The biology and conservation of elasmobranchs and chimaeras

Edited by
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The biology and conservation of elasmobranchs and chimaeras

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Editorial: The biology and conservation of elasmobranchs and chimaeras

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KEYWORDS

conservation, management, contemporary approaches, elasmobranchs, chimaeras

Editorial on the Research Topic
The biology and conservation of elasmobranchs and chimaeras

Encompassing a staggering array of species, elasmobranchs (sharks and rays) and chimaeras are ecologically vital creatures that have long played an important role in maintaining healthy marine ecosystems. Hailed by some as God-like creatures (Baughman, 1948), they have been feared by others due to their negative portrayal throughout history. Unfortunately, as is the case with many aquatic species, in recent years climate change, anthropogenic pressures, and habitat degradation have significantly threatened their populations. On top of this, their life traits and opportunistic feeding behaviors make them vulnerable to commercial fishing (Bengil and Basusta, 2018). As a result, today many species are endangered, some are data-deficient or nearly extinct and urgently need knowledge for their conservation (Dulvy et al., 2014, 2021). Sadly, the IUCN has already declared the first elasmobranch, Urolophus javanicus (Martens, 1864), the Java Stingaree, as extinct due to human activities (Constance et al., 2023). Therefore, producing information on elasmobranchs through scientific sampling or contemporary approaches, is crucial. Any contribution to their biology, ecology, distribution, migration and many other aspects is essential knowledge that will provide a basis for action, globally, regionally or locally.

But at what cost?

Conventionally, the methodologies commonly used to produce scientific information are mostly lethal but effective (Heupel and Simpfendorfer, 2010) and "convenient". However, does this justify lethal sampling? Traditionally, the primary objective of the majority of studies is not conservation but simply to produce scientific information. Such efforts target a few charismatic species, resulting in the "neglect" of Data-Deficient species while overstressing the focused populations (Ducatez, 2019). A recent study by Ducatez (2019) analyzed research efforts on 509 shark species, showing biases toward subjects, taxa, and species, and shedding some light on species and areas in urgent need of information. In addition to correctly addressing information gaps and planning "efficient" sampling -with minimal sample size but high information yield-, studies like this can minimize the pressure of lethal scientific sampling. Utilizing bycaught individuals can provide "samples" for further biological examinations if retained (Wosnick et al.), and if alive when released could provide ecological information.

Such opportunistic sampling has proven effective in some aspects of species physiology and bioecology, but has its pros and cons (Braccini et al., 2006; Bengil, 2020; Rosa et al.).

Is there any other way?

The diversity of elasmobranchs is increasing relatively quickly with new discoveries (Randhawa et al., 2015). Smartphones and the "to post" have provided a new digital database for scientists (Eryasar and Saygu, 2022) and aided these discoveries. People, whether members of the public, recreational divers, or fishermen, are eager to share on social media what they have observed, seen, or caught (Kabasakal and Bilecenoglu, 2020; Boldrocchi and Storai, 2021; Eryasar and Saygu, 2022; Saltzman et al., 2022; O'Keefe et al.). These "posts" reveal public perception, species distribution, morphology (in some cases), evidence of predation, or basically presence (Barnes et al., 2016; Roemer et al., 2016; Kabasakal and Bilecenoglu, 2020; Bengil et al., 2021; Boldrocchi and Storai, 2021; Saltzman et al., 2022). Additionally, utilizing local ecological knowledge (LEK) from fishers or on-board observations can provide information on reproduction, aggregation areas, general ideas about population trends, etc (Bengil, 2020; O'Keefe et al.). Citizen science, leveraging LEK and social media, is now pinpointing critical habitats for endangered species, like the recent discovery of new areas for guitarfish in the eastern Mediterranean (Bengil et al., 2018; Giovos et al., 2018; Bengil et al., 2020). Studies utilizing local news alongside social media and LEK have effectively tracked species biodiversity, status, habitat use, and public perception (Roemer et al., 2016; Kabasakal and Bilecenoglu, 2020; Boldrocchi and Storai, 2021; Papageorgiou et al., 2022; Saltzman et al., 2022, Rosa et al., O'Keefe et al.). Saltzman et al. (2022) have emphasized how social media posts have helped raise awareness of endangered elasmobranch species that have had conservation efforts implemented, which would otherwise have been unknown or less known. Data mining is also a good method to understand population status, trends, and shifts in addition to compiling, analyzing, and simplifying classic sources (Tsikliras and Stergiou, 2014; Colloca et al., 2017; Carpenter et al.).

One of the recently practiced non-invasive manual methods, which can also easily be performed by fishermen, is returning egg cases that have live embryos (Hof et al., 2018). This can provide information on the egg-laying grounds of some egg-laying species. Additionally, researchers have altered technologies or developed methodologies to determine maturity to provide information on reproductive status such as pregnancy without harming the individual (Carrier et al., 2003;

Awruch et al., 2008; McMillan et al., 2019; Campbell et al.; Hoyos-Padilla et al.) or from carefully stored samples (Anderson et al.). Acoustic monitoring (Simpfendorfer and Heupel, 2004), photo identification (Meekan et al., 2006), mark-recapture (Simpfendorfer et al., 2008), baited remote underwater video surveys (Brooks et al., 2011), mucus swabs for genetic sampling (Lieber et al., 2013), and diet composition identification with DNA metabarcoding from cloacal swabs (van Zinnicq Bergmann et al., 2021) are some other non-lethal methodologies.

On the other hand, the importance of scientific surveys cannot be disregarded because of their analytic value; however, we should keep our minds open to such contemporary approaches and possible new technological integrations. Nonetheless now more than ever these types of contemporary approaches for biological or ecological information are important as scientific surveys, are a destructive methodology in terms of fishing operations, in addition to being logistically difficult to obtain and expensive (Bengil and Basusta, 2018; Bengil, 2020). Furthermore, given their continued commercial value, there is an opportunity to test and refine new contemporary approaches in locations where they are fished sustainably. Local or regional knowledge gaps can be reduced by supplementing gathered knowledge with different data sources, allowing conservation measures or management plans to be implemented more quickly.

Author contributions

EB: Conceptualization, Methodology, Visualization, Writing – original draft, Writing – review & editing. NB: Conceptualization, Writing – original draft, Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Assessment of the physiological vulnerability of the endemic and critically endangered Daggernose Shark: A comparative approach to other Carcharhiniformes

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Introduction: The current Isogomphodon oxyrhynchus (Daggernose Shark) population status Q7points to 99% losses in the last decade due to certain biological traits, site fidelity, and historical high representativeness as bycatch in artisanal fisheries. This species is listed as Critically Endangered (CR), both in the IUCN and the Brazilian Red Lists. Its vulnerability is so high that its recovery potential requires protection from ongoing fishing pressure.

Objective: In this context, this study aimed to evaluate the health status of Daggernose sharks and their ability to cope with allostatic overload in a comparative analysis with other Carcharhinid and Sphyrnid sharks.

Methods: Sharks incidentally caught by the artisanal fleet in the state of Maranhão, on the Brazilian Amazon Coast, were sampled for blood, and serum was used to assess biochemical markers.

Results: The findings indicate significant differences in Daggernose Shark homeostatic capacity for ALP, ALT/GTP, creatinine, lactate, urea, total cholesterol, and triglycerides, pointing to lower health scores and recovery capacity when compared to other Carcharhiniformes inhabiting the same region.

Discussion and conclusions: It is possible that such vulnerability is a result of fisheries-induced evolution, leading to remaining populations with very low chances of fully recovering. Conservation planning is thus urgent, as current legislation based on fishing bans does very little for the species. International collaboration and longterm recovery measures are necessary, including the creation of MPAs specially designed for the species and captive maintenance aiming to monitor health status and carry out breeding attempts.

KEYWORDS

conservation physiology, Carcharhinidae, Sphyrnidae, capture stress, fisheries management

Introduction

The Daggernose Shark, *Isogomphodon oxyrhynchus* is the Carcharhiniformes representative exhibiting one of the narrowest geographic distributions, found only in coastal areas from Trinidad and Tobago and eastern Venezuela to the state of Maranhão, in northern Brazil (Lessa et al., 2016). It is one of the 24 carcharhinid sharks listed as Critically Endangered, displaying severe population declines of up to 99% in the past three generations, placing it as one of the sharks presenting the highest risk for extinction (Pollom et al., 2020). Threats to this species include intensive fishing pressure, mainly as bycatch of artisanal fleets targeting commercial teleost fish (Pollom et al., 2020), and habitat loss (Magris and Barreto, 2010). According to demographic analyses, the species' resilience to fishing is extremely low, with low genetic variability across extremely fragmented remaining populations (Lessa et al., 2016). Data on physiological vulnerability is non-existent. This is of particular concern, as nothing is known regarding capture survival rates and the potential success of release measures, if implemented.

An increasing interest in the potential effects of fishing-induced evolution on predatory fish has been noted (Enberg et al., 2011), mostly focused on sexual maturation size impacts and the reproductive outcomes of affected populations. Yet, the effects of reduced genetic diversity caused by overfishing on shark physiology remain poorly explored. In a recent review, Hollins et al. (2018) presented a physiological perspective on this topic, focusing on the effects of fisheries-induced evolution on the energy balance, swimming capacity, stress response, and sensory physiology of fishes. Possible outcomes, however, were treated only theoretically, and empirical studies are required to prove these hypotheses. As decreased genetic diversity causes a concomitant decrease in expressed phenotypes, it is plausible to infer that a population strongly affected by fishing will display less plasticity in the face of stressors, whether environmental or anthropogenic. Thus, low genetic diversity adverse effects can, for example, decrease capture resistance and the chances of post-release survival, leading to a cascade of mortality events that may not be reversed unless long-term conservation programs are established.

In this context, the present study aimed to carry out a novel assessment on the physiological profile of Daggernose Sharks incidentally caught off the Maranhão coast, in northern Brazil, focusing on evaluating the systemic health status of one of the remaining Daggernose Shark populations and its responses to capture stress. Furthermore, we also aimed to assess the status of energy stores mobilized in fight-or-flight situations, if the health of Daggernose sharks is compromised in relation to other Carcharhiniformes commonly caught under the same conditions and, finally, if this species is more sensitive to capture stress when compared to other representatives of the same order. Specific serological markers were chosen for this end, based on their shark roles and validation. More specifically, alkaline phosphatase (ALP) and alanine transaminase (ALT) activities were evaluated to test liver integrity and functionality, bilirubin was assessed to evaluate gallbladder function, serum creatinine was determined to evaluate kidney integrity and functionality, and the stress markers lactate, phosphorus, and urea were evaluated to assess potential allostatic overload caused by capture. Lastly, triglycerides and total cholesterol were assessed to test nutritional status.

Methods

Sampling

Five *I. oxyrhynchus* individuals incidentally captured by the artisanal fleet of the state of Maranhão, in northeastern Brazil, were sampled to assess physiological vulnerability. For comparative purposes, other Carcharhiniform sharks captured by artisanal fleets in the same region were also evaluated (Table 1). Animals were caught with surface longlines in fishing operations lasting about 10 h. Only recently deceased sharks were considered (score 1 for all categories), using a freshness index, considering the following variables: overall gill color (1 for reddish and 0 for pinkish or whitish coloration), ocular retraction level (1 for non-retracted and bright and 0 for retracted and opaque), blood clotting (1 for unclotted and 0 for partially or fully clotted), and *rigor-mortis* (1 for complete absence and 0 for partial or complete presence). Prior to necropsies, the individuals were measured and sexed and species identification was performed according to Compagno (2001).

All sharks were caught between August 2018 and May 2019. Blood samples (10 mL) were obtained by caudal venipuncture using an 18G needle attached to a 20 mL disposable syringe and immediately transferred to ultra-pure polypropylene microcentrifuge tubes (2 mL) (Tubes® 3810X, Eppendorf - Hamburg Germany). Samples were centrifuged for 7 min at room temperature (20°C) at 2,000 x g. The sera from all sharks were separated and frozen at - 20°C until analysis. Sampling was approved by the Brazilian Ministry of Environment (IBAMA/ICMBio-SISBIO #60306-1).

Serum assays

Shark sera were used to determine physiological markers indicative of systemic health and stress response upon capture. Dilutions (1:50) with ultrapure water were performed only for urea, according to a previously established protocol (Wosnick et al., 2017). ALP (Labtest – Brazil; catalog n. 40 wave-length 590 nm), ALT (catalog n. 108; wave-length 340 nm), bilirubin (catalog n. 31; wave-length 525 nm), creatinine (catalog n. 35; wave-length 510 nm), lactate (catalog n. 138-1/50; wave-length 550 nm), phosphorus (catalog n. 42; wave-length 650 nm), urea (catalog n. 27; wave-length 600 nm), triglycerides (catalog n. 87; wave-length 505 nm), and total cholesterol (catalog n. 76; wave-length 500 nm) were quantified colorimetrically (Visible UV Spectrophotometer Q898U2M5 Quimis, Brazil). All analyses were carried out following the manufacturer's instructions and employing previously sterilized material.

TABLE 1   Data on the shark specimens captured by artisanal fleets in the state of Maranhão, Brazil, evaluated in the present study.

Genus	Species	Total Length	Sex	Capture method	Location
Carcharhinus	C. leucas	168.5	F	Longline	MA
	C. leucas	175	M	Longline	MA
	C. leucas	210	F	Longline	MA
	C. limbatus	60.2	F	Longline	MA
	C. limbatus	62.6	M	Longline	MA
	C. limbatus	105	F	Longline	MA
	C. limbatus	101.3	F	Longline	MA
	C. limbatus	97.5	M	Longline	MA
	C. porosus	75	F	Longline	MA
	C. porosus	69	M	Longline	MA
	C. porosus	115.1	F	Longline	MA
	R. porosus	45	M	Longline	MA
	R. porosus	39	F	Longline	MA
	R. porosus	38.3	M	Longline	MA
Sphyrna	S. lewini	48	F	Longline	MA
	S. lewini	57.2	M	Longline	MA
	S. lewini	103	M	Longline	MA
	S. lewini	120	F	Longline	MA
	S. lewini	112.5	F	Longline	MA
	S. tudes	53	F	Longline	MA
	S. tudes	35	M	Longline	MA
	S. tudes	42.5	F	Longline	MA
	S. tudes	76	F	Longline	MA
	S. tudes	58.5	M	Longline	MA
Isogomphodon	I. oxyrhynchus	81	F	Longline	MA
	I. oxyrhynchus	122	F	Longline	MA
	I. oxyrhynchus	74	M	Longline	MA
	I. oxyrhynchus	150	F	Longline	MA
	I. oxyrhynchus	–	–	Longline	MA

Total length is presented in cm.

Statistical analyses

A Kruskal-Wallis test with a *post hoc* Dunn's test was used to assess serum markers differences among *Carcharhinus* spp., *Sphyrna* spp., and *I. oxyrhynchus*, considered adequate for assessments where different numbers of specimens are compared (López-Vásquez et al., 2009; Subotić et al., 2013; Páez-Rosas et al., 2018; Kehrig et al., 2022). A statistical significance of 0.05 was established for all tests. Analyses

were conducted and data were plotted using the SigmaPlot 12 software (Systat Software, San Jose, CA).

Results

Considering systemic health indicators, significant differences were observed for ALP activity, which was higher in *I. oxyrhynchus* compared to *Carcharhinus* spp. and *Sphyrna* spp. ($p = 0.002$) (Figure 1A). No difference was observed between *Carcharhinus* spp. and *Sphyrna* spp. Concerning ALT, significant differences were observed between all groups, higher in *I. oxyrhynchus*, followed by *Carcharhinus* spp. and *Sphyrna* spp. ($p = 0.001$) (Figure 1B). Regarding bilirubin concentrations, significant differences were observed only between *Carcharhinus* spp. and *Sphyrna* spp. ($p = 0.004$) (Figure 1C). Furthermore, significant differences were observed for creatinine concentrations, which were higher in *I. oxyrhynchus* compared to *Carcharhinus* spp. and *Sphyrna* spp. ($p = 0.001$) (Figure 1D). No difference was observed between *Carcharhinus* spp. and *Sphyrna* spp.

Concerning stress markers, significant differences were observed for lactate concentrations among all groups ($p = 0.001$), higher in *I. oxyrhynchus*, followed by *Sphyrna* spp. and *Carcharhinus* spp. (Figure 2A). With regard to phosphorus concentrations, significant differences were observed between *I. oxyrhynchus* and *Carcharhinus* spp. ($p = 0.001$) and between *Carcharhinus* spp. and *Sphyrna* spp. ($p = 0.002$) (Figure 2B). No difference, however, was observed between *I. oxyrhynchus* and *Sphyrna* spp., with the highest concentrations observed for both groups. Furthermore, significant differences were observed in urea concentrations for all groups ($p = 0.001$), lower in *I. oxyrhynchus*, followed by *Carcharhinus* spp. The highest concentrations were observed in *Sphyrna* spp. (Figure 2C).

Considering the determined energetic markers, significant differences were observed for total cholesterol, higher in *I. oxyrhynchus* when compared to *Carcharhinus* spp. and *Sphyrna* spp. ($p = 0.002$) (Figure 3A). No difference, however, was observed between *Carcharhinus* spp. and *Sphyrna* spp. Regarding serum triglyceride concentrations, significant differences were observed between all groups ($p = 0.001$), higher in *I. oxyrhynchus*, followed by *Carcharhinus* spp. The lowest concentrations were observed in *Sphyrna* spp. (Figure 3B).

Discussion

This is the first investigation of the physiological status of the Critically Endangered *I. oxyrhynchus*, focused on Maranhão's remaining population. Considering our first research question, results indicate that the studied Daggernose Shark population exhibits lower health scores when compared to other Carcharhiniformes, evidenced mostly by the higher activities of both ALP and ALT and higher circulating creatinine levels, all indicative of systemic health impairment in vertebrates (Gowda et al., 2010). An increase in enzyme flow to serum may result from increased cellular leakage due to structural damage or increased enzyme synthesis due to pathologies (Brancaccio et al., 2010). Flow rates are generally very expressive when enzyme leakage originates
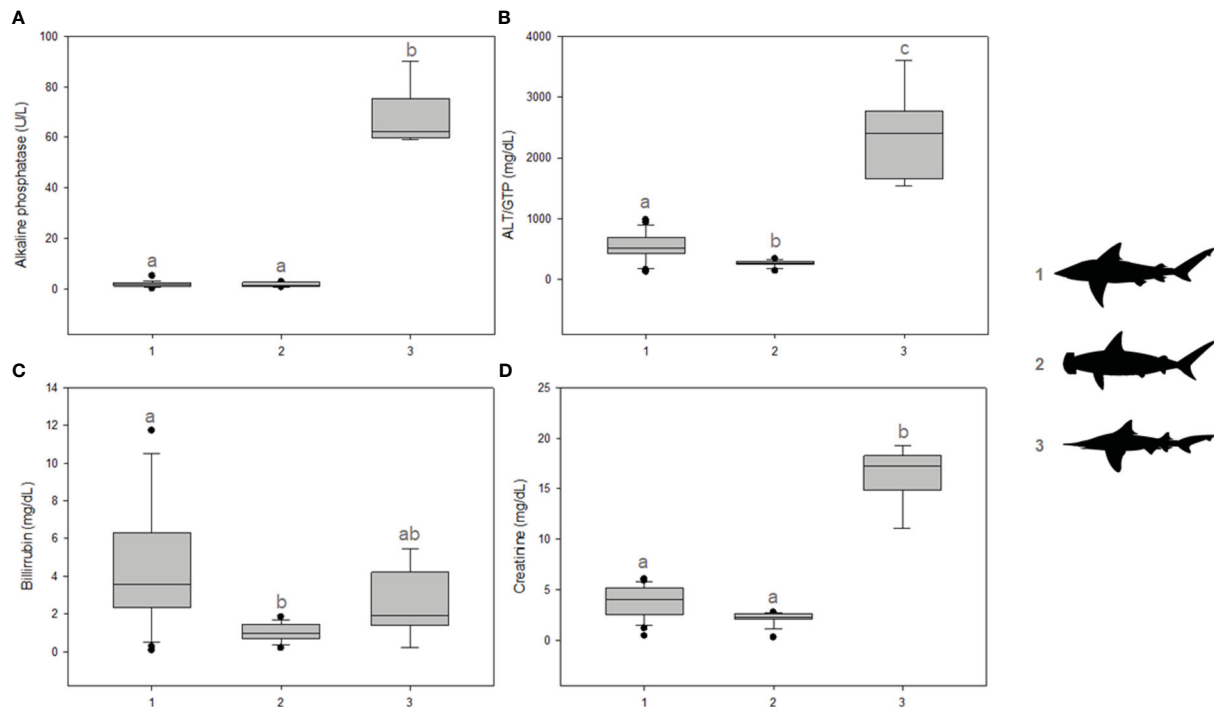
FIGURE 1
Serum markers indicating organ integrity and functionality. **(A)** Alkaline Phosphatase (ALP) activities; **(B)** Alanine transaminase (ALT) activities; **(C)** Bilirubin concentrations; **(D)** Creatinine concentrations. The shark groups are indicated in the graph by 1) *Carcharhinus* spp., 2) *Sphyrna* spp., and 3) *I oxyrhynchus*. Statistical differences are represented by lowercase letters.

from cellular damage and is closely associated to the severity of tissue damage (Melesse et al., 2011), and are widely deployed as a diagnosis tool. It is important to note that enzyme activity in sharks is very species-specific (Manire et al., 2001), preventing its use as a diagnostic tool for most species, as reference intervals are still lacking. Furthermore, as some enzymes can be expressed by several organs (e.g., ALP), it may be difficult to adequately track their origin and therefore, the causes related to increased activities.

In the case of ALP, in some vertebrates its increase in activity is closely related to hepatic damage (Boyd, 1983; Boone et al., 2005), while in others its origin may be cardiac or from skeletal musculature. Therefore, increased leakage to the circulation may be due not only to hepatic pathologies, but also cardiac impairment (Dasgupta et al., 2001). In the case of elasmobranchs, ALP is also a precursor of tesserae mineralization (Omelon et al., 2014), and for some sharks, its activity is related to kidney integrity (Johnson and Aubin, 2015). Thus, holistic approaches (*i.e.*, the assessment of several enzymes) become increasingly necessary to better understand enzyme dynamics in sharks. Our findings indicate that ALP activities were significantly higher in Daggernose sharks compared to other Carcharhiniformes, potentially indicating lower liver integrity in the studied population. However, as ALP may also increase due to capture stress in sharks (Manire et al., 2001), it is also possible that higher activity in *I. oxyrhynchus* indicates a lower allostatic overload resilience due to strenuous exercise (*i.e.*, skeletal musculature leakage).

Alanine Aminotransferase (ALT) activity/leakage was also higher in *I. oxyrhynchus* when compared to other Carcharhiniformes. As ALT expression is higher in vertebrates when liver damage is observed (Center, 2007; Yang et al., 2009) and detected in cases of hepatobiliary

impairment in Tiger sharks (*Galeocerdo cuvier*) (Wosnick et al., 2020), it seems likely that such a pattern may be another indication of poor liver integrity in the studied Daggernose sharks. No data on the effects of capture stress on ALT activity are currently available, leading us to believe that ALT is a promising marker to assess liver health, along with ALP (and other enzymes, whenever possible). However, as ALT exhibited no increase in a liver-damaged Sand Tiger Shark (*Carcharias taurus*), its sole use should be cautionary (Otway, 2015).

Higher circulating creatinine levels were also observed in *I. oxyrhynchus*. This marker is often used to assess kidney integrity in vertebrates, and an increase in serum levels may indicate structural damage to this organ due to the inability to properly excrete this compound (Hanedan et al., 2018). However, as creatinine is a byproduct of creatine phosphate activity in muscle (Volfinger et al., 1994), it is also possible that high circulating levels are a result of strenuous exercise during flight or fight behavior upon capture, although previous studies on capture stress have indicated no increase in creatinine levels in sharks (Manire et al., 2001). That being said, it is plausible to infer that kidney function on Daggernose sharks sampled in the present study was also impaired, at least when compared to other Carcharhiniformes caught in the same region.

Taken together, these results point out a higher systemic vulnerability of *I. oxyrhynchus*, potentially caused by several factors, such as lower genetic diversity, chronic exposure to environmental pollution (Brown et al., 2009), poor diet, and even higher evolutionary specialization (*i.e.*, hammerhead sharks, Gallagher et al., 2014a). Environmental pollution, in particular, may directly decrease organism resilience to other stressors, as it
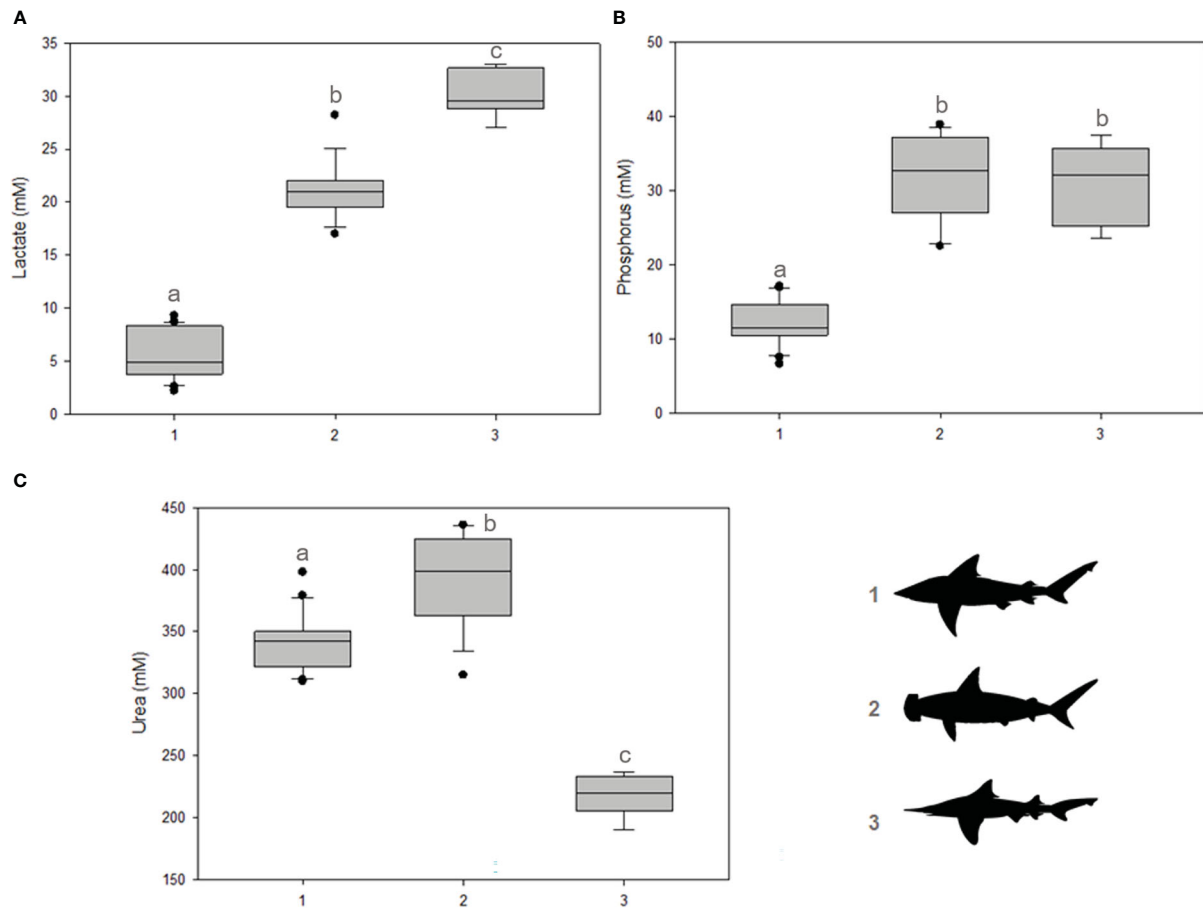
FIGURE 2
Secondary stress markers in shark serum. **(A)** Lactate concentrations; **(B)** Phosphorus concentrations; **(C)** Urea concentrations. The shark groups are indicated in the graph by 1) *Carcharhinus* spp., 2) *Sphyrna* spp., and 3) *I oxyrhynchus*. Statistical differences are represented by lowercase letters.

results in significantly decreased immune system responses in many vertebrates, including fish (Watts et al., 2001). In fact, in a study previously performed with sharks in the same region, including *I. oxyrhynchus*, results pointed to a negative metal bioaccumulation effect on their systemic health (Wosnick et al., 2021a) which, combined with fishing pressure, may explain the poor health status of the studied population. Thus, future studies on genetic structure, ecotoxicology, and molecular biology are necessary to better understand the underlying factors that are affecting the health and potentially the fitness of Daggernose sharks not only in the state of Maranhão, but among other fragmented populations. Such data is, in fact, imperative, as conservation planning is based on population viability, and health assessments are crucial to determine the chances a population has to thrive.

Based on our second research question, results also indicate that *I. oxyrhynchus* is more vulnerable to mortality due to capture stress than other Carcharhiniformes. To date, hammerhead sharks are considered the most sensitive to the negative effects of capture, exhibiting consistently high stress marker levels coupled with the highest mortality rates among studied sharks (Gallagher et al., 2014b; Butcher et al., 2015; Gulak et al., 2015; Jerome et al., 2018). It is believed that their evolutionary history is in part responsible for such vulnerability, as their extreme morphological alterations (*i.e.*, cephalofoil) make them less resistant to stressors (Gallagher et al.,

2014a). For this reason, Sphyrinid sharks were used in the present study, aiming to investigate if *I. oxyrhynchus* is as sensitive as hammerhead sharks. In fact, the results indicate similar, if not higher, vulnerability considering the employed stress markers.

In this regard, circulating lactate levels were higher in Daggernose sharks when compared to both *Carcharhinus* spp. and *Sphyrna* spp., although concentrations were also very elevated in the latter, consistent with previous studies (Gallagher et al., 2014b; Jerome et al., 2018). Lactate is the most reliable stress marker for elasmobranchs (Skomal and Mandelman, 2012), being consistently high in stressed animals as a byproduct of anaerobic metabolism due to strenuous exercise (Skomal and Bernal, 2010; Wosnick et al., 2017; Jerome et al., 2018). Lactate concentrations are very species-specific, and *post-mortem* data offers a more reliable picture than traditional reference intervals, as it is crucial to establish which values are lethal/ non-recoverable rather than which values represent a "non-stressed animal" (Wosnick et al., 2017). Lactate levels in *I. oxyrhynchus* were up to 30 mmol L^{-1}, indicating that putative control for Daggernose sharks should not exceed 70% of established lethal concentrations in order to ensure adequate recovery. However, lactic acidosis can be reverted by activating other compensatory mechanisms, such as ethanol conversion and carbonic anhydrase compensation (Shoubridge and Hochachka, 1980; Aspatwar et al., 2022). Thus, it is possible that individuals exhibiting higher lactate levels are still able
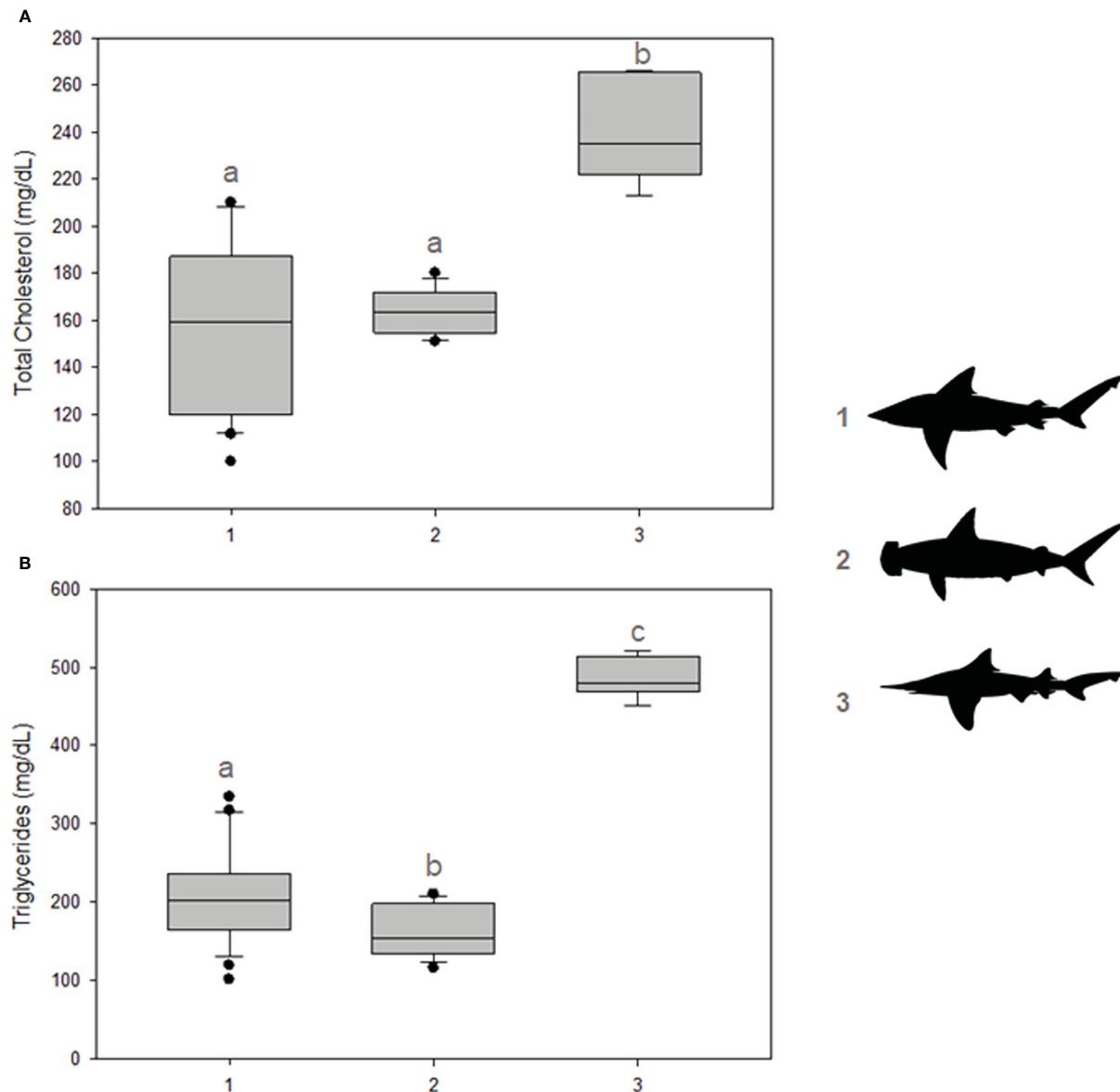
FIGURE 3
Energy metabolism indicators. **(A)** Total cholesterol concentrations; **(B)** Triglyceride concentrations. The shark groups are indicated in the graph by 1) *Carcharhinus* spp., 2) *Sphyrna* spp., and 3) *I oxyrhynchus*. Statistical differences are represented by lowercase letters.

to recover, indicating the need for further studies on other physiological parameters, including serum pH, pCO2, and carbonic anhydrase activity, to better understand how Daggernose sharks respond to systemic lactic acidosis and if the concentrations established in the present study are, in fact, always lethal.

