29	0.66
Crown size 501-600 $\mu$ m	-103.43	0.00	-59.45	1.25	-114.17	0.00	-44.16	1.25
Crown size 601-700 $\mu$ m	-44.95	NaN	37.45	0.70	-33.92	0.88	55.97	0.60
Crown size 701-800 $\mu$ m	-22.04	NaN	-86.69	0.00	19.93	0.00	-81.11	0.00
Crown size 801-900 $\mu$ m	2.26	0.00	-37.28	0.00	72.12	0.00	-34.24	0.00
Crown size 901-1000 $\mu$ m	-5.40	NaN	-2.23	NaN	19.87	0.00	-2.73	0.00
Crown size >1000 $\mu$ m	-11.84	0.00	-29.58	0.00	20.59	0.00	-83.44	0.00
Crown thickness	0.70	0.94	55.58	1.27	1.37	0.70	58.01	1.16

**Table B.4** Confusion matrix from the multinomial logistic regression model trained with our denticle reference collection ( $n = 213$ ) and used to classify a subset of the denticles extracted from the sediment samples ( $n = 574$ ), compared with visual classifications of the same denticles. The numbers indicate the proportions of visually identified denticles that were classified into each predicted group by the model. Each row sums to one, and values of one across the diagonal would indicate perfect alignment between the two classification methods. The largest proportion in each row is bolded. Overall correspondence between the two methods was 75%. Note that only one denticle was visually classified as defense.

Visually Identified Group	Predicted Group				
	Abrasion Strength	Defense	Drag Reduction	Ridged Abrasion Strength	Generalized Functions
Abrasion Strength	<b>1</b>	0	0	0	0
Defense	0	<b>0</b>	0	<b>1</b>	0
Drag Reduction	0	0.007	<b>0.729</b>	0.075	0.188
Ridged Abrasion Strength	0.157	0.014	0.029	<b>0.786</b>	0.014
Generalized Functions	0.091	0	0.273	0.068	<b>0.568</b>

**Table B.5** Weathering score criteria. Weathering was determined based on the state of the denticle base, crown, and peaks, which were each assigned a point value. Each value was multiplied by a scaling factor to give equal weight to the base, crown, and peaks. Scaled point values were then summed to yield the final score, which ranged from zero (pristine) to three (poorly preserved).

Character	Description	Points	Scaling Factor	Example (Fig. S3)
Base	Present	0	1/2	g, h
	Partially present	1	1/2	b
	Absent	2	1/2	a, c, d, e, f, i
Crown	Fully intact	0	1/3	a, b, d, g, h
	Light damage (90-99% intact)	1	1/3	c, e
	Damage (51-89% intact)	2	1/3	f
	Fragment ( $\leq 50\%$ intact)	3	1/3	i
Peaks	Intact	0	1	a, b, c, g, h
	Fractured	1	1	d, e, f, i

**Table B.6**  $N$ -mixture model comparisons to identify covariates influencing shark abundance ( $\lambda$ ) and detection probability ( $p$ ). AICc values were used for model selection given low sample size, and  $\hat{c}$  was used as an indicator of overdispersion, whereby values farther from one provide greater evidence of overdispersion. The top-ranked models ( $\Delta\text{AICc} < 2$ ) are bolded, and the asterisks indicate the best models selected using the two-step approach.

Model	AICc	$\Delta$ AICc	AICc Weight	Log Likelihood	No. Parameters	$\hat{c}$
<b><math>p(\text{method} + \text{effort}), \lambda(.)</math></b>	212.39	0	0.29	-98.37	6	1.20
<b><math>p(\text{effort}), \lambda(.)</math> *</b>	213.07	0.68	0.21	-101.74	4	1.26
<b><math>p(\text{effort}), \lambda(\text{year})</math> *</b>	213.49	1.1	0.17	-95.32	8	1.20
$p(\text{effort} + \text{method}), \lambda(\text{angle})$	215.06	2.68	0.08	-97.99	7	1.13
$p(\text{effort}), \lambda(\text{angle})$	215.45	3.06	0.06	-101.48	5	1.21
$p(\text{effort} + \text{method}), \lambda(\text{habitat})$	215.6	3.22	0.06	-98.26	7	1.21
$p(\text{effort}), \lambda(\text{habitat})$	215.65	3.26	0.06	-101.57	5	1.24
$p(\text{effort}), \lambda(\text{habitat} + \text{year})$	217.17	4.78	0.03	-95.08	9	1.22
$p(\text{effort}), \lambda(\text{angle} + \text{year})$	217.43	5.04	0.02	-95.21	9	1.20
$p(\text{effort}), \lambda(\text{habitat} + \text{angle})$	218.59	6.2	0.01	-101.47	6	1.26
$p(\text{effort} + \text{method}), \lambda(\text{habitat} + \text{angle})$	218.8	6.41	0.01	-97.97	8	1.18
$p(\text{effort} + \text{method}), \lambda(\text{year})$	220.34	7.95	0.01	-94.38	10	1.23
$p(\text{effort}), \lambda(\text{habitat} + \text{angle} + \text{year})$	221.74	9.35	0	-95.08	10	1.29
$p(\text{effort} + \text{method}), \lambda(\text{angle} + \text{year})$	225.35	12.97	0	-94.34	11	1.33
$p(\text{effort} + \text{method}), \lambda(\text{habitat} + \text{year})$	225.42	13.03	0	-94.38	11	1.29
$p(\text{effort} + \text{method}), \lambda(\text{habitat} + \text{angle} + \text{year})$	230.89	18.51	0	-94.27	12	1.42
$p(\text{method}), \lambda(.)$	284.33	71.95	0	-135.92	5	1.72
$p(\text{method}), \lambda(\text{habitat})$	286.35	73.96	0	-135.35	6	1.48
$p(\text{method}), \lambda(\text{angle})$	286.65	74.26	0	-135.5	6	1.63
$p(.), \lambda(.)$	286.72	74.33	0	-139.9	3	1.98
$p(.), \lambda(\text{angle})$	286.8	74.41	0	-138.6	4	1.49
$p(.), \lambda(\text{habitat})$	287.51	75.12	0	-138.95	4	1.39
$p(.), \lambda(\text{year})$	289.18	76.8	0	-135.05	7	1.73
$p(.), \lambda(\text{habitat} + \text{angle})$	289.55	77.16	0	-138.52	5	1.43
$p(\text{method}), \lambda(\text{habitat} + \text{angle})$	289.73	77.34	0	-135.32	7	1.55
$p(.), \lambda(\text{angle} + \text{year})$	291.02	78.63	0	-134.08	8	1.58
$p(.), \lambda(\text{habitat} + \text{year})$	291.91	79.52	0	-134.53	8	1.57
$p(.), \lambda(\text{habitat} + \text{angle} + \text{year})$	295.15	82.77	0	-134.08	9	1.64
$p(\text{method}), \lambda(\text{year})$	296.42	84.04	0	-134.71	9	1.88
$p(\text{method}), \lambda(\text{angle} + \text{year})$	299.02	86.63	0	-133.72	10	1.69
$p(\text{method}), \lambda(\text{habitat} + \text{year})$	299.79	87.4	0	-134.11	10	1.67
$p(\text{method}), \lambda(\text{habitat} + \text{angle} + \text{year})$	304.07	91.69	0	-133.7	11	1.73



**Table B.7** Estimates of coefficient values for covariates influencing shark abundance ( $\lambda$ ) and detection probability ( $p$ ) in the three top-ranked negative binomial  $N$ -mixture models.

Model	$\lambda$ (Int)	$\lambda$ (year2013)	$\lambda$ (year2014)	$\lambda$ (year2015)	$\lambda$ (year2016)	$p$ (Int)	$p$ (methodcpue)	$p$ (methoduvc)	$p$ (effort)	$\alpha$
$p$ (method + effort), $\lambda$ (.)	2.77	NaN	NaN	NaN	NaN	-3.65	0.35	2.01	2.10	1.42
$p$ (effort), $\lambda$ (.)	2.95	NaN	NaN	NaN	NaN	-2.89	NaN	NaN	1.29	1.34
$p$ (effort), $\lambda$ (year)	4.46	-1.68	-1.04	-1.01	-2.18	-3.56	NaN	NaN	1.85	1.96

**Table B.8** Predicted shark abundance and standard error (SE) at each site using the top-ranked  $N$ -mixture models.

Site	Habitat	Shark Abundance	SE
Western Terrace 1	backreef	40.58	3.04
Western Terrace 2	backreef	29.23	1.34
West Lagoon 1	lagoon	29.79	1.62
West Lagoon 2	lagoon	33.59	1.84
West Lagoon 3	lagoon	23.13	3.02
Central Lagoon 1	lagoon	19.01	4.38
East Lagoon 1	lagoon	11.82	6.40
East Lagoon 2	lagoon	16.21	5.28
East Lagoon 3	lagoon	21.20	3.49
East Lagoon 4	lagoon	35.78	1.86
Far East 1	backreef	35.78	1.70

**Table B.9** Sensitivity analysis using the mean, standard deviation ( $\pm 1\sigma$ ), maximum, and minimum sedimentation rate at each site. Spearman's rank correlation coefficients ( $\rho$ ), test statistics ( $S$ ), and adjusted  $p$ -values are shown for each correlation. The values in parentheses show the same correlation with the outlier removed for the hook and line (Far East 1) and baited remote underwater video (West Lagoon 1) surveys.

Sedimentation Rate Applied		Conventional Surveys								
		Underwater Visual Census			Hook and Line			Baited Remote Underwater Video		
		S	$\rho$	p-value	S	$\rho$	p-value	S	$\rho$	p-value
Denticle accumulation rate	Mean sedimentation rate	4.19	0.88	0.031	66.70 (10.54)	0.60 (0.91)	0.1 (0.0009)	31.56 (1.01)	0.44 (0.97)	0.49 (0.002)
	Mean sedimentation rate $+1\sigma$	4.19	0.88	0.031	99.80 (26.61)	0.40 (0.78)	0.39 (0.02)	31.56 (1.01)	0.44 (0.97)	0.49 (0.002)
	Mean sedimentation rate $-1\sigma$	10.56	0.7	0.18	54.67 (12.55)	0.67 (0.90)	0.05 (0.002)	31.56 (1.01)	0.44 (0.97)	0.49 (0.002)
	Maximum sedimentation rate	23.31	0.33	0.78	52.66 (18.58)	0.68 (0.85)	0.045 (0.006)	37.67 (7.19)	0.33 (0.79)	0.71 (0.09)
	Minimum sedimentation rate	2.06	0.94	0.008	128.89 (38.66)	0.22 (0.68)	0.82 (0.067)	33.60 (13.37)	0.40 (0.62)	0.56 (0.29)
Denticle abundance	None	4.19	0.88	0.031	109.83 (50.71)	0.33 (0.58)	0.52 (0.16)	35.63 (5.13)	0.36 (0.85)	0.63 (0.046)

**Table B.10** Output from the multiple regression models.

Response	Predictors	Estimate	Standard Error	t-value	p-value
Denticle accumulation rate	(Intercept)	-5.64	3.26	-1.73	0.12
	Shark abundance	0.22	0.09	2.50	0.037
	Habitat (lagoon)	4.15	1.7	2.44	0.041
Denticle abundance	(Intercept)	-36.75	24.72	-1.49	0.18
	Shark abundance	1.61	0.65	2.46	0.04
	Habitat (lagoon)	37.96	12.93	2.93	0.019

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## ***Appendix C: Chapter 4***

### Appendix C.1 Supplementary Methods

**Multinomial Logistic Regression for Denticle Classification.** Multinomial logistic regression was used to verify the visual denticle classifications since denticle functional morphotypes fall along a morphological continuum (1, 2). We first characterized the denticles in the reference collection, which comprises 37 shark species with a focus on reef-associated sharks (2), as well as the intact denticles recovered from the sediments ( $n = 572$ ). The crown, peaks, and ridges were measured using a stereo microscope. Denticle characters have been previously described in detail (2).

