



Dermal denticle shedding rates vary between two captive shark species

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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 2 shark species in a large aquarium over 9 mo. 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, bonnethead 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.

KEY WORDS: Shark · Dermal denticle · Fossil record · Denticle replacement · Abundance · Aquarium

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1. 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 dura-

bility 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 9 mo for zebra sharks *Stegostoma fasciatum* and bonnethead sharks *Sphyrna tiburo*, 2 warm-water, coastal species which differ in ecological traits such as habitat, body size, and activity patterns (Fig. 1, Table 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. MATERIALS AND METHODS

2.1. Setting

Denticle shedding was measured at the Aquarium of the Pacific (Long Beach, California, USA) in the

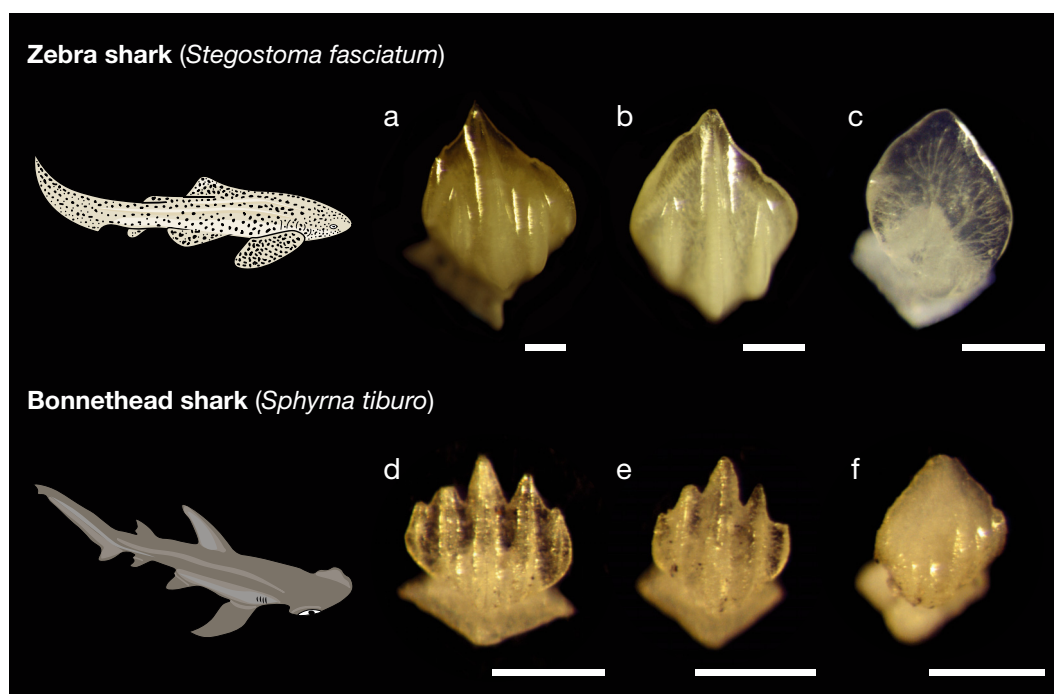


Fig. 1. Variation in denticle morphology between the 2 shark species in the Tropical Pacific Gallery at the Aquarium of the Pacific on the (a,d) body, (b,e) fins, and (c,f) fin edges. Zebra sharks *Stegostoma fasciatum* are characterized by *ridged abrasion strength* and *abrasion strength* denticles, 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 A. Diedenhofen. Scale bars = 200 μm

Table 1. Ecological traits (life mode) and denticle characteristics of the 2 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 denticle shedding rates

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

^aSource: Compagno et al. (2005); ^bModeled as an ellipse, using estimated body measurements from sharks in the Tropical Pacific Gallery; ^cMean proportional cover data were obtained from published morphometric studies of museum specimens (described in Section 2.3)

Tropical Pacific Gallery. The tank was ~1 325 000 l in volume and housed 5 captive sharks of 2 species: 3 zebra sharks and 2 bonnethead sharks (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/

[m682p153_supp.pdf](#)). All sharks were mature females apart from 1 juvenile female zebra shark, and they had been in captivity for ~5–15 yr. The tank also housed numerous other bony and cartilaginous (ray)

fish species. Seawater was maintained between 23.2 and 24.7°C (mean \pm SD; 23.9 \pm 0.1°C), and water quality was monitored to ensure animal health. The water turnover rate was approximately 1 h, 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⁻¹ and bonnethead sharks receiving 0.2 kg d⁻¹ of a mix of clam foot, sardines, mackerel, mahi mahi, capelin, herring, and market squid.