Interestingly, phosphorus concentrations were not significantly different between *I. oxyrhynchus* and *Sphyrna* spp., in both cases very elevated when compared to Carcharhinid sharks. As phosphorus is a predominantly intracellular ion, increased extracellular concentrations indicate cell disruption, leading to excessive leakage (D'Arcy, 2019). In this context, as elasmobranch mortality due to fishing is mainly caused by flight or fight responses, biomarkers indicative of cell rupture/damage have been proven reliable (Wosnick et al., 2017; Wosnick et al., 2021a), always increasing circulating levels upon allostatic overload, as in the case of lactate. That being said, our results indicate that *I. oxyrhynchus* exhibits similar vulnerability to capture stress when compared to the well-known

vulnerable hammerhead sharks, configuring another shark species heavily affected by fishing with little chances of post-release survival. Such a pattern is problematic, as captures are mostly incidental when targeting other fishes (Lessa et al., 2016), so compensatory release might not be the best strategy to reduce bycatch mortality as proposed for several elasmobranchs in both IPOA-sharks and the Brazilian NPOA (Pan-Tubarões).

In the present study, urea concentrations were significantly lower in *I. oxyrhynchus*, which may be related to their ability to make incursions in more dilute waters or even freshwater systems (Ballantyne and Robinson, 2010; Feitosa et al., 2019). As euryhalinity seems to be more common than traditionally proposed (Wosnick and Freire, 2013), records of individuals caught in different salinities alongside the lower urea concentrations observed in the present study are strong evidence that this is the case for Daggernose sharks. Therefore, the use of urea as an allostatic overload indicator should be cautionary. It is also important to consider the potential

effects of such plasticity on stress responses, as euryhaline elasmobranchs may respond differently depending on environmental salinity upon capture. It seems that environmental conditions may influence capture mortality, as in the case of reports of artisanal fishers capturing live Daggernose sharks and releasing them very responsive in higher freshwater input regions (personal communication, N. Wosnick), indicating a better ability to deal with allostatic overload under these conditions. However, studies are required to further investigate this influence, aiming to generate data that might benefit conservation measures based on the higher efficiency of compensatory release, for instance.

As for our third research question, results indicate higher metabolite mobilization in *I. oxyrhynchus* when compared to the other evaluated Carcharhiniforms. While triglycerides are mobilized during exercise, increasing survival chances (Ballantyne, 1997; Wang et al., 2018), total cholesterol levels may increase in serum as a result of energy mobilization or cellular rupture/membrane denaturation (Durstine et al., 1983; Brett et al., 2000; Fines et al., 2001). Thus, it is necessary to evaluate both metabolites when accessing the effects of capture stress on energy mobilization. For example, upon stress, when triglycerides levels are low and cholesterol levels are high, it is plausible to infer that dynamics are being dictated by cellular rupture/membrane denaturation (Leite et al., *in prep.*). When both are observed at high circulating levels, it is more likely that concentrations are being dictated by adaptive mechanisms, as observed in vertebrates that can cope with stressful situations (Pickering and Pottinger, 1995). As stated by Romero and Beattie (2022), higher circulating metabolite levels indicate a higher capacity/ adaptative response to cope with stressors. Based on such a premise, our results indicate that Daggernose sharks are energetically capable of dealing with allostatic overload, although such a response is not enough to ensure their survival, as some physiological impairments (e.g., systemic acidosis) cannot be reserved through energy mobilization itself. Furthermore, we cannot rule out the potential effects that lower health scores might have on metabolite mobilization, as hepatic impairments have the potential to alter metabolites dynamics, leading to higher circulating levels of both analyzed markers (Boyd, 1983; Wosnick et al., 2020). Hepatic damage may also result in decreased environmental contaminant metabolization and subsequent excretion, as the liver is the main detoxifying organ in vertebrates (Yao et al., 2019). This, in turn, leads to higher circulating pollutant levels and significant deleterious physiological effects, including oxidative stress, altered hepatocellular lipid metabolism, citotoxicity and, potentially, genotoxicity which have been reported for many taxonomic groups (Hui et al., 2017; Gabriel et al., 2020; de Farias Araujo et al., 2022), including sharks (Hauser-Davis et al., 2021; Wosnick et al., 2021a), potentially affecting animal health conditions due to altered physiological, systemic and, ultimately, behavioral aspects (Wosnick et al., 2021b; Willmer et al., 2022; Wosnick et al., 2022).

Taken together, our results indicate that the systemic health of the Daggernose shark studied population is impaired when compared to other Carcharhiniformes that inhabit the same region. In general, poor health conditions are related to low-quality prey, environmental pollution, or lower plasticity due to genetic limitations, including very fragmented populations and low gene flow (Neff et al., 2011; Murray et al., 2015; Sueiro et al., 2020). Exposure to constant stressors can also

compromise population health (Stott, 1981), which becomes a significant concern under the current climate change scenario, as this shifting condition is increasingly affecting aquatic ecosystems, resulting in altered water mass flows and the transport patterns of and consequent exposure to a myriad of environmental contaminants, such as metals and persistent organic pollutants (Teran et al., 2012; Hauser-Davis and Wosnick, 2022). Furthermore, certain climate change effects, such as higher temperatures in all environmental compartments (*i.e.*, water, soil, atmosphere) and ocean acidification have been indicated as significantly increasing pollutant bioavailability and toxicity (Ficke et al., 2007; Hauser-Davis and Wosnick, 2022) for many vertebrates, including fish. In this regard, environmental contaminant level effect assessments in elasmobranchs, such as different biochemical response assessments (*i.e.*, protein determinations, immune response assessments and gene expression evaluations) in particular, have been recommended in a multifaceted approach to better understand whole organism/ population fitness and responses to stressors in this group (Skomal and Mandelman, 2012). In addition, the physiology of several species is bound to be affected by climate change effects, with several authors indicating that the most probable response will comprise altered migration shifts, both in timing and established routes, and geographic distributions (Field et al., 2009), in turn making organisms more vulnerable, significantly aggravating ecological risks in a continuous cause-and-effect cycle.

In the case of the sharks evaluated herein, prey availability seems not the case, nor differentiated pollution gradients, as all assessed species share the same habit and are part of the same trophic chain. Although species-specific differences cannot be disregarded, the phylogenetic proximity among the sharks evaluated in the present study leads us to believe that the lower health condition of *I. oxyrhynchus* might be a result of low genetic diversity, leading to poorer physiological plasticity as a negative outcome of fisheries-induced evolution.

The same was observed for the Daggernose Shark capacity to overcome allostatic overload, indicating that their ability to cope with stress is now severely compromised. In fact, stress markers were as elevated as those observed in hammerheads, considered the most physiologically vulnerable sharks. Once again, low genetic diversity leading to limited physiological plasticity as a result of fisheries-induced evolution might explain the observed vulnerability. Of course, intrinsic lower species-specific capacity to cope with stressors cannot be ruled out, and further studies employing other markers along with population genetics investigations are necessary to shed light on how much fisheries-induced evolution may be affecting the remaining Daggernose sharks. Furthering our knowledge on the adverse effects of pollution in this species is paramount, to better understand how its survival/ability to cope with other stressors are being affected. Pathology investigations are also urgent, aiming to evaluate Daggernose shark health status as a whole.

Some conservation and fisheries management aspects must also be considered. First, no conservation planning has been developed for the Daggernose Shark to date. Although this species is highlighted in the Brazilian NPOA and specific fishing regulations prohibit their capture, transport, and commercialization, there are no management plans that consider the negative consequences

related to mortality due to commercial capture, or the overall health of the remaining populations. In fact, fishing bans do very little for Daggernose sharks, as most current captures are incidental, and even if release is adopted as a mitigating measure, the chances of survival appear to be low, resulting in little or no efficiency. Unfortunately, it is likely that the chances of reversing the current situation of this species are low, or practically nil, especially considering the low genetic diversity of the remaining populations coupled with their physiological vulnerability described herein. Thus, international efforts and long-term recovery programs are urgent. More specifically, it is possible that the only chances of the species rely on captive maintenance, which is a challenge for most shark species. However, this should not hinder joint efforts, not only to more effectively protect free-ranging individuals (e.g., Marine Protected Areas and Sanctuaries), but also to direct individuals to institutions that can maintain them under adequate human care and perform long term health assessments, aiming to monitor the real status of remaining individuals, as well as to advance assisted reproduction attempts.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the animal study because Only dead animals from commercial fishing were used, requiring only permission from the Brazilian government to collect samples (IBAMA/ICMBio-SISBIO #60306-1).

Author contributions

Acknowledgments

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Evidence for the first multi-species shark nursery area in Atlantic Africa (Boa Vista Island, Cabo Verde)

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This study describes the first potential multi-species shark nursery area in Atlantic
Africa (Sal Rei Bay − SRB, Boa Vista Island, Cabo Verde). From August 2016 to
September 2019, 6162 neonates and juveniles of 5 different shark species were
observed in SRB using beach gillnet-based bycatch surveys, namely milk
(*Rhizoprionodon acutus*; n= 4908), scalloped hammerhead (*Sphyrna lewini*; n=
1035), blacktip (*Carcharhinus limbatus*; n=115), Atlantic weasel (*Paragaleus
pectoralis*; n= 93) and nurse (*Ginglymostoma cirratum*; n= 12) sharks. Except for
nurse sharks, significant seasonal variations in shark relative abundance were
observed, with higher levels being recorded during summer and autumn. These
findings, together with local knowledge (interviews to fishermen), denote the
consistent use of SRB by juvenile sharks and its preference relative to other
areas in the region. Ensuring the protection and conservation of SRB nursery
area is especially relevant as, according to IUCN, all identified shark species are
threatened with extinction over the near-future − in particular, scalloped
hammerheads (critically endangered) and Atlantic weasel sharks (endangered).
The effective protection of SRB will not only support the conservation of shark
populations, but also of other charismatic fauna (e.g., loggerhead turtles) and
broader benthic and pelagic ecosystems.

KEYWORDS

elasmobranchs, juveniles, parturition area, marine conservation, Western Africa, sharks

Introduction

Most sharks occupy high trophic levels in marine ecosystems, thus exerting a key influence on their structure and function ([1990](#); Compagno, 1984). Yet, contrary to most fishes, sharks generally have a K-selected life history strategy, which means slow growth rates, late maturity age, low fecundity, long gestation period, few offspring, and long-life spans (Dulvy et al., 2014; Wheeler et al., 2020). This, allied to a general tendency for segregation by age and sex, makes them especially vulnerable to human impacts (Baum et al., 2003; García et al., 2008; Ferretti et al., 2010; Roff et al., 2016). In fact, shark populations have been plummeting over the past few decades, namely driven by intense fishing pressure, with key implications for their sustainability and conservation (Queiroz et al., 2019; Dulvy et al., 2021; Pacoureau et al., 2021).

Understanding the habitat-use patterns of sharks, namely through identification of key aggregation sites and nursery grounds, is essential to recognize the potential effects of human activities on these populations and design effective conservation and management strategies (Knipp et al., 2010; Speed et al., 2010; Diemer et al., 2011; Henderson et al., 2016; Queiroz et al., 2016; Heupel et al., 2019; Queiroz et al., 2019). Among several hypotheses concerning the role of nursery areas, it is generally accepted that such areas provide enhanced food availability and protection against predation (Springer, 1967; Branstetter, 1987; Heupel and Simpfendorfer, 2002; Heupel et al., 2007). According to Heupel et al. (2007), three criteria must be met so that a particular marine area can be considered as a shark nursery ground, namely: i) *preference* – sharks are found more often in the specific area than in neighboring ones, ii) *residency* – sharks tend to remain in the area (or return) for extended periods, and iii) *consistency* – the area or habitat is used repeatedly by sharks over the years.

While great efforts have been made to identify and describe sharks' nursery areas around the world, there are still strong climate, habitat, and taxonomic bias in the literature (Heupel et al., 2019). Moreover, and because the identification of such important nursery areas is often dependent on long-term sample size datasets, most studies do not comply with all three criteria defined by Heupel et al. (2007), fulfilling only one or two criteria. For the Atlantic African region in particular, seven potential shark nursery areas were described over the past decade, all of them being for single species (see Supplementary Table 1). These pertained to areas used by angel sharks (*Squatina squatina*) in the Canary Islands, leafscale gulper sharks (*Centrophorus squamosus*) in Mauritania and Namibia, and great white (*Carcharodon carcharias*), smooth hound (*Mustelus mustelus*), and blue (*Prionace glauca*) sharks in South Africa (see details and respective references in Supplementary Table 1).

Communal nurseries are locations where juveniles of multiple shark species occur and the adults are mostly absent (Simpfendorfer and Milward, 1993). Yet, in such nurseries, the juveniles face a tradeoff between lower predation risk and increased competition – while the latter is potentially reduced *via* partitioning of food resources (Kinney et al., 2011). Within this context, here we describe, for the first time, a potential multi-species/communal shark nursery area in the Atlantic African region. More specifically, we describe the first potential nursery of milk (*Rhizoprionodon acutus*), scalloped hammerhead (*Sphyrna lewini*), blacktip

(*Carcharhinus limbatus*), Atlantic weasel (*Paragaleus pectoralis*), and nurse (*Ginglymostoma cirratum*) sharks in Sal Rei Bay (SRB), Boa Vista Island, Cabo Verde. Here we assess: (i) the diversity of shark species occurring in the SRB; (ii) the size frequency distribution of juvenile sharks; (iii) inter and intra-year patterns in the relative abundance of juvenile sharks (catch per unit of effort data); and (iv) the spatial variation in species composition and abundance around Boa Vista Island based on interviews to local fishermen.

Material and methods

Temporal changes in juvenile shark relative abundance in SRB, Boa Vista Island (Cabo Verde)

Cabo Verde is a small archipelagic country, located in the Atlantic Ocean (Supplementary Figure 1), which has been long recognized as a global hotspot of marine biodiversity (Roberts et al., 2002; Freitas et al., 2019). Boa Vista Island is the easternmost (windward) island of the archipelago (Supplementary Figure 1), with a coastline mostly composed of sandy and rocky beaches and high-energy exposed shores (Gomes, 2019). While Boa Vista is a well-known nesting area for loggerhead turtles (*Caretta caretta*) in the eastern Atlantic (Marco et al., 2012; Martins et al., 2022), and breeding area for the endangered North Atlantic humpback whale (*Megaptera novaeangliae*) (Wenzel et al., 2020), biological knowledge on other marine taxa, namely on sharks, is largely absent. A particular bay in the island, the SRB (Supplementary Figure 2), comprises a marine area of c. 22 km^2, mostly of sandy substrate, and is locally known (e.g., local communities, artisanal fishermen) to bear a variety of shark juveniles. Therefore, from August 2016 to September 2019, in SRB, and with the help of local fishermen, beach gillnet-based bycatch was surveyed (4 cm square-mesh monofilament gill net, with 30 meters in length and 3 m deep) on a monthly basis. This type of artisanal fishing gear is used by the local fishermen to catch small pelagic fish (sparids, bigeye scad, tuna, among others). The gill net was always set perpendicular to the shore, with soak time ranging from 2 to 4 h, depending on tidal and weather (season) conditions. Juvenile sharks were identified to the species-level. To prevent post-release casualties, the net was regularly surveyed, and when necessary, the animals were manually moved through the water during release to promote recuperation. Catch per unit effort (CPUE) was calculated as the number of sharks caught per hour per net meter square (sharks h^{-1} m^{-2}).

Spatial differences in juvenile shark abundance

To understand if sharks are found more often in SBR than in other areas of the island, a short questionnaire was conducted close to local artisanal and semi-industrial fishermen. From a list of 92 licensed fishermen in Boa Vista Island, a total of 55 interviews were done (~60%). They were conducted in Cabo Verdean creole, by telephone, in August 2022. Prior to the interviews, fishermen were informed about the purpose of the survey, anonymity, and confidential treatment of the obtained data, and asked for verbal

consent to participating. The interview entailed the following 4 questions:

i) "Are you aware of any specific areas in Boa Vista Island where one can find higher abundance of shark juveniles?"

ii) "Among those areas, which one shows the highest values of abundance of shark juveniles"?

iii) "Which species do you find in that particular area"? and

iv) "Where do you usually fish"?

Total length distributions

Total length (TL) of juvenile sharks was measured to the nearest 0.5 cm. TL frequency distributions of milk (n= 2165), scalloped hammerheads (n= 404), blacktip (n=115), Atlantic weasel (n=94) and nurse sharks (n=12) were evaluated and compared with key biological information obtained from previous studies, namely species' length at first maturity and size at birth in the Atlantic Ocean (see respective data and references in Supplementary Table 2).

Statistical analyses

To evaluate changes over time generalized additive models (GAMs) per species were used, with a smooth of time (year) and a cyclic smooth of month. We considered the relative abundance (catch per unit of effort) response to be Gaussian, with a log link. To ensure that the cyclic nature of the variable month was respected we considered a cyclic basis for the month spline.

Models were implemented in the R library mgcv, following Wood (2017). The residuals of the fitted models were checked for temporal autocorrelation, and since no serious reasons for concern were found, we did not include an autocorrelation term in the models.

Results

Temporal changes in juvenile shark relative abundance in SRB

From August 2016 to September 2019, juveniles of five different shark species were observed during bycatch surveys in SRB, namely milk (n=4908), scalloped hammerhead (n=1035), blacktip (n=115), Atlantic weasel (n=93) and nurse (n=12) sharks (Figures 1, 2). The higher CPUE values were observed for the milk shark (reaching a maximum of 3.55 individuals h^{-1} m^{-2} in August 2017), followed by the hammerhead (a maximum of 0.52 individuals h^{-1} m^{-2} in September 2019), blacktip (a maximum of 0.06 individuals h^{-1} m^{-2} in November 2017), Atlantic weasel (a maximum of 0.03 individuals h^{-1} m^{-2} in June 2017), and nurse sharks (a maximum of 0.01 individuals h^{-1} m^{-2} in January 2019). Except for the nurse sharks, all other species revealed significant seasonal variations in CPUE values (Table 1), with highest values being observed during summer or autumn periods (Figures 1, 2).

Total length distributions

The size range of milk sharks (n=2165) was 30 to 70 cm total length (mode 40-50 cm interval), while for the scalloped
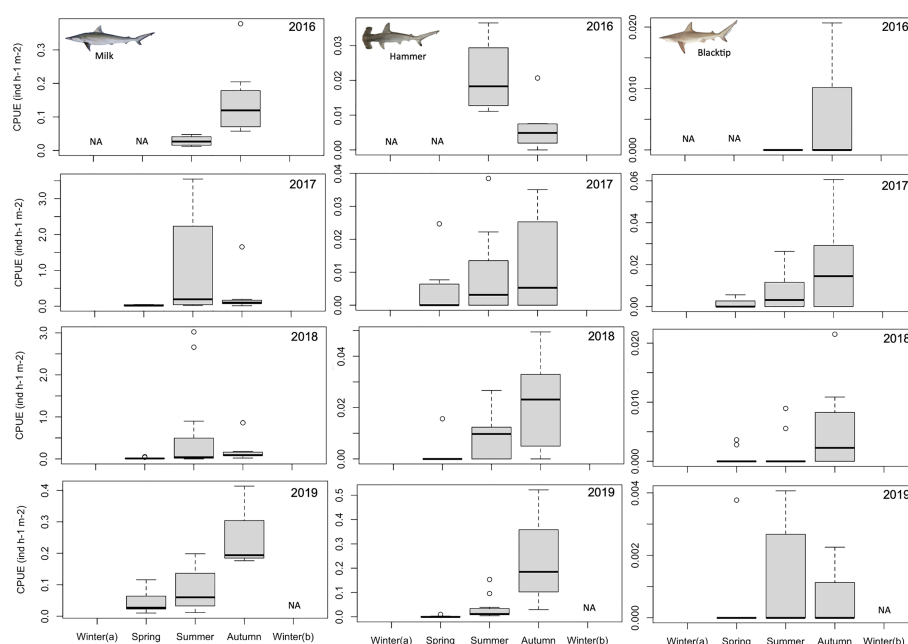
FIGURE 1

Temporal changes in the relative abundance (catch per unit of effort; number of individuals h^{-1} m^{-2}) of juvenile milk (*Rhizoprionodon acutus*), scalloped hammerhead (*Sphyrna lewini*), and blacktip (*Carcharhinus limbatus*) sharks in Sal Rei Bay, Boa Vista Island, Cabo Verde. NA, not applicable.
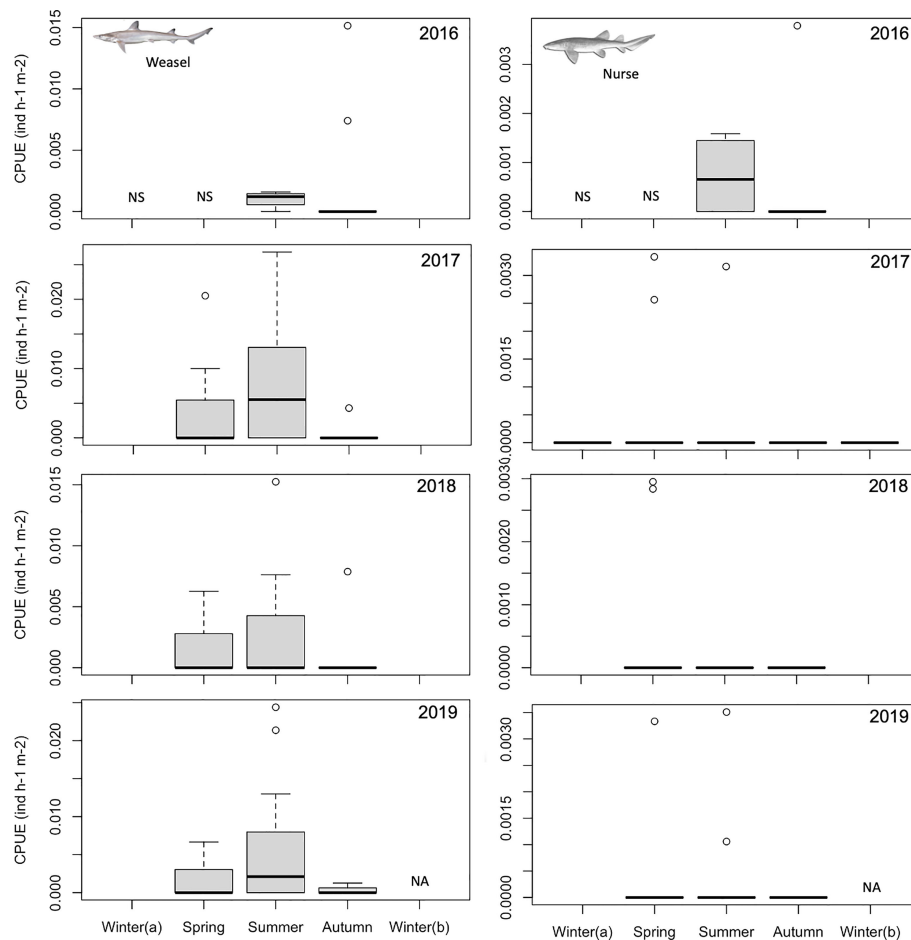
FIGURE 2
Temporal changes in the relative abundance (catch per unit of effort; number of individuals h^{-1} m^{-2}) of juvenile Atlantic weasel (*Paragaleus pectoralis*) and nurse (*Ginglymostoma cirratum*) juvenile sharks in Sal Rei Bay, Boa Vista Island, Cabo Verde. NS, not sampled.

hammerheads (n=404) was 30 to 65 cm (mode 50-60 cm interval). The blacktip sharks (n=115) presented a size range of 58 to 110 cm (mode 70-80 cm interval), the Atlantic weasel sharks (n=94) of 43 to 98 cm (mode 50-60 cm interval), and the nurse sharks (n=12) of 43 to 140 cm (mode 50-60 cm interval). All sampled individuals revealed sizes below species' length at first maturity, except to two weasel shark individuals (with 96 and 98 cm total length; Figure 3).

Spatial differences in juvenile shark abundance

Local fishermen identified 11 areas of occurrence of juvenile sharks around the island. Yet, SRB collected the highest level of agreement by far, with 78% of respondents identifying it as an area of juvenile sharks' occurrence, and 60% as *the area* with the highest number of juvenile sharks in the entire island (Figures 4A, B). Some areas within the SRB

TABLE 1 Effects of time (year) and cyclic month effects on the catch per unit of effort (CPUE) for each of the five shark species.

Species	Time		Month		R^2	deviance
	df	p-value	df	p-value		
Milk (*Rhizoprionodon acutus*)	8.99	<0.001	2.54	< 0.0001	96.2	96.2
Scalloped hammerhead (*Sphyrna lewini*)	5.27	<0.001	4.21	< 0.0001	85.5	85. 6
Blacktip (*Carcharhinus limbatus*)	4.67	<0.0001	3.96	< 0.0001	47.2	48.8
Atlantic weasel (*Paragaleus pectoralis*)	9.00	<0.0001	4.76	0.0110	62.5	64.6
Nurse (*Ginglymostoma cirratum*)	1.00	0.3398	4.31	0.7769	68.3	67.7

Results presented correspond to outputs for Generalized Additive Models (Gaussian family with a log link) depicting the smooth effects (estimated degrees of freedom and p-value associated with testing the need for the term to be included in the model) of time and cyclic smooth effect of month on CPUE per each shark species. Also shown the R^2 and the % of deviance explained.
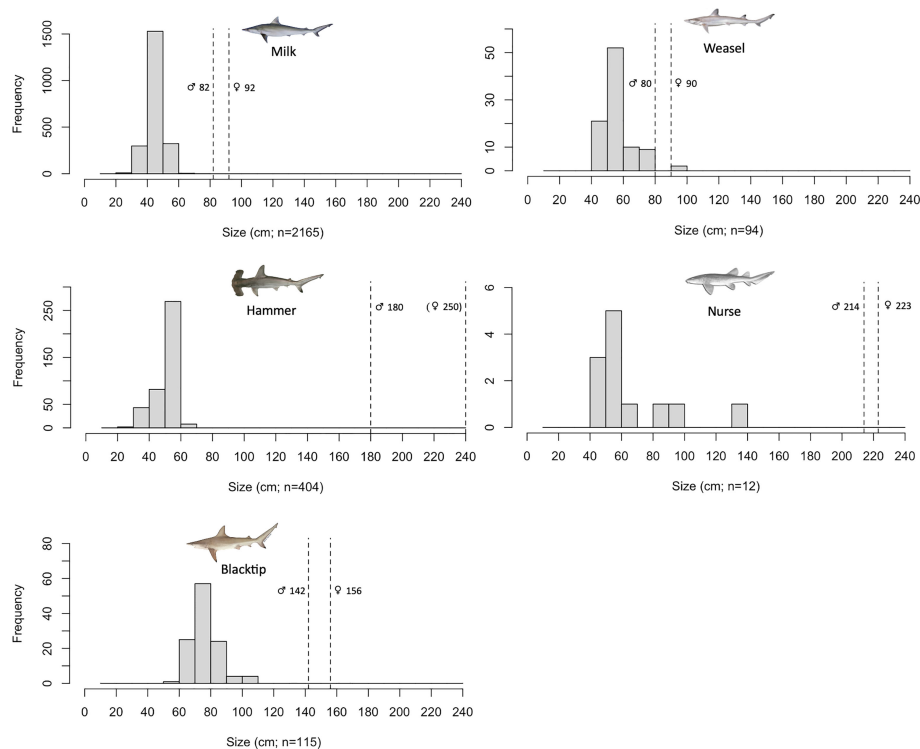
FIGURE 3
Length distributions of juvenile milk (*Rhizoprionodon acutus*), scalloped hammerhead (*Sphyrna lewini*), blacktip (*Carcharhinus limbatus*), Atlantic weasel (*Paragaleus pectoralis*) and nurse (*Ginglymostoma cirratum*) sharks in Sal Rei Bay, Boa Vista Island, Cabo Verde. Vertical dashed lines represent species' length at first maturity for both males and females (see respective references in Supplementary Table 2).

were further emphasized by fishermen, such as "Djeu", "Praia do Estoril", "Morro de Areia", "Praia de Chaves", or "Caramboa" (n=14). Ervatão and Santa Mónica were also identified as areas of juvenile sharks' occurrence (24% and 22%, respectively), and Ervatão and Esgata as having the highest number of juvenile sharks, only to a lower extent (11% and 7%, respectively; Figures 4A, B). When asked about what species were present in the area with the highest number of juvenile sharks, 16% of respondents identified blacktip sharks (Figure 4C), 20% identified hammerhead sharks (Figure 4D), and 60% identified "cação" – the latter is the common name used locally to refer several species, including milk and Atlantic weasel sharks (Figure 4E). In all cases, SRB was the area that collected most responses for each species (from 64% to 92%; Figures 4C–E). This preference was irrespective from respondents fishing grounds, as 80% of the fishermen that selected SRB do not use it as a fishing ground (Supplementary Figure 3). Only a very small percentage of respondents did not provide any information on shark juveniles (2 fishermen were not knowledgeable on areas with shark juveniles, and 1 did not want to share information). Most fishermen identified only one type of shark (n=45, 82%; Supplementary Figure 4). Only a small number of fishermen identified two types of sharks simultaneously (n=4) or was not able to identify any particular species (n=3; Supplementary Figure 4).

Discussion

The present study shows that SRB is used by juveniles of, at least, 5 threatened shark species. According to the IUCN Red List of Threatened Species (IUCN, 2022), *S. lewini* is designated by as

"Critically Endangered", *P. pectoralis* as "Endangered", and *R. acutus*, *C. limbatus* and *G. cirratum* as "Vulnerable". Furthermore, although not caught during the present surveys, information acquired through citizen science and preliminary data based on baited remote underwater videos (BRUVs), suggest the presence of juveniles of other shark species, namely spinner (*Carcharhinus brevipinna)* and lemon (*Negaprion brevirostris*) sharks (see Supplementary Figure 5).

The high consistency of the results obtained based on local fishermen's knowledge clearly showcases that shark juveniles are found more often in SRB than in other coastal areas of the island (Figure 4). This preference is potentially related to the fact that SRB is a shallow, relatively wave-protected area (mostly with less than 10 m of depth). Because the predominant direction of waves in Boa Vista Island is from the Northeastern quadrant (Gomes, 2019) under the influence of the Canary Current (Peña-Izquierdo et al., 2012), SRB has relatively low wave action, especially during summer months. Moreover, there is a small islet at the north side of the bay that provides further protection (Supplementary Figure 2). By contrast, most other areas in Boa Vista Island are high-energy exposed shores without noticeable barriers (e.g., reefs or small islets) (Gomes, 2019).

SRB is repeatedly used by juvenile sharks over the years (Figures 1, 2), with higher CPUE values during summer and autumn periods. With this information, we are confident that SRB fully meets both criteria I (i.e., sharks are found more often in the specific area than in neighboring ones) and III (i.e., the area is used repeatedly by sharks over the years), as defined by Heupel et al. (2007). While no definite conclusions can be drawn regarding criterion II (i.e., sharks tend to remain in the area for extended

FIGURE 4
(A) Areas of occurrence of juvenile sharks in Boa Vista Island, Cabo Verde, according to local fishermen. Eleven areas were identified based on dedicated interviews (n=55) to local registered commercial and artisanal fishermen; six of these areas were considered as *the* areas with the highest number of juvenile sharks in Boa Vista Island. **(B)** Spatial distribution of the identified areas around the island of Boa Vista. Sal Rei Bay (SRB) collected the highest level of agreement 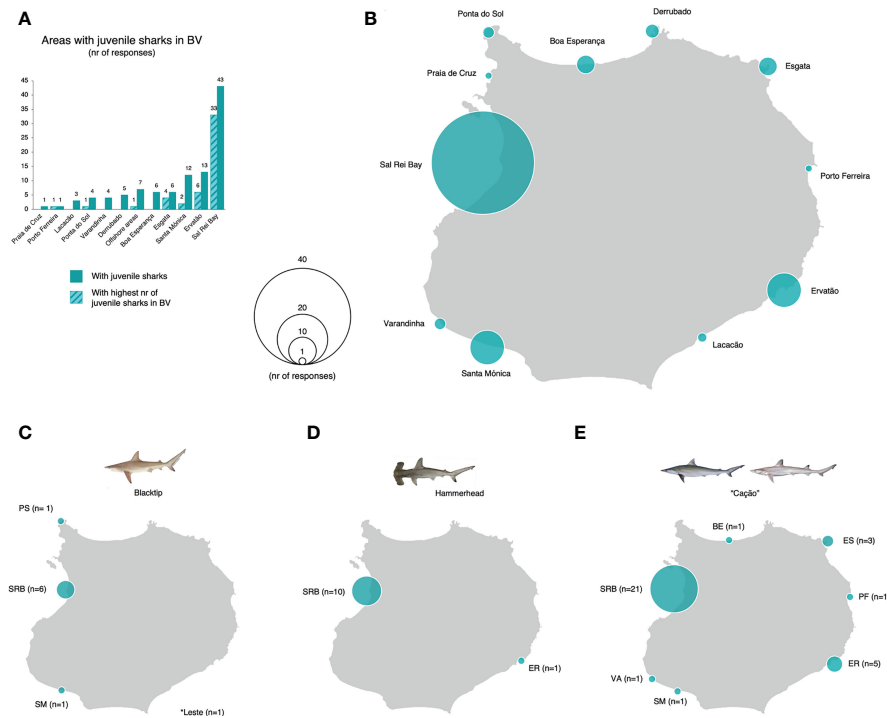among fishermen, both as an area with juvenile sharks (n=43; 78%) and as the area with the highest number of juvenile sharks (n=33; 60%). Respondents identified **(C)** areas of occurrence of juvenile blacktip sharks (n=9; 16%), **(D)** juvenile hammerhead sharks (n=11; 20%), and **(E)** juvenile "cação" (n=33; 60%). "Cação" is the common name used locally to refer to both milk and Atlantic weasel sharks – only two fishermen referred to Atlantic weasel sharks specifically, and one to "boca cumprido" referring to milk sharks. Identified offshore fishing grounds include "Leste", "Rio de Janeiro", "Costa de Mar", "Txom Branco", "Verde", and "West" (these are large areas off the coast, locally known as "pesqueiros"). BV, Boa Vista.

periods), the observation of neonates with umbilical scars at different healing stages (preliminary findings) together with the general range of sizes observed (Figure 4) suggest that these animals are likely to make use of the bay for at least a few weeks after birth, and, thus, likely to meet criteria II. In this context, and although direct efforts to definitively confirm criterion II are still required (e.g., mark-recapture studies, remote tracking, stable isotope analysis), we argue that the importance of the SRB as a potential nursery area for multiple threatened shark species must not be understated.