The multinomial logistic regression model, built using the R package *nnet*, was trained and validated on the reference collection denticles ( $n = 213$ ) to determine its accuracy (Table C.8). After using variance inflation factors to detect multicollinearity, the model was built with ten denticle characters as predictors of functional morphotype (Table C.9). The denticles were split into  $n$  groups ( $n = 2, 3, 4, 5, 6, 7, \text{ or } 8$ ), and either  $n$  or  $n-1/n$  randomly selected specimens were used to train the classification of the remaining denticles (following ref. 3). Accuracy increased with training set size, so we report results from a training set of 186 denticles (tested on 27 denticles). The classification algorithm was run 2,000 times, and the overall mean accuracy was 85% (Table C.8).

The multinomial logistic regression model was then trained with all the reference collection denticles and used to classify the denticles recovered from the sediments (Table C.9). There was a 79% correspondence between the visual classifications and the model predictions (Table C.10). This correspondence was relatively consistent across denticles recovered from the mid-Holocene (80%) and modern (78%) reefs.

The visual classifications were used in all reported analyses. However, the classifications derived from the multinomial logistic regression model produced similar patterns, including strong support for the selective loss of drag reduction denticles. Using the predicted classifications, drag reduction accumulation rates declined by 79%, whereas ridged abrasion strength and abrasion strength accumulation rates only declined by 74% and 69%, respectively. Furthermore, the shift in denticle assemblage composition between the mid-Holocene and modern time periods was still statistically significant (PERMANOVA  $F =$

3.07,  $p = 0.023$ ). Our results were therefore not overly sensitive to whether the visual classifications or multinomial logistic regression predictions were used.

**Temporal Context and Reef Accretion Rates.** Radiometric dates were used to establish the temporal context at each site. To estimate the age and accretion rate of the mid-Holocene reef, we used Uranium–Thorium (U–Th) dates on seven *Acropora cervicornis* coral pieces in addition to published calibrated radiocarbon ( $^{14}\text{C}$ ) dates on six *Acropora palmata* coral pieces (4) (Table C.1). Corals were collected from trenches excavated at three localities on the mid-Holocene reef, which spanned an elevation of -1.1 m to -3.4 m from mean sea level (see ref. 4 for stratigraphic sections). The elevations of these dated corals overlapped with those of the bulk samples, which covered a stratigraphic depth of ~10 cm at elevations ranging from ~-0.5 to -1.5 m from mean sea level. On each modern reef, we used published U–Th dates on *Porites* sp. pieces recovered from the top ~50 cm of reef matrix cores (5), with the assumption that the denticle record did not truncate alongside the termination in reef accretion that occurred at the core tops [e.g. the uppermost U–Th dates were not modern at two of the three sites (5)].

Reef accretion rates were estimated using linear interpolation between the radiometric dates after removing age reversals (Fig. C.13). On the mid-Holocene reef, we calculated the accretion rate between consecutive dates in each trench (Table C.1), using the differences in depth (elevation) and age. The radiocarbon dates on pieces of *A. palmata* defined a faster reef accretion rate than the U–Th dates on pieces of *A. cervicornis* (Fig. C.13), potentially due to differences in how these two coral growth forms accrete, the larger analytical uncertainty of radiocarbon dates, or spatial and temporal variability in the marine reservoir correction values (6, 7). Due to these methodological differences and because radiometric dates were not available at all sampling localities, the overall mean accretion rate across the three trenches was used to determine the denticle accumulation rate in each mid-Holocene sample. On each modern reef, we calculated the accretion rate using the uppermost two U–Th dates from the reef matrix cores after accounting for compaction (Table C.1). The amount of time encompassed by a stratigraphic depth of ~10 cm (sampling depth), based on these accretion rates, was used to correct the denticle abundances in each mid-Holocene and modern sample to yield denticle accumulation rates, or the number of denticles accumulating per kilogram sediment per year. On average, the modern samples

encompassed ~3–18-fold more time than the mid-Holocene samples, consistent with the slowdown in reef accretion documented over the last couple of millennia in the region (5).

Finally, we conducted a sensitivity analysis to assess the influence of our reef accretion calculations and analytical age uncertainties on the declines reported in denticle accumulation, particularly since the contrasting accretion rates of the mid-Holocene and modern reefs were found to dictate the magnitude of the calculated difference in denticle accumulation rates. The maximum and minimum number of years that each bulk sample could encompass (based on the reef accretion rates and assuming little mixing or reworking) was determined from the  $2\sigma$  errors on each radiometric date, and the analyses were re-run to calculate the percent change in denticle accumulation over time and to test for statistical significance (Table C.2).

**Uranium–Thorium Dating Methods.** Coral samples were prepared for U–Th dating and measured on a Nu Plasma Multi-Collector Inductively Coupled Mass Spectrometer (MC-ICP-MS) in the Radiogenic Isotope Facility at the University of Queensland. Sample preparation protocols have been previously provided in detail (8). Briefly, corals were stripped of surficial detritus and encrusting organisms using a hand-held Dremel saw with a diamond blade. Sub-samples were crushed to ~sand size in an agate mortar and pestle and soaked in 15%  $\text{H}_2\text{O}_2$  overnight to remove organic contaminants. Samples were then ultrasonicated multiple times in Milli-Q water (~18.2  $\text{M}\Omega\cdot\text{cm}$  resistivity) and dried on a hot plate set to  $60^\circ\text{C}$  overnight. Approximately 150 mg of the crushed and cleaned samples were hand-picked under a binocular microscope to select for the best aragonite (no detritus or alteration). Sample spiking with  $^{233}\text{U}$ – $^{229}\text{Th}$  mixed tracer, digestion, and U–Th column chemistry procedures are available in previous publications (8, 9). U–Th data were calculated using Isoplot 3.76 (10). Activity ratios were calculated from atomic ratios using the following decay constants:  $\lambda^{238} = 1.55125 \times 10^{-10} \text{ y}^{-1}$  (11),  $\lambda^{234} = (2.8262 \pm 0.0057) \times 10^{-6} \text{ y}^{-1}$ , and  $\lambda^{230} = (9.158 \pm 0.028) \times 10^{-6} \text{ y}^{-1}$  (12). U–Th ages were corrected for non-radiogenic or detrital  $^{230}\text{Th}$  contributions using a two-component mixing equation (available in ref. 9).

**Interpreting Denticle Accumulations as a Proxy for Shark Abundance.** One important methodological consideration when using the denticle record to reconstruct shark communities is the relationship between denticle accumulation and shark abundance. The interpretation of denticle assemblages is complicated by their disarticulated, multi-element

nature, whereby a single shark leaves a scattered composite of many isolated denticles in sediments. In these assemblages, it is challenging to count individuals and determine absolute abundances.

Previous work has established a positive correlation between denticle accumulation and shark abundance across low-energy sampling sites (representing a shark density gradient) for reef shark communities in aggregate, demonstrating the ecological fidelity of denticle assemblages as a proxy for relative shark abundance (13). Following from this ground truthing work, we measured absolute denticle accumulation (corrected by an age-depth model) as well as the relative abundance of each functional morphotype in this study. Calculating both absolute and relative denticle abundances provides a robust depiction of community assembly through time. For example, a relative change could be caused by either a shift in the absolute abundance of a taxon of interest or, alternatively, by shifts in the absolute abundances of other taxa in the assemblage (14). Measuring absolute denticle abundances can help distinguish between a meaningful ecological change and an artifact of proportional math. Yet, interpreting these absolute denticle abundances as absolute shark abundances requires additional information.

To generate shark density estimates from denticle accumulations, we would need at a minimum:

- 1) Measurements of denticle shedding rates, either at the species or functional level, to quantify and constrain variation in sharks' input into the sediment record;
- 2) Denticle densities (denticles  $\text{mm}^{-2}$ ), which vary across shark species (15), and shark body sizes;
- 3) Denticle identifications at the same taxonomic or functional level as the denticle shedding rate and density data;
- 4) Taphonomic analyses to account for selective preservation or other taphonomic biases which could distort measurements of abundance (16);
- 5) Assessments of reef accretion (or sedimentation) rates and time-averaging; and
- 6) Additional calibration work measuring denticle accumulations across regions with different known shark densities.

Given the investment and challenges associated with obtaining such information, potential variability and uncertainty in these parameters, and the influence of sedimentological processes and preservational controls on perceived abundances in the



fossil record, we recommend exercising caution when inferring shark densities from measurements of denticle accumulation. Rather, we maintain a conservative interpretation of the denticle record in this study, rooted in the relationship between denticle accumulation and relative differences in shark abundance.

**Exploring Changes in the Perceived Ecological State of Sharks.** Records of sharks in Caribbean Panama were compiled using backward and forward searches in Google Scholar and Google Books using the following keywords and their Spanish translations: “shark\*”, “fin”, “nurse”, “hammerhead”, “requiem”, “Ginglymostoma\*”, “Sphyrn\*”, and “Carcharhin\*”. Both modern and historical place names were examined in the texts to ensure that any observations could be geolocated to the Caribbean coast of Panama. A total of 91 records from 41 unique sources were recovered (Table C.5; see below for the full list of sources). These records were separated into seven cultural periods in Panama’s history, which were described in terms of resource use (Table C.6). Cultural periods were not derived from the state of marine resources to avoid circularity (following ref. 17). Earlier cultural periods were longer than later cultural periods.

To evaluate the disparate data types compiled in our literature search, we applied established criteria (17–19) to assign a semi-quantitative ecological state to each cultural period based on perceived shark abundance (Table C.7). Ecological states were determined using the data in aggregate for each cultural period, and they were based on the most frequent state given the potential for variation in perceptions of shark abundance. The ecological state of sharks in the pre-human cultural period was assigned to be pristine (following ref. 17). We used the same evaluation criteria as previous studies to enable comparison, and the sources analyzed were largely independent (e.g. only eight overlapping sources with ref. 17).