2.2. Denticle shedding rate measurements

To measure denticle shedding rates, we deployed 6 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. S1) for intervals of 25–42 d at 6 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 (~2 cm² openings) to prevent disturbance from animals and reduce turbulence that could displace the substrate (Gardner 1980). We deployed the trays in 3 locations within the tank, with 2 replicates per location (Fig. S1). Four trays were placed within a webbed mesh enclosure (~0.5 cm² openings) under the main viewing window, which allowed denticles and small shark teeth, but not animals, to enter the immediate area, and 2 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 ~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 μ m to 2 mm size fractions were quartered using a splitter (subsample mean \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 μ 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. 1; Table S1). Only 3 % (n = 218) of the denticles recovered could not be reliably identified (Fig. S2) and were excluded from the species-level analyses. Denticle shedding rates were calculated as the number of denticles accumulating per day per m² 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 3 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 1) because the 2 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 2.5).

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. 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 5 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 2 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 (dorso-lateral, 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 \times 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).

2.4. 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 0 (pristine) to 3 (poor preservation) and was based on visual inspection of the crown, peaks, and base (Dillon et al. 2020) (Table S2). 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 1 mo of being shed.

2.5. Shark behavioral surveys

To determine how behavior differed between the 2 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. S1) on 7 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 hap-

hazardly 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). Because shark space use was not homogeneous throughout the tank, denticle shedding rate measurements were corrected by the frequency of 2 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.

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). 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 S3), and likelihood ratio tests were used to calculate p-values. All analyses were performed in R (R Core Team 2019, Wickham et al. 2019).

2.6. 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 m² 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). The

Table 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	Traveling 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 Bottom third	Shark space use could influence the location of denticle accumulation
Horizontal position in the tank	Every 30 s	Outer third 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

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.

3. RESULTS

3.1. Denticle shedding rates

Denticles were frequently shed by sharks in the tank, with upwards of 33 000 denticles accumulating in the $\sim 0.6 \text{ m}^2$ 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. S3A), although rates varied 1.1- to 2.3-fold across locations during each deployment (Fig. S3B). Between 14 and 54 denticles accumulated per day in each tray, indicating that the 5 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 3 to 5 order of magnitude difference between the number of denticles and teeth possessed by these species at any point in time.

Denticle shedding rates differed significantly between the 2 shark species in the tank. The mean shedding rate of bonnethead sharks (denticles shed $\text{shark}^{-1} \text{ d}^{-1} \text{ m}^{-2}$) was 3.6 times higher than that of zebra sharks ($\chi^2_1 = 73.80$, $p < 0.001$; Fig. 2), 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 3). Notably, correcting by denticle quantities did not have a large effect on the magnitude of difference in mean shedding rate between these 2 species, as their contrasting body sizes were offset by opposite patterns in denticle density (Table 1). As a result, the 2 bonnethead sharks in the tank produced more denticles than the 3 zebra sharks.

Denticle shedding rates fluctuated across the monthly deployments. In aggregate, shedding rates varied 1.6-fold across deployments on average (Fig. S4), although the pattern of temporal variation differed between the 2 shark species. The mean shedding rate of bonnethead shark denticles decreased from June to March by an overall factor of 2.5 (Fig. 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 2 deployments (Fig. 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. S5). 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 ± 242 denticles kg^{-1} . Given the mean addition of 135 g tray^{-1} of grain sizes $< 3 \text{ mm}$, we predict that ~ 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 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.

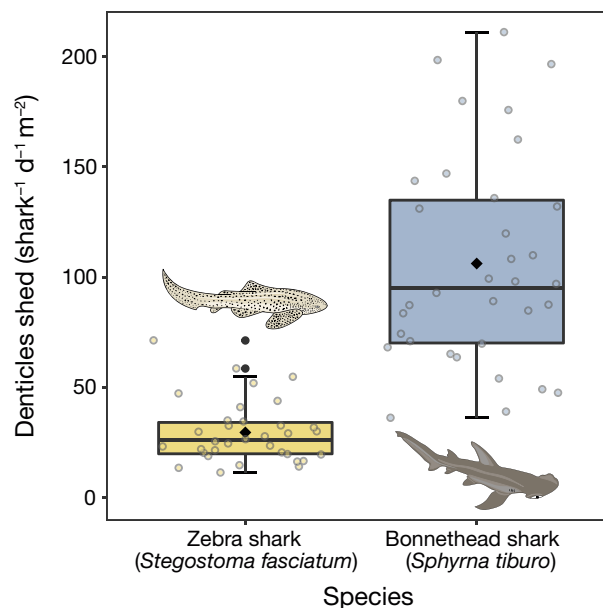


Fig. 2. Denticle shedding rates for zebra sharks *Stegostoma fasciatum* (yellow) and bonnethead sharks *Sphyrna tiburo* (blue), aggregated across the 9 mo long study. Shedding rates were measured for each collection tray as the number of denticles accumulating $\text{shark}^{-1} \text{ d}^{-1} \text{ m}^{-2}$ ($n = 34$). 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

Table 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 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 2 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)	χ^2	p
Raw data	106.1	29.5	3.6× (1.4–6.5)	73.80	<0.001
(1) Non-experimental input	103.0	27.3	3.8× (1.4–7.1)	70.70	<0.001
(2) Denticle quantity	9.3×10^6	2.9×10^6	3.2× (1.3–5.8)	68.82	<0.001
(3) Space use	89.3	37.7	2.4× (1.0–4.3)	45.35	<0.001
(4) All corrections	7.6×10^6	3.4×10^6	2.2× (0.9–4.2)	34.96	<0.001

3.2. 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. S6). Most zebra shark denticles in the trays were classified as *ridged abrasion strength* (mean \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.