Ensuring the effective protection of the SRB potential nursery area is of the utmost relevance as all identified shark species – in particular scalloped hammerheads and Atlantic weasel sharks – are threatened by anthropogenic pressures, and vulnerable to extinction over the near future (IUCN, 2022). There are many types of potential marine protected areas (MPAs), from full to minimal protection, from the ones that exist in practice (implemented) to the ones only on paper (Grorud-Colvert et al., 2021). The SRB is partially encompassed by two of the Boa Vista Island protected areas, however these do not focus on the marine realm, and are not MPAs but "natural reserves" – over 80% of their extension corresponds to terrestrial area (Cabo Verde Parliament, 2014a; 2014b). Indeed, the *Boa Esperança Natural Reserve* and the *Morro de Areia Natural Reserve* do include "peripheral areas for marine protection" that extend up to 300 m offshore (Cabo Verde Parliament, 2014a; 2014b). Yet, the latter represent only a small fraction of the SRB (10%), with an area of c.

2.7 km^2 (see Supplementary Figure 2). At the same time, these natural reserves are still pending implementation, which means that they do not yet have any management instruments in place (e.g., management plans) (Boa Vista Municipality, 2022). The latter is especially significant as benefits of MPAs are highly dependent on their effective implementation and management (Grorud-Colvert et al., 2021). Also, the lack of enforcement further undermines the effectiveness of MPAs, as observed in other islands of Cabo Verde (Vasconcelos et al., 2015).

New opportunities and risk also arise from the recently approved coastal and marine spatial plan of Boa Vista Island – *Plano de Ordenamento da Orla Costeira e Mar adjacente da ilha da Boa Vista* (POOCM) (Cabo Verde Parliament, 2020). The POOCM establishes a planning unit for SRB (the *Sal Rei Bay Integrated Management Area*) whose general goals are to: (1) minimize the risk of environmental impacts; and (2) regulate fishing, nautical sports, and other recreational activities to make them compatible with the protection and valorization of marine ecosystems. Still, human activities such as artisanal fisheries, aquaculture, renewable energy, and maritime transportation are generally allowed in the planning unit (Cabo Verde Parliament, 2020). At the same time, while the POOCM has several references to the protection of sea turtles and marine mammals, no references are found for sharks, reflecting the lack of attention to this particular taxonomic group. Also, the POOCM is to be further implemented by dedicated management

plans and regulations that are still to be developed, and which sometimes take long to be put in place because of social-political factors (Frazão Santos et al., 2021). Finally, the close proximity to two "tourism development zones" (the *Integral Tourism Development Zone of Chave* and *Integral Tourism Development Zone of Morro de Areia*) makes the SRB further vulnerable to human pressures and impacts (Cabo Verde Parliament, 2008; 2009).

Further action is therefore needed to ensure the effective conservation of shark populations, and broader benthic and pelagic ecosystems, in the SRB. Such protection will largely depend on the specific conservation measures and monitoring plans that are put in place, but also on capacity building and awareness raising actions targeting national and international ocean users and stakeholders. Only then will it be possible to ensure an equitable and sustainable use and conservation of the SRB.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the animal study because It entailed surveys on local artisanal bycatch fishery.

Author contributions

RR conceptualized the study. RR, EN, VP, CS, JV, JP, MB, TR, JC and CF collected field data. RR and TM performed data analyses. RR and CF wrote the original draft of the manuscript. All authors reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1077748/ full#supplementary-material

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Multi-decade catches of manta rays (*Mobula alfredi*, *M. birostris*) from South Africa reveal significant decline

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Manta rays (*Mobula alfredi* and *M. birostris*) are poorly understood in South Africa, despite their ecological importance and charismatic appeal. This study analyzed a 41-year dataset from the KwaZulu-Natal bather protection program to investigate catch per unit effort between 1981-2021. We used Generalized Additive Models and the probability of encounter to assess annual and seasonal trends, as well as the effect of location and moon phases on catch rates. We also evaluated the size composition and demographics of caught manta rays using the same dataset. Our analysis revealed a significant decline in overall manta ray catches since the late 1990s (p<0.0001), with increased catch rates during summer, suggesting seasonal visitation to South African waters. We found that manta rays were caught at least once in all 46 netted beaches along the 350 km span of coastline, but with significantly more catches in the Central Area, between Anstey's beach in the north and Mtwalume in the south. We also observed that moon phase had an effect on manta ray presence, with significantly more catches during spring tides at new and full moon phases. Over half of the caught individuals were juveniles, and a total of 841 individuals (52% of the total catch) belonged to the confirmed juvenile size class (1400-2500 mm disc width). We further found that a greater proportion (70%) of juveniles were caught in the southernmost sampled area, from Hibberdene in the north to Mzamba in the south. These findings highlight the importance of South African waters as a seasonal habitat for manta rays along the southern African coastline. The significant decline and spatial-temporal patterns we observed have critical implications for management and conservation efforts. Our study provides valuable baseline data for future research and underscores the need for continued monitoring and protection of these iconic marine species.

Introduction

Manta rays (Family Mobulidae) are pelagic planktivores that aggregate in regions supporting high zooplankton densities and cleaning stations, where symbiotic fish remove parasites from them (Feder, 1966; Couturier et al., 2012; Stevens, 2016; White et al., 2017). Being large filter feeders, manta rays spend their lives in proximity to where plankton blooms occur, these being elicited by temporal and spatial environmental cues (Sims et al., 2005; Armstrong et al., 2021). The great variability and transience of regional plankton likely drive their foraging behavior, prey sources, and habitat use (Stewart et al., 2017; Barr and Abelson, 2019; Putra et al., 2020).

The oceanic manta ray, *Mobula birostris* (Walbaum, 1792), has circumglobal distribution, and generally occurs more offshore than the smaller, more coastal reef manta ray, *Mobula alfredi*, (Kreft, 1868), which is semi-circumglobal and restricted to tropical and subtropical waters (Marshall et al., 2009; Burgess et al., 2016; Armstrong et al., 2020). These are the two largest of all ray species (*M. birostris*; 8 m maximum disc width (DW); *M. alfredi*; 5.5 m maximum DW) and are both slow-growing, with late maturation and low fecundity (Marshall et al., 2009; Marshall and Bennett, 2010; Stevens et al., 2018). Due to these life history characteristics, as well as the exploitation of mobulids for the gill plate trade, both manta ray species are listed on the IUCN's Red List of Threatened Species (*M. birostris* as Endangered and *M. alfredi* as Vulnerable) (Marshall et al., 2009; O'Malley et al., 2016; Marshall et al., 2018a; Marshall et al., 2018b). Although directly fished and caught as bycatch in Mozambique (Couturier et al., 2012; Croll et al., 2016), one of the sources of fishing mortality for *M. birostris* and *M. alfredi* in the south-west Indian Ocean is the KwaZulu-Natal (KZN) bather protection program in South Africa. Although not a fishery in the conventional sense, this is the only shark fishing operation in South Africa documented to catch these species as a means to protect public bathers (Dudley and Cliff, 1993; Marshall et al., 2008; Croll et al., 2016).

Both *M. birostris* and *M. alfredi* are known to migrate, with current recorded ranges of >1400 km for oceanic manta rays (Hearn et al., 2014) and 1150 km for reef manta rays (Armstrong et al., 2019). Despite such extensive horizontal movements, manta rays display affinity to certain locations such as inshore reefs, seamounts, or foraging sites, for example, which the same individuals have been found frequenting for up to 30 years (Dewar et al., 2008; Couturier et al., 2014; Couturier et al., 2018; Venables et al., 2020).

Manta ray movement patterns in southern Africa may be driven by temporal and spatial patterns of zooplankton abundance (Sims et al., 2006; Rohner et al., 2017; Stewart et al., 2019). On the east coast of South Africa, the narrow continental shelf (Martin and Flemming, 1988) and shifting seasonal water temperatures and currents (Walker, 1990; Roberts et al., 2010) allow numerous elasmobranch species, such as the tiger shark, *Galeocerdo cuvier*, and the diamond ray, *Gymnura natalensis*, to exploit a wide range of habitat and area (Connell, 2001; Wetherbee, 2004; Dicken et al., 2006; Daly et al., 2018; Daly et al., 2022). Acoustic telemetry revealed a reef manta ray that traveled up to 90 km in a single day in Mozambique (Venables et al., 2020). At monitored locations

in southern Mozambique, manta ray habitat use is seasonal; sightings increase in Tofo during austral summer (November to February), (Marshall et al., 2011) while more sightings occur from July to November in Závora, which is 90 km further south (Carpenter et al., 2022). Oceanic manta ray sightings peak in April in Tofo (Rohner et al., 2013). Despite contrasting temporal patterns, oceanic and reef manta rays in southern Mozambique overlap in their use of cleaning and foraging habitats, which may be a result of resource availability in the area (Kashiwagi et al., 2011).

While manta rays have been studied for two decades in Mozambique, they remain relatively understudied in South Africa, despite sightings from KwaZulu-Natal (KZN) and the availability of suitable habitat, including cleaning stations (Carpenter, unpublished data). Genetic analysis and photo identification studies suggest that there is a single breeding population of reef manta rays common between the two countries (Venables et al., 2021; Marshall et al., 2022), and it is likely that KZN coastal waters may serve as critical habitat for southern African manta ray populations.

Given the migratory nature of manta rays and limited information about the species in South African waters, we evaluate baseline trends in encounters, similar to other studies on ray species in KZN (Daly et al., 2021; Daly et al., 2022). We use 41 years of catch data from the KZN bather protection program to investigate long-term trends in manta ray occurrence, body size and demographic composition. We determine the influence of environmental variables on manta ray occurrence using Generalized Additive Models (GAMs), and describe patterns of temporal and spatial habitat use.

Materials and methods

Study area

The marine environment of the KwaZulu-Natal (KZN) Province, on the east coast of South Africa, is subtropical and dominated by the southward-flowing Agulhas Current (Lutjeharms et al., 2000). Two ecoregions have been described by Sink et al. (2019) within KZN borders: 'Maputaland', which extends from the Mozambique border southwards to Cape Vidal, and 'Natal', from south of Cape Vidal to the Eastern Cape (Sink et al., 2005; Griffiths et al., 2010). However, within the Natal region there is variation in the flow of the Agulhas Current and how it interacts with the continental shelf (Lutjeharms et al., 2000; Roberts et al., 2010). This variation is largely due to the presence of the Natal Bight, a 160 km long and 50 km wide coastal offset located between Cape St. Lucia and immediately south of Durban (160 km), which interrupts the strong, stable flow of the Agulhas Current evident along most of the coast (Fennessy et al., 2016). South of the Natal Bight, the continental shelf break becomes narrower and closer to shore, extending southwards to the Eastern Cape (Fennessy et al., 2016). Therefore, for the occurrence analysis in this study, the Natal region is further divided into three areas to allow for the possibility of the heterogeneity of ocean processes along the coastline. The study area extended approximately 350 km from Richard's Bay in the North, to

Mzamba Beach in the South (Figure 1 and Table S1). The three areas (North, Central, and South) from North to South measure 84.9 km, 84.6 km, and 86.1 km, respectively (Figure 1) and are broadly consistent with designated regions defined by previous local studies (Dicken et al., 2006; Dudley and Cliff, 2010).

Catch analysis

KwaZulu-Natal bather protection program

The KwaZulu-Natal bather protection nets are large-mesh gill nets installed year-round at public recreational beaches since 1952 to mitigate shark-human interaction (Cliff and Dudley, 1992). The nets are 214 m long, 6.3 m deep, and set parallel to, and 300-500 m from the shore, in a water depth of 10-14 m (Cliff and Dudley, 1992; Daly et al., 2022). The nets were deployed at a maximum of 46 fixed locations throughout the study, and are currently installed at 37 locations along the KZN coastline (Table 1 and Figure 1). The deployed nets are regularly inspected, whereby trained field staff visit each net by boat, a process called 'meshing'. Meshing usually occurs at first light, between 17-19 times per month (Dudley and Cliff, 2010). The monthly average number of nets per day per location multiplied by the average net

length was used as a measure of the unit effort. Statistically reliable bycatch data (in this case mobulids) from the bather protection nets began in 1981, therefore data prior to that were excluded. Observers were trained to distinguish between devil ray and manta ray species; but we excluded individuals with a Disc Width (DW) less than 1.4 m from the analysis, as these could be *Mobula kuhlii* or *Mobula eregoodoo* specimens (Cliff, pers. comm.). Due to the relatively recent speciation of manta rays (Marshall et al., 2009) and limited access to training, observers could not distinguish between manta ray species. Therefore, the two manta ray species were pooled together. All individuals used in the study were measured in the field. When an individual manta ray was caught, the individual was sexed using the presence or absence of claspers, and the DW was measured to the nearest mm as the straight-line distance between pectoral fin tips.

In an effort to reduce bycatch of non-target species there was substantial removal of nets at 34 of the 37 beaches in the early 2000's, which were replaced by drumlines (Cliff and Dudley, 2011; Dicken et al., 2016; Dicken et al., 2018). Each drumline is anchored adjacent to the nets and consists of a single Mustad 4480DT 14/0 J hook (Gjøvik, Norway) suspended 4 m beneath a large float (Dudley et al., 1998; Cliff and Dudley, 2011). The hooks were baited and checked every weekday (weather permitting) and re-baited, as
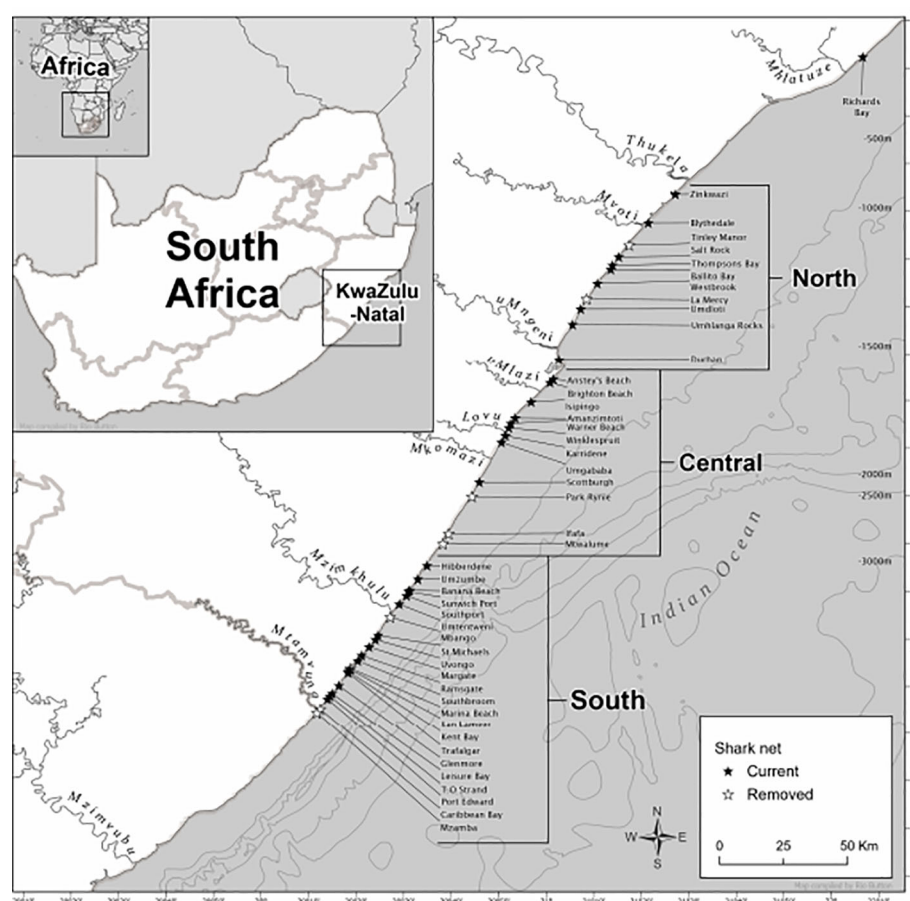

FIGURE 1
Map of KwaZulu-Natal showing sites where bather protection nets were deployed and defining the three designated coastal areas that were used in the study between 1981-2021. Also shown are depth contour (500 m intervals) and defined major river systems. Black stars indicate nets that remain as of 2022 and white starts indicate currently removed nets.

TABLE 1 Deviance table documenting the relative importance of the explanatory variables included in the GAM model to assess manta ray catch trends from the KwaZulu-Natal Bather protection net dataset in South Africa between 1981-2021.

Variable	Df	Deviance	% Deviance explained	Pr(>F)	Significance
NULL		17654			
Year	40	17266	71.4	<2.2e^{-16}	***
Month	42	17237	5.2	<6.6e^{-06}	***
Area	45	17126	20.5	<2.2e^{-16}	***
Moon Phase	47	17111	2.8	0.00009	***

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

necessary. In 2007, a total of 79 drumlines replaced almost half (4 km) of the nets at 17 of the 18 protected beaches along the Hibiscus Coast (Hibberdene, beach 25, to Port Edward, beach 44; Figure 1), An additional 28 drumlines were installed between Zinkwazi and Ballito in 2015, and an additional 70 drumlines between Tongaat and Umgababa in 2019. The 177 drumlines currently in operation were deployed at a replacement ratio of four drumlines to one net. Specifics of the drumline deployments are given in Dicken et al. (2016).

Overall catch per unit effort (CPUE) was measured at each beach by calculating the total catch divided by the total of the monthly average number of nets multiplied by the average net length used at each location between 1981-2021. This is because the number of nets and net length varied at each beach throughout the study period (Table S1). Means (± Standard deviation, or, 'SD') were calculated to assess the following: the average annual number killed as a result of catch, the average number of nets and net length at each location, and the average size of each individual caught.

Standardizing probability of encounter

We used Generalized Additive Models (GAMs) to examine the relationships between the Probability of Encounter (PE) (0 = absent, 1 = present) of manta rays and predictor variables assuming a binomial error distribution. All analyses were conducted in R software (R Core Team, 2021). Probability of encounter is preferred over count distributions when a species is rarely captured, as overdispersion is accounted for. Furthermore, simulation testing has shown that if PE decreases below a certain threshold, the information provided by non-zero observations is minimal and the relationship between PE and abundance becomes approximately linear (Parker et al., 2016; Kerwath et al., 2019). Daily moon phase data were extracted from the 'suncalc' package (Thieurmel and Elmarhraoui, 2019). Effort was treated as an offset in natural logarithmic scale which included the average number of nets and net length and each location.

The full GAM included the smoothing functions for the variables *month* and *moon phase* as follows:

$$\text{logit}(p) = \alpha + \text{offset}(\log(\text{effort})) + \text{Year} + \text{Area} + s1(\text{Month})$$
$$+ s2(\text{Moon phase})$$

where logit denotes the binomial link function, p is the probability of catching at least one individual per net deployment, α is the intercept, s1-2 denotes cyclic cubic smoothing functions for *Month* and *Moon phase* (Wood, 2006). *Year* and *Area* were treated as categorical variables.

Sequential F-tests were used to determine the covariates that contributed significantly (p < 0.001) to the deviance explained and GAMs were fitted in R statistical software using the 'mgcv' and 'nlme' (Wood, 2006). The annual value of PE was standardized by fixing all covariates other than *Year* in the prediction dataset. Drumline data were excluded from GAM analysis because of the short time-frame of their deployment and low catches of manta rays compared to the nets.

Size and sex composition

All manta rays caught were sexed based on the presence or absence of external claspers, and measured, using DW (Marshall and Bennett, 2010). While a threshold of ≥6 m DW was used to identify oceanic manta rays, the majority of catch data included unknown manta species. Nevertheless, detection of juveniles versus adults was possible using known sizes of maturity for both reef and oceanic manta rays (Table S2). Juvenile and adult maturity status for an individual was determined by a DW between 1400-2500 mm and 3801-8000 mm, respectively (Table S2). Individuals that had a DW between 2501-3800 mm were recorded as being of unknown maturity. Sex ratios were calculated using an exact binomial test in the 'stats' package in R (R Core Team, 2021) with a significant difference in sex ratio accepted at p < 0.05.

Results

Catch and effort

Between 1981-2021, 1,602 manta rays were caught in the nets. Between 2007-2021, 10 were caught in the drumlines and therefore excluded from statistical analysis. Manta rays were caught throughout the year, with more caught in austral summer (Dec-Feb; n=534), accounting for 33% of the total catch, compared to the austral winter (Jun-Aug; n=302), which accounted for 19% of the total catch. The size of mantas ranged from 1400-8000 mm Disc Width (DW). An average of 40 rays (±29 SD) were caught per year of which approximately one third (n=527) were found dead, the remaining 70% being released, thus resulting an average of 13 (±11 SD) confirmed mortalities per year. Annual mortality ranged from one (9% of annual catch) in 2017 to 38 (49% of annual catch) in 2001. The majority of catches were single individuals, with a maximum of two manta rays caught in a single deployment. The total net length (Figure 2A) and manta ray catches (Figure 2B) exhibited considerable variation, but there was an overall decrease across the 41-year period.

Spatially, the Central Area had the largest number of catches throughout the study period (n=649), followed by the South (n=528) and then the North Area (n=414), with 11 additional catches at Richard's Bay (R.B). Amanzimtoti beach (AMA), within the Central Area (Figure 1), had the highest total catch over the entire period (n=120; 7% of total catch) (Figure 3). Only two other beaches reported total catches exceeding 100, these being Scottburgh (SCO) and Zinkwazi (ZIN). AMA and SCO are within approximately 35 km of each other in the Central Area, whereas Zinkwazi is the northernmost beach in the North Area (Figure 3). When incorporating the unit of effort (the total of the monthly average number of nets multiplied by the average net length), the highest CPUE occurred at Winklespruit (0.0046) followed by Park Rynie (0.0036), Caribbean Bay (0.0035) and Ifafa (0.0034), three of these beaches being in the Central Area (Figure 3).

Generalized additive models

A total of 1,423 captures were included in the Generalized Additive Models. Month, moon phase, area, and year were significant predictors for manta ray capture. This model was offset with the logarithmic of effort. Year explained 71.4% of total deviation, followed by area (20.5%), month (5.2%), and moon phase (2.8%) (Table 1). There was an increase in manta ray catches up until the year 2000 where there was a significant temporal decline ($p < 2.2e^{-16}$) (Figure 4A). This is especially true when viewing year in numerical form, whereby the probability of capture is lowest in 2015-2021 (Figure S1). The probability of catching manta rays peaked in the summer months of December-February and was lowest in winter, between June-August (Figure 4B). The probability of capture was highest in the Central Area and lowest in the North Area (Figure 4C). Moon phase had a significant effect on the probability of manta ray capture, with increased catch during new and full moon phases (Figure 4D).

Size and sex composition

Most of the caught rays were sexed (62%, n=997) and of these, 56% were female (n=563) and 44% male (n=434), while 38% were recorded as unknown sex (n=605) and hence were excluded from the analysis of sex ratio. There was no significant difference in sex ratio (p=0.67, exact binomial test). This sex ratio with slightly more females remained similar when assessed by area (F:M North Area 1.28:1.0; Central Area 1.38:1.0; South Area 1.26:1.0).
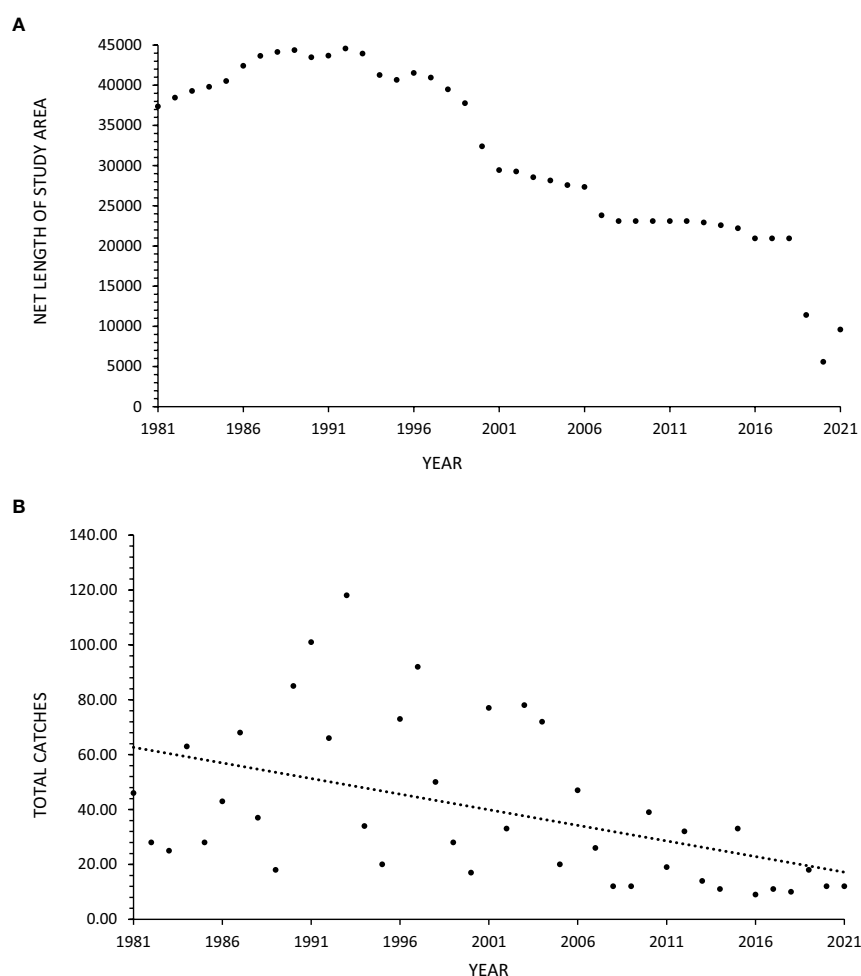
FIGURE 2
(A, B) Total annual number of bather protection nets (A) and total annual manta ray catches (B) in these nets in KwaZulu-Natal, South Africa, between 1981-2021. The dashed lines represent linear regression fitted to the data.
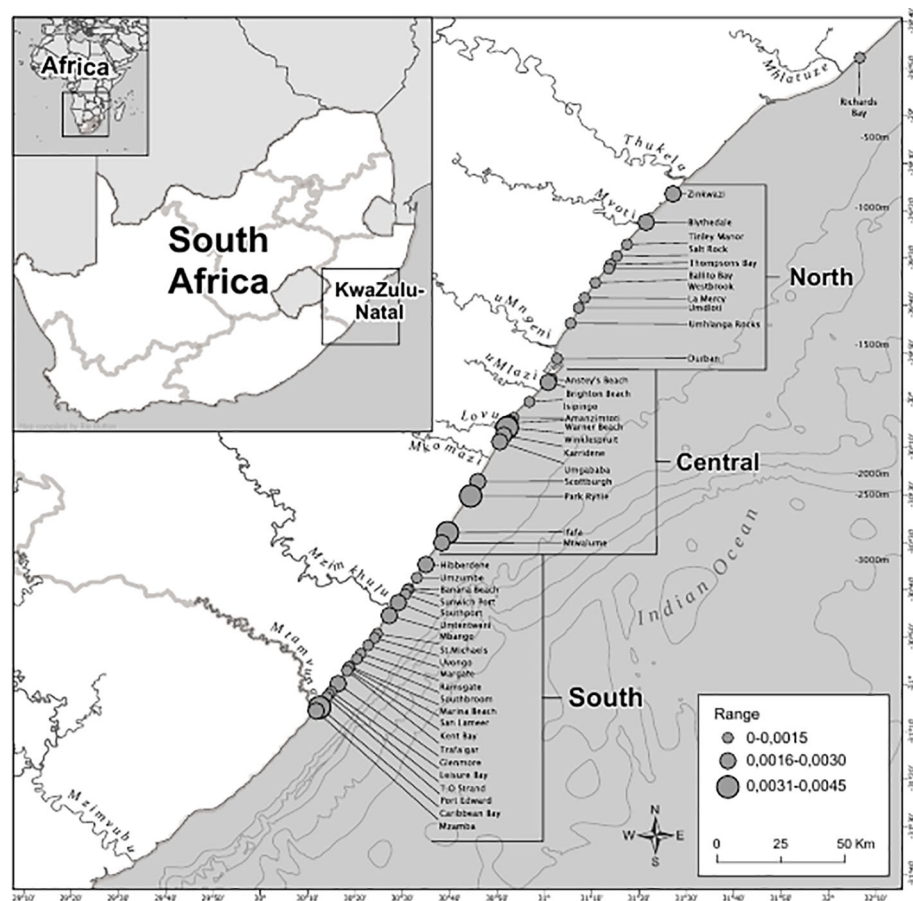
FIGURE 3
Average manta rays caught and standardized by the average net length at the particular beach (catch per unit effort, or CPUE) in the bather protection nets, KwaZulu-Natal, South Africa between 1981-2021. Catch per unit effort was divided into three ranges, the lowest being between 0.000-0.0015, up to the highest being 0.0031-0.0045.

Averaged across the entire study period, caught rays had an average DW of 2796 mm (± 1368 SD). More than half (52%, n=841) of caught individuals belonged to the juvenile size class, between 1400-2500 mm DW (Figure 5). There was an overall ratio of 1:1.8 adults (n=474) to juveniles (n=841) captured, although 18% (n=287) were recorded at unknown maturity due to the overlap in maturity sizes between oceanic and reef manta rays (a size range of 2501-3800 mm) (Figure 5). The South Area had the highest proportion of juveniles in the catch (70%) (Figure 6), comprising 44% of all juveniles caught throughout the study. Of these, 145 individuals were between 1400-1600 mm in size, the known size at birth. Confirmed adult manta rays of both species (3800-8000 mm) were caught in the highest numbers in Amanzimtoti (AMA; n=43), followed by Zinkwazi (ZIN; n=39), Scottburgh (SCO; n=38), Park Rynie (PAR; n=36), Winklespruit (WIN; n=36), and Durban (DUR; n=31); four of these locations (AMA, SCO, PAR, WIN) being within 35 km of one another. A total of 70 rays were 5501-8000 mm DW, confirming that they could only have been oceanic manta rays, and more than half of these were caught within the Central Area (53%, n=37) (Figure S2). Three individuals were measured to be 8000 mm DW: two from Hibberdene (HIB) in 1987 and 2019, and one from Brighton (BRI) in 1981 (Figure S2). This confirms the

maximum size of oceanic manta rays in South Africa to reach at least 8000 mm DW. Both adult and juvenile manta rays were caught throughout the year, with numbers for both peaking in summer, between December-February (Figure 7).

Discussion

Using a 41-year dataset, we describe broad spatial-temporal trends of manta ray distribution and abundance in South Africa for the first time. We found an overall significant decline in catches between 1981-2021 and South Africa to be important habitat for manta rays, especially in summer (December-February), and in the Central and South Areas. When accounting for variation in effort, as well as other possible environmental influences, the standardized probability of capture shows a peak in the late 1990s, followed by a marked decline thereafter. Further, nominal probability of capture has consistently remained below the annual mean since 2007. This supports the majority of studies from Mozambique which report that manta ray encounters have generally declined over time (Rohner et al., 2013; Venables, 2020).

Manta ray populations in the southern African region are of immediate conservation concern (Tibiriçá et al., 2011; Peel, 2019;
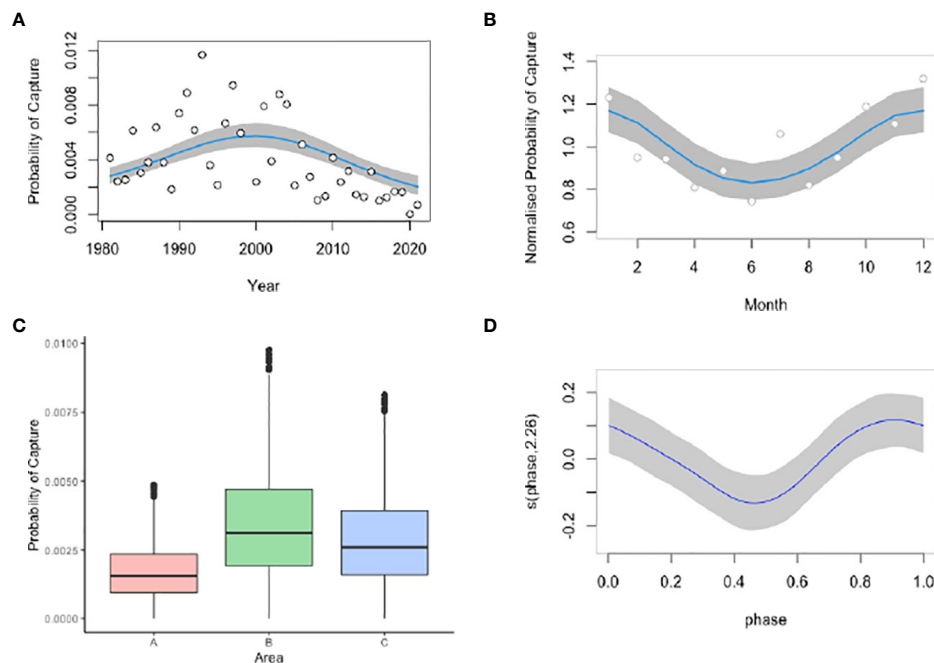
FIGURE 4
Significant predictors for the probability of manta ray capture in the KwaZulu-Natal bather protection nets between 1981-2021 including year **(A)**, month **(B)**, area **(C)**, and moon phase **(D)**. Year and month plots include both numerical and factor models. South Africa austral summer occurs between December-February and winter between June-August.