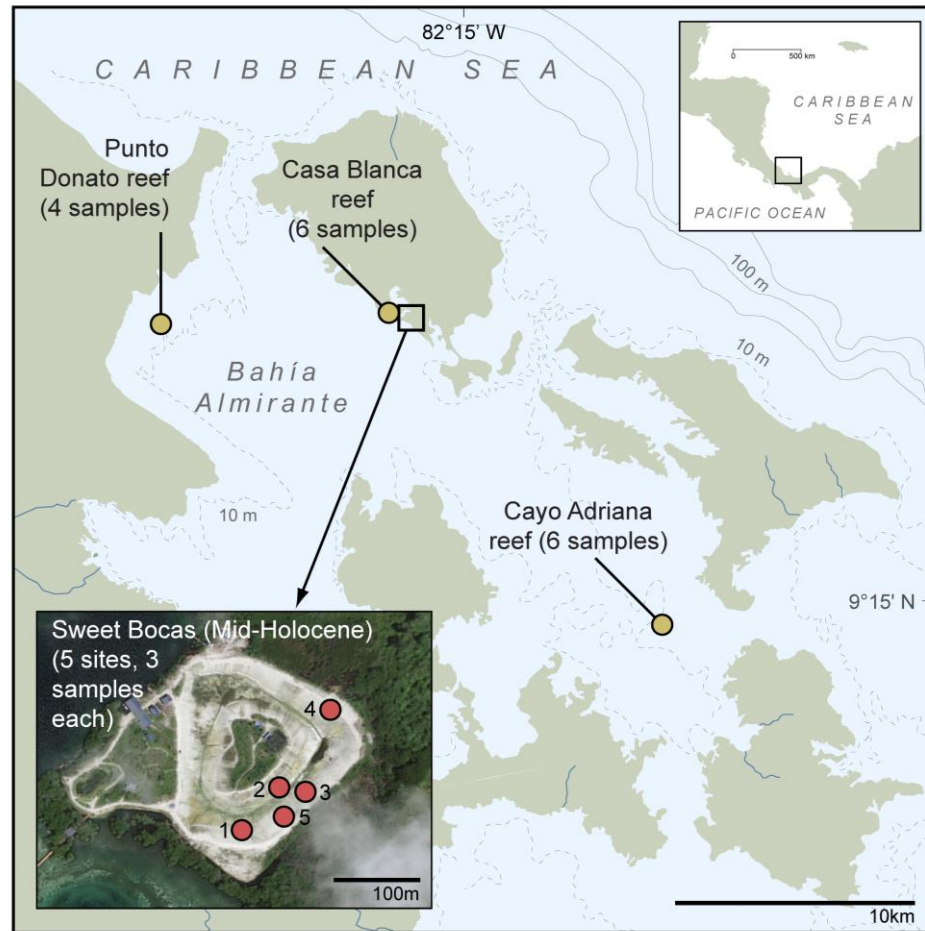
To constrain personal biases when interpreting the accounts, they were reviewed by 17 individuals, most of whom identified as having a background in ecology, environmental science, paleobiology, or the geosciences (IRB Protocol #3-20-0211). Identifying information was removed from the metadata prior to evaluation, and the cultural periods were blinded (following ref. 20). Respondents were asked to spend a maximum of 60 min reviewing the documents, which was determined to be a reasonable time limit via several preliminary trials, and they took an average of 52 min to complete the evaluation ( $SD = 10$

min). Respondents were also asked to report how confident they were in each of their responses (high, neutral, or low) and to provide a short justification for each response.

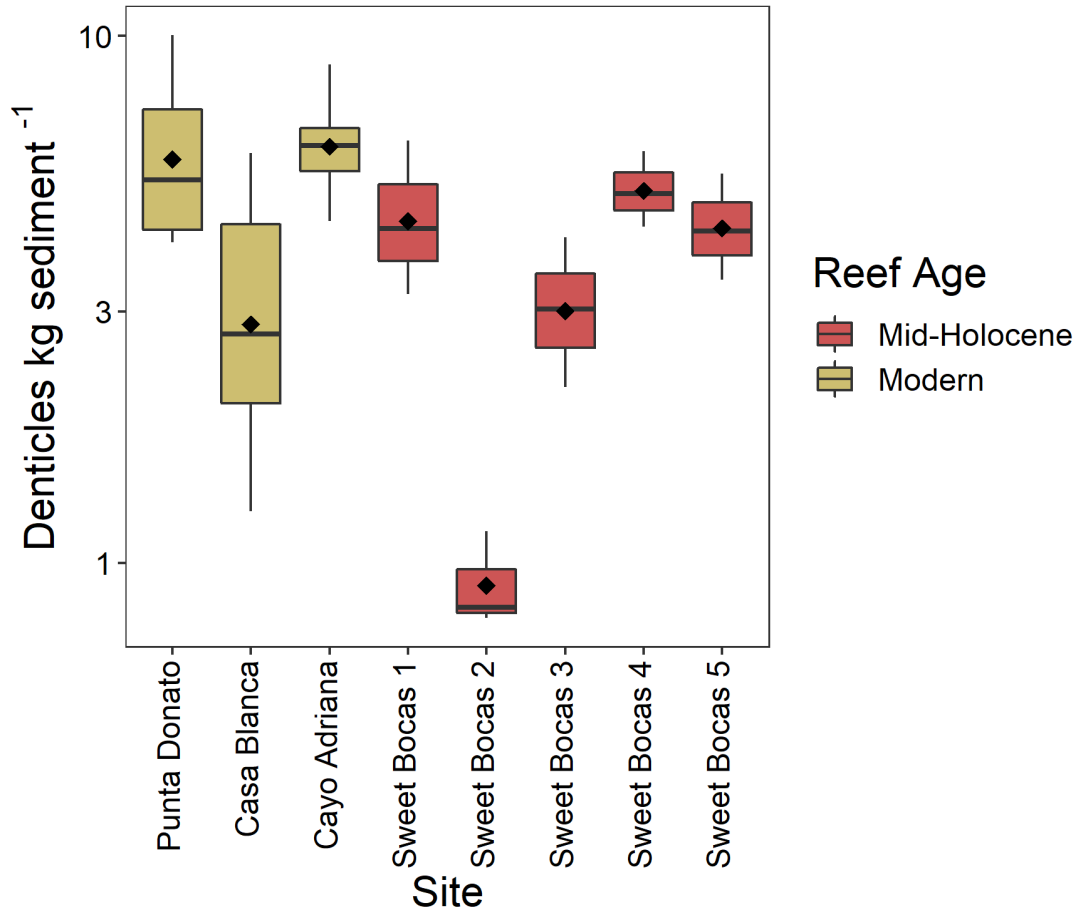
**Survey of Shark Catches in Colón, Panama.** To assess the composition of shark catches in Caribbean Panama, we surveyed shark landings at the Los Costeños pier, located in the city of Colón. This pier represents one of the main landing sites in the Colón province, which borders Panama's Caribbean coast. The pier was surveyed two to three times per month from January to October 2018 and from February to April 2019. In total, the surveys spanned 13 months, with a mean of 2 surveys per month ( $n = 28$  surveys, representing 280 fishing hours). We conducted the surveys during the unloading and processing of the catch, which usually began between 07:30 h and 09:00 h. During this time, sharks on each boat were counted and identified to the lowest taxonomic resolution possible (usually species) using published keys (21–23).

Fishing gear and effort were also described during the survey period. Artisanal fishermen in the region typically use gillnets (often ~182 m in length and 3 m high, with a mesh size of 7.9 cm), and the fishing day is ~10 h long, from the time the boats leave (18:00 h) until they return the next day (07:00 h). The fishery is multi-specific and mainly targets teleost fish, including snappers (Lutjanidae), snooks (Centropomidae), scombrids (Scombridae), and corvina (Sciaenidae). Many of the sharks landed and recorded in these surveys were caught as bycatch.

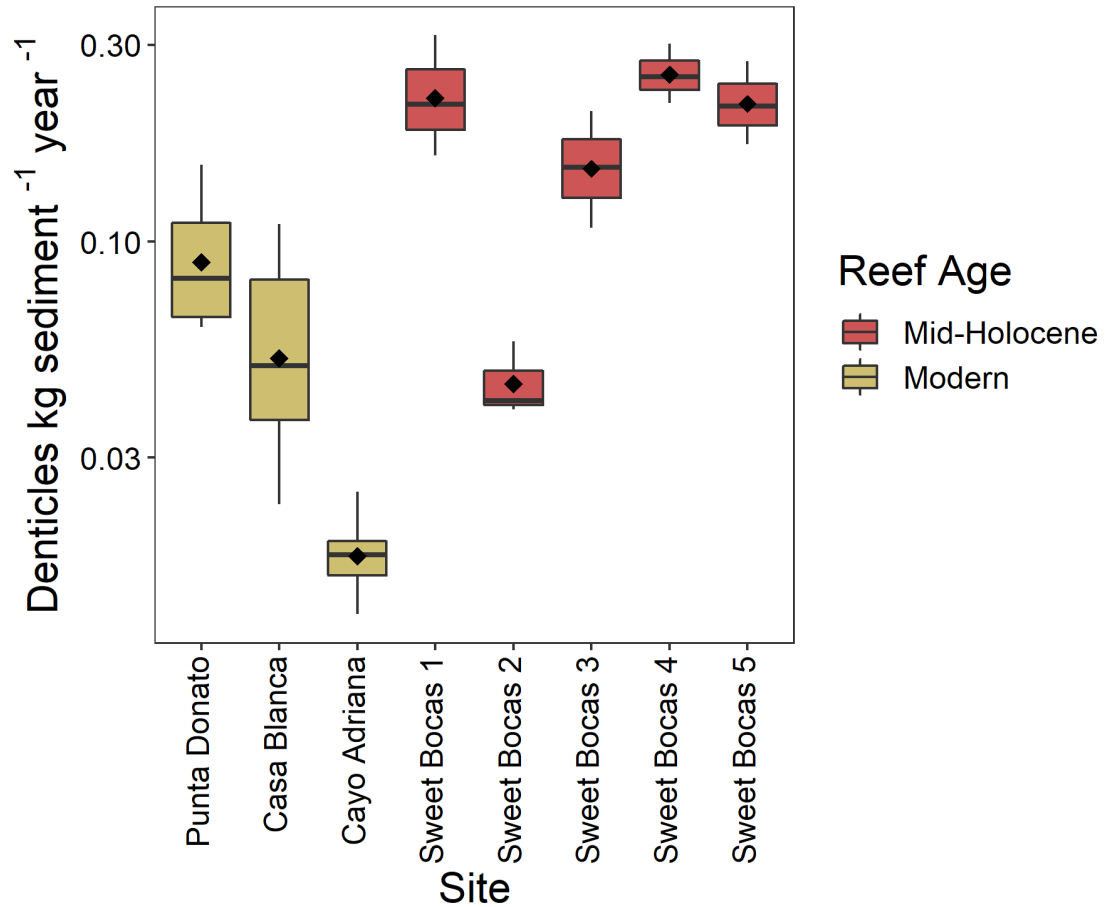
Appendix C.2 Supplementary Figures



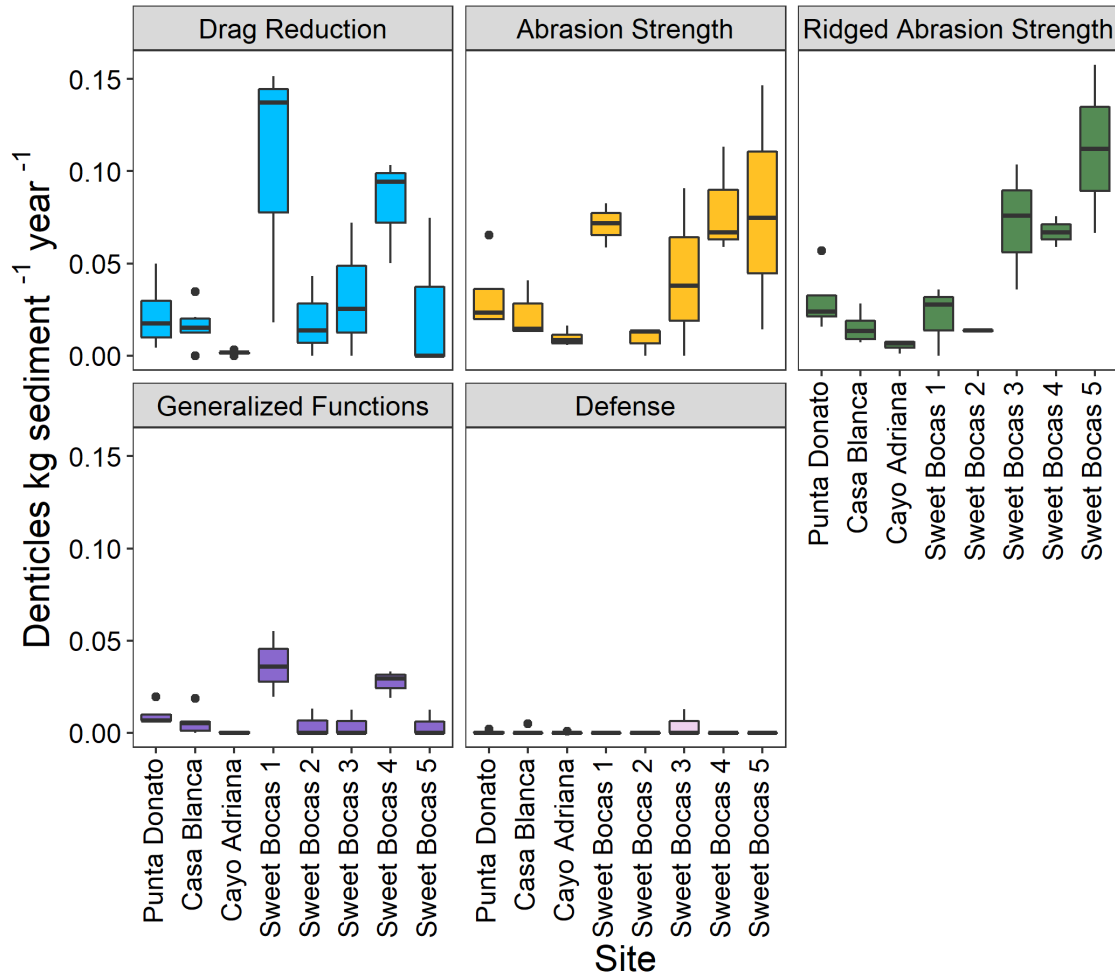
**Figure C.1** Map of modern (gold circles) and mid-Holocene (inset red circles) sampling sites in Almirante Bay, Bocas del Toro, Caribbean Panama. Sample sizes describe the number of ~9 kg bulk sediment samples collected at each site. Map is centered on 9°15' N and 82°15' W. Inset satellite image credit: Copyright © 2009 Microsoft Corporation/Bing Maps.