3.3. Denticle weathering

Denticles recovered from the trays were better preserved (median weathering score \pm median absolute deviation; 1.0 ± 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

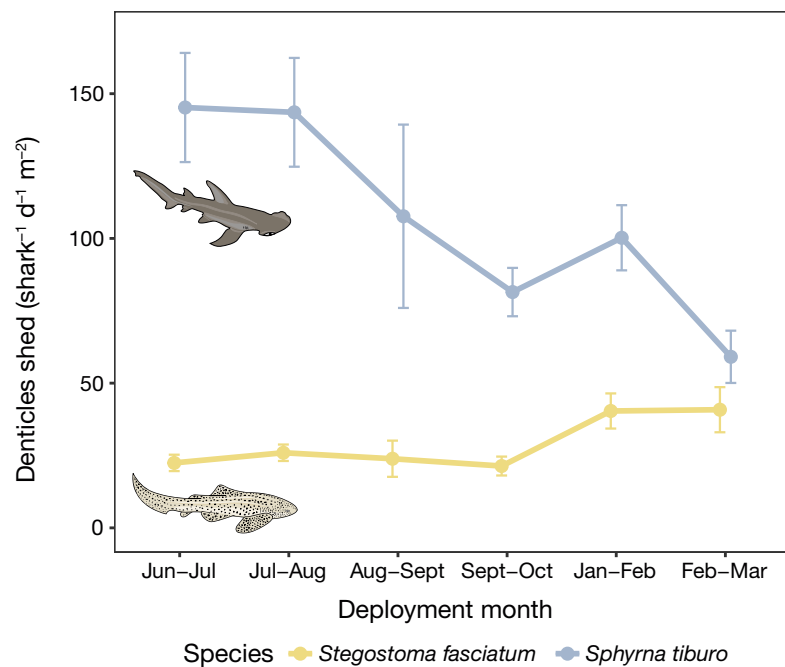


Fig. 3. Temporal variation in denticle shedding rates across the 6 deployments, which spanned a 9 mo long period from June to March. The mean shedding rate of bonnethead shark *Sphyrna tiburo* denticles (blue) decreased 2.5-fold during the study, whereas the mean shedding rate of zebra shark *Stegostoma fasciatum* denticles (yellow) remained relatively consistent across the first 4 deployments before increasing 1.7-fold in the final 2 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])

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 ± 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. S7) and were more frequently observed with fragmented peaks (58%) than the other functional morphotypes (29%). Nevertheless, bonnethead shark denticles in aggregate (1.0 ± 1.5) had similar, although more variable, preservation relative to zebra shark denticles (1.0 ± 0.7).

3.4. 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. S8 & S9). 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 (mean \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.

3.5. 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. 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 2 metrics varied with community composition (Fig. 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 character-

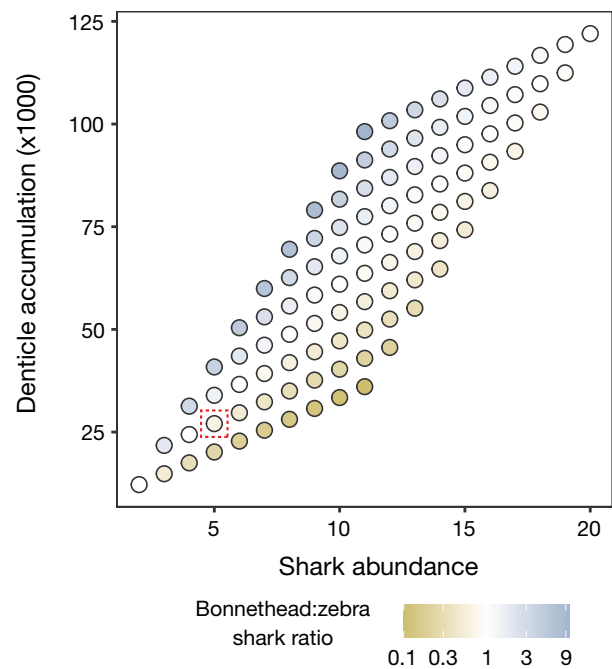


Fig. 4. Denticle accumulations simulated using the empirically measured denticle shedding rates over a 90 d period in a 1 m^2 area. The denticle accumulation produced by a given number of sharks varied with the ratio of fast-shedding (bonnethead sharks, in blue) to slow-shedding (zebra sharks, in yellow) 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

ize zebra sharks, outnumbered *drag reduction* denticles only when zebra sharks were several times more abundant than bonnethead sharks (Fig. S10). 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.

4. 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 2 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 2 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 hypothe-

size 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. 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, 2-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 2 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 5 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. S3). 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 2 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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