Venables, 2020). Venables (2020) stated that annual landings in an artisanal fishery of 20-50 individuals per year over 16 years could have resulted in the detected abundance decline in Tofo, Mozambique; from 836 in 2004 to less than 100 since 2013 (Marshall et al., 2011; Temple et al., 2018). The 88% decline in sightings of reef manta rays observed in Tofo between 2003-2011 further supports this (Rohner et al., 2013). Manta ray populations cannot withstand fishing mortality due to their low fecundities (one pup per two years), even from small artisanal fisheries, or as bycatch from destructive fishing practices (Croll et al., 2016; Lawson et al., 2017; Parton et al., 2019). Given that *M. alfredi* in South Africa and Mozambique comprise a single breeding population, it is crucial to ensure that these mobile, threatened species are adequately safeguarded in both countries.

The catch numbers found in this study suggest that South Africa encompasses important habitat for manta rays, the extent of the visitation to that habitat which differs across seasons. Though manta rays were caught throughout the year, catches were highest in summer (Dec-Feb). In KZN, summer is associated with higher rainfall and north-easterly winds that drive the Durban Eddy, both of which increase upwelling and riverine output, and subsequent primary productivity and abundance and diversity of marine taxa (Woodson et al., 2012; Guastella and Roberts, 2016). Increased copepod and chaetognath abundance occur during summer in

FIGURE 5
Disc width frequency distribution of manta rays caught in the KwaZulu-Natal shark nets between 1981–2021. Dashed lines indicate the division between juvenile, unknown maturity, adult, of unknown species, and confirmed *Mobula birostris* individuals based on size.

FIGURE 6
Catch and maturity status of manta rays from each area (North Area=Zinkwazi to Durban; Central Area=Anstey's Beach to Mtwalume; South Area=Hibberdene to Mzamba) from bather protection net catch data in KwaZulu-Natal, South Africa 1981-2021.

FIGURE 7
Monthly comparison of catch and maturity status of manta rays from bather protection net catch data in KwaZulu-Natal, South Africa over the period 1981-2021.
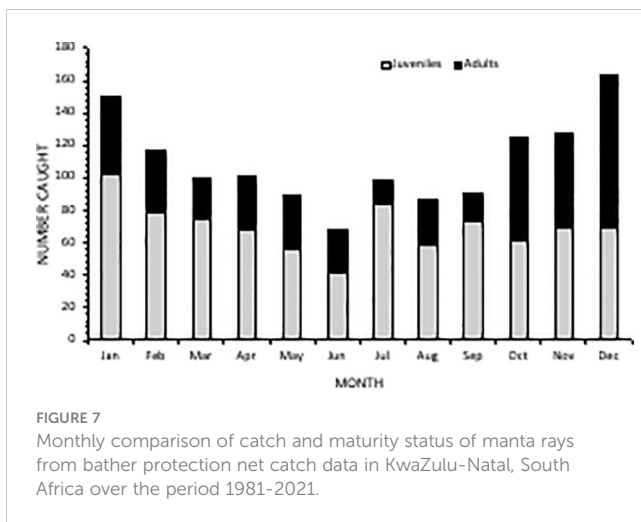
KZN, these being known prey of manta rays (Schleyer, 1985; Couturier et al., 2013; Bennett et al., 2016; Peel, 2019). Therefore, the increased manta ray catches may be due to the increased peaks of phytoplankton and subsequent zooplankton blooms during summer. These results indicate that manta rays may be present year-round in the region but with seasonal peaks, which suggests migration from other parts of the coast driven by life stage, reproduction, food availability, or individual movements.

There were significantly higher catches of manta rays from the area between Anstey's beach and Mtwalume (Central Area), nearby the Aliwal Shoal Marine Protected Area. The Aliwal Shoal Marine Protected Area is an important offshore habitat for elasmobranchs (Dicken et al., 2006; Dicken and Hosking, 2009; Dicken et al., 2016). It was declared a Marine Protected Area in 2004 (Government Gazette No. 26433) with fishing prohibited in the controlled zone, however, bather protection nets are also permitted at Scottburgh Beach, which is located five kilometers southwest. Despite historically high catches, few manta rays have been observed at Aliwal Shoal Marine Protected Area between 2020-2022 (Carpenter, unpublished data).

With at least one catch from every beach, this study provides further evidence that manta rays utilize the expansive continental coastline year-round from the Eastern Cape (approximately 175 km south of Mzamba) (Marshall et al., 2022) northwards into southern Mozambique. However, the intricacies of habitat use remain unknown in KZN, for instance, the specific hotspots for each species, and how often they move in and out of various areas. Full and new moons were significant with manta ray capture, a known predictor of manta ray sightings (Rohner et al., 2013; Fonseca-Ponce et al., 2022). This may be due to tidal effects on zooplankton availability (Rohner et al., 2013; Barr and Abelson, 2019), or the efficacy of nets in capturing manta rays during the full tidal range. The variability in manta ray catches during this study are thus likely consequences of physical processes that drive resource availability and/or net efficacy (Graham et al., 2012; Braun et al., 2014; Jaine et al., 2014; Stewart et al., 2016). Further in-water surveys and telemetry studies would allow for the determination of the possible hotspots for manta rays in KZN, and the visitation patterns associated with these sites.

A greater proportion of juvenile manta rays (DW of 1400-2500) were found in the South Area, from Hibberdene to Mzamba. A total of 9% of individuals (n=145) caught were at the estimated birth size (1400-1600 mm) (Stewart et al., 2018), and most were caught at Mzamba (MZA) (n=14), the most southerly location in the present study. Initial observations in Port St. John's, Eastern Cape, roughly 93 km south of Mzamba, reported six juvenile individuals sighted during winter (Marshall et al., 2022). Further, 52% of total catches (n=841) were within juvenile size for either manta ray species, with almost half of these (43%; n=365) from the South Area. Aggregations in Mozambique monitored for 11 - 20 years have reported small numbers of juveniles (roughly 5% of the photo-identified population in Tofo and Závora and 3% in Bazaruto) (Venables, 2020; Carpenter et al., 2022). Our results fit two of three of the criteria outlined by Heupel et al. (2007): juveniles were more common in a certain area and the habitat was repeatedly used across multiple years; however, we could not validate one criterion; this being if individuals remain or return to the area for extended periods. In contrast, overall, larger mantas were caught in the North and Central Areas, from Zinkwazi (ZIN) to Mtwalume (MTW), where the most confirmed oceanic manta rays (based on size class) were also caught, which may be reflective of a possible oceanic manta ray aggregation. More research is needed to confirm this as it is possible that the nets are incapable of holding large adults.

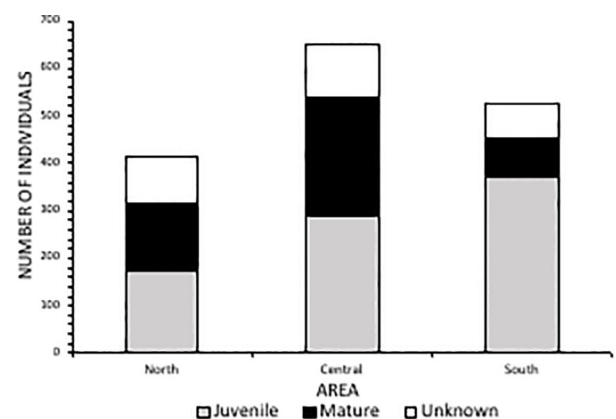The primary caveat in this study is that it reports on relatively low sampling coverage over an expansive area. Considering their depth and habitat ranges, both manta ray species are likely to be spending the majority of time outside the limits of bather protection nets or recreational diving in KZN. Further, we did not distinguish between species in the catch data, due to the overlap in size and potential confusion with species identification. Both species are known to overlap in habitat use (Marshall et al., 2009; Kashiwagi et al., 2011) and both have been identified in various locations amongst the KZN coast (Carpenter, unpublished data; Marshall et al., 2022). Therefore, the pooling of species needs to be considered when interpreting the results, as this describes trends of the two species. In further studies species identification and data quality could be improved via team training or the implementation of photographic records for each captured animal, whether dead or alive (and released). Nevertheless, our results are informative for baseline spatial-temporal habitat use, and can be used to inform policymakers on the impacts of bycatch mortality and the need for development of local conservation management plans.

Both manta ray species are protected in South Africa through international agreements; the Conservation of Migratory Species (CMS) and the Convention on the International Trade of Endangered Species (CITES, 2013; Lawson et al., 2017); and national protection including oceanic manta rays under the Biodiversity Act of 2004 in South Africa, and reef manta rays listed under Threatened or Protected Species (TOPS) regulations (Notice No. 40875 under No. 476 of the Biodiversity Act, 10 of 2004, 2017). An increased network of Marine Protected Areas would benefit manta rays and other threatened species, as South Africa has not yet reached the Ocean Economy and Sustainability Goals of the United Nations of 10% by 2020.

Identifying sources of mortality of manta rays within the southern African region is key to mitigating impacts. Though the scientific knowledge gained from the bather protection nets has been unprecedented, including pioneering studies on numerous elasmobranch species in KZN (Cliff and Dudley, 1991; Dudley and Simpfendorfer, 2006; Kock et al., 2022), the current design may impact manta ray populations (an average of 13, up to 38 confirmed annual catch fatalities). Manta rays have one of the lowest reported population growth rates (median r_{max} of 0.116 year^{-1} 95th percentile [0.089–0.139]; Dulvy et al., 2014) of 106 assessed elasmobranch species. Efforts to reduce bycatch have been implemented by the KZN bather protection program, such as reducing the number of nets and drumlines (Guyomard et al., 2019), and the removal of gear at three of the four beaches with the highest manta ray CPUE. However, due to the bycatch mortality of many vulnerable species, efforts should continue in seeking solutions to mitigate catches even further, especially at beaches installed within already established species refuges (i.e., Marine Protected Areas). This would help reduce impact to the southern African manta ray populations and facilitate their conservation into the future.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors upon reasonable request.

Ethics statement

The KwaZulu-Natal Sharks Board is granted permission to catch animals as part of its operational activities as per its permit conditions listed in OCS/TOPS/STANDINGPERMIT/2022/02. This permit is issued by the Department of Forestry, Fisheries and the Environment in terms of regulation 7(1) of the Threatened or Protected Marine Species Regulations, 2017 (Government Gazette Notice No. 40876 of 30 May 2017) (the Regulations) read with the National Environmental Management: Biodiversity Act, 2004 (Act no.10 of 2004) (the Act).

Author contributions

MC and MD conceived the paper. Data was collected by MD. Data analysis was conducted by MC and DP. Written principally by MC with input and editorial comments from all other authors. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1128819/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Significant decline in the probability of manta ray capture over time (numerical) in the KwaZulu-Natal bather protection nets between 1981-2021.

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Potential negative effects of the Brazilian Space Program on coastal sharks

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The Brazilian government recently announced its first partnerships with the private sector, including American and Canadian companies, to use the Alcântara Space Center (Maranhão, northern Brazil). This center is known for its privileged location, saving up to 30% of fuel in launches. Its operationalization is an offshoot of the Technological Safeguards Agreement, which is important for the Brazilian Space Program due to greater space sector investments and environmentally relevant projects. In 2003, a major fire at the Alcântara Space Center destroyed a rocket and killed 21 workers, halting Brazilian Space Program activities. Recently, our research group reported serious environmental consequences of this accident, i.e., extremely high amounts of Rubidium (Rb) in apex predatory sharks near the Alcântara Space Center. This element is used in fuels and in space propulsion systems and is potentially toxic, displaying bioaccumulating and biomagnifying capabilities. The observed concentrations are the highest ever detected in any living organism (up to 24.65 mg kg-1 dry weight). The launch base is located on the Brazilian Amazon Coast, and population recruitment impacts may compromise biota conservation and biodiversity. Local shark meat consumption is also worrying, as consumers may be exposed to Rb, whose effects in humans are unknown. We, therefore, indicate an urgent need for biomonitoring efforts in the area, as the Alcântara Space Center is about to operate at its maximum capacity.

KEYWORDS

space race, rocket launching, environmental disaster, public policies, Brazilian Amazon Coast

The space sector has undergone major transformations worldwide in the last few years, mainly due to the ongoing privatization of space activities, aiming at space tourism and "the race to Mars", in turn mostly possible due to the availability of professionals fired by NASA, after successive cuts in public funds in the last 10 years. This trend is also observed in other

countries (e.g., Russia and Kazakhstan) (Kovalev et al., 2019). In Brazil, the Brazilian Space Agency is an autonomous agency belonging to the Ministry of Science, Technology, and Innovation and is responsible for the Brazilian Space Program. This agency has ensured the country's prominence in the South American space race, making Brazil an International Space Station project partner (Gouveia, 2003). Initially, under the command of the military, the agency was transferred to civilian control in 1994 (Figure 1A). Since then, the Brazilian Space Program has pursued a policy of joint technology development with more advanced space programs,

including BRICS members, Ukraine, and the United States, depending on the space race agenda of the political party in power (AEB, 2015) (Figure 1B).

The Alcântara Launch Center is the second launch base under the command of the Brazilian Air Force, located in the state of Maranhão, on Brazil's northern Atlantic coast. The Alcântara Launch Center Nucleus was activated on March 1, 1983, considered the official opening date (FAB, 2017a) (Figure 2). However, only in November 1989 did the Alcântara Launch Center become effectively operational, as many families, including
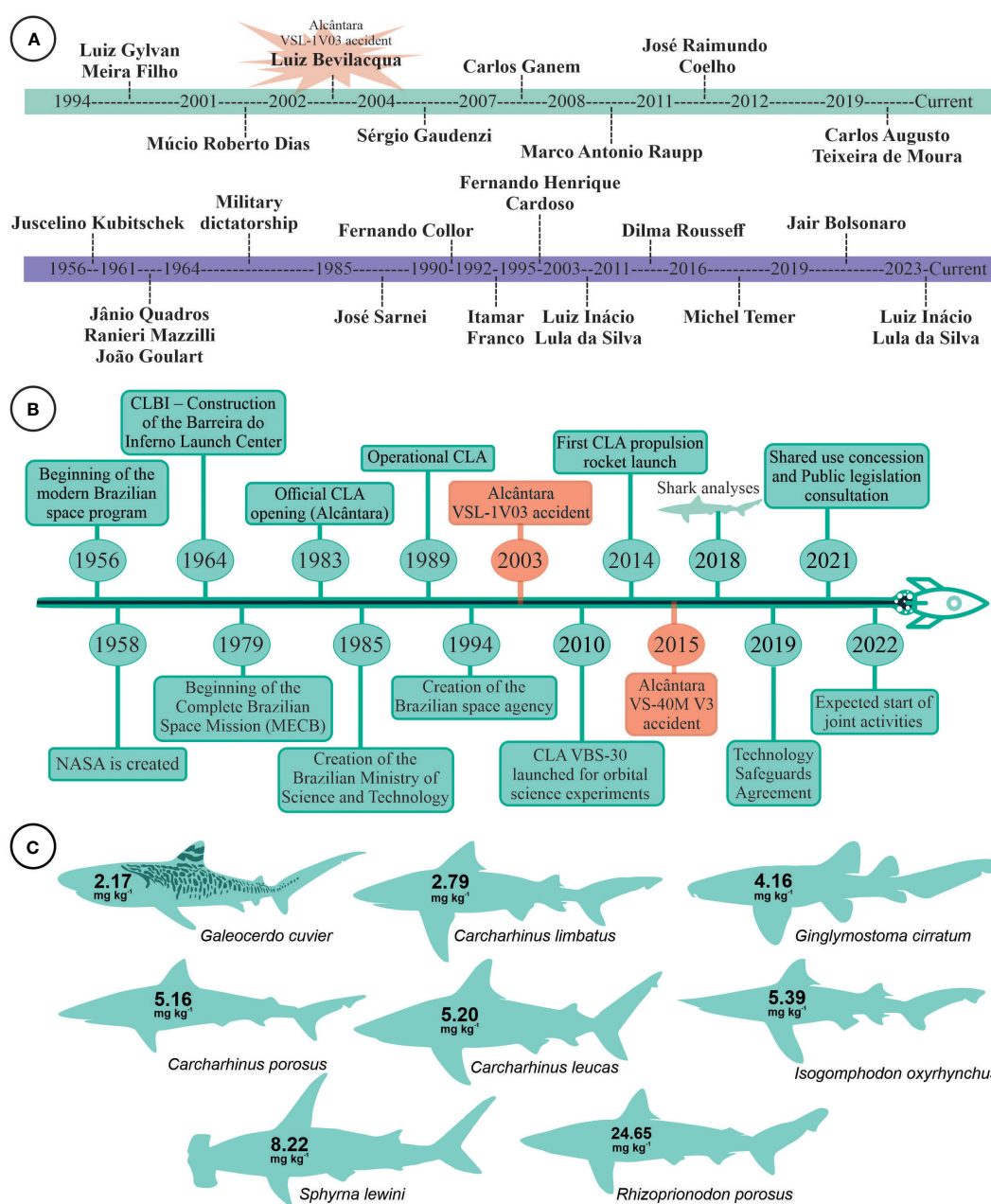
FIGURE 1
(A) Timeline of the Brazilian authorities relevant to the present study. The green band indicates the authorities responsible for the Brazilian space program, while the purple band depicts Brazilian presidents in the same period. (B) Timeline summarizing the Brazilian Space Program activities since its inception, along with the main events related to the national space race, including the two main disasters at the Alcântara Launch Center (orange). (C) Mean of Rubidium concentrations found in muscle tissue of eight shark species sampled around the Alcantara Launch Center.

FIGURE 2
The Alcântara Launch Center, located in the state of Maranhão, on Brazil's northern Atlantic coast.

traditional "quilombolas" (direct Afro-Brazilian descendants of African slaves, residents of settlements first established by escaped slaves in Brazil), who lived on the island of Alcântara had to be relocated, an issue still not solved by the State Government. The first launch carried out at the Alcântara Launch Center was called "Mission Pioneer", and aimed to launch the first SBAT-type rocket carrying payloads for biological and physical tests (FAB, 2017b). The center was built as an alternative to the Barreira do Inferno Launch Center, located in the state of Rio Grande do Norte, as high-density urban areas did not allow for base expansions (Figure 1B). The Alcântara Launch Center is known for its privileged location, allowing for savings of up to 30% in fuel consumption used for rocket and satellite launches. Its proximity to the Equator allows for launches to any orbit and constant local weather conditions allow for launches to take place almost all year round without delay (FAB, 2017c). All of these characteristics have transformed the Alcântara Launch Center into the largest Brazilian Space Center and boosted the Brazilian space conquest. Since then, more than 44 launches have been carried out, including probes, sounding rockets, training rockets, and rocket launch vehicles (i.e., VLS). This is, however, a significant cause of concern, as these activities are known to result in high environmental contamination levels due to the employed fuel compounds, resulting in both direct and indirect environmental and human health risks (Carlsen et al., 2008). In fact, territorial integrity and national heritage concerning the effects of space race are fundamental, as Brazil is the largest country in South America and the fifth-largest in the world, being susceptible to accidents resulting from the fall of space debris on its soil (Vieira et al., 2021).

In 2003, a major fire followed by explosions at the Alcântara Launch Center led to the destruction of the Brazilian VLS-1 V03 rocket and the death of 21 civilian technicians, resulting in a major controversy and halting Brazilian Space Program activities (Figure 1B) (G1, 2016). The VLS-1 V03 was loaded with two types of fuel, a solid propellant mixed with chemical additives

responsible for combustion (i.e., metal catalysts) and a liquid propellant, responsible for increasing buoyancy force and preventing the rocket from rotating around its axis. The exact composition of both propellants was not disclosed in the official report. The objective of the mission, named "Operation São Luís", was to place a SATEC meteorological microsatellite from the Brazilian National Institute for Space Research and a UNOSAT nanosatellite from the University of the North of Paraná in an equatorial circular orbit at an altitude of 750 km (Brazilian Space, 2009). The accident occurred three days before the scheduled launch date, on August 22, 2003. At the time of the accident, the president of the Brazilian Space Agency was in the midst of announcing the agreement signed between Brazil and Ukraine concerning Alcântara Launch Center use. According to the official report, the ignition process occurred ahead of schedule, so the launch tower was not removed in time, becoming the main fire cause. In 2015, another explosion occurred, during the launch of the VS-40M V3 suborbital rocket in the "Operation São Lourenço" (Figure 1B). The entire rocket was lost and the launch structure was damaged (G1, 2015). Fortunately, no deaths occurred, although this accident may have been an additional source of environmental contamination in the region.

On April 26, 2021, the Brazilian Space Agency published Public Consultation Notice No. 3, a public call to update Ordinances No. 5 (AEB, February 21, 2002) and No. 182 (AEB, May 28, 2020), both aimed at regulating Brazilian space activities. Despite representing an advance, public suggestions hardly reflect the complexity of government measures that must be adopted to prevent future accidents from causing environmental impacts, as discussed in the next sections. Furthermore, no normative or ordinances concerning potential environmental disasters due to the Brazilian space race have been implemented to date, no environmental studies are required to establish launch bases, and no regular inspections are mandated.

For example, primary pollutants that can result from space fuel burning may undergo transformation processes (i.e., secondary

pollutants), displaying the potential for accumulation and biomagnification processes (Carlsen et al., 2008). In this regard, our research group has recently reported serious environmental consequences of the Alcântara Launch Center accident, in the form of extremely high amounts of Rubidium (Rb) in the vicinity of Alcântara Island (Wosnick et al., 2021). This element is used in fuels and as part of space propulsion systems and, although rarely detected in wild animals, appears to be toxic, mainly to the reproductive system (Yamaguchi et al., 2007), displaying bioaccumulating and biomagnifying capabilities throughout trophic networks (Campbell et al., 2005; Anandkumar et al., 2019). Rubidium was, in fact, detected in the muscle tissue of several other top marine predators in the vicinity of the Alcântara Launch Center following the same methodology applied in the aforementioned study (see Wosnick et al., 2021 for further details) including tiger sharks (*Galeocerdo cuvier*), bull sharks (*Carcharhinus leucas*), Atlantic nurse sharks (*Ginglymostoma cirratum*), smalltail sharks (*Carcharhinus porosus*), blacktip sharks (*Carcharhinus limbatus*), Atlantic sharpnose sharks (*Rhizoprionodon porosus*), daggernose sharks (*Isogomphodon oxyrhynchus*) and scalloped hammerhead sharks (*Sphyrna lewini*) (Figure 1C), considered promising sentinel species, as higher trophic level species are adequate environmental contamination bioindicators, reflecting the biological effects of environmental disasters (Torres et al., 2014). It is important to note the fact that no other sources of Rb exist in the state of Maranhão, and that the detected Rb concentrations are the highest ever reported in any living organism, ranging from 2.17 to 24.65 mg kg-1 dry weight (Figure 1C). This is of particular concern, as the Alcântara Launch Center is located within the Brazilian Amazon Coast, an area that boasts of great fauna richness and biological relevance. In addition, it is also a hotspot for endemic and threatened sharks and their relatives (Dulvy et al., 2014), and any impacts on population health and recruitment may irreversibly compromise local fauna conservation.

Rubidium has also been detected in Arctic sharks, albeit at much lower concentrations (Pacific sleeper shark, 0.79 mg kg-1; Greenland shark, 0.66 mg kg-1) (McMeans et al., 2007). This is interesting, as rocket stages from SS-19 intercontinental missiles re-purposed for launching satellites into the Arctic Sea were dropped by Russia in the area on ten occasions since 2002. As a result of these drops, toxic space fuel components (e.g., unsymmetrical dimethylhydrazine) have been identified in the region (Byers and Byers, 2017), potentially the source of the detected Rb in Arctic sharks. However, as the sharks were sampled before 2002, it is possible that the Rb sources originate from other activities or even from rocket stage drops performed prior to the published assessments.

Besides Rb, several other potential residual contaminants from this accident may have been discharged in the surrounding aquatic ecosystem. These include many organic compounds, such as ammonium nitrate, potassium chlorate, ammonium chlorate, hydrocarbons, kerosene, alcohol, hydrazine and its derivatives and liquid hydrogen, as well as inorganic compounds, i.e., boron, lithium, aluminum and magnesium, all a part of rocket fuel according to the National Aeronautics and Space Administration (NASA, 2023). All are toxic to aquatic biota, both invertebrates and vertebrates, in general to some degree. Hydrazine in particular, is a confirmed animal carcinogen (ACGIH, 2000) and has been

reported as causing cytotoxicity and reproductive alterations in fish (Rajagopal et al., 2019). Hydrocarbons are also cyto- and genotoxic to many aquatic biota representatives, and display the ability to bioaccumulate and, in some cases, biomagnify throughout trophic food webs, depending on their chemical class (Trowbridge and Swackhamer, 2002). Metallic elements, even essential ones like B, Li and Mg, may be toxic depending on their concentration (Jaishankar et al., 2014), while Al has been noted as mostly toxic to aquatic organisms (Sparling and Lowe, 1996). Thus, further monitoring efforts in this region are warranted to evaluate potentially deleterious impacts on locally exposed biota.

Apart from a conservationist point of view, the high consumption of shark meat across the Brazilian Amazon Coast (Barbosa-Filho et al., 2019) is also a call for concern, as consumers may be exposed to high Rb concentrations, whose effects in humans are unknown. In fact, Brazil is one of the largest shark meat consumers worldwide (Bornatowski et al., 2018), and even with increasing indications of high contaminant concentrations in shark meat (Souza-Araujo et al., 2021), no sanitary surveillance programs are currently in force, and no safe consumption levels for Rb have been established. In fact, to date, most countries that rely on space launches have not established environmental disaster monitoring and prevention programs, including Brazil. Fortunately, biomonitoring methods and computational modeling methodologies are available to assess the potential effects of space fuel combustion, as well as gas behavior when released into the environment, allowing for real-time assessment of the impacts of both orbital and suborbital launches (Conn et al., 1975; Carlsen et al., 2008).

To date, the most significant sources of marine pollution through environmental disasters in Brazil consist of oil extraction/ transportation activities, with many studies carried out on the affected fauna and environment (Michel, 2000; Ruoppolo et al., 2017; Craveiro et al., 2021). Both domestic and international legislation tailored to environmental disasters are, of course, required in these situations and must be coupled with government efforts to inspect public and private enterprises before accidents occur. Unfortunately, Brazil's reality hardly reflects this ideal disaster prevention model, with thousands of human and non-human lives paying the price (Brum et al., 2020). Furthermore, even with high media attention, most environmental disasters in the country, even if recurrent, remain without punishment to those involved, and usually, no robust environmental studies and compensatory measures are applied. Furthermore, environmental compensations are rarely carried out voluntarily, even less so imposed by Brazilian courts, and, when they are, the guilty parties simply do not pay up.

When accounting for the potential impacts of rocket explosions, this is even more concerning. However, as noted in other disasters in the country, the guilty parties were not held accountable in the case of the Alcântara explosion. Astonishingly, this impunity is supported by the Brazilian space legislation which sustains through decree established based on in the "Convention on International Liability for Damage Caused by Space Objects" in 1972. This document states that: "(a) the term "damage" means loss of life, personal injury or other impairment of health, beyond loss or damages of properties;" That is, any environmental impacts will

not be classified as damage, making the application of appropriate measures unattainable. As a result of environmental neglect, the Alcântara Launch Center has operated without an environmental license and without an environmental impact study for about 40 years, making it impossible to identify the possible impacts generated during these years of activity (MABE, 2019).

Recently, the Brazilian Space Agency announced the second public call for private companies to use along the northeast coast, focusing on attracting companies with the capacity to carry out larger launches, in addition to exploring the nine thousand hectares of the base (Agência Brasil, 2021). The first call provided the sending of documentation from 14 companies, with nine final proposals from joint partnerships between Brazilian and foreign companies. The arrival of private companies represents a Brazilian Space Program boost, as budget restrictions have recently been applied to space operations. More specifically, in the beginning of 2021, the Brazilian Space Agency suffered a 1.2 million reais cut in its budget, which also affected the Alcântara Launch Center. On April 29th 2021, the Brazilian government announced its first partnership with foreign private companies, including Hyperion, Orion AST, Virgin Orbit (USA), and C6 Launch (Canada), to make use of the Alcântara Launch Center (TecMundo, 2021). According to the Brazilian Space Agency, each company will be responsible for operating a space center unit in Alcântara. The VLS platform system will be operated by Hyperion, the suborbital launcher, by Orion AST and the Alcântara airport will be maintained under the control of Virgin Orbit. The C6 Launch was chosen to manage the Profiler Area (Agência Brasil, 2021). Nanosatellite launches will be one of the priorities from these new agreements. This operationalization is an offshoot of the Technological Safeguards Agreement between Brazil and the United States, signed by Jair Bolsonaro and Donald Trump in 2019 (G1, 2019). This agreement is extremely important for the Brazilian Space Program, endorsing projects of significant environmental relevance, such as the Amazonia-1 satellite launched from the Indian Space Research Organization (ISRO), in February 2021 during the PSLV-C51 mission, which will monitor Amazon deforestation rates. In the agreement, the government of the United States of America ensures that North American Representatives or North American Licensees are allowed to provide the Brazilian government with information related to the presence of radioactive material or any substances defined as potentially harmful to the environment or human health. However, the Brazilian government may only carry out any study or photographic record if authorized and monitored by the Government of the United States of America, and even then, it shall take all necessary measures to avoid public disclosure of any information collected.

To date, no agreement to reallocate financial resources to monitor potential impacts of future launches has been disclosed. Furthermore, Brazilian legislation does not provide for any punitive measure for international private companies that use the Alcântara Launch Center facilities, pointing to a scenario very similar to that observed in previous agreements between the Brazilian government and multinationals involved in major environmental disasters. It is worth mentioning that the Alcântara Launch Center security area covers the entire coast of Alcântara, during the rocket launch period, the region is restricted, the community is prohibited from fishing for up to 40 days and there is no compensatory measure during this period of prohibition.

The constant advances of the space race require permanent incorporation of best practices, ensuring the safety of space activities not only in Brazil, but in all countries engaged with launch programs. In this scenario, it is clear that the Brazilian space race requires public policies to guide and monitor upcoming activities from private companies' that will make use of Alcântara Launch Center facilities, and the Brazilian government must become aware of the potential impacts that increasing space activities may cause. It is also imperative that inspections be carried out at the Alcântara Launch Center, preferably within the Brazilian Ministry of the Environment and the State Secretariat for the Environment. The agreements that are being signed should also contain specific clauses for the implementation of environmental disasters monitoring and prevention efforts, as well as guaranteed funds in case of new disasters. Lastly, it is paramount that the domestic legislation on space activities be revised, relying not only on public and specialists in space activities, but also environmental specialists to provide scientific consultancy and to direct mitigation measures, if necessary.

Considering the reported impacts, it is also paramount that compensation measures be adopted, directing public resources to neutralize the effects of chemical compounds released in the vicinity of Alcântara. Furthermore, from a public health perspective, initiatives concerning the monitoring of fish quality (particularly shark meat, as regional characteristics make this one of the most consumed meats in the Alcântara Launch Center region) should be implemented, preferably comprising joint activities between both State Fisheries and Health Departments.

The environmental risks of space exploitation to Earth's environment has been highlighted recently by Napper et al. 2023. The authors suggest that there needs to be a global treaty focussing on Earth's orbit, with the agreement including measures to implement producer and user responsibility for satellites and debris, from the time they launch, through-life impacts on the night-sky and at the end of life. It was also stated that enforcement of collective international legislation should be put in place, including fines and other incentives to ensure accountability. Finally, the treaty should require that any countries with plans to use Earth's orbit commit to global cooperation. That being said, it is important to ensure any impacts to the biodiversity on Earth will also be included in such a treaty.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Author contributions

Acknowledgments

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Using reproductive hormones extracted from archived muscle tissue to assess maturity and reproductive status in porbeagles *Lamna nasus*

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While lethal sampling can be the most effective technique to collect critical reproductive data for elasmobranchs, non-lethal techniques need to be validated for future use. Concentrations of reproductive hormones in plasma and muscle have been found to correlate to sexual maturity and/or reproductive cycles in oviparous as well as yolk-sac, placental, and histotrophic viviparous elasmobranchs, offering a potentially non-lethal technique to study reproduction. However, reproductive hormone analysis is scant for oophagous sharks. This study utilized muscle tissues from porbeagles *Lamna nasus* that were dissected for other life history studies and were stored frozen for up to 37 years to quantify testosterone (T) and estradiol (E_2) concentrations in relation to previously-known maturity and reproductive stage. A total of 207 samples (92 males, 115 females) from porbeagles ranging in size from 80 to 256.5 cm fork length were analyzed. Muscle T and E_2 concentrations were related to maturity and reproductive stage in porbeagles, with the highest T concentrations found in mature males during the spermatogenic season (summer) and the highest E_2 concentrations found in gravid females. These results suggest muscle hormone concentrations have the potential to serve as a non-lethal proxy of reproductive stage in oophagous sharks. This study also demonstrates the value of specimen sharing and the potential for continued use of stored vertebral muscle tissue for reproductive hormone analysis in order to optimize the amount of data gained from biological samples. Future use of these methods would be particularly valuable for threatened species for which lethal sampling is restricted.