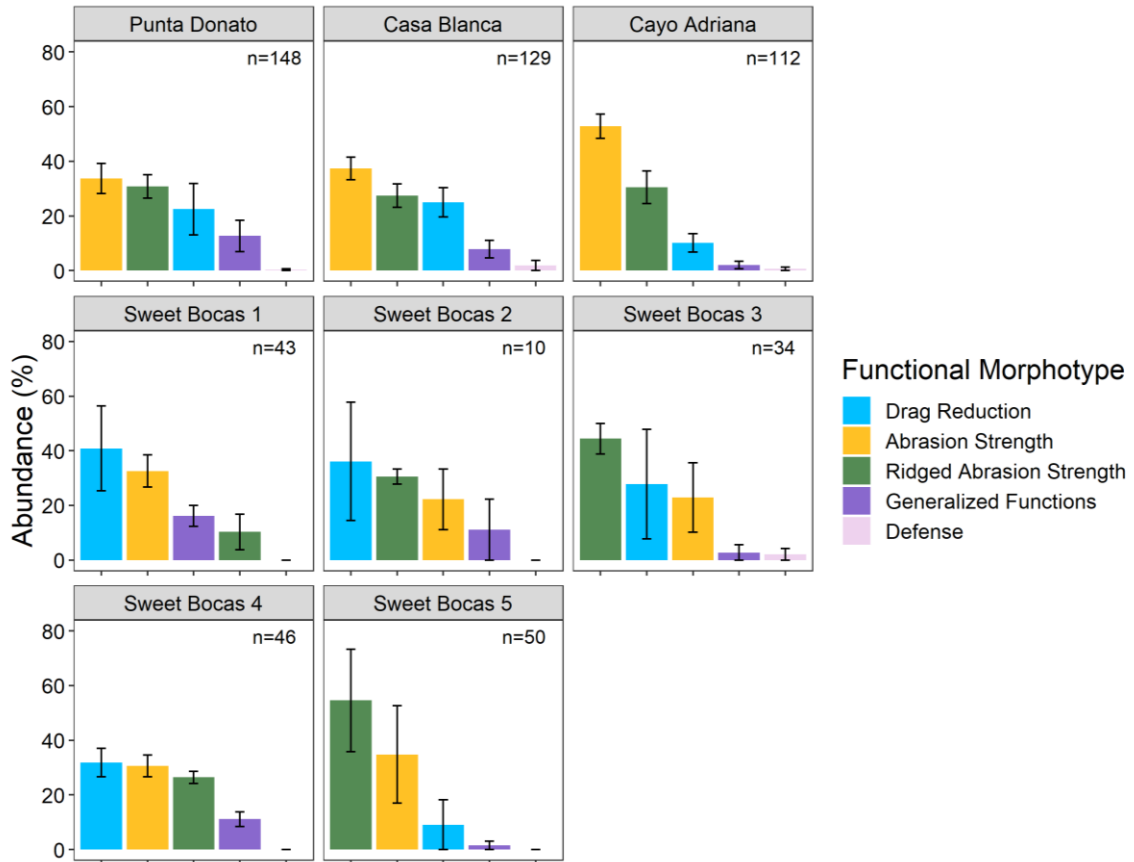
**Figure C.2** Denticle abundances varied by a factor of 1.9 across the modern (gold) sites and by a factor of 5.6 across the mid-Holocene (red) sites ( $\chi^2 = 26.94$ ,  $p < 0.001$ ). Although more denticles were found in the modern samples ( $n = 389$ ) than in the mid-Holocene samples ( $n = 183$ ), the mid-Holocene sample weights were smaller on average, producing similar denticle abundances. One fossil site (Sweet Bocas 2) contained few denticles per kilogram sediment despite having good preservation. Rather, denticle abundances at that site might have been low due to heterogeneity in shark habitat use or sedimentological processes. Each boxplot shows the median (dark bar), mean (diamond), and interquartile range (box), and outliers are displayed as points. The vertical axis is log<sub>10</sub> transformed.



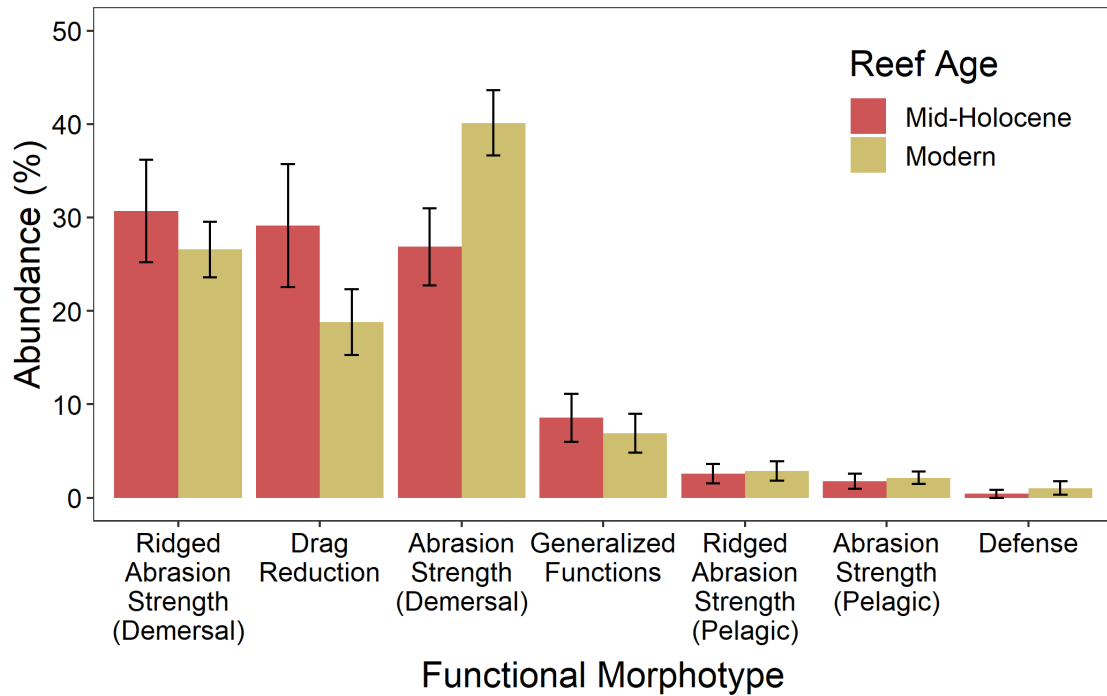
**Figure C.3** Denticle accumulation rates varied by a factor of 5.4 across the modern (gold) sites and by a factor of 5.6 across the mid-Holocene (red) sites ( $\chi^2 = 33.88, p < 0.001$ ). Each boxplot shows the median (dark bar), mean (diamond), and interquartile range (box), and outliers are displayed as points. The vertical axis is  $\log_{10}$  transformed.



**Figure C.4** Accumulation rates of each denticle functional morphotype across sites. Patterns differed across sites, particularly among the mid-Holocene localities. Generalized functions and defense denticles were rare at most sites. Each boxplot shows the median (dark bar) and interquartile range (box), and outliers are displayed as points.

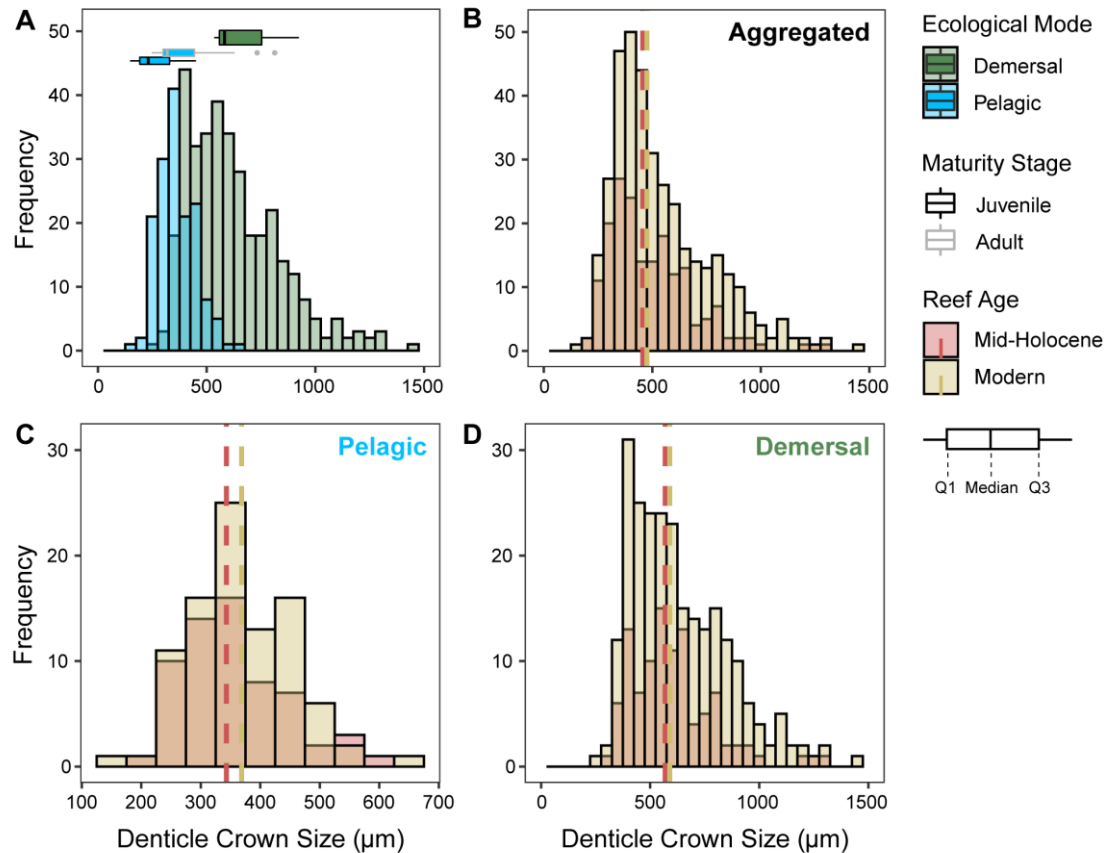


**Figure C.5** Relative abundances of each denticle functional morphotype, ranked in order of abundance at each site. The rank order was consistent across the modern reefs (top row), with abrasion strength and ridged abrasion strength denticles being the most abundant, followed by drag reduction denticles. The rank order varied across the mid-Holocene localities (middle and bottom rows), although drag reduction denticles were the most abundant at three of the five localities. Sample sizes indicate the number of intact denticles recovered from each site. Error bars denote the standard error around each mean.

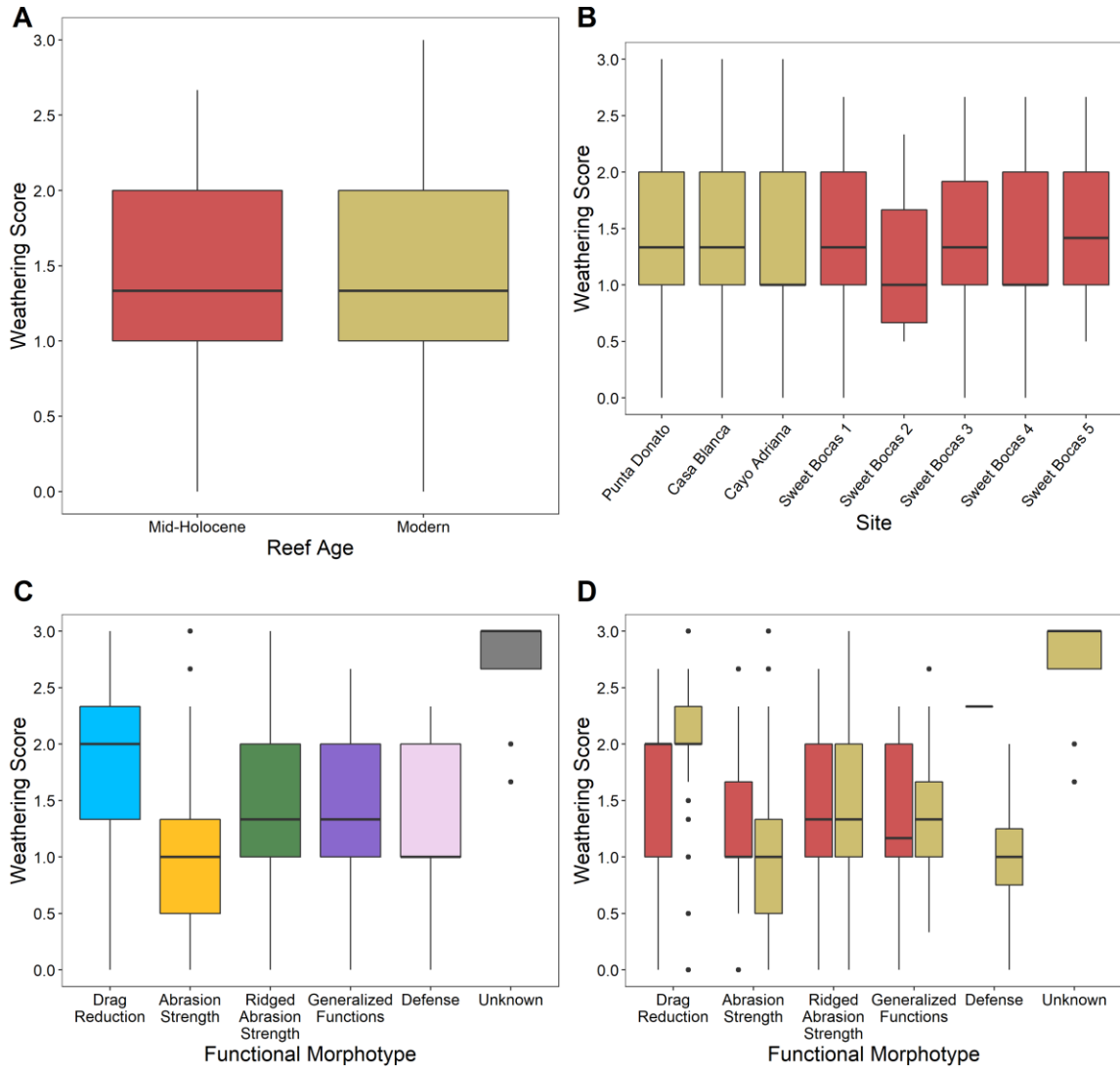


**Figure C.6** Relative abundances of each denticle functional morphotype, colored by time period. Abrasion strength and ridged abrasion strength morphotypes were categorized by shark ecological mode (pelagic or demersal). Most of the denticles belonging to these two morphotypes were characteristic of demersal sharks (i.e. the nurse shark *Ginglymostoma cirratum*). The relative abundance of abrasion strength and ridged abrasion strength denticles belonging to pelagic sharks (e.g. families Carcharhinidae and Sphyrnidae) represented <10% of the assemblage in each time period. Error bars denote the standard error around each mean.

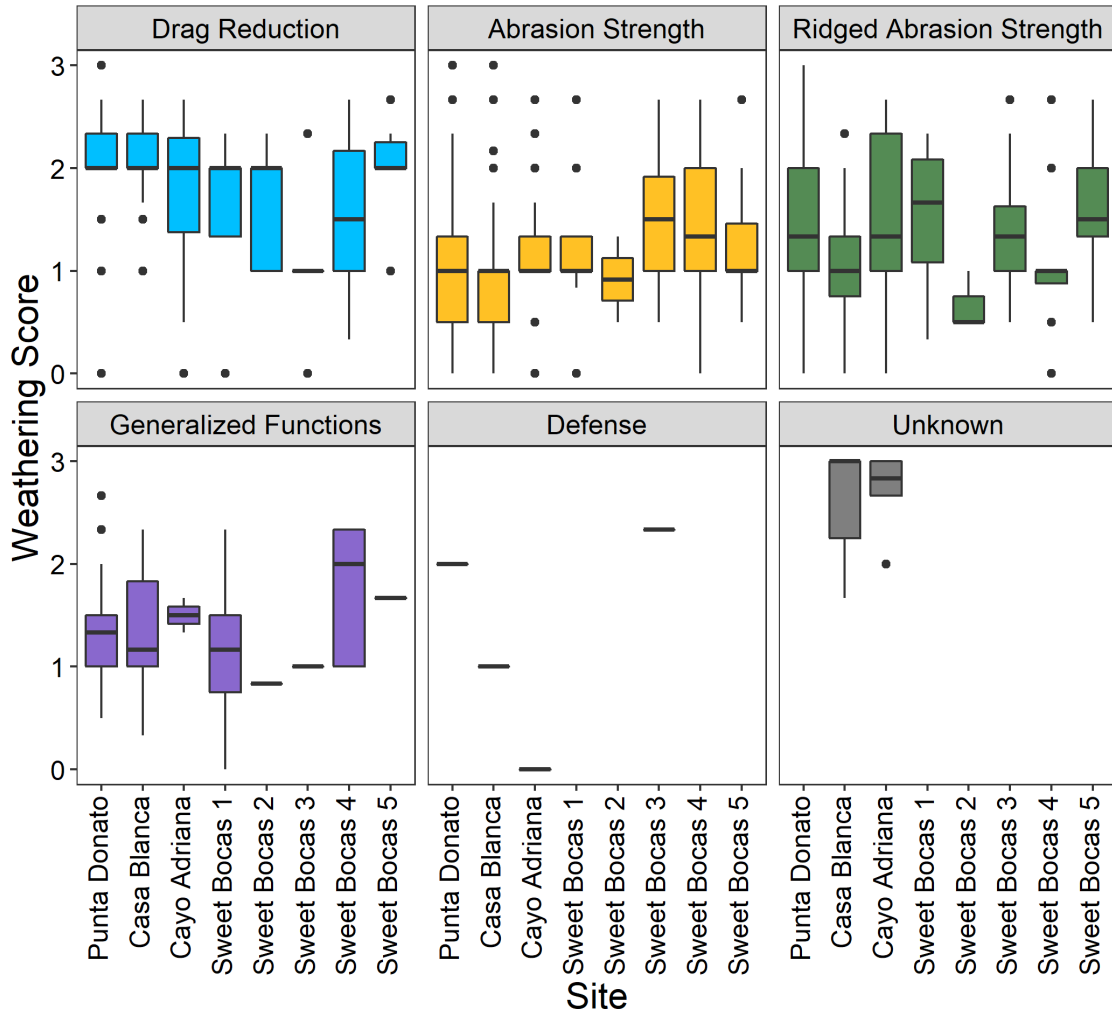




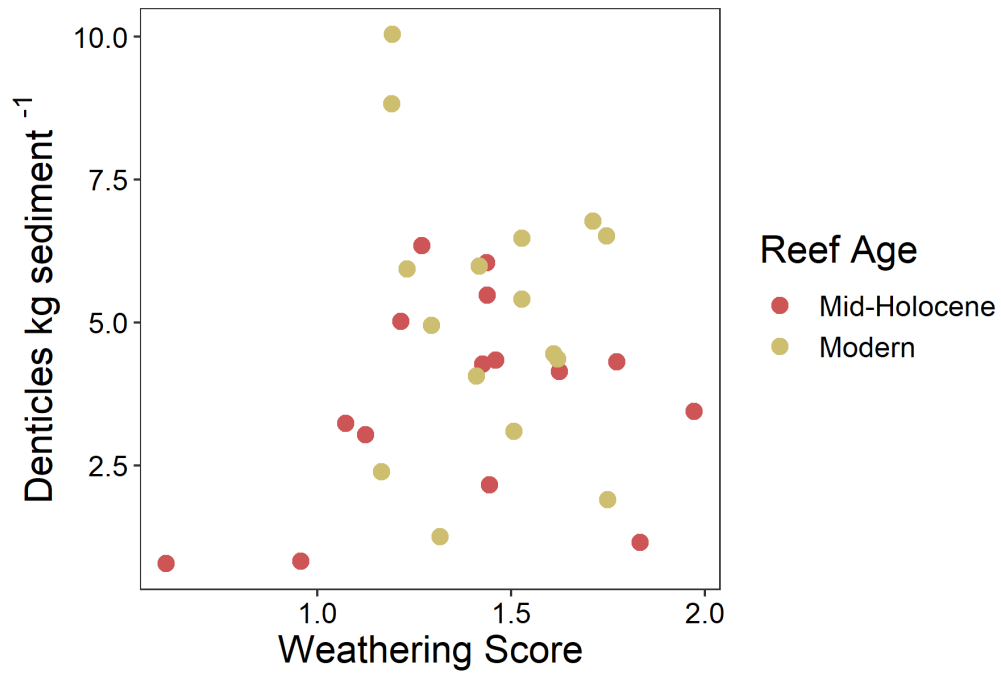
**Figure C.7** Denticle size-frequency distributions, disaggregated by time period and shark ecological mode. (A) Denticles belonging to demersal sharks (green) were, on average, larger than denticles belonging to pelagic sharks (blue), consistent with previous observations (1, 2). For context, the overlaid boxplots show the size of denticles in the reference collection (2) and previous morphometric work (1, 24) for a subset of species documented in Caribbean Panama ( $n = 15$  species,  $n = 70$  sharks). Shark ontogeny was based on estimated size at maturity (21). Data from adult demersal sharks were not available. Around 40% of denticles belonging to pelagic sharks fell within the 75<sup>th</sup> percentile (Q3) of the range represented by juveniles. However, the large amount of morphological variation suggests that these denticles had been shed by multiple species, portraying a community rather than a species-specific aggregation. Size-frequency distributions for (B) all denticles combined, (C) pelagic denticles, and (D) demersal denticles recovered from the mid-Holocene (red) and modern (gold) reefs. Crown size was measured as the square root of the product of the crown width and length, which scale allometrically with shark length. Median crown size (dashed lines) and distributions were not significantly different between the two time periods ( $p > 0.05$ ). Distribution attributes are reported in Table C.3.