KEYWORDS

oophagy, reproduction, shark, testosterone, estradiol

1 Introduction

Effective conservation and management of elasmobranchs requires a comprehensive understanding of a species life history, including age and size at sexual maturity, reproductive cycles, and habitats used for reproduction (Walker, 2005; Awruch, 2013). Such information can be used to determine sustainable harvesting rates, understand a population's potential for timely recovery, and/or develop protected areas or strategies for bycatch avoidance (Awruch, 2013). Although lethal sampling of a relatively large number of specimens has historically been the approach for studying the reproductive biology of elasmobranchs (Heupel and Simpfendorfer, 2010), sacrificing threatened species is in direct opposition to the conservation and management goals science is intending to support (Hammerschlag and Sulikowski, 2011). Given this nuance, non-lethal alternatives for collecting reproductive data should be validated and prioritized whenever possible (Hammerschlag and Sulikowski, 2011). One promising technique that can be used to study the reproduction (reproductive cycles, maturity, reproductive habitats) of elasmobranchs is the quantification of sex steroids (reproductive hormones) in muscle tissues (Prohaska et al., 2013; Verkamp et al., 2021). This technique may be most practical for large elasmobranch species for which other non-lethal sampling collections (i.e., a blood sample for plasma hormone analysis) are logistically challenging, as muscle samples can be collected from free-swimming animals (Prohaska et al., 2013; Verkamp et al., 2021). Muscle reproductive hormone concentrations have been found to correlate to plasma concentrations as well as dissection-verified reproductive stage (preovulatory, early, mid, late gestation) in an oviparous (little skate *Leucoraja erinacea*), yolk-sac viviparous (spiny dogfish *Squalus acanthias*) and placental viviparous (Atlantic sharpnose shark *Rhizoprionodon terraenovae*) species (Prohaska et al., 2013). Muscle hormone concentrations were also used non-lethally (not verified by dissection) to gain preliminary insight into the possible reproductive role of an aggregation site for white sharks *Carcharodon carcharias* (Verkamp et al., 2021). Most studies conducting reproductive hormone analysis for elasmobranchs have quantified estradiol (E_2), progesterone (P_4) and/or testosterone (T) (Becerril-Garcia et al., 2020). In general, in female elasmobranchs E_2 has primarily been related to vitellogenesis and maturation, while P_4 has been related to ovulation and the maintenance of early pregnancy (Awruch, 2013). In males, T is associated with spermatogenesis (Awruch, 2013).

The Northwest Atlantic (NWA) porbeagle *Lamna nasus* is overfished and is a population of conservation concern (ICCAT, 2020). This population's life history is characterized by late age (8 and 13 years for males and females, respectively; Natanson et al., 2002) and large size (162-185 cm fork length (FL) for males and 210-230 cm FL for females; Jensen et al., 2002) at maturity as well as low fecundity (average of 4 pups; Jensen et al., 2002). The population was originally thought to have an annual reproductive cycle (Jensen et al., 2002) but more recent evidence suggests at least a portion of the population reproduces biennially (Natanson et al., 2019). The embryos are nourished by consuming unfertilized eggs ovulated by the mother throughout much of gestation (oophagy; Jensen et al., 2002). Mating occurs in September through November and pupping from April through June (Jensen et al., 2002).

While the life history of the NWA porbeagle is relatively well studied, information on reproductive hormones is absent for this species and is scant for all oophagous sharks, particularly for mature females (Tribuzio, 2004; Sulikowski et al., 2012; Verkamp et al., 2021). Most information of shark reproductive endocrinology is provided by direct assessment of plasma hormone concentrations. However, because blood samples are logistically difficult to obtain in the large specimens of porbeagles, and currently are unavailable, the assessment of reproductive hormone concentrations in muscle tissue could be a useful method to evaluate reproductive endocrinology in this species, providing further the possibility of a non-lethal methodology. In this sense, a large collection of stored vertebral specimens or muscle tissues from NWA porbeagles that were dissected between 1985 and 2019 for other life history studies (Natanson et al., 2002) offers the opportunity to have available samples to assess reproductive endocrinology in the species. The objectives of this study were therefore to 1) determine if reproductive hormones (T, E_2) could be quantified from shark muscle tissue that was stored frozen for up to 37 years, 2) determine if muscle T and E_2 concentrations were related to size or maturity in the NWA porbeagle, and 3) determine if muscle T and E_2 concentrations were related to reproductive stage in the NWA porbeagle.

2 Method

Porbeagles were sampled between 1985 and 2019 onboard commercial and research longline vessels in U.S. and Canadian waters between Massachusetts and the Grand Banks. The majority of samples were collected after 1990 and came from vertebral columns used to study age and growth in the U.S. (Natanson et al., 2002). Additional samples were collected during a Canadian fishery-dependent porbeagle survey in 2017. Capture date, geographic location, and sex was recorded and over the body fork length (FL; cm) was measured and recorded to the nearest mm. All Canadian survey samples had associated information on sexual maturity and reproductive stage and reproductive data were also taken from U.S. samples when possible (74% of U.S. samples). Determination of sexual maturity status and reproductive stage from both the U.S. and Canadian sampling followed established methodology based on morphometric measurements (including: clasper length, testes length and width, oviducal gland width, uterus length and width) and observations of the reproductive tract (including: ovary, uteri, vaginal membrane, testes, clasper calcification, etc.; Jensen et al., 2002; Natanson et al., 2019). In brief, males were grouped into immature, transitional (immature but maturing), and mature, with transitional males exhibiting lengthening claspers and initial development of the rhipidion (Jensen et al., 2002). Females were grouped into immature, transitional (immature but maturing), mature non-gravid, and mature gravid, with transitional females exhibiting the presence of a vaginal membrane and a thin tubular uterus indicating no

previous mating but an ovary similar in appearance to mature non-gravid females (Natanson et al., 2019). While it is important to note that transitional sharks are technically classified as immature, they were separated from other immature sharks for this study due to predicted changes in reproductive hormone concentrations associated with the maturation process that may begin prior to reaching maturity (Barnett et al., 2009). For the U.S. samples, vertebral columns were stored frozen and muscle tissues used for reproductive hormone analysis were scraped from these stored vertebral columns in 2021. For samples collected during the 2017 Canadian survey, muscle tissues were excised from vertebral columns of porbeagles immediately upon dissection after capture. All muscle tissues were stored frozen and were shipped on ice to Arizona State University, where they were kept frozen at -20°C until processing.

Hormones were extracted from the muscle tissue following ether extraction protocols modified from Verkamp et al. (2021). Protocol modifications included adjusting the phosphate buffered saline (PBS) to muscle ratio and adding an additional extraction (as described below). Samples were thawed on ice and muscle was excised, weighed, and transferred to a conical centrifuge tube. Phosphate buffered saline (PBS) was added in a ratio ranging from 1 g:1 ml to 1 g:4 ml of muscle to PBS. While most samples (69%) were resuspended in a 1 g: 1 ml of muscle to PBS following Verkamp et al. (2021), initially processed samples and drier samples (31%) were resuspended in a higher ratio of PBS. Samples were homogenized using a Kinematica Polytron PT 10-35. Approximately 0.5 g of homogenate was transferred to a borosilicate tube in duplicate (if sample size allowed) and spiked with approximately 1000 counts min^{-1} of the appropriate tritiated hormone (1, 2, 6, 7, ^{3}H-T for males or 2, 4, 6, 7, 16, 17, ^{3}H-E_2 for females; Perkin Elmer Life Sciences) in order to calculate the percent recovery of hormone during the extraction process. The ~0.5 g spiked homogenate aliquots were then extracted 4-5 times with 5 ml of diethyl ether (ACS grade) and snap frozen in a dry ice and acetone (ACS grade) bath. A fifth extraction with diethyl ether was added during the study to improve hormone recovery during the extraction procedure. The ether phase was decanted into a second borosilicate tube and the diethyl ether evaporated at 37°C under a stream of nitrogen. Dried isolated hormones were reconstituted in 250 µl of PBS with 0.1% gelatin (PBSG) and stored at 4°C.

T and E_2 concentrations were quantified for males and females, respectively, following radioimmunoassay methods described in Prohaska et al. (2013). Antibodies (provided by Dr. Gordon Niswender, Colorado State, Fort Collins, CO) used to bind hormones for quantification were diluted in PBSG to final concentrations of 1:24,200 and 1:54,000 for T and E_2, respectively. A Tri-Carb 4910TR liquid scintillation counter (Perkin Elmer Life Sciences) was used to quantify radioactivity. Final concentrations were corrected for procedural loss using individual sample hormone extraction recoveries. Any sample that had a hormone concentration below the detection limit (6.25 pg g^{-1} for T, 5 pg g^{-1} for E_2) of our assay was assigned the minimum detection limit for that hormone. The inter-assay coefficients of variation were 15% and 9%, and the average intra-assay coefficients of variation were 7% and 6% for T and E_2, respectively.

Muscle T and E_2 concentrations were grouped by sampling season (summer = June-August; fall = September-December; spring = March-May) and then plotted by FL for males and females, respectively. Muscle T concentrations were then grouped and boxplots were plotted by reproductive stage (immature, transitional, and mature) for males and average inner clasper lengths (mm) were also plotted for a subset of these individuals for which reproductive measurements were available (n = 26). For males that were not formally assessed for maturity based on internal reproductive morphology, maturity was predicted based on size at 50% maturity (174 cm FL; Jensen et al., 2002). Mature males were further divided by reproductive seasonality, with mature males sampled in the summer (June-August; predicted to be undergoing spermatogenesis) separated from those sampled during the other times of year (September-May; predicted to not be undergoing spermatogenesis) based on previously established timing of spermatogenesis in male sharks (i.e., Manire and Rasmussen, 1997; Verkamp et al., 2022). Average (± standard error) muscle T concentrations were also plotted by month of the year for males grouped by reproductive stage to evaluate the relationship with reproductive seasonality. For this reproductive seasonality plot, males that were not formally assessed for maturity were plotted as separate groups based on whether they were smaller or larger than the size at 50% maturity. For females, muscle E_2 concentrations were grouped and boxplots were plotted by reproductive stage (immature, transitional, mature non-gravid, and gravid) for only the females that were formally assessed for maturity and pregnancy. While many internal reproductive characteristics have been found to relate to maturity in this species (Jensen et al., 2002), average oviducal gland widths (mm) were plotted for a subset of these individuals given this was the morphological measurement with the largest sample size available (n = 19). One oviducal gland measurement from a gravid porbeagle that did not have a muscle sampled to analyze for E_2 was included for reference. Average (± standard error) muscle E_2 concentrations were also plotted by month of the year for females grouped by reproductive stage to evaluate the relationship with reproductive seasonality. For this reproductive seasonality plot, females that were not formally assessed for maturity were plotted as separate groups based on whether they were smaller or larger than the size at 50% maturity. Muscle T and E_2 concentrations were tested for correlations with FL using Kendall 's tau rank correlation tests given data violated assumptions of parametric regression even following transformation. Muscle T and E_2 concentrations were compared between different reproductive stages by Kruskal Wallis tests followed by pairwise Dunn tests with a Bonferroni correction for multiple testing. All tests were considered significant at α = 0.05.

3 Results

Muscle samples were analyzed from a total of 207 porbeagles (92 males, 115 females), ranging in size from 85 to 246 cm FL for

males and 80 to 256.5 cm FL for females (Table S1). Average hormone recoveries from muscle tissue during the extraction process were 67.0% and 48.6% for T and E_2, respectively. Hormone recovery (%) during the extraction process was not impacted by the age of the sample (Figure S1) nor the muscle to PBS ratio (Figure S2). For males, muscle T concentrations ranged from 6.25 to 735.15 pg g^{-1}. For females, muscle E_2 concentrations ranged from 5 to 954.32 pg g^{-1}. Hormone concentrations were not impacted by the age of the sample (Figure S3) nor the muscle to PBS ratio (Figure S4) when considering the additional factor of reproductive stage.

Muscle T concentrations in males generally increased with increasing shark size (Figure 1), yet the overall range was greater in larger or mature males (6.25-735.15 pg g^{-1}) compared to smaller, immature males (6.25- 427.32 pg g^{-1}; Figure 1). When considering the time of year of sampling, the increase in T concentration with increasing shark size was most visible for sharks sampled in the summer (June-August), with less consistent trends for sharks sampled in the fall (September-December) and spring (March-May; Figure 1). Overall however, muscle T was not significantly correlated with FL in males (Kendall's tau; z = -0.653; p = 0.514). In regards to reproductive stage, immature male porbeagles had T concentrations ranging from 6.25 to 427.32 pg g^{-1} (average = 163.09 pg g^{-1}) and inner clasper lengths ranging from 82 to 167 mm (average = 137.3 mm; Figure 2A). Males that were considered to be in a transitional state had T concentrations ranging from 6.25 to 308.88 pg g^{-1} (average = 120.17 pg g^{-1}) and inner clasper lengths ranging from 29.5 to 305 mm (average = 217.4 mm). Mature males that were sampled in seasons porbeagles are predicted to not be undergoing spermatogenesis (fall through spring) had T concentrations ranging from 6.25 to 439.89 pg g^{-1} (average = 81.89 pg g^{-1}) and there were no clasper measurements available for this group. Finally, mature males that were sampled in the predicted spermatogenic season (summer) had T concentrations

FIGURE 1
Muscle testosterone (T) concentration (pg g^{-1}) as a function of fork length (FL; cm) in male porbeagles. The shape represents the sampling season with seasons grouped into summer (June-August), spring (March-May), and Fall (September-December). The size at 50% maturity (174 cm FL; Jensen et al., 2002) is identified by the vertical dashed line and the minimum detection limit of the assay (6.25 pg g^{-1}) by the horizontal dashed line.

ranging from 96.68 to 735.15 pg g^{-1} (average = 360.70 pg g^{-1}) and had inner clasper lengths ranging from 199 to 363 mm (average = 322.3 mm). Muscle T concentrations were significantly different between reproductive stages (Kruskal Wallis; χ^2 = 28.026, p < 0.001; Figure 2A). Pairwise comparisons indicated mature males sampled during the summer had T concentrations that were significantly higher than concentrations in transitional males (Dunn test; z = 2.80, p = 0.031) and mature males sampled in predicted non-spermatogenic seasons (Dunn test; z = 5.24, p < 0.001). However, T concentrations were not significantly different between any other groups (p > 0.05). When reproductive seasonality was considered for males (Figure 2B), a seasonal trend was clear for mature males (including males that were not formally assessed for maturity but were larger than the size 50% maturity). Average muscle T concentrations for mature males were low to moderate during the spring (March through May), increased and were highest during June and July, and then decreased for the remainder of the year. Lowest concentrations occurred during the mating season of September through November, while lowest variability in muscle T concentrations occurred in October through December (Figure 2B). Seasonal trends were less consistent among immature, transitional, and males that were not formally assessed for maturity but were smaller than the size at 50% maturity.

For females, muscle E_2 concentrations were significantly correlated with shark size (Kendall's tau; z = 6.233; p < 0.0001); E_2 showed a clear increase in sharks above the size at 50% maturity (218 cm FL; Jensen et al., 2002) (Figure 3). However, similar to males, there was a greater range in muscle E_2 concentrations in larger or mature females (5-954.32 pg g^{-1}) compared to smaller, immature females (5-90.11 pg g^{-1}) (Figure 3). When considering the time of year sharks were sampled, E_2 was elevated in females sampled in the fall (September-December; Figure 3). In regards to reproductive stage, all immature females, except for one individual (which had an E_2 concentration of 90.11 pg g^{-1}), had muscle E_2 concentrations below the minimum detection limit (5 pg g^{-1}, average = 6.81 pg g^{-1}) and oviducal gland widths ranging from 2.9 to 46 mm (average = 9.6 mm; Figure 4A). Females that were considered to be in a transitional stage had E_2 concentrations ranging from 5 to 152.78 pg g^{-1} (average = 54.26 pg g^{-1}), yet there were no oviducal gland measurements available for this group. Females that were confirmed to be mature but were non-gravid had E_2 concentrations ranging from 5 to 152.18 pg g^{-1} (average = 49.60 pg g^{-1}) and oviducal gland widths ranging from 21 to 37 mm (average = 29 mm). Finally, females that were confirmed to be mature and gravid had the highest E_2 concentrations, ranging from 5 to 954.32 pg g^{-1} (average = 196.47 pg g^{-1}). A gravid porbeagle that did not have associated hormone data had an oviducal gland width of 42.2 mm. Muscle E_2 concentrations were significantly different between reproductive stages (Kruskal Wallis; χ^2 = 58.248; p < 0.001; Figure 4A). Pairwise comparisons indicated that E_2 concentrations were significantly higher in gravid females compared to immature females (Dunn test; z = 7.6, p < 0.001). However, E_2 concentrations were not significantly different between any other groups (p > 0.05). There were no trends in reproductive seasonality for immature, transitional, or sharks that were not formally assessed for maturity
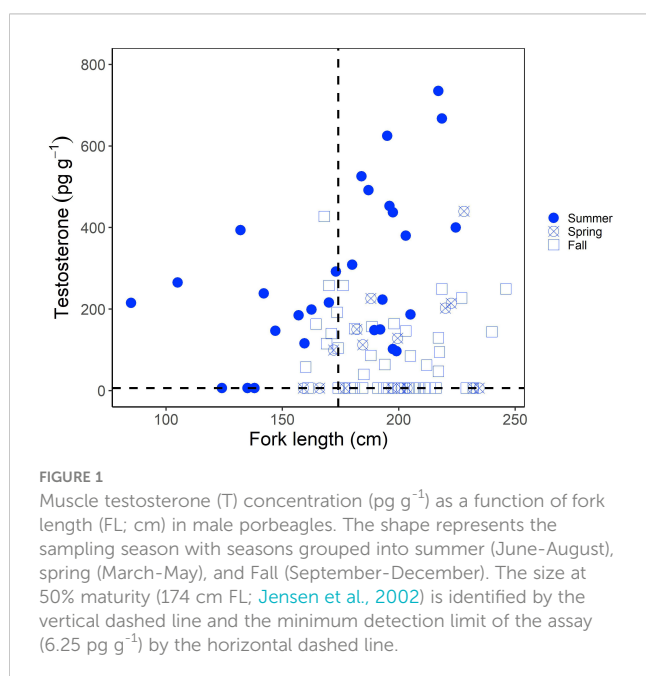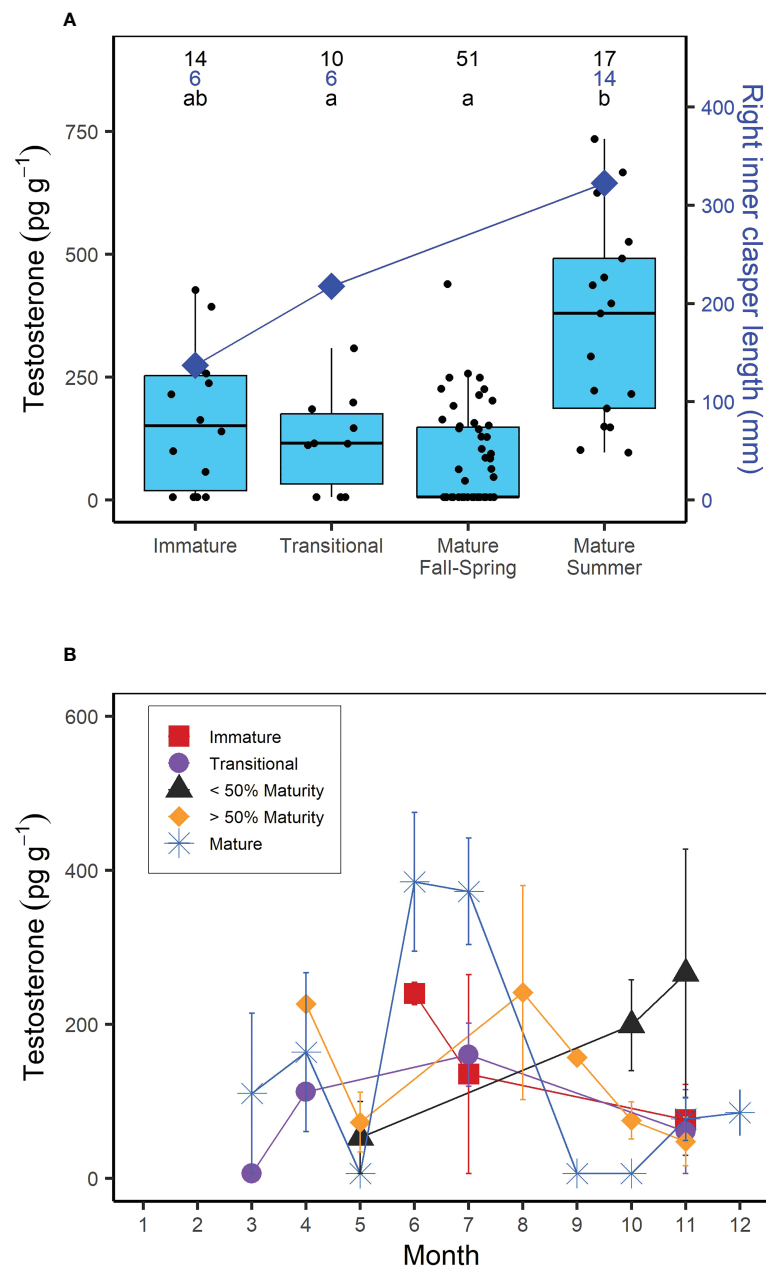
FIGURE 2
(A) Boxplots of muscle testosterone (T) concentration (pg g^{-1}) and average right inner clasper length (mm) as a function of reproductive stage in male porbeagles. For males that were not formally assessed for maturity based on reproductive morphology, reproductive stage was predicted based on size at 50% maturity (174 cm FL; Jensen et al., 2002). Mature males were further divided into summer (June-August; predicted to be undergoing spermatogenesis) or Fall-Spring (September-May; predicted to not be undergoing spermatogenesis) based on previously established timing of spermatogenesis in male sharks (i.e., Manire and Rasmussen, 1997; Verkamp et al., 2022). Numbers represent sample size for T concentrations (black) and right inner clasper length (blue) and letters identify significant differences in T concentrations among groups. **(B)** Average muscle T concentration by month in male porbeagles grouped by reproductive stage. Sharks that were not formally assessed for maturity were grouped based on whether they were smaller or larger than the size at 50% maturity. Error bars represent standard error.

(Figure 4B). Samples for females that were confirmed to be mature but were non-gravid were limited to the months of July, September, and November. E$_2$ was low in these females that were sampled in July and September but was elevated in the single mature non-gravid female that was sampled in November. Samples for gravid females were limited to October through December. E$_2$ concentrations increased from October to December for gravid female samples.

4 Discussion

Not only can reproductive hormones be quantified from muscle samples stored frozen for up to 37 years, new insights into hormonal control of reproduction are evident for this oophagous shark species. Although validated through relatively few samples, this study indicates measurable relationships between hormones and sexual maturity and reproductive stage in porbeagles. These

FIGURE 3
Muscle estradiol (E_2) concentration (pg g^{-1}) as a function of fork length (FL; cm) in female porbeagles. The shape represents the sampling season with seasons grouped into summer (June-August), spring (March-May), and Fall (September-December). The size at 50% maturity (218 cm FL; Jensen et al., 2002) is identified by the vertical dashed line and the minimum detection limit of the assay (5 pg g^{-1}) by the horizontal dashed line.

data indicate muscle T concentrations could most easily differentiate between reproductive stages in the summer, with mature males sampled during the summer having higher T than transitional males and mature males sampled in other times of the year. Muscle E_2 concentrations could most easily differentiate between gravid females and immature females. However, it is important to note that there was overlap in hormone concentrations among stages for both sexes, suggesting there is potential for misclassification of individuals if muscle hormone concentrations are used as the sole predictor of reproductive stage. This overlap is likely associated with individual level variation in hormone concentrations (i.e., Verkamp et al., 2022) rather than the age (Prohaska et al., 2018; Figure S3) or dryness of the sample (Figure S4) given concentrations were found to be variable (from below the detection limit to hundreds of pg g^{-1}) among samples that were collected from animals in the same reproductive stage, in the same year, and homogenized with the same ratio of PBS.

Muscle T concentrations appeared to be higher in many larger male porbeagles compared to smaller conspecifics, suggesting a possible role of T in the maturation of the male reproductive tract in this species. Moreover, although sample size was small and did not include mature individuals sampled during seasons in which male porbeagles are not predicted to be undergoing spermatogenesis, T appeared to be related to reproductive morphology, as T concentrations were highest in males that had the largest claspers. A relationship between plasma T concentration and size or maturity has been documented in males of other shark species (Awruch et al., 2008; Awruch et al., 2014). However, it is important to note that there was a much larger range in T concentrations in larger or mature males compared to immature males. This variability in T concentrations among mature males of the same size was suggested to be attributed to sharks being sampled at varying stages of the reproductive cycle (Awruch et al., 2008). For example, muscle T concentrations were highest in mature males sampled in June through August, which likely

corresponds to when individuals actively undergo spermatogenesis in the months prior to mating (Manire and Rasmussen, 1997; Verkamp et al., 2022). Muscle T concentrations began to drop and were lowest in mature males sampled during the known mating season (September through November; Jensen et al., 2002) when these sharks are likely undergoing testicular regression (Manire and Rasmussen, 1997; Verkamp et al., 2022). Collectively, these findings suggest that male porbeagle muscle T concentrations are related to the *combined* influences of maturity and reproductive seasonality. This highlights the importance of considering reproductive seasonality relative to when samples were collected when predicting sexual maturity based on T concentrations, as has been suggested in previous work on male elasmobranchs (Awruch et al., 2008). In the case of porbeagles, predicting sexual maturity based on T concentrations would be most applicable during the summer months when mature males undergo spermatogenesis.

Female muscle E_2 concentrations had a more distinct relationship with maturity in the porbeagle. All females (with the exception of one individual) that were confirmed to be immature based on internal morphology had E_2 concentrations below the detection limit of our assay and immature females had the smallest average oviducal gland width. This finding was expected given undetectable or very low E_2 concentrations are commonly observed in immature female sharks of other species (Awruch, 2013; Verkamp et al., 2021), including the related oophagous white shark (Verkamp et al., 2021). In mature female sharks, E_2 is associated with the follicular phase and vitellogenesis and is thus typically found to be elevated prior to ovulation (i.e., Awruch, 2013). However, our study found that gravid female porbeagles had elevated E_2 compared to mature non-gravid females. This finding may be related to multiple factors. First, elevated E_2 in gravid compared to non-gravid females is likely unique to oophagous species that continue follicular development and ovulation throughout gestation (Gilmore, 1993; Tribuzio, 2004). Second, it is possible that the non-gravid females analyzed in this study, most of which had E_2 concentrations comparable to females in a transitional stage, were post-partum (if sampled in summer) or in a resting phase of the reproductive cycle. These non-gravid mature females analyzed in this study could be part of the portion of female porbeagles that reproduce biennially in the NWA (Natanson et al., 2019). Low E_2 concentrations have been observed in other sharks during resting phases when not actively undergoing vitellogenesis (Tribuzio, 2004; Prohaska et al., 2013), including the closely related, oophagous, salmon shark *L. ditropis* (Tribuzio, 2004). These conclusions are consistent with our limited data on oviducal gland widths. Mature non-gravid females had oviducal gland widths comparable to porbeagles found to be in a post-partum or resting phase (Natanson et al., 2019), while the gravid female had a larger oviducal gland, comparable to other gravid porbeagles (Jensen et al., 2002).

Overall, the hormonal trends observed in this study are consistent with the current understanding of the role of reproductive hormones during sexual maturation and reproductive stages in sharks. It appears muscle reproductive hormones have the potential to serve as a non-lethal proxy of reproductive stage in the porbeagle and potentially other oophagous sharks, albeit with limitations regarding overlap in concentrations among reproductive stages. It is possible that this
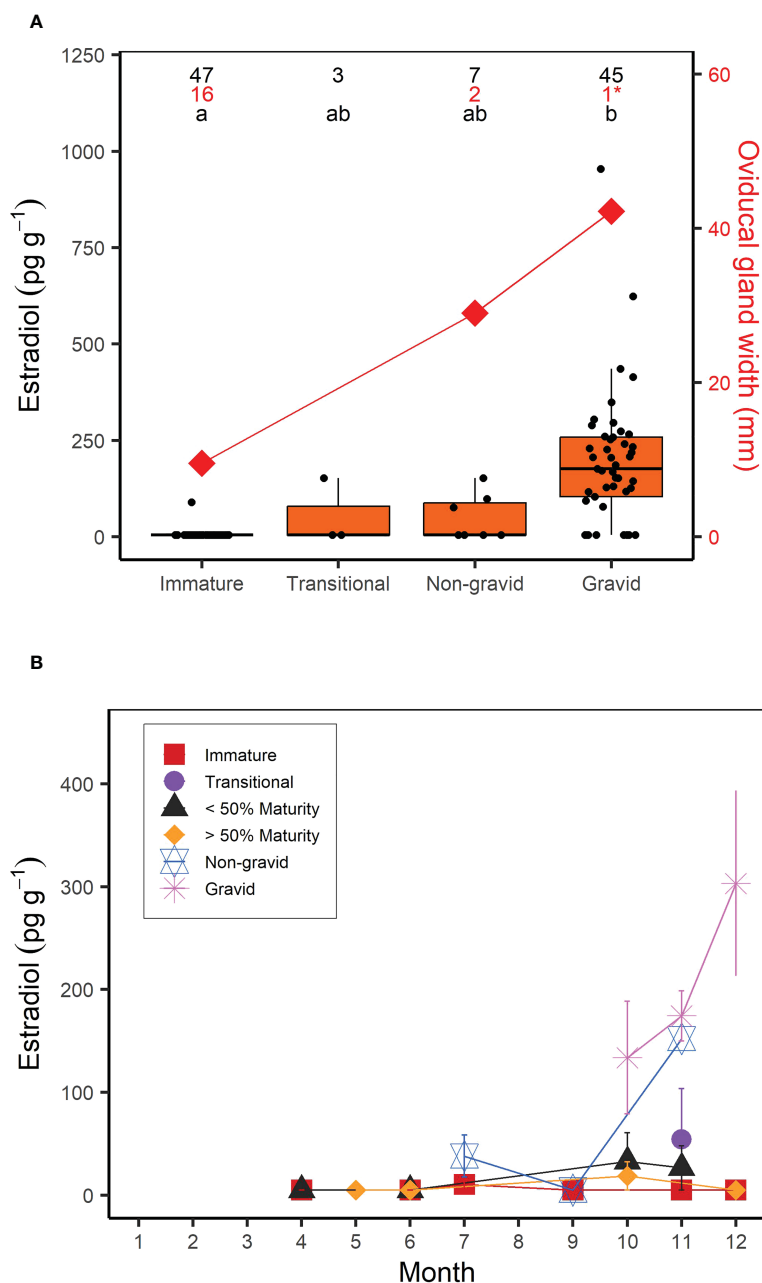
FIGURE 4
(A) Boxplots of muscle estradiol (E_2) concentration (pg g^{-1}) and average oviducal gland width (mm) as a function of reproductive stage in female porbeagles that were formally assessed for maturity and pregnancy. Numbers represent the sample size for E_2 concentrations (black) and oviducal gland width (red) and letters identify significant differences in E_2 concentrations among groups. *Indicates an oviducal gland width measurement taken from a gravid porbeagle that was not analyzed for muscle E_2 but was included for reference. **(B)** Average muscle E_2 concentration by month in female porbeagles grouped by reproductive stage. Sharks that were not formally assessed for maturity were grouped based on whether they were smaller or larger than the size at 50% maturity. Error bars represent standard error.

limitation may be overcome by including additional non-lethal assessments of reproductive stage, such as examinations of claspers for males and ultrasonography for females. Moreover, while the size of muscle samples available for this opportunistic study precluded the quantification of additional reproductive hormones (i.e., P_4, 11-ketotestosterone), it is possible that the inclusion of other hormones may improve the differentiation of reproductive stages, and this is a potential avenue of future research. Another limitation of this study was the lack of samples available from mature females in the winter

and spring, which precluded the ability to assess changes in reproductive hormones across the entirety of the reproductive cycle, such as late gestation. Nevertheless, the success of this work has wide-reaching applications for elasmobranch reproductive research. We demonstrated for the first time that reproductive hormones could be successfully extracted and quantified from shark muscle tissues that have been stored frozen for decades and found no impact of sample age or dryness on hormone recovery or concentration. Therefore, similar work could be done for other species that have archived

biological samples in order to increase the amount of scientific information gathered from lethal sampling. This would be especially relevant for threatened species for which lethal sampling is restricted and reproductive data is especially needed for conservation and management decisions.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by Arizona State University Institutional Animal Care and Use Committee.

Author contributions

Study design was led by JAS, LJN, MSP, and HDB. Sample collection was led by LJN, MSP, and HDB. Laboratory analyses were completed by BNA, JK, CH, AE, LD, and HM. Data analyses were completed by BNA and JK. Manuscript was written by BNA and edited by JAS, LJN, MSP, HDB, JK, CH, AE, LD, and HM. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1176767/full#supplementary-material

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Connectivity between white shark populations off Central California, USA and Guadalupe Island, Mexico

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Marine animals often move beyond national borders and exclusive economic zones resulting in a need for trans-boundary management spanning multiple national jurisdictions. Highly migratory fish vulnerable to over-exploitation require protections at international level, as exploitation practices can be disparate between adjacent countries and marine jurisdictions. In this study we collaboratively conducted an analysis of white shark connectivity between two main aggregation regions with independent population assessment and legal protection programs; one off central California, USA and one off Guadalupe Island, Mexico. We acoustically tagged 326 sub-adult and adult white sharks in central California (n=210) and in Guadalupe Island (n=116) with acoustic transmitters between 2008-2019. Of the 326 tagged white sharks, 30 (9.20%) individuals were detected at both regions during the study period. We used a Bayesian implementation of logistic regression with a binomial distribution to estimate the effect of sex, maturity, and tag location to the response variable of probability of moving from one region to the other. While nearly one in ten individuals in our sample were detected in both regions over the study period, the annual rate of trans-regional movement was low (probability of movement = 0.015 yr^{-1}, 95% credible interval = 0.002, 0.061). Sub-adults were more likely than adults to move between regions and sharks were more likely to move from Guadalupe Island to central California, however, sex, and year were not important factors influencing movement. This first estimation of demographic-

specific trans-regional movement connecting US and Mexico aggregations with high seasonal site fidelity represents an important step to future international management and assessment of the northeastern Pacific white shark population as a whole.