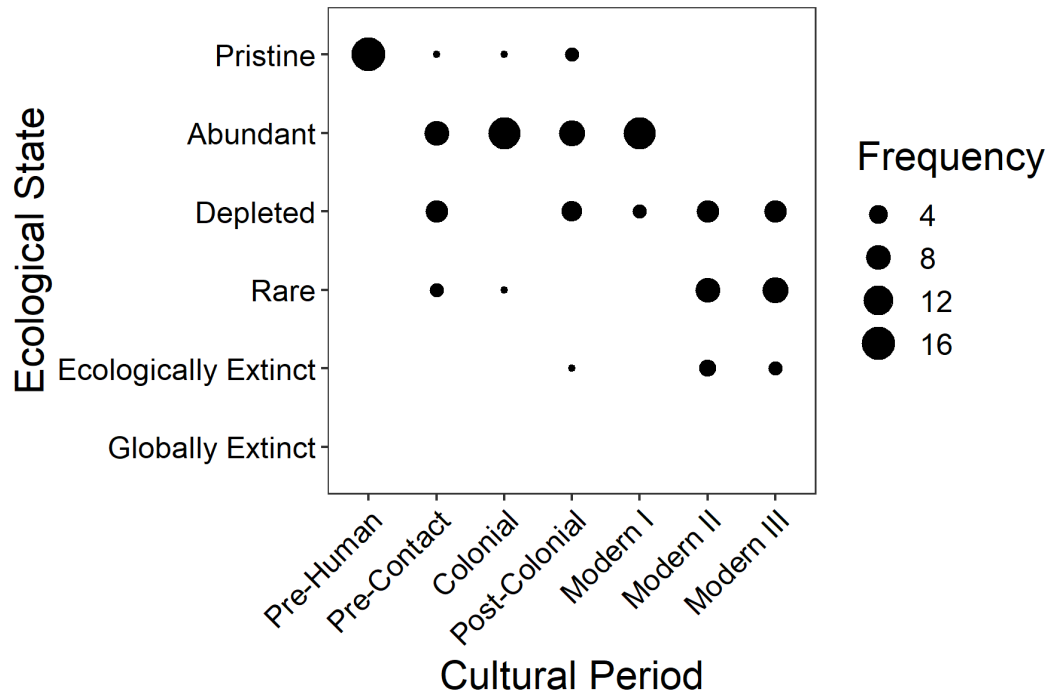
**Figure C.8** Denticle weathering scores across (A) time periods, (B) sites, (C) functional morphotypes, and (D) functional morphotypes colored by time period. Unidentified and fragmented denticles were included in the weathering analyses ( $n = 590$ ). Fragments comprised only 1% of the mid-Holocene assemblage and 4% of the modern assemblage. Lower scores indicate better preservation. Weathering scores did not differ across time periods ( $p = 0.59$ ) or sites ( $p = 0.79$ ), although drag reduction denticles were more weathered than the other morphotypes in both time periods ( $p < 0.001$ , Dunn's test  $p < 0.05$ ).



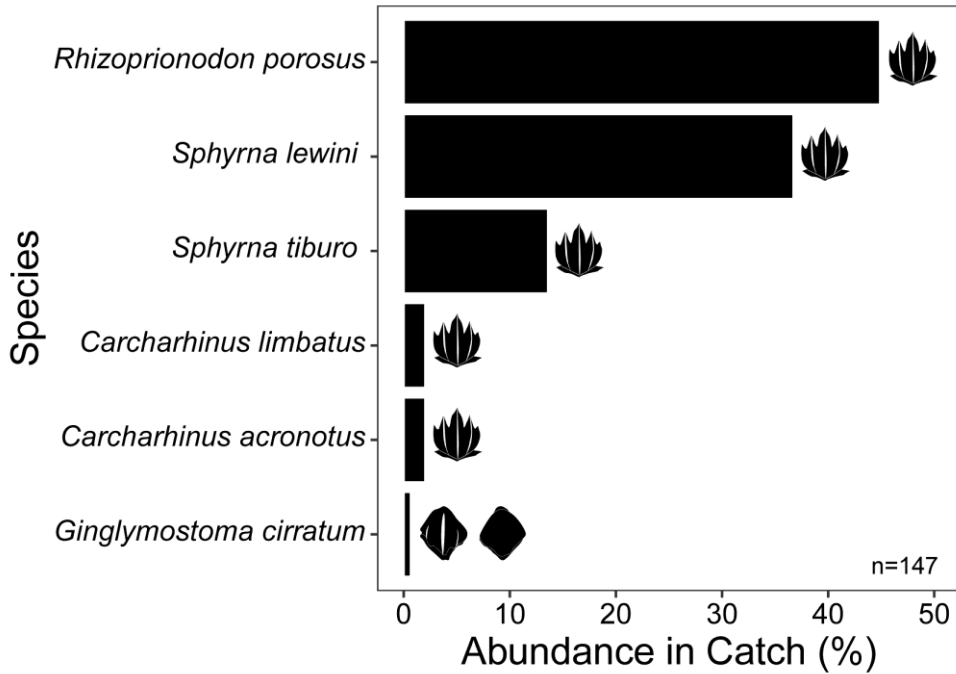
**Figure C.9** Patterns in denticle weathering scores for each functional morphotype across sites. Lower scores indicate better preservation. Median weathering scores for each morphotype were relatively consistent across sites, although patterns in preservation varied across morphotypes. Each boxplot shows the median (dark bar), and interquartile range (box), and outliers are displayed as points.



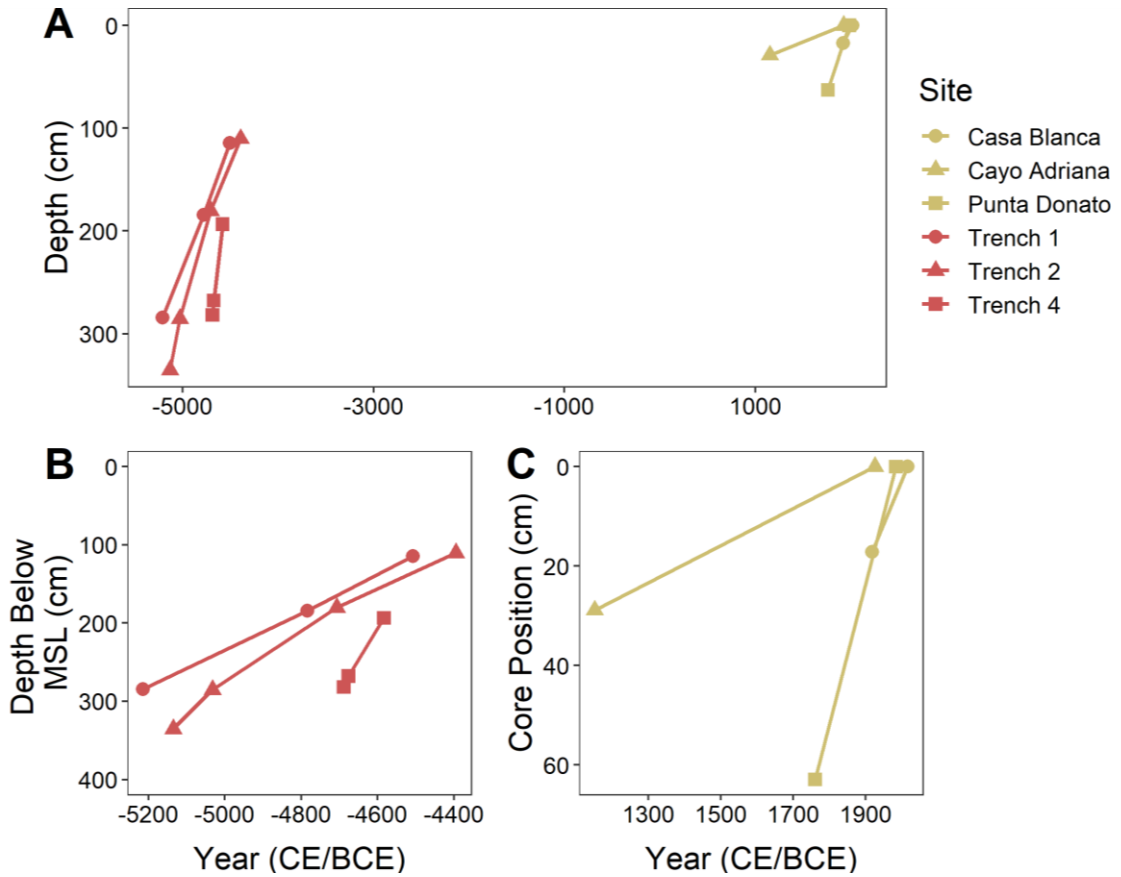
**Figure C.10** Correspondence between denticle weathering scores and abundances. There was no correlation between weathering scores and denticle abundances (Spearman  $r = 0.08$ ,  $p = 0.65$ ). Each point represents a bulk sample, where denticle abundance was measured at the level of sample and weathering scores were averaged across the denticles within that sample.



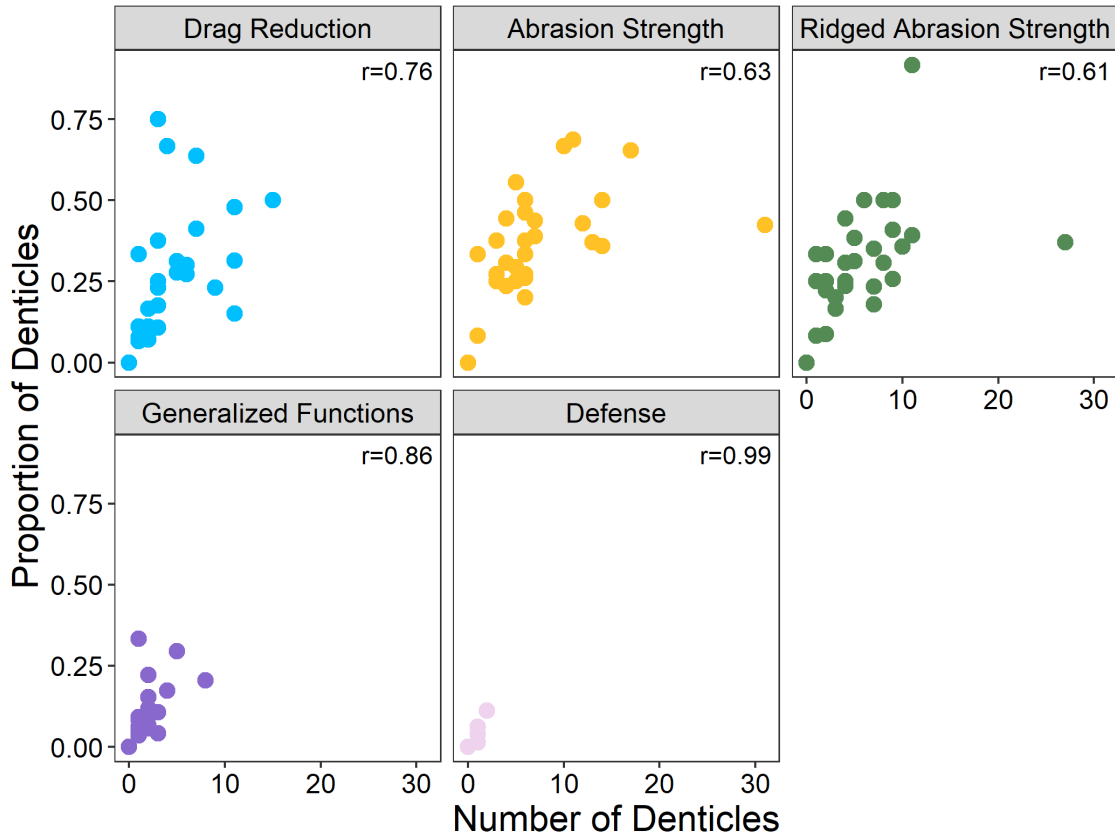
**Figure C.11** Frequency of ecological state scores for each cultural period in the history of Caribbean Panama ( $n = 17$  respondents). The variation across responses varied by cultural period, but there was a general trend of degradation over time. The pre-human period was assigned to be pristine (following ref. 17).