1 Introduction

Informed wildlife resource use and management decisions are derived from an understanding of population structure and life-history parameters. Demarcating populations of marine animals on ocean-basin scales can be difficult due to large home ranges and inability to observe animals directly in the ocean habitat. However, advances in tagging technologies have increased our capacity to observe movements and ocean-scale migratory paths for multiple species across taxa (Block et al., 1998; Block et al., 2005; Block et al., 2011). These technologies have provided bounds on many highly mobile species, indicating predictable species-specific migratory pathways and high-use areas where focused sampling efforts can provide important population characteristic data.

The white shark (*Carcharodon carcharias*) is an apex marine predator with a circumglobal distribution. Tagging studies in tandem with genetic techniques provide evidence that natal philopatry is strong (Jorgensen et al., 2010), which results in discrete population structure with clearly defined population segments that have been identified in South Africa (Pardini et al., 2000), Australia-New Zealand (Blower et al., 2012), the northeast (Jorgensen et al., 2010; Bernard et al., 2018) and northwest Pacific Ocean (Tanaka et al., 2011), the Mediterranean Sea (Gubili et al., 2010), and the northwest Atlantic Ocean (O'Leary et al., 2015). Within the northeastern Pacific segment, estimated to be isolated from other Pacific populations for some 200k years (Jorgensen et al., 2010; Bernard et al., 2018), evidence for further genetic structure is mixed. Indications of mitochondrial DNA structure between Central California, USA and Guadalupe Island, Mexico reported in one study (Oñate-González et al., 2015) run contrary to a second study which found no Mitochondrial DNA structure (Santana-Morales et al., 2020). A third transcriptome-derived microsatellite study also reported a lack of support for NEP substructure (Bernard et al., 2018) leaving the question of connectivity across the U.S. – Mexico regions inconclusive.

Off the western U.S. coast, white sharks in the northeastern Pacific are thought to give birth in the Southern California Bight (area between Point Conception and San Diego). This assumption is based on incidental catches of neonate and young-of-the-year white sharks (120 -150 cm) in near-shore waters (<3 nm from shore,<50 m depth) from gill-net fisheries that target other fishes such as California Halibut (*Paralichthys californicus*), White Seabass (*Atractoscion nobilis*), and Pacific Angel Shark (*Squatina*

californica) (Klimley, 1985; Lowe et al., 2012; Lyons et al., 2013). Just to the south in Baja, Mexico, a nursery ground has been described in Bahia Sebastian Vizcaino (Oñate-González et al., 2017). Additional nursery areas in the Gulf of California have been proposed based on the movement of large females, although captures of young-of-the-year sharks there are rare (Galván-Magaña et al., 2011; Domeier and Nasby-Lucas, 2013) White sharks are hypothesized to spend ~three years in these nursery grounds feeding upon cephalopods, teleosts and elasmobranchs until they reach lengths of ~ 200-250 cm. As larger juveniles and sub-adults these white sharks recruit primarily to the north of Point Conception, CA and tags along with photo ID indicates they are present in central California or to Guadalupe Island ~250 km offshore of northern Mexico, with large aggregations of pinnipeds residing in both regions (Weng et al., 2007a; Oñate-González et al., 2017).

Multiple tagging studies (Boustany et al., 2002; Weng et al., 2007a; Domeier and Nasby-Lucas, 2008; Jorgensen et al., 2010; Domeier and Nasby-Lucas, 2012) have provided evidence that sub-adult and adult white sharks undertake predictable seasonal migrations in the northeastern Pacific between three main focal areas: (i) North American shelf waters, (ii) the slope and offshore waters of the Hawaiian archipelago, and (iii) the offshore white shark Café, located ~ 1500 km offshore between Baja, Mexico and Hawaii, USA (Weng et al., 2007a; Domeier and Nasby-Lucas, 2008; Jorgensen et al., 2010; Andrzejaczek et al., 2022). While sharks from both coastal regions overlap in the White Shark Café and Hawaii, satellite and acoustic tagged white sharks have been shown to consistently return to their respective tagging region in central California or Guadalupe Island (Domeier and Nasby-Lucas, 2008; Jorgensen et al., 2010; Chapple et al., 2016).

Adults from both locations predictably travel offshore and have similar timing for departure from and return to coastal areas (Domeier and Nasby-Lucas, 2008; Jorgensen et al., 2010). Sub-adults in Guadalupe Island, however, mostly remain coastal (Hoyos-Padilla et al., 2016), and sub-adults that do travel offshore do not appear to have offshore movements that are synchronized with seasonal departures by adults to offshore areas (Domeier, 2012). The onset of migratory behavior remains largely unknown for the sub-adult phase of life for white sharks in central California due to a scarcity of research on this specific transition phase. In both regions, transition of sub-adults making temporally predictable and direct migrations remains unclear but could be a cumulative

experience of annual attempts to hone in on an efficient migratory pattern.

Prior to recruitment at adult aggregating sites, tagging indicates that some juvenile white sharks seasonally migrate back and forth between Vizcaino Bay, Mexico and Southern to central California (Weng et al., 2007a; Benson et al., 2018). Additionally, five acoustically tagged sub-adult sharks were detected in both central California and Guadalupe Island (Jorgensen et al., 2012a; Hoyos-Padilla et al., 2016). To date, a comprehensive analysis of tagging data has been lacking across both regions to quantify the extent of the connectivity, its frequency, and the potential demographic factors (e.g. sex, year, size).

To date, assessments of the NEP white shark population have been conducted separately for central California (Chapple et al., 2011; Kanive et al., 2021) and Guadalupe Island (Sosa-Nishizaki et al., 2012). To date, assessments of the entire NEP segment, along with the potential for source/sink or rescue effects between these two regions remains an important conservation goal. Estimating abundance for the combined northeastern Pacific region could be artificially inflated if individuals use both regions and were counted twice. Therefore, it is important to accurately define annual rates of overlap between both regions to accurately quantify multi-regional population parameters.

Quantifying the connectivity between these two transboundary regions needs to be considered when making informed management decisions about the overall regional population size.

Additionally, jurisdictional differences between Mexico and the US are important to consider in monitoring the population structure and trajectory of this protected species. In this study, we conducted a comprehensive analysis of acoustic tagging data collected over 12 years of two populations that bound international waters. Through collaboration between several research groups using parallel tagging methods and technology, we quantify movement between these two important aggregations in USA and Mexico and explore demographic-specific migration rates and drivers of movement for sub-adult and adult white sharks.

2 Materials and methods

2.1 Study area and tagging methods

From 2008 to 2018, we acoustically tagged sharks at known sub-adult and adult aggregation regions in California, USA (Southeast Farallon Island, Año Nuevo Island, and Tomales Point) and Guadalupe Island, Mexico (Figure 1) during periods (September – February) of peak coastal residency (Klimley, 1985; Jorgensen et al., 2010). White sharks were attracted to a research boat using a seal decoy, and motivated to circle the boat with a small (<2kg) piece of salvaged marine mammal blubber tethered against the boat at the water line (Kanive et al., 2015) in central California. In Guadalupe Island similar techniques are used but the attraction is a whole
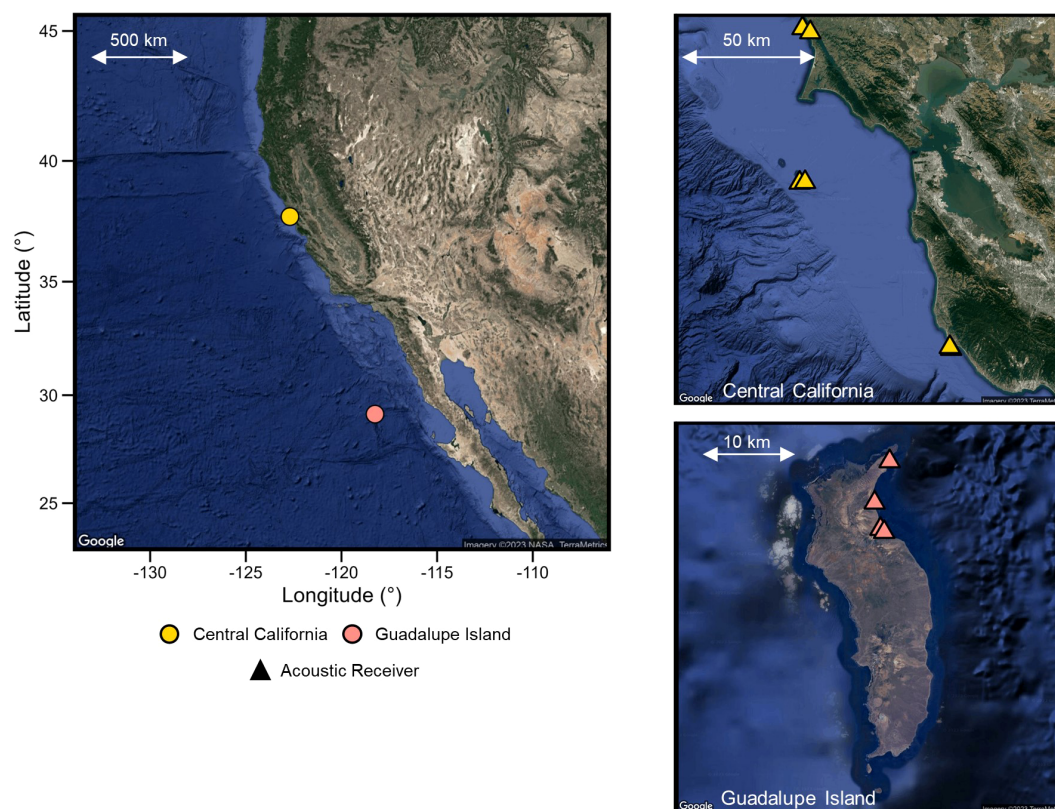
Map of the Northeast Pacific that includes the seasonal coastal aggregation sites of white sharks off central California, USA (yellow) and Guadalupe Island, Mexico (red). Circles represent coastal/island aggregation sites. Triangles indicate receiver locations at each study site.

yellowfin tuna or portions of the fish. Individual sharks were identified from photo and video images of the natural and unique patterns on the trailing edge of their dorsal fin as fin morphological patterns have been validated for stability over periods exceeding 25 years (Anderson et al., 2011). Total lengths of the sharks were estimated from one to three experienced researchers using the known length of the research vessel as a reference measurement as the shark swam close to the vessel. If there was a disparity in an estimate of the total length among the researchers, the mean was used as the estimate. Maturity was assigned based on published values where adult males ≥ 380cm total length (TL), sub-adult males < 380cm TL, adult females ≥ 440cm TL, and sub-adult females < 440cm TL (Francis, 1996; Pratt, 1996). Sex was determined by the presence (male) or absence (female) of claspers.

Free-swimming sharks were tagged with individually coded acoustic transmitter tags (V16-4H; and V16-6H; Innova Sea, Inc formerly Vemco, Halifax, Nova Scotia) in central California using a 59 mm titanium dart with an 18-20 cm 136 kg test monofilament leader protected by hollow braided Dacron and shrink-wrap (Jorgensen et al., 2010; Wilson et al., 2015). The darts were inserted into the dorsal musculature using a tagging pole (Boustany et al., 2002; Weng et al., 2007a) as the shark swam by the research vessel. In Guadalupe Island, sharks were tagged with individually coded acoustic transmitter tags (V16-4H; Vemco, Halifax, Nova Scotia) using a stainless steel dart and a steel leader with shrink-wrap.

Acoustic receivers (Vemco; VR-2, VR-3UM, VR-4UM) were placed at known white shark aggregation sites in central California and Guadalupe Island (Figure 1). These receivers archived the acoustic detections of each shark with an individual acoustic number as they swam within ~500 m of the receiver. The data from the receivers could then be downloaded *in situ* (VR-3), physically retrieved (VR-2) or remotely transmitted (VR-4UM).

2.2 GLMM analysis

We modeled the probability of transitioning from one region (Guadalupe Island or central California) site to the other using logistic regression with a binomial distribution for the response variable (moved from one aggregation site [central California or Guadalupe Island] to the other) and a logit-link between the response variable and covariates of interest. We used a Bayesian approach to implement one general model that included five features that we predicted might be associated with variation in an individual's probability of movement. These included sex, maturity class, and the aggregation site at which it was observed at the start of the binomial trial. Because some individuals provided data for multiple binomial trials and because multiple individuals were studied within a year, we also included random effect of individual and year in our models. As the annual migratory pattern consists of an offshore phase followed by a coastal phase, our model defines a 'movement' as either (i) a shark migrates offshore from one region and returns to the coast at the other region or (ii) a shark returned to where it departed at previous time step, then moved to the other region within the season. Individual sharks were coded as

'1' if the shark moved or '0' if the shark did not move between sites in a given season. Therefore, the probability of 'moving' to the other site at least one time in a season can be defined as:

$$logit(\text{moved}) = \alpha + \beta s \times \text{sex} + \beta m \times \text{maturity} + \beta l \times \text{location}$$

$$+ \hat{s}^2_{\text{ind}} \times \text{ind} + \hat{s}^2_{\text{year}} \times \text{year}$$

We implemented the model in the R software (R Core Team, 2019) environment using the 'rstanarm' (Goodrich et al., 2020) and 'shinystan' (Gabry, 2018) packages (Muth et al., 2018). We ran four chains using diffuse priors and ran each chain for 1,000 iterations after a burn-in of 1,000 iterations was completed and discarded (Gelman et al., 1996). We used the standard priors set by 'rstanarm'. We used normal distributions for the priors for random effects of individual and year (random effects were $\sim N[0, \hat{s}^2_{\text{individual}}]$ and $\sim N[0, \hat{s}^2_{\text{year}}]]$). After 1,000 burn-in iterations, we ran an additional 1,000 iterations per chain, resulting in 4,000 total samples from the posterior distribution. We assessed model convergence by inspecting the trace plots and Geweke diagnostics (Geweke, 1991) and evaluating whether the Gelman-Rubin statistic, $\hat{R}$, was<1.1 for each monitored parameter (Gelman and Rubin, 1992). We evaluated how well predicted values from the fitted model corresponded with actual observations using posterior predictive checks (Gelman et al., 2000). Specifically, we evaluated how well distributions of predicted values corresponded to the mean, standard deviation, density, and 2.5% and 97.5% quantiles of the observed data. We also examined the distribution of discrepancies between predicted and observed values to evaluate whether there was evidence that some observations were not predicted well by the model.

3 Results

In California, from 2008 to 2018, a total of 249 tags were deployed onto 210 individual white sharks (some sharks were tagged multiple times) that ranged in estimated total length from 240 cm to 550 cm in total length (mean = 401.75 cm; SD = 75.14). Of the sharks tagged, 81 were adult males, 46 were sub-adult males, 43 were adult females, and 40 were sub-adult females.

In Guadalupe Island, from 2008 to 2018, 122 acoustic tags were deployed onto 116 sharks that ranged in estimated total length from 200 cm to 570 cm in total length (mean = 374.27 cm; SD = 80.44). Of those sharks tagged, there were 26 adult males, 33 sub-adult males, 18 adult females, and 39 sub-adult males.

The mean length of time that acoustic tags remained attached to a shark and had a functional battery that transmitted a signal was 572 days (SD = 508; range = 6 to 2983 days). Over the course of the study, we recorded a total of 6,190,866 detections from receivers placed at both locations in central California and Guadalupe Island. In central California, there were a total of 666,560 detections from receivers (VR-2, VR-3, and VR-4) and 5,524,306 detections from receivers (VR-2) in Guadalupe Island (Figure 1). A summary of the number of sharks with functioning acoustic tags for each year of the study can be found in Table 1.

TABLE 1 Summary of white shark movements throughout the study period, 2008-2019.

Year	2008	2009	2010	2011	2012	2013
Non-movers	55	72	72	92	92	71
Movers	1	4	3	3	4	3
Total Tags	56	76	75	95	96	74
PPN. Moved	0.018	0.053	0.040	0.032	0.042	0.041
Year	2014	2015	2016	2017	2018	2019
Non-movers	70	53	35	74	55	28
Movers	3	1	1	6	8	3
Total Tags	73	54	36	80	63	31
PPN. Moved	0.041	0.019	0.028	0.075	0.127	0.097

Non-movers are the number of sharks that stayed in their respective tagging location (central California or Guadalupe Island). Movers are the number of sharks detected that have transitioned to the other location (central California or Guadalupe Island). Total tags are the number of active tags detected on receivers each year of the study. PPN moved is the proportion of tagged sharks to the total tags that either moved from central California to Guadalupe Island or Guadalupe Island to central California.

Of the 326 total sharks tagged in the study, 210 (64.42%) were tagged in central California while the other 116 (35.58%) were tagged in Guadalupe Island. Of the 326, 30 (9.20%) were detected in both locations, and 296 (90.80%) were detected only at the aggregation sites (central California or Guadalupe Island) where the tag was originally applied. Of the 30 sharks detected at both locations, 20 (66.67%) were tagged in central California and included four adult males, seven sub-adult males, two adult females, and seven sub-adult females (1.22 males for every female). The 10 (33.33%) sharks tagged in Guadalupe Island that were detected at both locations included one adult male, five sub-adult males, one adult female, and three sub-adult females (1.5 males for every female). The proportion of sharks tagged at each region was similar to the proportion of sharks that moved from each region.

Of the 30 sharks that moved, 20 made one move to the other region and apparently remained there for the remainder of tag function. There were eight sharks that made two movements, meaning they moved to the other region and then returned to the original location. In addition, one shark made three movements and one shark made four movements between the regions (Figure 2).

There were three sharks that made more than one transition within a season (shark 53916, 350-cm male; shark 46104, 450-cm female; shark 32539, 300-cm female). The straight-line distance from the southernmost location in central California to Guadalupe Island is ~ 950 km. The mean length of time of within season

FIGURE 2
Detections of white sharks by class at receivers in central California, USA and Guadalupe Island, Mexico over the course of the study period. The Tag ID is the acoustic code of the tag applied to the individual shark.
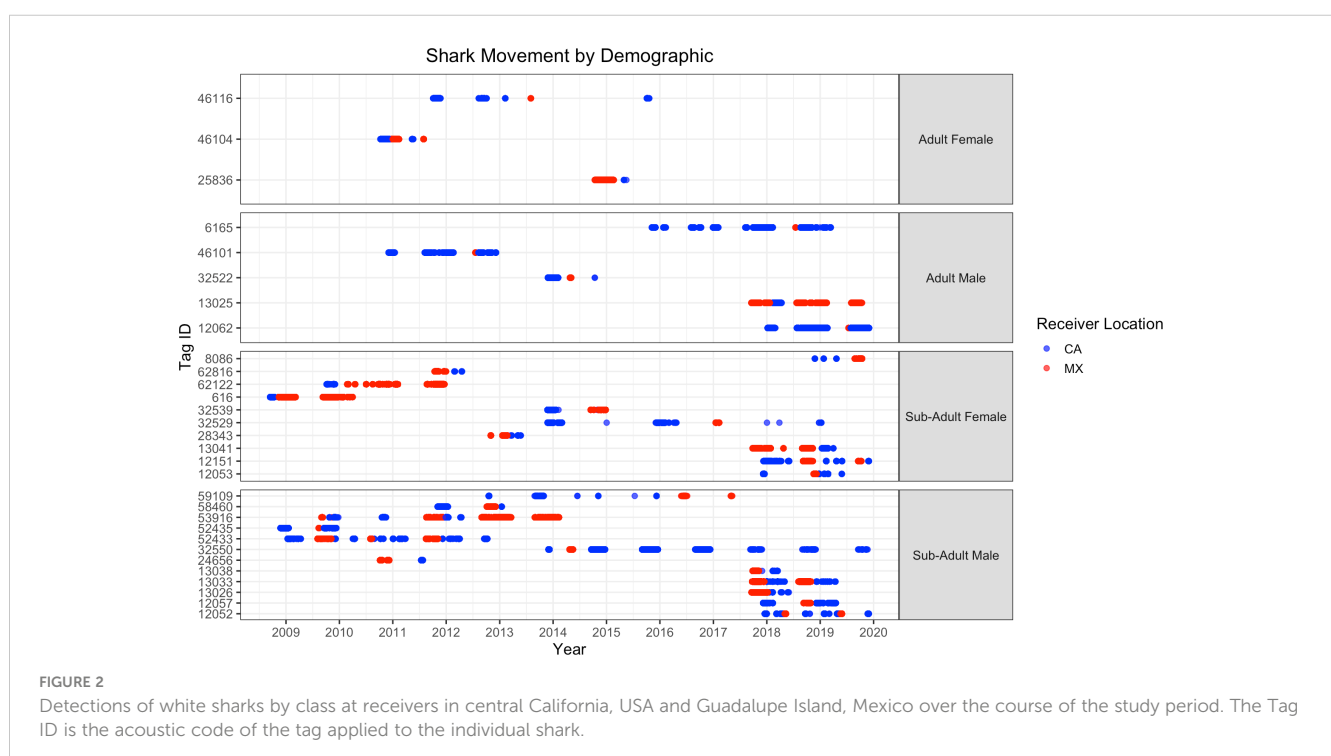
movement between central California and Guadalupe Island ranged from 8 to 91 days (mean = 32.56 days; SD = 24.81). Therefore, the fastest minimum rate of travel was estimated to be 133 km/day at 1.31 m/s.

3.1 Model results

The model converged successfully, and the posterior distribution for model coefficients generated predicted values that corresponded well with features of the observed data. The model results indicated that the predicted probability of moving in between central California and Guadalupe Island (regardless of direction) was rare (α = -4.16; SE = 0.86; 90% credible intervals [CI] = -6.11 to -2.72). The model indicated that males and females were quite similar with respect to movement probability (β_s = 0.15; SE = 0.63; 90% CI = -1.15 to 1.37). Adults were less likely to move than sub-adults (β_m = -2.10; SE = 0.71; 90% CI = -3.63 to -0.79), and sharks were similar in movement probability given their starting location (β_l = 0.55; SE = 0.51; CI = -1.54 to 0.47). The estimated variance term for the random effect for individual sharks was larger ($\hat{s}^2_{ind}$ = 7.65; 90% credible intervals = 2.68 to 16.67) than the random effect for years ($\hat{s}^2_{year}$ = 0.12; CI = 0.00 to 0.64). As only 30 of the 326 sharks in this 12-year study moved to the other region, accordingly, the individual random effect-outcomes predicted for all but those 30 sharks were small and below 0, whereas the remaining 30 tended to be small and positive with only a very few sharks predicted to have positive random effects that notably increased their probability of movement. Predicted probabilities of moving between central California and Guadalupe Island for each of the 30 individuals that moved averaged 0.39 (SE = 0.22), whereas movement probabilities for the other individuals averaged<0.02 (SE = 0.05).

While all demographics from both locations moved to the other location at some point during the study period, the sub-adult demographic from both central California and Guadalupe Island had the higher probabilities of movement to the other location (Table 2). For sub-adults, these probabilities ranged from 0.073 (90% CI = 0.000 to 0.25) to 0.155 (90% CI = 0.000 to 0.405) compared to the lower probabilities of movement for the adult demographic that ranged from 0.017 (90% CI = 0.000 to 0.088) to 0.031 (90% CI = 0.000 to 0.141). Interestingly, sub-adult males tagged in Guadalupe Island had the highest probability of moving 0.155 (90% CI = 0.000 to 0.779) and the adult males tagged in Guadalupe Island had the lowest probability of moving 0.006 (90% CI = 0.000 to 0.080).

4 Discussion

The purpose of this study was to better understand the connectivity and movement of sub-adult and adult white sharks between the two main aggregations that comprise the northeastern Pacific population. Our results indicate that the majority of sharks have high annual fidelity to their respective foraging aggregation site where they were first tagged. Over the 12-year study, ~10% of sharks utilized both aggregation sites, yet the overall probability of movement was relatively low. Understanding the rate of exchange will be important to inform how to best integrate mark-recapture data from each site and an help understand ambiguity and genetic structure.

Individuals from all classes (male and female sub-adult and adults) were shown to have moved to the other location from where they were tagged. Sub-adult sharks were more likely than adults to be using or exploiting both aggregation sites. These results are consistent with previous studies that have found sub-adult sharks visiting both major aggregations (Jorgensen et al., 2012a; Hoyos-Padilla et al., 2016). Sub-adult connectivity has also been hypothesized to be a source of genetic mixing between the two aggregations (Domeier, 2012). In this study, however, we also detected eight adult white sharks (five male, three female) making transregional movements, a first for this population and a finding that puts aside an early hypothesis that only sub-adults move between the regions (Domeier, 2012). The mechanisms driving a small number of individual sharks to exploit both areas remain unknown, but could be associated with low prey availability and/or high density of white sharks at a respective aggregation area leading to increased intraspecific competition.

TABLE 2 Summary of results from the GLMM analysis of movement probabilities for each demographic at central California, USA and Guadalupe Island, Mexico.

Group by Location	#Annual Transitions	#Moved	Prop. Moved	Mean Prob. of Moving	SD	90% Credible Intervals	
GI SAF	71	5	0.070	0.087	0.113	0.000	0.320
GI SAM	59	10	0.169	0.154	0.128	0.000	0.405
GI AF	42	2	0.048	0.028	0.052	0.000	0.132
GI AM	47	1	0.021	0.031	0.055	0.000	0.141
CC SAF	101	8	0.079	0.073	0.091	0.000	0.251
CC SAM	131	11	0.084	0.084	0.086	0.000	0.253
CC AF	97	2	0.021	0.024	0.046	0.000	0.110
CC AM	262	4	0.015	0.017	0.036	0.000	0.088

SAF, sub-adult female; SAM, sub-adult male; AF, adult female; AM, adult male.

Mating locations and dynamics in the NEP have not been confirmed and thus the potential population-level implications for these movements detected by tagging remain unknown. Mating likely occurs at either coastal locations (Domeier, 2012; Domeier and Nasby-Lucas, 2012; Domeier and Nasby-Lucas, 2013) or in the White Shark Café (Jorgensen et al., 2012b), ~1500 km between Baja, Mexico and Hawaii, USA. However, a single migrant per generation over time can be sufficient to homogenize genetic structure between two otherwise isolated populations (Morjan and Rieseberg, 2004; Hartl and Clark, 2006). During the 12-year period of this study we documented 30 individuals (of 329 tagged) moving between Guadalupe Island and central California. Therefore, this study supports the findings of Bernard et al. (2018) and Santana-Morales et al. (2020), which detected no genetic structure (nuclear and mitochondrial DNA respectively) between these regions, rather than with those of Oñate-González et al. (2015), which indicated significant mitochondrial DNA structure. Until future studies resolve the genetics of the population conclusively as well as the mating dynamics, the relationships between the individual trans-regional movement and overall population structure in this case remains uncertain.

Sub-adult white sharks are a demographic of which relatively little is known. During this life stage, white sharks undergo an ontogenetic shift in diet and habitat (Tricas and Mccosker, 1984) and transition to aggregations where adult white sharks are well established and have been successfully exploiting these areas for food resources (Weng et al., 2007b). There is likely intense intraspecific competition for recruiting sub-adult sharks prospecting for new resources around pinniped prey concentrations to fulfill their increased physiological demand in the colder nearshore waters. It has been shown that the smaller size classes in central California have substantially lower apparent survival rates than larger conspecifics (Kanive et al., 2019). However, since mortality is confounded with permanent emigration in apparent survival, this could mean that smaller sub-adults may fail to recruit at adult aggregating sites resulting in either mortality or emigration to secondary locations. Grievous wounds have been observed on multiple sub-adult sharks that appear to have been inflicted by a larger white shark (Kanive et al., 2019). Sub-adults could be motivated to explore other habitats if they experience such aggression while attempting to recruit at an established, predominately adult hunting ground. The results of this study where the sub-adults have the highest degree of movement support these possibilities.

This study provides critical information for a future estimation and assessment of the overall abundance of the northeastern Pacific that includes both groups, central California and Guadalupe Island, that to date have largely been considered separately. This study confirms connectivity between the two main aggregation of both sub-adults and adults.Consequently, simply summing independent estimates from both locations to estimate overall population characteristics would include a percentage of 'double-counted' individuals. Furthermore, a joint method of 'marking' or identifying individuals, such as using the long-lasting (>26 years) natural and unique patterns on dorsal fins (Anderson et al., 2011) is needed to incorporate data into a mark-recapture framework, an established method to identify population vital rates (Kanive et al., 2015; Chapple et al., 2016; Kanive et al., 2019) and abundance estimates (Chapple et al., 2011; Towner et al., 2013; Kanive et al., 2021). Lastly, continued and expanded mutually beneficial collaborative efforts and shared information are needed to enable researchers to estimate robust population parameters that can be used for international management.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by Montana State University Institutional Animal Care and Use Committee.

Author contributions

PK, JR, SJ, MH, and TC contributed to conception and design of the study. PK, SJ, TC, SA, MH-P, FG-M, and BB tagged white sharks in the field and downloaded receivers. PK organized the database. PK and JR performed the statistical analysis. PK wrote the first draft of the manuscript. SA and AK helped with figures. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The successful use of a submersible ultrasound to confirm pregnancy on free swimming bull sharks, *Carcharhinus leucas*, in a provisioned shark site

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A prototype, fully submersible, high definition ultrasound was used to determine the reproductive state of wild, free-swimming bull sharks, *Carcharhinus leucas*, at a provisioned shark diving site in Playa del Carmen, Mexico. During two opportunistic dives, the presence of embryos was confirmed in three female sharks (greater than 2.0 m total length) and emphasizes the importance of developing and linking emerging technologies with shark diving sites for the conservation of elasmobranch species.

KEYWORDS

emerging technologies, gestation ground, conservation, management, ecotourism

1 Introduction

Diving with sharks has become popular throughout the world in recent decades (Gallagher and Hammerschlag, 2011) and in Mexico has been suggested to contribute approximately 12.4 million in United States dollars (USD) per year to local economies (Cisneros-Montemayor et al., 2013). In addition to an economic importance, observational studies at such shark tourism sites can also provide fishery-independent scientific information to improve population level assessments for certain species (Clua et al., 2010), as not only do these sharks often form seasonal aggregations, they also can be individually identified (Photo-ID; Pierce et al., 2018) and potentially monitored over multiple years. The next stage in the evolution of linking this type of tourism to scientific data collection, is the ability to characterize life history stages to the observed animals.

One particular life history stage that is critical for establishing site-based conservation strategies, such as marine protected areas or time/area closures is an understanding of

where gravid sharks spend their time gestating/and or give birth (Chapman et al., 2013; Sulikowski et al., 2016). However, historically, such reproductive data was collected from sacrificed animals from fisheries, which is problematic for species that have been classified as threatened and in the case of shark diving tourism, can also have a negative socioeconomic impact (Hammerschlag and Sulikowski, 2011). Thus, new approaches to studying elasmobranchs increasingly include non-lethal sampling methods (Penfold and Wyffels, 2019). For example, the use of ultrasounds and short-term restraint for biological sampling has continued to advance this field of research in both wild caught and aquarium elasmobranchs (Carrier et al., 2003; Sulikowski et al., 2016, Murakumo et al., 2020). However, the use of ultrasound technology on free swimming sharks in the wild, has only been documented on one species (Murakumo et al., 2020), and has yet to be applied to sharks aggregating at dive sites.

To fill this knowledge gap, a prototype, submersible, high definition ultrasound developed by EI Medical (Inc.) was field tested on free swimming bull sharks, *Carcharhinus leucas*, within a provisioned dive site in the coast of Playa Del Carmen to determine if any of sharks were gravid at this location.

2 Materials and methods

Opportunistic sampling occurred during two, 20 min dives, approximately 1 km of the coast of Playa del Carmen, in Quintana Roo state, Mexico (Figure 1). Water depth was 24 m and the substrate comprised of sandy bottom. A fully submersible ultrasonography was performed on the abdominal region of free-swimming female bull sharks to assess reproductive stage (Figure 2). Here, a topside Ibex EVO III portable ultrasound (E.I. Medical Imaging) was redesigned and encased in waterproof housing (via proprietary specifications) for use at depths greater than 30 meters, herein termed the Aquanaut (Figure 3). The Aquanaut was equipped with a 60 mm curved linear array 2.5 to 5 MHz transducer (model eCL3) capable of a 30 cm scan depth which was connected to the unit with a 3 m cord. The Aquanaut recorded one continuous video until the dive ended. Bull sharks were fed by a professional shark handler with chunked, local fish species, and led

single file so that the transducer could make contact as the sharks slowly swam by (Figure 2). Due to limited bottom dive time (20 min), the largest sharks estimated visually, were targeted for ultrasonography. In addition, due to the frequency of dives performed (two to three per day for four to five months over the course of a dive season) individual bull sharks were able to be identified by the dive operators. Once targeted, a second diver recorded a video of the scanning event for each shark as it moved away from the handler. Each video recording of the scanning event was time stamped and later coordinated with the internal clock on the ultrasound to link an image of the gravid female to her recorded embryos. Scanning was performed primarily on the lateral surface from the pectoral to the pelvic fin in either transverse or longitudinal orientation to obtain cross sectional and lengthwise images, respectively (Figure 2). Each scan lasted between one to three seconds. Collected video saved on the Aquanaut were used to create still images of observed embryos (Sulikowski and Hammerschlag, 2023). Stills from the video were then used to measure (via proprietary software pre-installed on the Aquanaut) pup diameter (cm) along the transverse axis (Sulikowski et al., 2016).

3 Results

Over the course of the two 20 minute dives, individual sharks made several passes and based on the analysis of the Aquanaut video, three sharks were identified to be gravid (Figure 4) by the presence of identifiable embryos within the uterus (Sulikowski and Hammerschlag, 2023). Proprietary software measurements from still images indicated the embryos measured were approximately 4 cm in circumference. While the exact location and orientation of the embryos could not be determined, given the short duration of transducer shark contact, the observed sizes are similar to those reported from a gravid female captured on November 22nd-2022 by local fishermen in Isla Mujeres, Quintana Roo (a nearby location to Playa del Carmen), during a fishing survey realized by Save Our Sharks staff.

4 Discussion

The use of ultrasonography as a non-invasive methodology has proven to be a useful tool for the study of reproductive aspects in both captive and wild caught sharks (Walsh et al., 1993; Daly et al., 2007; Sulikowski et al., 2016; Inoue et al., 2022; Santos et al., 2022; Sulikowski and Hammerschlag, 2023). However, the use of this technology adapted for use underwater on free swimming wild animals is scant, with only one published study to date on whale sharks (Matsumoto et al., 2023). The study herein adds to this limited body of work and shows the utility of this technology to identify gravid females in a provisioned dive site. The positive outcome has several implications for the preservation of specific life history stages for sharks and potentially other elasmobranchs. For example, it is well established that critical component of successful conservation of wild populations is an understanding of a species'

FIGURE 1
Location of the study area, Playa del Carmen, Quintana Roo, Mexico.
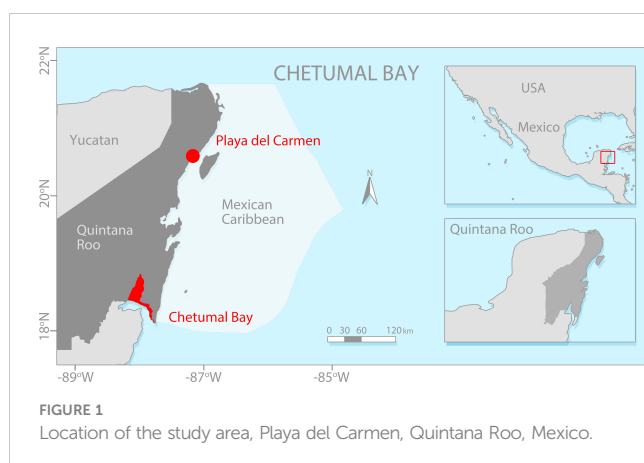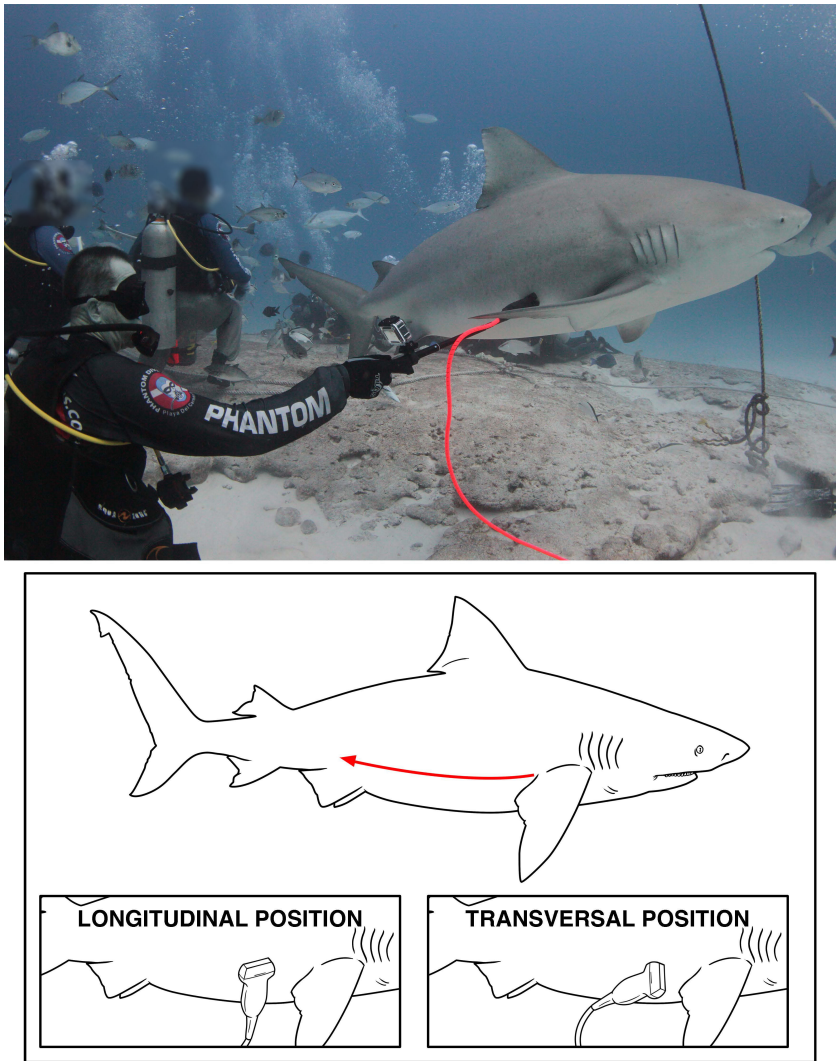
FIGURE 2
Representative distance from and lateral scanning of a pregnant bull shark. The Aquanaut underwater ultrasound was connected to 60 mm curved linear array 2.5 to 5 MHz transducer. Scanning produced either a cross section or lengthwise orientation of embryos within the uterus.
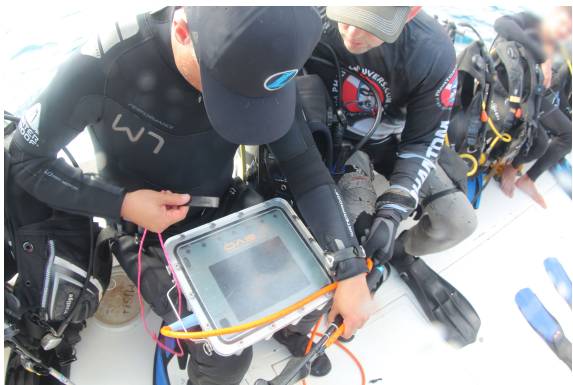
FIGURE 3
Topside Ibex EVO III Aquanaut portable ultrasound (El Medical Imaging) encased in waterproof housing.

FIGURE 4
Representative transverse ultrasound images of bull shark *Carcharhinus leucas* obtained from gravid females with identified embryos *in utero*. Arrows point to the embryo.

reproductive biology (Natanson et al., 2019) as it can assist in stock assessments thereby informing management decisions related to protected area designation (Awruch, 2013).

The identification of pregnant bull sharks in Playa del Carmen, suggests that this area may serve as a gestation ground and as such provides critical habitat for this life history stage. A similar phenomenon has also been observed in tiger sharks within Tiger Beach, a provisioned dive site within Bahamas (Sulikowski et al., 2016). Anecdotal evidence from Playa del Carmen dive operations suggest female bull sharks regularly migrate into the shallow waters of the Playa del Carmen beginning in November and stay until March when water temperatures are above 26°C (78.8°F). Bull sharks are absent from this area by April when waters are well above 26°C. The absence of bull sharks by April suggests that similar to tiger sharks at Tiger Beach (Sulikowski et al., 2016), environmental or ecological drivers are responsible for the observed movement out of the dive site (Chapman et al., 2013). For pregnant females observed herein, seeking a suitable habitat for parturition provides a likely explanation (Chapman et al., 2013). For example, Blanco-Parra et al. (2022) have recently demonstrated that Chetumal Bay (Figure 1) serves as a nursery area for bull sharks in the Mexican Caribbean and reported the presence of neonates beginning in May.

Whether the gravid bull sharks analyzed in this study travel to Chetumal Bay (approximately 360 km from Playa del Carmen) requires further investigation *via* other methodologies (Sulikowski and Hammerschlag, 2023). Regardless, the results of the current study emphasize the importance of developing and linking emerging technologies with ecotourist sites for the conservation of elasmobranch species.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by ASU Protocol Number: 23-1955R Protocol Title: Using innovative and non-lethal techniques to study the biology, ecology, and habitat utilization of sharks Institutional Animal Care and Use Committee (IACUC) Office of Research Integrity and Assurance Arizona State University.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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The influence of light on elasmobranch behavior and physiology: a review

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In this review, we summarize the state of knowledge of the influence of light on the activity and physiology of elasmobranchs (sharks, skates, rays, and sawfish). These are a diverse group with great economic and ecological importance. The long-term success of a species is largely determined by its ability to respond to changes in its environment. Light plays an important role for many marine species in signaling rhythmic environmental changes which are part of daily and annual cycles. Behavioral and physiological changes by organisms in response to these signals have evolved enabling them to maximize survival and reproductive success. In an environment with increased levels of artificial light at night (ALAN), deleterious changes in activity and physiology can occur. By summarizing what is known about the influence of light on elasmobranch activity, it can be concluded that ALAN is likely to have a negative impact on elasmobranchs at the individual and population level. We also discuss the example of intentional nocturnal light pooling by the tourism industry to attract whale sharks (*Rhincodon typus*) and manta rays (*Mobula* spp.) and recommend regulation of this activity.

Introduction

Light is a fundamental signal for living organisms to organize processes ranging from the molecular scale to coordinated behaviors across entire populations (Mishra and Kumar, 2017; Lincoln, 2019; Yan et al., 2020). The role of rhythmic variation in natural light sources, primarily the sun, in regulating activity such as feeding and breeding is well established for many clades (Guh et al., 2019). It is likely, if currently under researched, that such rhythmic variation plays a similar role for elasmobranchs (sharks, skates, rays, and sawfish). This clade contains a diverse range of species fulfilling important ecological roles as top predators, prey, and scavengers (Myers et al., 2007; Ferretti et al., 2010; Dulvy et al., 2017). Many elasmobranchs also have a direct value for humans as a food source (Dulvy et al., 2017; Glaus et al., 2019) and, increasingly, through ecotourism (Gallagher and

Hammerschlag, 2011; Cisneros-Montemayor et al., 2013; Huveneers et al., 2017; Mieras et al., 2017). Due in part to their long generation time and low fecundity, many elasmobranch species are currently at risk of population decline or extinction (García et al., 2008; Lucifora et al., 2011; Dulvy et al., 2014).

Organisms have evolved with light being a reliable predictor of environmental cues (Hut and Beersma, 2011; Ashton et al., 2022). Disruption of such cues impacts daily and seasonal biology (Falcón et al., 2020; Fishbein et al 2021).The introduction of artificial light at night (ALAN) in elasmobranch habitats therefore has the potential to compound other threats to elasmobranch populations as daily and seasonal rhythms are disrupted. On land, the influence of ALAN has been characterized for many clades (Gaston et al., 2017; Falcón et al., 2020; Sanders et al., 2020; Burt et al., 2023). In the ocean, however, its influence is less well known. In 2017, 22% of coastal areas were exposed to ALAN. This has induced changes in the physiology of marine organisms (Davies et al., 2014; Marangoni et al., 2022). The area exposed to ALAN is known to be increasing by more than 2% each year (Kyba et al., 2017). Understanding the likely influence of both intentionally and unintentionally introduced ALAN on elasmobranchs requires an understanding of the role of light in establishing rhythms in physiological and behavioral activity.

Much ALAN is the unintentional outcome of human activities (Levin et al., 2020), however there is increasing occurrence of 'light pooling.' Here, multiple bright lights, exceeding 4000 Watts, are shone on the ocean surface leading to an increase in biological activity and the attraction of both micro and macrofauna, including sharks and rays, with larger species feeding on smaller species (Jauharee, 2014; Zareer, 2022). Light pooling is conducted to attract whale sharks (*Rhincodon typus*) and manta rays including *(Mobula birostris)* by the tourism industry in Hawaii, Palau, and the Maldives (Shaahunaz, 2017; Passoni and Saponari, 2019; Passoni, 2021). Whale shark tourism is an important source of income in many countries, including the Maldives, where it was valued at 9.4 million USD to the Maldivian economy in 2014 (Cagua et al., 2014). The global value of manta ray tourism was estimated at 140 million USD in 2013 (O'Malley et al., 2023). Despite the conservation benefits of macrofauna focused ecotourism, such activities can also be damaging when unregulated (Harvey-Carroll et al., 2021; Gayford et al., 2023). The impacts of newly introduced activities such as light pooling, should thus be investigated.

Biological rhythms are highly conserved across taxa (Kumar and Sharma, 2018). Rhythmic activity can broadly be split into diurnal or diel (daily) and seasonal (annual) activity (Lincoln, 2019). Although many studies refer to diel, diurnal, and circadian rhythms interchangeable, there is an important distinction to be made. Where both diel and diurnal rhythms refer to activity which follows a 24-hour cycle, circadian rhythms refer to activity which is endogenous and can be demonstrated to follow an internal control (Vitaterna et al., 2001; Vetter, 2018). Thanks in part to the influence of external cues, circadian rhythms generally also follow a 24-hour cycle. They are, however 'free running' meaning they persist when cues are removed, and lose synchrony with the external environment (Golombek and Rosenstein, 2010; Cox and Takahashi, 2019). Circadian rhythms are the endogenous

'pacemaker' controlling diel activity while circannual rhythms control seasonal behavior. For the purposes of this review, we use the term 'diel rhythm' to refer to any activity with a 24-hour cycle to avoid confusion with the term diurnal behavior, which refers to activity which peaks during daylight hours.

To make sensible decisions about priority areas of ALAN mitigation and minimize negative anthropogenic impacts on elasmobranch species, it is first necessary to understand how light controls behavior and physiology in the subclass. In this review, we synthesis the current state of knowledge about how light influences elasmobranch behavioral and physiological activity. Through a systematic review, we investigate the known occurrences of diel and circadian rhythms in elasmobranchs, and how light affects them. We discuss the case of light pooling by the tourism industry as a case study for an emerging source of ALAN and recommend that this practice be regulated.

Methods

A systematic review of primary literature on the influence of light on elasmobranch activity was carried out using the Web of Science Database (Clarivate, 2022) and Google Scholar (Google, 2022). The terms Elasmobranch AND ("photoperiod" OR "entrainment" OR "zeitgeber" OR "circadian organization" OR "clock gene*" OR "extra-ocular photoreceptor*" OR "deep brain photoreceptor*" OR "artificial light at night" OR "ALAN" OR "pineal gland*" OR "light pollution" OR "circadian oscillator" OR "light exposure" OR "light pulse" OR "circadian rhythm*" OR "circadian organization" OR circadian) were searched and all resulting peer reviewed literature evaluated for relevance. Within reviews, cited literature was searched and evaluated for relevance.

Results

The initial review of primary literature identified 54 unique studies from Google scholar and the Web of Science Database which reported an impact of either light intensity or photoperiod on elasmobranch activity or physiology. A further five relevant studies were identified by searching cited literature in reviews (Table 1).

The influence of light on elasmobranch behavior

In four studies (Nelson and Johnson, 1970; Finstad and Nelson, 1975; Gleiss et al., 2017; Kelly et al., 2020), a definitive influence of light on elasmobranch behavior in isolation of other factors was reported. Under controlled laboratory conditions an individual nocturnal horn shark (*Heterodontus francisci*) (n = 1) was shown by Nelson and Johnson (1970) to exhibit diel rhythms in locomotor activity influenced by light exposure. This activity became arrhythmic in the absence of light or under constant light and was re-established under a 12-12 light-dark (LD) cycle. Under

TABLE 1 Summary of findings of the systematic review into the impact of light on elasmobranch behavioural and physiological activity.

Finding	Number of studies	References	Number of species	Study species
Isolated impact of light on shark behaviour	4	(Nelson and Johnson, 1970; Finstad and Nelson, 1975; Gleiss et al., 2017; Kelly et al., 2020)	5	*Heterodontus portusjacksoni, Cephaloscyllium isabellum, Pristis pristis, Heterodontus francisci, Cephaloscyllium ventriosum,*
Isolated impact of light on shark physiology	4	(Demski, 1990; Mandado et al., 2001; Mull et al., 2008; Mull et al., 2010)	3	*Scyliorhinus canicular, Raja montagui, Urobatis halleri*
Diel Rhythms	35	(Sciarrotta and Nelson, 1977; Casterlin and Reynolds, 1979; Nixon and Gruber, 1988; Nelson et al., 1997; Graham et al., 2005; Vaudo and Lowe, 2006; Wilson et al., 2006; Whitney et al., 2007; Andrews et al., 2009; Fitzpatrick et al., 2011; Cartamil et al., 2012; Kneebone et al., 2012; Brunnschweiler and Barnett, 2013; Gleiss et al., 2013; Nosal et al., 2014; Tyminski et al., 2015; Barnett et al., 2016; Gallant et al., 2016; Bouyoucos et al., 2017; Robinson et al., 2017; Brewster et al., 2018; Legare et al., 2018; Kadar et al., 2019; Coffey et al., 2020; Meese and Lowe, 2020; Bass et al., 2021; Byrnes et al., 2021; Lavender et al., 2021; Lear et al., 2021; Niella et al., 2021b; Vedor et al., 2021; Rodrigues et al., 2022; Spaet et al., 2022; Wheeler et al., 2022)	27	*Alopias vulpinus, Carcharhinus leucas, Carcharhinus limbatus, Carcharhinus plumbeus, Carcharias taurus, Carcharodon carcharias, Dasyatidae* rays (pooled to the family level), *Dipturus intermedius, Galeocerdo cuvier, Hemiscyllium ocellatum, Heterodontus francisci, Heterodontus portusjacksoni, Hexanchus griseus, Isurus oxyrinchus, Megachasma pelagios, Mustelus Canis, Negaprion acutidens, Negaprion brevirostris, Prionace glauca, Rhincodon typus, Rhynchobatus australiae, Somniosus microcephalus, Sphyrna lewini, Sphyrna mokarran, Triaenodon obesus, Triakis semifasciata, Urobatis halleri*
Seasonal behaviour	11	(Gordon, 1993; Grubbs et al., 2007; Heupel, 2007; Kneebone et al., 2012; Dudgeon et al., 2013; Kessel et al., 2014; Nosal et al., 2014; Kajiura and Tellman, 2016; Ayres et al., 2021; Bangley et al., 2021; Niella et al., 2021a)	8	*Rhinoptera bonasus, Carcharhinus limbatus, Stegostoma fasciatum, Carcharias taurus, Negaprion brevirostris, Carcharhinus plumbeus, Triakis semifasciata, Carcharhinus leucas*
Seasonal physiology	5	(Sumpter and Dodd, 1979; Crow et al., 1999; Valls et al., 2016; Sueiro et al., 2019; Wyffels et al., 2020)	4	*Notorynchus cepedianus, Carcharias taurus, Triaenodon obesus, Scyliorhinus canicular.*
Sensing light	3	(Hamasaki and Streck, 1971; Davies et al., 2012)	3	*Callorhinchus milii, Etmopterus spinax, Scyliorhinus caniculus*

constant light, locomotive behavior was diminished, whereas under constant darkness, near continuous locomotion occurred. When subjected to both one- and seven-hour phase shifts, locomotion activity patterns were immediately changed to match the corresponding light levels. The entrainment speed and lack of rhythmicity during constant photoperiods indicated no circadian endogeneity.

This study also provides proof of endogenous circadian rhythms cued by light in an elasmobranch; the nocturnal swell shark (*Cephaloscyllium ventriosum*). An individual swell shark (n = 1) shifted to constant darkness maintained a 24-hour cycle in locomotor activity, however this began to drift with peak activity shifting by 0.6-hours each day resulting in a nine-hour phase shift following 15 days of constant darkness. The reintroduction of 12-12-hour LD cycle resulted in the slow reestablishment of the 24-hour cycle, taking three days for locomotion to be synchronized with light periods. Following one week on a 12-12-hour photoperiod, the shark was held under constant light conditions for 18 days. This resulted in a shift in activity, with a seven-hour shift in peak activity by day 18, characteristic of true endogenous circadian behavior. Unlike synchronization following exposure to constant darkness, synchronization to an LD cycle following continuous light conditions was immediate. A one-hour shift in the LD cycle resulted in a corresponding shift in peak activity, which

generally anticipated the dark phase. Furthermore, the sharks were able to track a seven-hour light shift.

The re-establishment of rhythmicity in behavior matching that of the photoperiod was evident for both the horned and swell shark when they were returned to a 12-12-hour LD conditions. This is clear evidence for an influence of light on shark activity although the study was limited in sample number and consideration of long-term effects. Importantly, in this study no food was given to isolate the effect of light as a zeitgeber (a stimulus capable of entraining biological rhythms).

Finstad and Nelson (1975) found that wild horn shark movement activity (leaving cave count) peaked 60-90 minutes after sunset; corresponding to 0.03 lux environmental levels. Under laboratory conditions, with a 12-12-hour LD cycle (light = 8 lux) horn sharks (n = 2) displayed cyclic activity, (passing sensors, binary) with anticipation of dark periods. When moved to constant darkness, all rhythmicity was immediately lost, and activity became irregular.

Three sharks were then held under constant lighting conditions: 0.2 lux for days 3-18. 0.13 lux for days 19-25 and finally during days 26-30, sharks were held in complete darkness. Marked differences were identified between individuals, as has been observed in other taxa (Guyomarc'h et al., 1998). When held under constant light conditions of 0.2 lux, behavioral rhythmicity of all sharks was found

to drift, demonstrating a phase advance of activity. This would be expected of an endogenous circadian clock. Continual exposure to 0.13 lux resulted in individual differences. A loss of rhythmicity occurred for 2/3 sharks. Interestingly, one of the two sharks with initial complete loss of rhythmicity began to establish slight rhythmicity of behavior during the final days of constant 0.13 lux conditions. The final shark maintained complete rhythmicity, which appeared to drift by one hour earlier each day. When the three sharks were moved to complete darkness an immediate and complete loss of rhythmicity occurred (Finstad and Nelson, 1975). This experiment demonstrated the importance of light in controlling behavior in elasmobranchs, regardless of the presence of endogenous circadian rhythms.

In freshwater, Gleiss et al. (2017) showed that the crepuscular and night-time movement activity of sawfish (*Pristis pristis*) tagged with accelerometers (n = 13) is driven by light. Sawfish activity was shown to be elevated prior to twilight. In addition, they investigated the influence of water temperatures on diel vertical migrations (measured by using Time Depth Recording (TDR) devices). These were found to respond to alterations in water temperatures independently of circadian accelerometer activity. This is noteworthy as the only study in this review to show a decoupling between two aspects of elasmobranch behavior and the influence of photoperiod and water temperature.

Kelly et al. (2020), studied swimming (distance and time) of two shark species; the Port Jackson (*Heterodontus portusjacksoni*) (n = 8) and draughtsboard (*Cephaloscyllium isabellum*) (n = 8) shark under a 12-12-hour LD cycle, 6-6-hour LD cycle, constant light, and constant darkness. Under the 12-12-hour LD cycle, swimming activity in both species peaked during the dark phase. Under a 'force desynchrony' paradigm of a 6-6-hour LD cycle, peak in swimming activity of the Port Jackson shark closely followed dark phases, however, a 12-12-hour circadian pattern in activity was still detected during the first and third day under these conditions. Regardless of underlying circadian rhythms, sharks were found to swim more during dark phases. This indicates that activity is entrained by external light, however elasmobranchs may have a reduced capacity to follow light cycles shorter than 24-hours. Similarly, under a 6-6-hour LD cycle, draughtsboard sharks displayed higher swimming activity during the dark phase with no increase during the light phase reported. The swimming rhythmicity of Port Jackson sharks was disrupted after 48-hours in either constant light or darkness. Port Jackson sharks retained an attenuated circadian activity rhythm (activity levels dramatically decreased) for the first 24-hours of constant conditions. Draughtsboard sharks appeared to maintain rhythmic behavior under constant conditions. During these experiments, animals were fed every 72-hours, with the timing of feeding coinciding with the second day of each lighting regime. Feeding has the potential to act as a strong entrainment factor (Shibata et al., 2010; Carneiro and Araujo, 2012; Trzeciak and Steele, 2022), potentially influencing results. The short time frame of this study (72-hours for each lighting regime) also limits a full assessment of the longer-term effects of light on elasmobranch activity.

20 other studies implicated light as a cue for diel or seasonal rhythms across 22 species of elasmobranch but did not isolate light

from other environmental cues such as sea surface temperature, seafloor water temperature, wind speed, or tides (Table 1). Diel rhythms in movement have been associated with daily photoperiods include based on accelerometer (Kneebone et al., 2018; Kadar et al., 2019; Byrnes et al., 2021) and TDR (Nelson et al., 1997; Andrews et al., 2009; Gallant et al., 2016; Kneebone et al., 2018; Byrnes et al., 2021) tagging studies. An influence of photoperiods on movement has also been inferred based on broad scale trends in shark abundances, such as daily aggregations (Brunnschweiler and Barnett, 2013; Nosal et al., 2014), rate of movement (Cartamil et al., 2003), and bycatch rates (Niella et al., 2021b). Seasonal behaviors associated with solar and lunar photoperiods include aggregation (Grubbs et al., 2007; Nosal et al., 2014; Kajiura and Tellman, 2016; Ayres et al., 2021; Niella et al., 2021a), migration (Kessel et al., 2014; Bangley et al., 2021), site fidelity (Vaudo and Lowe, 2006; Grubbs et al., 2007; Kneebone et al., 2012; Dudgeon et al., 2013; Nosal et al., 2014), residency (Kneebone et al., 2012; Kessel et al., 2014), and diving (Andrews et al., 2009).

The influence of light on elasmobranch physiology

Demski (1990) proposed that gametogenesis and reproductive behavior in elasmobranchs is controlled *via* photic input to the retina and pineal gland, which is analogous to other vertebrates (Bertolucci and Foà, 2004; Golombek and Rosenstein, 2010; Cassone, 2014). They collated data on elasmobranch photic neural projections and endocrine systems and documented the overlap of projections from both the retina and pineal gland to areas of the brain involved in sex steroid production, including gonadotropin-releasing hormone (GnRH). They proposed that this effects gonad physiology and indicated strong evidence for the role of the pineal gland in the production of pituitary gonadotrophins (GTHs). GnRH is the major neuropeptide modulating reproduction in vertebrates (Gorbman and Sower, 2003; Chen and Fernald, 2008; Roch et al., 2011) including elasmobranchs (Awruch, 2013). Extensive projections of pineal neurons throughout the brain of skate (*Raja montagui*) and dogfish (*Scyliorhinus canicula*) have since been mapped by (Mandado et al., 2001). Projections were found to be wide reaching, and largely conserved between teleosts, amphibians, and elasmobranchs. Pineal projections were identified in the only area of the dogfish brain producing GnRH. The authors concluded that the midbrain sGnRH immunoreactive nucleus is a core part of pineal pathways and heavily involved in photic induced control of brain function of the pineal gland (Figure 1) (Mandado et al., 2001).

A direct influence of light on aspects of elasmobranch physiology have been reported for three species (Mull et al., 2008; Mull et al., 2010; Waltrick et al., 2014), although this influence is difficult to disentangle from other environmental conditions (e.g. water temperature). Waltrick et al. (2014) reported concentrations of the reproductive hormone, 17β-estradiol, and ovarian follicle size to be positively correlated with day length and water temperature in the Australian sharpnose shark (*Rhizoprionodon taylori*). Mull et al., 2010 found that progesterone concentrations in female
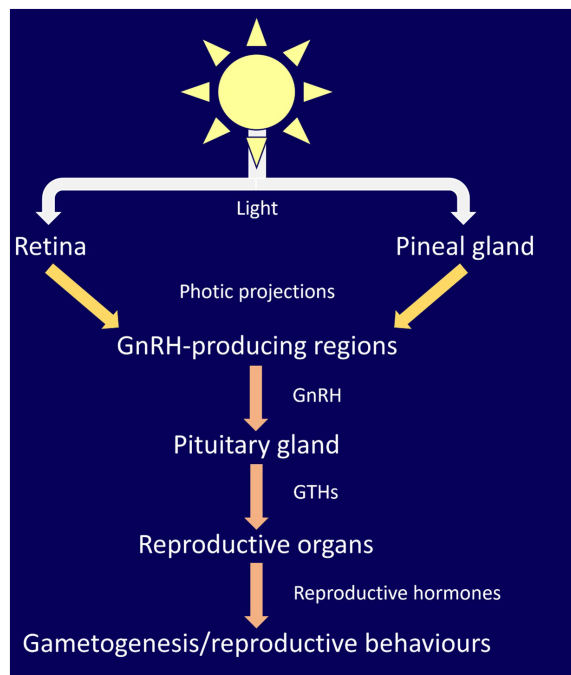
FIGURE 1

An updated version of the pathway for light dependent control of reproductive behavior proposed by (Demski, 1990). Support for this pathway has been presented in (Mandado et al., 2001; Awruch, 2013). Light is detected by photoreceptors in the retina and pineal gland. Photic projections signal gonadotropin-releasing hormone (GnRH)-producing regions of the brain, stimulating the production and release of GnRH, which in turn stimulates production and release of gonadotrophins (GTHs). Within reproductive organs (testes and ovaries), GTHs stimulates the production/release of reproductive hormones, such as progestins, androgens and estrogen, which in turn lead to gametogenesis and reproductive behaviors.

round stingrays (*Urobatis halleri*) were significantly positively correlated with day length and water temperature. In males of the same species, Mull et al., 2008 reported gonadosomatic index (GSI) and plasma 11-ketotestosterone levels to be significantly negatively correlated with photoperiod, with an additional influence of an undefined change in day length on 11-ketotestosterone. Plasma testosterone levels were negatively correlated with both photoperiod and temperature, with photoperiod demonstrating a stronger influence. These three studies present findings that are consistent with photoperiodic regulation of seasonal behavior in other taxa, such as birds and mammals (Dawson et al., 2001; Hazlerigg and Wagner, 2006).

More broadly, seasonal rhythms in shark physiology have been observed in five species (Table 1). Concentrations of the reproductive hormone, T4, were found to follow seasonal rhythms in whitetip reef sharks (*Triaenodon obesus*) by (Crow et al., 1999). Blood cholesterol levels were found to follow seasonal rhythms in small-spotted catshark (*Scyliorhinus canicula*) by (Valls et al., 2016). Seasonal changes in sevengill shark (*Notorynchus cepedianus*) immune function indicators (lymphocyte and heterophil counts along with granulocyte to lymphocyte ratio) have also been documented (Sueiro et al., 2019). The highest testosterone and sperm motility has been reported in captive sand

tiger sharks (*Carcharias taurus*) when environmental conditions mimic natural seasonal photoperiods and temperatures (Wyffels et al., 2020). Finally, Sumpter and Dodd (1979) report that pituitary gonadotropin (involved in photic control of reproduction in non-mammalian vertebrates (Pérez, 2022)) concentrations in mature female lesser spotted dogfish (*Scyliorhinus canicula*) are up to 100 times higher between February and April than other months, coinciding with peak egg-laying and highest levels of GSI.

The prevalence of diel rhythms in elasmobranchs

The rhythmicity of animal behavior is largely influenced by light, alongside temperature and food availability (Häfker and Tessmar-Raible, 2020). In wild non-model organisms, it is often a challenge to disentangle the influence of different environmental cues. This can be compounded by the fact that studies of captive sharks often neglect to record water temperatures (e.g., Casterlin and Reynolds, 1979). Some inference must therefore take place when considering the influence of light on elasmobranch activity. It is likely that the prevalence of diel rhythms in sharks should be considered an initial indication of a light cued activity, although other factors such as water temperature or prey activity are likely involved. The diurnal and nocturnal activity of sharks has been reviewed by (Hammerschlag et al., 2017).

In literature recovered during this review, 33 elasmobranch species were reported to display some form of diel rhythm (Table S1). There were no reported instances in which diel rhythms were absent. 15 species of elasmobranch were reported to be nocturnal; three species were reported to be diurnal, and crepuscular activity was reported in seven species. Rhythmicity in either depth or presence at a location was reported for seven species (Table S1).

Diel behavior can be directly inferred from the observation of behavior or physiological markers or indirectly through, for example, bycatch reports. Diel rhythms in diving activity have been reported for 12 species and in swimming speed for two species of elasmobranch; the blue (*Prionace glauca*) and common thresher (*Alopias vulpinus*) sharks (Sciarrotta and Nelson, 1977; Cartamil et al., 2012). Elasmobranch physiology was also reported to follow diel rhythms by four studies with diel rhythms in metabolic rates reported for three species (Table S1). One bycatch study demonstrated that the blue shark (*Prionace glauca*) and shortfin mako (*Isurus oxyrinchus*) are largely caught during the night and between 00:00 and 04:00 respectively (Rodrigues et al., 2022).

Sensing light

Vertebrates use external light cues to modulate diel and seasonal rhythms (Figure 2, Tosini et al., 2001; Cowan et al., 2017; Mishra and Kumar, 2017; Liddle et al., 2022). The mechanisms of detection of external light varies across taxa, but the result (rhythmic hormone production) is highly conserved. In mammals, light stimulation is restrained to the retina, whereas in birds, teleosts,
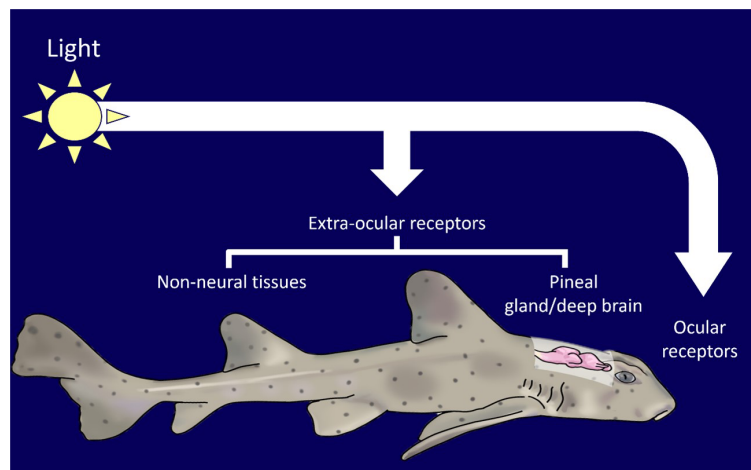
FIGURE 2
Elasmobranchs can detect light through a variety of ocular and extra-ocular receptors in tissues such as the pineal and skin.

amphibians, and reptiles, light input is transduced by both ocular and non-ocular photoreceptors (Aschoff et al., 1982; Katherine Tamai et al., 2003; Nishiwaki-Ohkawa and Yoshimura, 2016). The mechanism for modulation of light cued rhythms in elasmobranchs is not fully established. Only the elephant shark (*Callorhinchus milii*) and the lantern shark (*Etmopterus spinax*) have been screened for, and found to possess, extra-ocular photoreceptors (Davies et al., 2012; Delroisse et al., 2018). The presence and responsiveness of non-ocular photoreceptors suggests that non-ocular control of rhythmic activity can occur.