**Figure C.12** Percent catch during the 13-month-long survey of sharks landed at the Los Costeños pier in Colón, Panama between 2018–2019 ( $n = 147$  sharks total over 280 fishing hours). The functional morphotypes that characterize each shark species are shown. Only one nurse shark (*Ginglymostoma cirratum*), which possesses both abrasion strength and ridged abrasion strength denticles, was landed during the survey. All other shark species landed are characterized by drag reduction denticles. Denticle illustrations credit: Ashley Diedenhofen (artist).



**Figure C.13** Age-depth plot showing reef accretion rates at (A) all sites, (B) the trenches dated on the mid-Holocene (red) reef, and (C) the modern (gold) reef sites. Age reversals were excluded from linear interpolations of accretion rates. Steeper slopes indicate faster reef accretion rates. Corresponding depths—either elevation below mean sea level (MSL) or core position—and ages are available in Table C.1.



**Figure C.14** Correspondence between denticle counts and proportional abundance. Relative abundances of denticles were positively correlated with absolute denticle abundances for each functional morphotype ( $p < 0.001$ ), demonstrating that relative abundance is a reliable proxy for the raw counts. Spearman’s rank correlation coefficients are shown for each relationship.



## Appendix C.3 Supplementary Tables

**Table C.1** Radiometric dates used in this study. Uranium–Thorium (U–Th) and calibrated radiocarbon dating ( $^{14}\text{C}$ ) were used to calculate reef accretion rates on the mid-Holocene and modern reefs and estimate the site ages. Coral pieces were collected from trenches excavated at three localities on the mid-Holocene reef, and elevations are reported here in terms of meters from mean sea level (MSL). Trenches were located adjacent to bulk sampling localities: Trench 1 was located between Sweet Bocas 2, Sweet Bocas 3, and Sweet Bocas 4; Trench 2 was located near Sweet Bocas 3; and Trench 4 was located near Sweet Bocas 2. On the modern reefs, coral pieces were extracted from reef matrix cores. Ages are reported as years before present (years BP), where the present is 1950 CE. Accretion rates were calculated by linear interpolation between consecutive dates at each site. Duplicate  $^{14}\text{C}$  dates were run for each piece of *Acropora palmata*.

Site	Locality	Sample	Type	Core Position (cm from top)	Re-expanded Core Position (cm from top)	Elevation (m from MSL)	Method	Medium	Year BCE/CE	Age (years BP)	95% CI (years)	Ref.
Cayo Adriana	-	VC13-4-1	Core	0	0	-	U-Th	<i>Porites</i> sp.	1926 CE	24	4	[5]
Cayo Adriana	-	VC13-4-1	Core	20	29	-	U-Th	<i>Porites</i> sp.	1152 CE	798	7	[5]
Punta Donato	-	VC13-3-1	Core	0	0	-	U-Th	<i>Porites</i> sp.	1984 CE	-34	3	[5]
Punta Donato	-	VC13-3-1	Core	53.5	63	-	U-Th	<i>Porites</i> sp.	1761 CE	189	4	[5]
Casa Blanca	-	AT17-13-1	Core	7.5	17	-	U-Th	<i>Porites</i> sp.	1919 CE	31	3	This Study
Sweet Bocas	Trench 1	AT13-2-10	Bulk	-	-	-1.142	U-Th	<i>Acropora cervicornis</i>	4507 BCE	6457	10	This Study
Sweet Bocas	Trench 1	AT13-2-9	Bulk	-	-	-1.842	U-Th	<i>Acropora cervicornis</i>	4784 BCE	6734	16	This Study
Sweet Bocas	Trench 1	AT13-2-1	Bulk	-	-	-2.842	U-Th	<i>Acropora cervicornis</i>	5214 BCE	7164	16	This Study
Sweet Bocas	Trench 2	AT13-3-14	Bulk	-	-	-1.103	U-Th	<i>Acropora cervicornis</i>	4395 BCE	6345	17	This Study
Sweet Bocas	Trench 2	AT13-3-13	Bulk	-	-	-1.803	U-Th	<i>Acropora cervicornis</i>	4706 BCE	6656	14	[4]
Sweet Bocas	Trench 2	AT13-3-11	Bulk	-	-	-2.853	U-Th	<i>Acropora cervicornis</i>	5032 BCE	6982	22	This Study
Sweet Bocas	Trench 2	AT13-3-10	Bulk	-	-	-3.353	U-Th	<i>Acropora cervicornis</i>	5135 BCE	7085	18	This Study
Sweet Bocas	Trench 4	AT13-56A	Hand	-	-	-1.934	$^{14}\text{C}$	<i>Acropora palmata</i>	4583 BCE	6533	-	[4]
Sweet Bocas	Trench 4	AT13-56A	Hand	-	-	-1.934	$^{14}\text{C}$	<i>Acropora palmata</i>	4583 BCE	6533	-	[4]
Sweet Bocas	Trench 4	AT13-53A	Hand	-	-	-2.676	$^{14}\text{C}$	<i>Acropora palmata</i>	4676 BCE	6626	-	[4]
Sweet Bocas	Trench 4	AT13-53A	Hand	-	-	-2.676	$^{14}\text{C}$	<i>Acropora palmata</i>	4676 BCE	6626	-	[4]
Sweet Bocas	Trench 4	AT13-51A	Hand	-	-	-2.818	$^{14}\text{C}$	<i>Acropora palmata</i>	4688 BCE	6638	-	[4]
Sweet Bocas	Trench 4	AT13-51A	Hand	-	-	-2.818	$^{14}\text{C}$	<i>Acropora palmata</i>	4688 BCE	6638	-	[4]

**Table C.2** Sensitivity analysis results. Generalized linear mixed models were run with denticle accumulation rates calculated using the 95% confidence intervals on the radiometric dates to test how sensitive our results were to the age uncertainty. The “upper” and “lower” values represent the maximum and minimum number of years that a bulk sample could encompass based on the 95% confidence intervals, respectively. The amount of decline in denticle accumulation rates varied from 31% to 87%.  $\chi^2$  and  $p$  values were obtained using nested likelihood ratio tests.

Time Encompassed by the Samples	Decline in Denticle Accumulation Rate	$\chi^2$	$P$
Upper	31%	0.55	0.46
Mean	71%	4.68	0.030
Lower	87%	9.27	0.002

**Table C.3** Denticle size-frequency distribution attributes. Central tendency metrics and standard deviation are reported in terms of micrometers. Positive skewness indicates that the distribution is right-skewed, and negative skewness indicates that the distribution is left-skewed. Positive kurtosis indicates that the distribution is leptokurtic and negative kurtosis indicates that the distribution is platykurtic. All distributions are visualized in Fig. C.7.

Ecological Mode	Reef Age	Mean ( $\mu\text{m}$ )	Median ( $\mu\text{m}$ )	Standard Deviation ( $\mu\text{m}$ )	Skewness	Kurtosis	Fig. S7 Panel
Aggregated	Mid-Holocene	496	456	198	1.3	2.4	B
	Modern	544	475	227	1.1	1.0	B
Pelagic	Mid-Holocene	357	343	85	0.8	0.1	C
	Modern	372	369	85	0.3	-0.02	C
Demersal	Mid-Holocene	606	569	191	1.3	2.3	D
	Modern	635	590	223	0.9	0.4	D

**Table C.4** Example records from each cultural period in Caribbean Panama’s history. One example is provided for each cultural period except the pre-human period.

Cultural Period	Example Observation	Location	Date (CE)	Data Type	Ref.
Pre-Contact	Midden remains included 7 teeth (family Sphyrnidae), representing at a minimum 2 individuals. Specimens were young juveniles, likely ranging from 1-5 kg in total weight. Authors estimated a weight of ~1.3 kg per shark (2.7 kg biomass total), which could have yielded 2.4 kg of usable meat.	Bocas del Toro, Panama	550; 880-1250	Archaeological Study	[25]
Colonial	"... we had two days of calm during which so many sharks surrounded the ship that it was frightening, especially to those who believe in omens. For just as some say that vultures recognize the presence of a corpse by its smell many leagues away, so some believe that sharks have the same divinatory power. These beasts seize a person's leg or arm with their teeth and cut it off as clean as if with a knife, because they have two files of saw-like teeth. We made carnage among them with a chain hook until we could kill no more, and they still followed us making turns in the water, so voracious are these beasts that not only would they eat carrion but one can catch them by simply attaching a piece of red cloth to the hook."	Portobelo, Panama	1502	Anecdotal & Ethnographic Account	[26]
Post-Colonial	"During our stay here we heard some wondrous shark stories. They were such as I had never before heard, or even read of, and it so excited our curiosity, that we afterwards endeavored to test what amount of truth they contained by introducing the subject apparently in an inadvertent manner, in the several districts we afterwards visited. But there was no apparent contradiction in any of the several stories heard; they all agreed in the most important particulars, and were lastly confirmed by the Captain of a trading schooner who could not possibly know what we had previously been told, and therefore, if I give his description it will suffice for all. He told us, that when sailing his schooner on the coast, that on a calm clear day in a smooth sea, he was seated in the afterpart of his vessel, when he saw over the side, what at first appeared to be a shoal; in consequence he started up for the purpose of altering his course, when the object was discovered to be an enormous shark. The schooner was over 40 feet in length and he declared to us, that the shark was of equal dimensions. It came alongside, rubbing itself against the side of the schooner for several minutes. He said to us, that it struck him that he could kill it with a magazine rifle he had on board, but on second thoughts, he decided not to trouble the gentleman, as it might injure his schooner. In a short time afterwards it gradually disappeared as quietly as it had come, and to his great joy, was seen no more... From this and the other stories, it would appear to be almost certain that sharks of a large size abound on the coast, and are at times rather troublesome neighbours. The season of the year they are most frequent is at the time that the Hawk's-bill turtle abound. The flesh of this reptile is not used for food by the turtle-fishers, and their carcasses are, when stripped of the shell, thrown into the sea, and it is this carrion that probably attract the sharks at one particular season."	Bocas del Toro, Panama	19th century	Anecdotal & Ethnographic Account	[27]
Modern I	"Sharks, skates, saltwater catfishes, half-beaks, houndfish, or gars, parrotfish, saltwater eels and many others, which are seldom seen in American markets, are sold daily both in the Colón and Panama City markets. Not only small sharks are sold for food, but large ones also, and it is not unusual to see fishermen carry to market on their backs the carcass of a large shark, from which the skin and internal organs have been removed and which has been cut into halves or quarters after the manner in which beef animals are butchered. The meat of the large sharks is usually sold in slices like steaks."	Colón, Panama	early 20th century	Anecdotal & Ethnographic Account	[28]
Modern II	"[Sr. Samudio] borrowed \$200,000 from a development bank and constructed a stainless steel shark processing plant on Carenero Cay near the southwest end of the island, closest to Bocas town. To get sharks, he chartered and sub-contracted with five small boats that fished for sharks from Isla Colón to Punta Valiente area [...] Sr. Samudio was really proud of his first product and showed it to me and anyone who would look at it. He had a big debt to pay to the bank but was not able to make payments because shark catches were too minimal. In other words, there were very few sharks being caught, even with five working boats. After a couple of years or so, the bank foreclosed and sent their man to salvage what he could from the shark factory. Meanwhile, Samudio took selected materials and re-organized a smaller facility for lobster processing on the Carenero beach on the opposite side of the island [...] This venture also failed and Samudio moved on after several years."	Bocas del Toro, Panama	1980s	Anecdotal & Ethnographic Account	[29]
Modern III	Observed <i>Sphyrna lewini</i> (50% of identifiable sharks), <i>Rhizoprionodon porosus</i> (18%), <i>Sphyrna tiburo</i> (12%), <i>Carcharhinus limbatus</i> (9%), <i>Ginglymostoma cirratum</i> (9%), and <i>Squalus cubensis</i> (3%), plus 2287 unidentified sharks in the fish market over 4 months; "Shark meat is sold for \$0.75 per Lb [...] People in Colón consume 1500-2000 Lb of shark per week. According to local traders and personal observation of transactions between vendors and buyers, I estimate an average weight of 4.93 Lb per shark. Assuming this valuable to be real, traders in the Colón market sell between 300-400 sharks per week. That means an average of 16000-21000 sharks per year since shark demand and supply appears constant during the year."	Colón, Panama	2003-2004	Fishery Report	[30]

**Table C.5** Records used to reconstruct the ecological state of sharks, summarized by data type and cultural period. A total of 91 records were extracted from 41 unique sources. Each record constitutes one description from one publication or one fisheries or ecological survey data set from one source. In some cases, multiple records were obtained from a single source, and in rare cases, these records constituted different data types.

Type of Record	Number of Records	Number of Unique Sources
By data type		
Archaeological Studies	15	5
Anecdotes & Ethnographic Accounts	47	25
Ecological Surveys	12	7
Fisheries Reports	17	7
By cultural period		
Pre-Human	-	-
Pre-Contact	15	5
Colonial	7	6
Post-Colonial	6	6
Modern I	16	7
Modern II	26	11
Modern III	21	8

**Table C.6** Cultural periods in the history of Caribbean Panama. Periods were defined by resource use and encompass key historical events that occurred in Bocas del Toro and Costa Arriba, Panama.

Cultural Period	Dates	Description	Refs.
Pre-Human	>6 ka	This period predated the earliest evidence of human occupation and resource use along the Caribbean slope of Panama.	[31–35]
Pre-Contact	~6 ka-1500 CE	This period preceded the arrival of European explorers to the region, during which people practiced agriculture, "garden hunting," subsistence harvesting, and early trade.	[25, 31, 35–37]
Colonial	1501-1821 CE	This period began with the arrival of Rodrigo de Bastidas in 1501 and Christopher Columbus in 1502. It is defined by colonial occupation, exploration, and trade as well as depopulation of indigenous settlements and the spread of Western culture. Panama's independence from Spain in 1821 marked the end of this period.	[26, 38–40]
Post-Colonial	1822-1903 CE	During this period, Panama joined the Republic of Colombia (Gran Colombia) and subsequently the Republic of New Granada (which later became the Granadine Confederation, United States of Colombia, and then the Republic of Colombia). Key events include the construction of the city of Colón (1850), the completion of the Panama Railroad (1855), and the introduction of banana cultivation in Bocas del Toro. The Hay–Bunau-Varilla Treaty, which established the Canal Zone, and the formation of the Republic of Panama in 1903 are designated as the end.	[29, 41, 42]
Modern I	1904-1958 CE	Early industrial period in Panama's history, defined by technological advances, infrastructure development, and trade, coupled with rapid population growth in Bocas del Toro and Costa Arriba. Key events include the construction of the Panama Canal (completed in 1914), the creation of the free trade zone in Colón (1948), the consolidation of banana cultivation interests into the United Fruit Company in Bocas del Toro (1905), and the later use of industrial fertilizers and agrochemicals (1950s).	[29, 41, 43–45]
Modern II	1959-1999 CE	Late industrial period in Panama's history, defined by continued infrastructure development and trade as well as the advent of oil extraction and industrial fishing. Key events include the General Fishing Law (1959) and development of industrial fisheries, the paving of a road over the continental divide (1984-1990s), the oil refinery built at Bahía Las Minas (1960s) and oil pipeline built in Bocas del Toro (1982), and the increased use of agrochemicals in Bocas del Toro. Tourism in Bocas del Toro became popular toward the end of this period. United States military bases were turned over to Panama in 1999 via the Torrijos–Carter Treaties, marking the end of this period.	[29, 46–48]
Modern III	2000-2020 CE	Most recent period in Panama's history, defined by foreign investment in trade, construction, and finance alongside a rise in tourism, real estate, and the services sector. In Bocas del Toro, tourism and land speculation have led to increased coastal development and infrastructure, which have facilitated access to the region.	[48–50]

**Table C.7** Criteria used to determine the ecological state of sharks. Published criteria were used to evaluate disparate data types using a common methodology to assign an ecological state score on an ordinal scale (17–19).

Ecological State	Criteria for Assessment
1. Pristine	No evidence of human presence, use, or damage.
2. Abundant/Common	Human use with no evidence of reduction of marine resource. <u>Archaeological</u> : No shifts in relative abundance of organisms or size of organisms harvested <u>Anecdotal &amp; Ethnohistoric</u> : Observations lack any evidence of significant reduction at the population level; individual accounts describe taxa as common <u>Ecological &amp; Fisheries</u> : Evidence of use or harvest shows increasing catch; most economically valuable and highest-value species are the major focus of harvesting efforts
3. Depleted/Uncommon	Human use and evidence of reduced abundance (number, size, biomass, etc). <u>Archaeological</u> : Observed shift in assemblages to smaller sized organisms, decrease in abundance, or changes in the proportional representation of species <u>Anecdotal &amp; Ethnohistoric</u> : Observations of consistent harvesting or use of resource with some indication of use beyond the subsistence scale <u>Ecological &amp; Fisheries</u> : Persistent use of resource; catches are level, with no major increases or decreases
4. Rare	Evidence of severe human impact. <u>Archaeological</u> : Observations of decreases in size and abundance in assemblages over larger geographic ranges; significant reductions in sizes, abundances or diversity of organisms in assemblages; harvesting of pre-reproductive individuals <u>Anecdotal &amp; Ethnohistoric</u> : Observations of commercial or industrial scale harvesting, large-scale removal, or verbal accounts of significant declines in species availability for subsistence fishing <u>Ecological &amp; Fisheries</u> : Rate of resource extraction exceeds natural replenishment; catch records are declining
5. Ecologically Extinct	Rarely observed & further reduction would have no further environmental effect. <u>Archaeological</u> : Local extirpation of populations; decreases in size or abundance followed by absence in archaeological assemblages; absence observed in the majority of midden deposits reviewed <u>Anecdotal &amp; Ethnohistoric</u> : Rarely observed or described as having disappeared (local extinctions); observation of individual species worthy of publication <u>Ecological &amp; Fisheries</u> : Closure of fishery due to overfishing; fishery not economically viable; species considered worthy of special protection via statute
6. Globally Extinct	No longer in existence.

**Table C.8** Confusion matrix from the multinomial logistic regression model trained and validated using the denticle reference collection. The numbers indicate the proportions of cross-validated denticles that were classified into each predicted group ( $n = 186$  training,  $n = 27$  testing). Each row sums to one, and values of one across the diagonal would indicate perfect performance. The largest proportion in each row is bolded. Overall model accuracy was 85%, although accuracy varied across functional morphotypes.

Actual Group	Predicted Group				
	Abrasion Strength	Defense	Drag Reduction	Ridged Abrasion Strength	Generalized Functions
Abrasion Strength	<b>1</b>	0	0	0	0
Defense	0	<b>1</b>	0	0	0
Drag Reduction	0	0	<b>0.917</b>	0	0.083
Ridged Abrasion Strength	0	0.167	0	<b>0.833</b>	0
Generalized Functions	0.167	0.167	0	0	<b>0.667</b>

**Table C.9** Estimates of coefficient values (Coeff) and standard errors (SE) for denticle characters used to predict the functional morphotype using the multinomial logistic regression model. The model was trained with our denticle reference collection ( $n = 213$ ) and used to classify intact denticles recovered from the sediment samples ( $n = 572$ ). Coefficients were estimated using a maximum likelihood estimation approach and are expressed here as log odds. The abrasion strength functional morphotype is the reference level and likelihoods are expressed with respect to that outcome. Denticle characters (predictors) have been previously described (2).

Predictor	Defense		Drag Reduction		Ridged Abrasion Strength		Generalized Functions	
	Coeff	SE	Coeff	SE	Coeff	SE	Coeff	SE
(Intercept)	20.37	0.83	-47.99	1.52	-56.82	1.42	-23.47	1.28
Crown shape: circular or elliptical	-49.60	NA	-5.07	3.41	-30.06	1.39E-08	-6.85	3.19
Crown shape: lanceolate or teardrop-shaped	-16.07	2.32	37.15	1.06	-10.13	2.10	44.79	1.21
Crown size	-0.20	0.02	-0.11	0.02	-0.08	0.01	-0.10	0.02
Crown thickness	2.92	1.77	10.82	1.41	8.08	1.11	10.72	1.41
Peak type: rounded peaks or single V-shaped peak	-0.78	1.40	6.23	1.23	22.28	0.93	3.76	1.89
Peak type: distinct serrated peaks	-31.94	6.24E-05	-4.00	0.94	-13.89	4.89E-10	2.74	0.94
Peak type: peak edges curve inward to form single tip	14.30	1.09	-39.02	0.96	25.86	0.79	-36.58	0.89
Complete ridges	9.15	1.76	23.62	2.64	14.72	1.24	8.48	2.45
1 to 100 $\mu$ m ridge spacing	29.80	0.86	38.50	0.91	42.23	0.60	19.19	0.50
>100 $\mu$ m ridge spacing	115.60	0.46	81.28	1.10	86.02	1.54	62.99	1.22

**Table C.10** Correspondence between the multinomial logistic regression predictions and visual classifications of denticles recovered from the sediment samples. Confusion matrix from the multinomial logistic regression model trained with our denticle reference collection ( $n = 213$ ) and used to classify intact denticles recovered from the sediment samples ( $n = 572$ ), compared with visual classifications of the same denticles. The numbers indicate the proportions of visually identified denticles that were classified into each predicted group by the model. Each row sums to one, and values of one across the diagonal would indicate perfect alignment between the two classification methods. The largest proportion in each row is bolded. Overall correspondence between the two classification methods was 79%.

Visually Identified Group	Predicted Group				
	Abrasion Strength	Defense	Drag Reduction	Ridged Abrasion Strength	Generalized Functions
Abrasion Strength	<b>0.856</b>	0	0	0.024	0.12
Defense	0.2	<b>0.8</b>	0	0	0
Drag Reduction	0	0.025	<b>0.95</b>	0	0.025
Ridged Abrasion Strength	0.047	0.123	0.094	<b>0.585</b>	0.152
Generalized Functions	0.045	0	0.136	0.045	<b>0.773</b>



#### Appendix C.4 Supplementary References

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## Appendix C.5 Historical Ecology Analysis References

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