In non-mammalian vertebrates proteins, called opsins, have been linked to photic control of the endocrine system, such as breeding, circadian behavior and locomotion (Pérez et al., 2019; Dekens et al., 2022). Es-encephalopsin, a non-visual ciliary opsin has been identified in the in the ventral skin of the velvet belly lantern shark (*Etmopterus spinax*, (Delroisse et al., 2018). Melanopsins are a class of extensively studied non-visual opsin. They exist in two main classes: *opn4m* (mammalian-like) and the *opn4x* isoform (xenopus like). In mammalian vertebrates, *opn4ms* are found exclusively within the eye and are implicated with circadian rhythm regulation and melatonin production. In non-mammalian vertebrates, *opn4x* and *opn4ms* are present in the retina, pineal gland, skin, and deep brain regions (Davies et al., 2012). Davies et al. (2012) reported three melanopsin genes in the elephant shark (*Callorhinchus milii*). Two of these belonged to opn4m class (*opn4m1* and *opn4m2*) and the third was the *opn4x* class. All melanopsins were found to be expressed in elephant shark eyes. *Opn4m2* was found to be expressed in the fin, gills, hypothalamus, liver, skin, and testes. *Opn4x* was found throughout the brain, fin, gills, hypothalamus, kidney, liver, snout, skin, and testes. It has been proposed that melanopsins are involved in photoentrainment of circadian behavior, displaying different spectral sensitivity for deep-sea bioluminescence and bright-light environments (Davies et al., 2012). The wide expression of opsins in elasmobranchs is analogous to that seen in teleosts, which are capable of photoentrainment (Frøland Steindal and Whitmore, 2019; Steindal and Whitmore, 2020).

(Hamasaki and Streck, 1971) demonstrated light sensitivity of the pineal gland in dogfish (*Squalus acanthias*). Following exposure to as little as 4.3×10^{-4} lumens for 1 second, distinctive neuronal activity was detected through electrophysiology. This gland has extensive neuronal connections throughout the brain and humoral outputs, indicating the importance of photic influenced brain function. The pineal projections seen in elasmobranchs are largely similar to those found in teleosts, who are thought to display photic controlled breeding (Mandado et al., 2001).

Discussion

Elasmobranchs are diverse and many aspects of their behavior, physiology, and role in ecosystems are understudied. This presents challenges for drawing firm general conclusions about their activity and conservation needs. It is clear, however, that light is a strong driver in establishing and regulating diel rhythms across elasmobranch taxa, which in turn likely controls seasonal behavior. Given the importance of light for modulating activity, it is of critical importance that the impact of anthropogenic alterations to natural light cycles be assessed. Among the literature reviewed, there is a clear absence of this research. Consequently, recommendations for minimizing the impact of light pollution on elasmobranchs must be based on inference from known aspects of elasmobranch ecology and other taxa.

Exposure to light outside of natural cycles is highly likely to disrupt rhythmic physiological and behavioral activity of individual elasmobranchs. The metanalysis conducted by (Sanders et al., 2020) demonstrated wide ranging effects of ALAN on organisms' life history traits, physiology, population structure, and activity patterns resulting from as little as 1 lux in both terrestrial and marine ecosystems. Extensive studies have demonstrated that ALAN can have catastrophic effects on physiology and behavior as it disrupts the immune and endocrine system leading to impairments in reproduction and health (Sanders et al., 2020; Bumgarner and

Nelson, 2021; Liu et al., 2022). For example, it has been demonstrated that, when subjected to ALAN, 0% of clownfish (*Amphiprion ocellaris*) eggs hatch (Fobert et al., 2019). A 36% decrease in survival, 51% weight reduction, and significant changes to metabolism have been identified in damselfish (*Chromis viridis*) and juvenile orange-fin anemonefish (*Amphiprion chrysopterus*) exposed to ALAN (Hillyer et al., 2021; Schligler et al., 2021). Similarly, multiple reviews have demonstrated unpredictable light regimes can disrupt circadian rhythms, negatively impacting health by disrupting multiple physiological systems, leading to disease and lowering offspring survival rates (Vetter, 2018; Chellappa et al., 2019; Maury, 2019; Rijo-Ferreira and Takahashi, 2019; Hou et al., 2020; Fishbein et al., 2021; Fatima et al., 2022; Lane et al., 2022). Cumulatively, disruption of life history in many individuals leads to population level effects such as reduced population growth rate and resilience to exploitation, hampering conservation efforts (Longcore and Rich, 2004; Davies et al., 2014; Gaston et al., 2017).

The case of light pooling being used to attract whale sharks and manta rays by the tourism industry should be considered as an emerging source of ALAN. Light pooling is reported in Hawaii, Palau and the Maldives (Shaahunaz, 2017; Passoni and Saponari, 2019; Passoni, 2021). It may also occur in other locations. During 2023, light pooling excursions were run by multiple operators for several hours at a time in the South Ari Atoll Marine Protected Area (SAMPA) in the Maldives throughout the night (7pm-8am). The frequency and duration of light pooling events has yet to be quantified.

Whale sharks and manta rays exhibit strong diel rhythms (Graham et al., 2005; Wilson et al., 2006; Brunnschweiler and Sims, 2011; Robinson et al., 2017; Lassauce et al., 2020; Andrzejaczek et al., 2021). Manta rays are thought to dive deeper at night than during the day (Lassauce et al., 2020; Andrzejaczek et al., 2021). In shallow waters whale sharks have been found to dive deeper during the day and shallower at night. There is evidence that this pattern may be reversed in deeper waters however this is not always the case

(Tyminski et al., 2015). Furthermore accelerometer readings have demonstrated strong crepuscular activity in whale sharks (Gleiss et al., 2013). A single pulse of light is known to be sufficient to disrupt circadian rhythm in a plethora of organisms (Leloup and Goldbeter, 2001) while white light exposure during the night can lead to decreases in melatonin and gonadotrophin levels in European perch (*Perca fluviatilis*) (Brüning et al., 2016). There have been suggestions that whale shark diving behavior is determined by prey availability rather than abiotic environmental cues (Gleiss et al., 2013). Prey availability is likely to be strongly influenced by ALAN. Demersal zooplankton, which form a large component of whale shark and manta ray diets, are known to exhibit skototaxis (movement towards darkness) (Rohner et al., 2013; Couturier et al., 2013). Light pooling is therefore likely to alter the diet of target species. It is likely then that light pooling leads to physiological stress in whale sharks and manta rays as a result of the mismatch between internal physiology and the environment caused by alterations to two major zeitgebers; light and food (Figure 3).

Outside of direct impacts to individuals and cumulative impacts to populations, light pooling may alter the ability of elasmobranchs to regulate populations on lower trophic levels. There are also likely to be impacts on numerous nontarget species, such as Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) and other elasmobranchs, such as nurse sharks (*Ginglymostoma cirratum*) which have all been documented during light pooling excursions.

Traditionally, emerging human activities harmful to wildlife have been permitted to occur until enough proof can be gathered of negative impacts to influence policy (Wilson et al., 2011). The precautionary principal in conservation counters this harmful dynamic by promoting the regulation of new practices before they have an opportunity to become harmful, based on what data is available (Meyers, 1993; Fisher et al., 2006; Wilson et al., 2011; Cooney and Dickson, 2012). As an endangered species and source of over 9.4 million USD to the Maldivian economy in 2014 (Cagua et al., 2014; Pierce and Norman, 2016), conservation of whale sharks
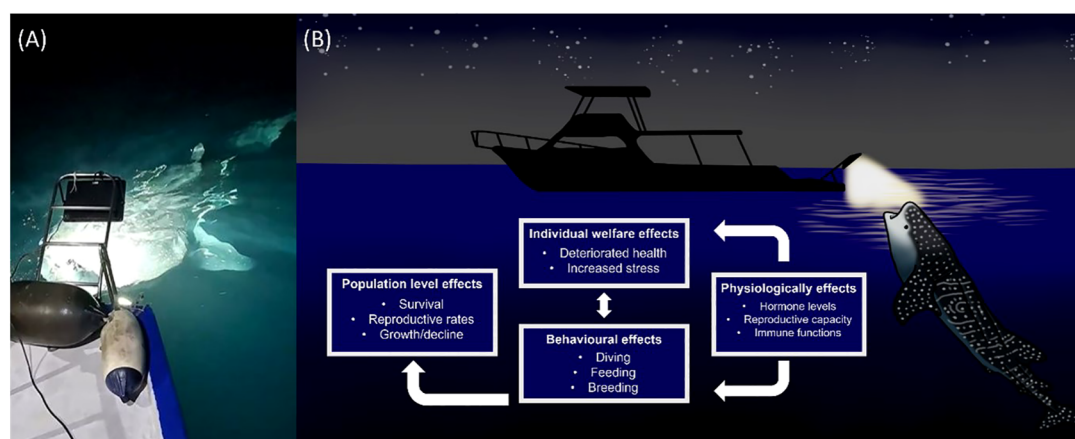
FIGURE 3
(A) In the context of wildlife tourism, light pooling involves the intentional shining of bright lights (> 4000 watts) into the ocean at night (Photo source: Marloes Otten, photographer/videographer). This practice is currently unregulated and the impact on elasmobranch behavior and physiology are unknown. **(B)** Based on a review of available literature, we predict artificial light at night (ALAN) to have multiple negative impacts on elasmobranch individuals and populations.

should be a priority when designing and regulating tourism activities. It is not currently possible to predict how light pooling activities can be conducted in a manner that will allow individuals sufficient time between encounters to recover. We therefore recommend a precautionary approach to safeguard the species. In this way the issue of harmful new practices outpacing regulation can be avoided (Cooney and Dickson, 2012).

We recommend that the frequency and duration of light pooling events by the tourism industry be quantified and that targeted research should be conducted into the impact of light pooling at different intensities and pulse durations on elasmobranch health and physiology. This should be done opportunistically, making use of currently unregulated light pooling activities. The endorsement of light pooling by conservation and research bodies should also be avoided until further evidence can be gathered. The output of such research could be used to conduct a risk assessment for various management strategies allowing recommendations for best practice to be made and minimizing the negative impacts of light pooling on elasmobranch health.

Until such research has been conducted, we recommend that incidents of light pooling be reduced and tightly regulated to avoid negative impacts for individuals and/or the population. We recommend that i) both the frequency and duration of light pooling encounters be limited to the hours immediately proceeding sunset and preceding sunrise, leaving animals with some level of natural darkness each night, ii) light pooling be prohibited in marine protected areas (MPAs), iii) tour operators offering light pooling be required to hold a licence which demonstrates they have been informed of the potential negative impact of the practice on wildlife, and iv) white lights be switched off and the encounter proceed under red lights when sharks or manta rays are present. Light of longer wavelengths (red, above 639 nm) has been shown to have lesser impacts on the circadian system when compared to higher wavelengths (blue light, under 465 nm) (Brainard et al., 2008; Rahman et al., 2008; Park et al., 2013; Di Rosa et al., 2015; Sánchez-Vázquez et al., 2019). It is important to note that red light likely still induces some circadian disruption (Dauchy et al., 2015; Bonmati-Carrion et al., 2017).

Conclusions

Light is a clear driver of behavior and physiology across the elasmobranch subclass. There are a great deal of questions remaining regarding the exact mechanisms of this control, how this varies between taxa, and the complex interactions between light and other environmental factors. In combination with well-established research into how the disruption of natural light rhythms effects all other taxa, we predict that artificial light at night (ALAN) is likely to have multiple disruptive and negative impacts on elasmobranch behavior and physiology. Taxa specific research should be conducted to confirm this in cases where economically important species, such as the whale shark and

manta rays, are experiencing increasing levels of ALAN. The emergence of light pooling has the potential to confound current elasmobranch conservation efforts. We recommend that a precautionary approach be taken and light pooling by the tourism industry be regulated.

Author contributions

Both authors contributed equally. JH-C conceived the study which was further developed in collaboration with DC. JH-C prepared Table 1 and illustrations. DC prepared Figures 1–3. JH-C and DC wrote the manuscript and contributed to revisions. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1225067/full#supplementary-material

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○ Check for updates

Diversity and distribution of elasmobranchs in the coastal waters of Cyprus: using bycatch data to inform management and conservation

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Introduction: Loss of biodiversity in marine ecosystems is a globally acknowledged problem. To address this and avoid extinctions, improved conservation is required to protect and restore our oceans. Elasmobranch species are considered the second most threatened vertebrate lineage, with overfishing considered the most important driver of declines. The Mediterranean Sea is a hotspot for elasmobranchs, with the eastern basin considered a data-poor area.

Methods: This study sought to address that deficiency, using bycatch data to assess the diversity and distribution of elasmobranch species caught as a result of commercial fishing in Northern Cyprus from 2018 to 2022.

Results: Thirty-six elasmobranch species were recorded, including 24 new records for Northern Cyprus, representing 41% of all species recorded in the Mediterranean. Of these 36 species, 61% are listed on the IUCN Red List as globally threatened (Critically Endangered, Endangered or Vulnerable). Of the species recorded, 71% of sharks (n=17), and 53% of batoids (skates and rays; n=19) are listed as threatened. We present data on the distribution of captures and size classes and infer life stage from these data.

Discussion: Our study demonstrates the importance of the coastal waters of Cyprus for multiple life stages of a broad range of elasmobranch species. As most elasmobranchs caught are retained for bait, subsistence or trade, our study has highlighted the need to update the regulations for this small-scale fishery, and led to a recent amendment to prohibit trade in all species listed under the Barcelona Convention.

KEYWORDS

shark, batoid, skate, ray, mediterranean, fisheries

1 Introduction

Anthropogenic impacts continue to drive loss of function and degradation in marine ecosystems. Biodiversity within these systems has declined over time, and we are now facing the extinction of some of the world's most important and charismatic species. Elasmobranchs (sharks, rays, skates and chimeras) are showing population declines globally, with more than 30% of species globally threatened with extinction (IUCN, 2022), and oceanic elasmobranch populations are thought to have decreased by at least two-thirds between 1971 and 2020 (Pacoureau et al., 2021). Elasmobranchs overall have a higher extinction risk than most other vertebrates, in part owing to their low fecundity, late maturation and long generation times, with large shallow water-dwelling species most at risk (Dulvy et al., 2014).

Although these declines are due to a cumulative range of anthropogenic impacts – habitat degradation, pollution and climate change, for example – overfishing, resulting in both targeted and incidental catch, is considered the most important driver (Dulvy et al., 2014; Dulvy et al., 2021). Across the world, elasmobranchs are fished by artisanal, recreational and commercial fisheries for their fins, livers and meat (Oliver et al., 2015; Tiralongo et al., 2018a) and it has been estimated from reported landings, unreported landings and discards that the global catch of sharks in 2010 was 1.41 million tonnes (Worm et al., 2013). As well as targeted fishing, a major problem facing elasmobranch populations is bycatch, the incidental capture of non-target species (Oliver et al., 2015). It has been estimated that bycatch makes up 40% of annual global marine catch (Davies et al., 2009). Bycaught elasmobranchs are increasingly being retained by fishers, due to their market value and decreases in target species catch, but a lack of management and legislation persists (Oliver et al., 2015; Pacoureau et al., 2021). Even if species are released alive, there can be sub-lethal impacts on individuals that could, in turn, lead to population impacts, as well as post-release mortalities (Wilson et al., 2014; Pacoureau et al., 2021).

Within the Mediterranean Sea, 88 species of elasmobranchs have been recorded (Serena et al., 2020), and many are facing declines in population size and range (Dulvy et al., 2016). Here, due to increased relative fishing pressure, 50% of skates and rays (hereafter referred to as batoids) and 56% of sharks, have regional International Union for Conservation of Nature (IUCN) Red List conservation status assessments that are elevated compared to their global populations (Dulvy et al., 2016). Historically, elasmobranch species diversity and richness were considered greatest in the western Mediterranean Sea, but in recent years there have been a greater number of local extinctions in the west and an increase in threats throughout the Mediterranean (Dulvy et al., 2016; Serena et al., 2020). There have been fewer studies of elasmobranch diversity in the eastern Mediterranean, although Türkiye alone has over three quarters of all Mediterranean elasmobranch species (Bengil and Basusta, 2018), with known nursery areas for *Carcharhinus plumbeus* (Nardo 1827, sandbar sharks), *Rhinobatos rhinobatos* (Linnaeus 1758, common guitarfish) and *Glaucostegus cemiculus* (Geoffroy St. Hilaire 1817, blackchin guitarfish) in the coastal waters of Türkiye (Bengil et al., 2020; Basusta et al., 2021).

Elasmobranchs make up just 1% of the annual fisheries catch in the Mediterranean (Bradai et al., 2018), however, fishing mortality due to bycatch is a major threat for this group (Bengil and Bengil, 2018). In most Mediterranean countries, elasmobranch bycatch is not reported by species, leading to underestimations of catches and poorly informed conservation efforts (Cashion et al., 2019; Giovos et al., 2021a). Many elasmobranch studies use data from large industrial fishing vessels such as trawlers and longliners (Molina and Cooke, 2012). Given that at least eighty percent of Mediterranean fishing vessels are small-scale vessels (<12m total length; FAO, 2020), and small-scale fisheries (SSF) have recently been identified as the second greatest contributor to bycatch of threatened elasmobranchs in the Mediterranean (after longline vessels; Tiralongo et al., 2018a; Carpentieri et al., 2021), understanding bycatch within the Mediterranean SSF fleet is urgently needed.

A checklist of 60 chondrichthyans was recently reported by a study which reviewed available data, studies and citizen science reports for the island of Cyprus (Giovos et al., 2021b). A published record of *Hexanchus nakamurai* (Teng 1962, bigeyed sixgill shark, now *H. vitulus*, Springer and Waller 1969, Atlantic sixgill shark) has since been retracted (Bengil et al., 2021), while a *Cetorhinus maximus* (Gunnerus 1785, basking shark) previously reported for Northern Cyprus (Kabasakal, 2013), and for the whole island (Giovos et al., 2021b) was based on a newspaper report which was later found to have been inaccurate, instead being *Alopias superciliosus* (Lowe 1841, bigeye thresher shark, Hakan Kabasakal pers. comm.). This brings the total species count for the entire island to 58 (30 sharks and 28 batoids). Most of the contributing records, however, are from studies focused in the area of the island under the effective control of the Republic of Cyprus (RoC), and until our current study, just fourteen species had been documented in Northern Cyprus (Turkish Republic of Northern Cyprus (TRNC); Oray and Karakulak, 2005; Çoker and Akyol, 2014; Akbora et al., 2019) a self-declared state recognised only by Türkiye and considered by the international community to be part of the Republic of Cyprus (hereafter referred to as Northern Cyprus).

The commercial fishing fleet of Northern Cyprus includes 300-400 SSF vessels [Northern Cyprus Department of Animal Husbandry (DAH)], predominantly fishing with gill nets, trammel nets and longlines. Interactions with marine vertebrate species, including elasmobranchs, marine turtles, Mediterranean monk seals (*Monachus monachus*, Hermann 1779) and dolphins are common, with an estimated 1,000 marine turtles caught in this fishery each year (Snape et al., 2013; Snape et al., 2018b; Beton et al., 2021). Trawl fishing is not permitted and there are no industrialised longline vessels. Although, marine protected areas (MPAs) have been established to protect sea turtles, monk seals, seabirds and some key habitats across more than a quarter of the coast of Northern Cyprus, (Snape et al., 2018a), few fisheries restrictions are implemented within or outside of these MPAs. Until May 2023, there were no formal protection measures for any elasmobranch species from commercial trade, other than for *C. plumbeus* and *C. maximus* of which catching or landing were prohibited (Fisheries

Law 27-2000, 2022). This was partly as a result of the lack of data available for policy makers, however, as a result of data from this current study, in May 2023 the fisheries regulations were updated to prohibit trade of all species listed on Annex I of the Barcelona Convention. The authorities have also maintained a blanket ban on industrialised fishing, with no trawling permitted since the 1990s (Ulman et al., 2015), thus, habitats may be in favourable condition compared with other parts of the Mediterranean, where bottom trawling persists.

This study aimed to describe the diversity, distribution and life stages of elasmobranch species caught in the fisheries of Northern Cyprus, through deploying onboard observers and engaging small-scale fishers in data collection, to inform fisheries management and conservation action in line with global, regional and national biodiversity conservation strategies.

2 Materials and methods

2.1 Study area

Cyprus is the third largest island in the Mediterranean Sea, divided into two geopolitical subregions by the United Nations-monitored buffer zone, the Green Line (Sabri and Sakallı 2021). This study focuses on the coastal waters of Northern Cyprus (Figure 1), where small-scale coastal fisheries operate using wooden boats of <12m length with inboard diesel engines (Ulman et al., 2015), using bottom-set gillnets and trammel nets, benthic longlines, with a small number of pelagic longlines (Snape et al., 2013). Fourteen fishing harbours are maintained and managed by the DAH and all commercial fishing vessels are registered to these ports (Figure 1). The informal shelter at

Apostolos Andreas (Ap Andreas; Figure 1) was also included in our study as it is frequently used by fishers during the summer months. Active fishing vessel data were available from the DAH for coastal zones 1, 2 and 3 (Zone 3 was subdivided into three areas (a, b and c) for finer spatial resolution resulting in a total of five areas; Figure 1) and were used to plan observer trips and recruit self-reporting fishers to ensure our data were representative of the fleet.

2.2 Data collection

Data on elasmobranch bycatch were collected between 04/01/2018 to 19/06/2022 through the Cyprus Bycatch Project (an island-wide project to understand and mitigate bycatch of vulnerable species) in the following three ways.

2.2.1 Onboard observers

Trained onboard observers broadly followed the General Fisheries Commission for the Mediterranean (GFCM) protocol (FAO, 2020). For every set observed, the time of deployment and GPS coordinates at deployment and hauling were taken. When elasmobranch bycatch occurred, individual specimens were removed from the nets or hooks by either the fishers or onboard observers. Where possible, individuals were measured using a flexible tape measure; for batoids, disc width and total length were taken and for sharks total length (FAO, 2020). Tails of Dasyatidae were often removed by fishers prior to handling; instances of tail removal were noted where possible and these individuals were removed from total length distributions. Photographs were taken for records and to confirm identification. Identification was made to the lowest possible taxonomic group using Otero et al. (2019), and other literature and expert knowledge
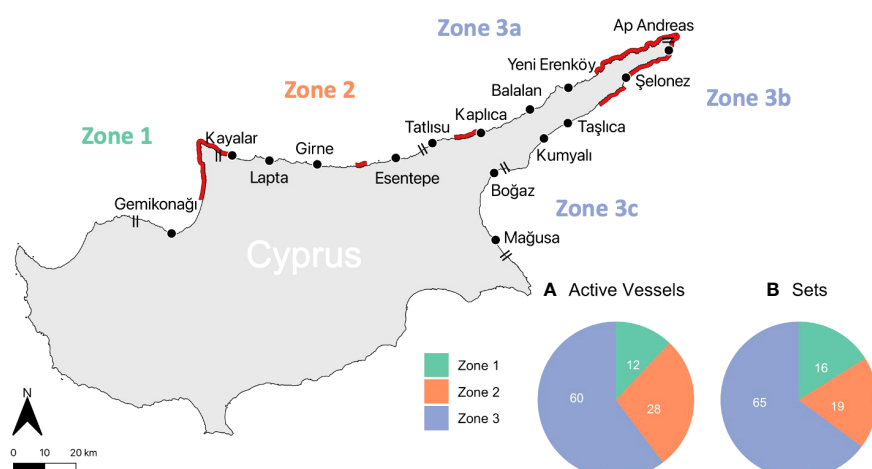
FIGURE 1
Study area, with 15 harbours, in three coastal zones, regularly used by fishers in Northern Cyprus. Double black lines show the division of harbours into coastal zones used by the authorities 1, 2 and 3; single black lines show further division of Zone 3 into three separate areas (a, b and c) in this study. Inset: Pie chart A shows proportion of the total active fishing vessels operating from each coastal zone in 2018/2019 (n = 340), with data provided by Department for Animal Husbandry for 2019 (the most recent available data); pie chart B, the proportion of all sets (n=1,899) from which departure port was recorded during 2018-2022, divided into coastal zone. Location of designated Marine Protected Areas (MPAs) is also shown. Greek place names (west to east) are Karavostasi, Orga, Lapithos, Kyrenia, Agios Amvrosios, Akanthou, Davlos, Platanissos, Gialousa, Apostolos Andreas, Chelones, Neta, Koma tou Gialou, Trikomo, Famagusta.

as required. Observers were not always able to assess maturity from external characteristics, therefore, maturity was estimated using L_{50} values – the total length at which 50% of the population are sexually mature – found in the peer-reviewed literature and using data from the Mediterranean where available or the lowest L_{50} estimations available globally (Supplemental Tables 1, 2; Supplemental References). The conservation status and common names for species were taken from the IUCN RedList (IUCN, 2022) for both global and regional status. For species endemic to the Mediterranean, that only have a global Redlist status, this was also included for the Mediterranean status. We recorded whether animals were alive or dead at hauling and from this calculated at-vessel mortality score for each species (AVM; percentage of individuals dead on hauling). Observers always encouraged and trained fishers to release live individuals where possible.

2.2.2 Self-reporting

In 2018 and 2019, prior to the COVID-19 pandemic, commercial fishers were trained to report catch and bycatch, using data sheets provided, in return for a subsidy per fishing trip. Fishers shared photographs of species caught *via* telephone applications and completed a simplified form which was the same as the onboard observer forms except individual fish measurements and the status of individuals on hauling were not recorded. A fisheries liaison officer was employed to visit fishers regularly during this period, to collect and replenish forms every two months during active fishing. In some cases where specimens were retained for trade, observers attended the port to take measurements and maturity was estimated using L_{50} values.

2.2.3 Opportunistic data

Further data were collected opportunistically using local newspaper, social media posts and direct information from fishers who were not part of the self-reporting study, including amateur and sports fishers who represent a much broader demographic and for whom no data on fishing effort and distribution are estimated. Photographs were provided for identification, and where possible, measurements and locations of capture were collected from follow up conversations with the fisheries liaison officer. Fishers could also make contact regarding elasmobranch bycatch, and when able, observers met the fishers to measure the individual, and take photographs, samples or the whole specimen, and again, maturity was estimated using L_{50} values.

2.3 Spatial analysis

Using the median location calculated from the start and end coordinates of onboard observer sets, the spatial distribution of (1) number of observed sets, (2) number of sets with one or more individuals of each species, and (3) bycatch per unit effort (BPUE), were mapped and presented using a 100 km^2 tessellating hexagonal polygon matrix. BPUE was calculated as the number of individuals bycaught per 1,000 m for set nets or per 1,000 hooks for longlines.

3 Results

A total of 1,702 sets were recorded, 730 by onboard observers and 972 by self-reporting fishers. These included 1,367 demersal net sets, 282 demersal longline sets and 2 pelagic (surface/epipelagic) longline sets, with average set depths of 34.2 m (SD: 23.7 m, range: 1.0-250.0 m), 53.5 m (SD: 39.7 m, range: 6.0-296.0 m) and 162.2 m (SD: 92.4 m, range: 96.8-227.5 m), respectively. The number of observed sets by year and month together with the proportion of these that had elasmobranch bycatch is shown in Supplementary Figure 1.

3.1 Species composition

A total of 1,205 individual elasmobranchs were recorded (2018: 294, 2019: 591, 2020: 140, 2021: 63, 2022: 117), of 36 different species (batoid n=19; shark n=17; Figure 2), including two of the three endemic to the Mediterranean (*Raja polystigma*, Regan 1923, speckled skate and *Raja radula*, Delaroche 1809, rough skate). During the study period, 160 (13%) individuals of 28 species were recorded opportunistically. Of these, seven were only recorded through opportunistic observations (*R. rhinobatos*; *Prionace glauca*, Linnaeus 1758, blue shark; *Mobula mobular*, Bonnaterre 1788, spinetail devil ray; *Oxynotus centrina*, Linnaeus 1758, angular roughshark; *A. superciliosus*; *Hexanchus griseus*, Bonnaterre 1788, bluntnose sixgill shar; *Odontaspis ferox*, Risso 1810, smalltooth sand tiger). In addition, for completeness, we have included a further two unique species records from opportunistic data from outside the study period (*Carcharhinus brachyurus*, Günther 1870, copper shark; *Carcharodon carcharias*, Linnaeus 1758, white shark), recorded in 2015 and 2017 respectively.

Of the species encountered, 61% are listed on the IUCN RedList as globally threatened (17% Critically Endangered; 25% Endangered; 19% Vulnerable; Figure 3) whereas 50% of recorded species are listed as regionally threatened in the Mediterranean [28% Critically Endangered; 17% Endangered; 6% Vulnerable (Supplementary Figure 2, note values rounded up within categories)]. Twenty-four species had not previously been recorded in Northern Cyprus (Tables 1, 2).

The most abundant species recorded was *Dasyatis pastinaca* (Linnaeus 1758, common stingray, n=350), followed by *Squalus blainville* (Risso 1827, longnose spurdog, n=299) and *Scyliorhinus canicula* (Linnaeus 1758, smallspotted catshark, n=48; Figure 2). Fifteen species caught throughout this study are listed by the Barcelona Convention, and banned from fishing and retention in the Mediterranean by the GFCM (Carpentieri et al., 2021; Figure 2). Six species are listed on Appendix II (international trade monitored and controlled) of CITES (Convention on the Trade in Endangered Species of wild fauna and flora): *A. superciliosus*, *C. carcharias*, *M. mobular*, *Isurus oxyrinchus*, (Rafinesque 1810, shortfin mako), *G. cemiculus* and *R. rhinobatos* (Figure 2). All extant Mediterranean representatives of the critically endangered *Squatina* spp. (angelsharks) were recorded in this study, namely *Squatina squatina* (Linnaeus 1758, angelshark), *Squatina oculata*
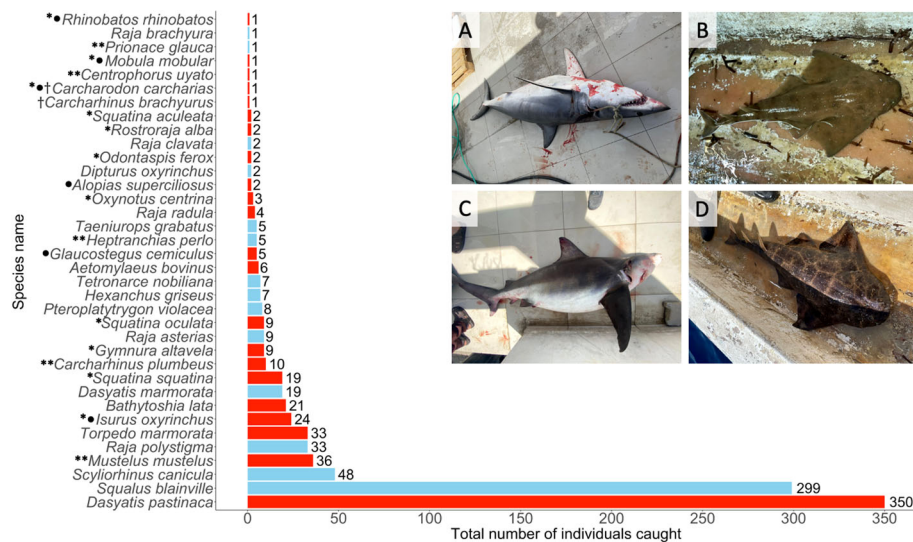
FIGURE 2
Total individuals (n = 988) that could be identified to species level caught as bycatch. Numbers on bars show individual count per species, red bars indicate species with critically endangered, endangered and vulnerable IUCN Red List status. * denotes species included in Annex II and ** denotes species in Annex III of the SPA/BD Protocol covered by GFCM/36/2012/3 and GFCM/42/2018/2, • denotes species listed in Appendix II of the CITES, † denotes species recorded outside of 2018-2022 data collection. Inset pictures show examples of critically endangered species observed as bycatch: **(A)** *I. oxyrinchus*, **(B)** *S. oculata*, **(C)** *C. plumbeus*, **(D)** *S. squatina*.

(Bonaparte 1840, smoothback angelshark) and *Squatina aculeata* (Cuvier 1829, sawback angelshark; Figure 2).

3.2 Life stages and mortality

Size class distributions of a selective group of threatened batoid and shark species/families are presented in Figure 4, and by individual species, where available, in Supplemental Figures 3, 4. The largest individual measured was from an opportunistic record of *I. oxyrinchus* at 270 cm in total length, however, some individuals that were opportunistically recorded but were not measured, appeared larger than the maximum recorded from photographs, such as a *A. superciliosus* caught on the west coast estimated at >4 m. The smallest individuals was a *D. pastinaca* that had a total length of 16 cm (Supplementary Table 1). Most species with total length measurements and L_{50} estimates were likely dominated by juveniles, except for *Torpedo marmorata* (Risso 1810, marbled torpedo ray), *D. pastinaca* and *S. blainville* for which 66.7%, 65.9% and 56.7% of females and 85.7%, 66.7% and 66.7% of males exceeded their sex specific L_{50s}, respectively.

The large number of *S.blainville* (n=137) caught in one set, were mostly of juvenile size, but included pregnant females. In addition, one fisher provided video footage of a *S. oculata* birthing on the west coast, while a large *Aetomylaeus bovinus* (Geoffroy Saint-Hilaire 1817, duckbill eagle ray) was filmed birthing on the deck of a boat on the north coast. Also worthy of note is that 10 out of the 25 *I. oxyrinchus* recorded were newborn or 1 - 2 year old and two were sexually mature, possibly pregnant, females (according to obtained length information and photos of the individuals).

At vessel mortality was higher for sharks (28%) than batoids (7%) with some species such as *Heptranchias perlo* (Bonaterre

1788, sharpnose sevengill shark), *I. oxyrinchus* and *Rostroraja alba* (Lacepède 1803,white skate) having 100% mortality on hauling, although sample sizes are low (Tables 1, 2).

3.3 Spatial distribution

Figure 5 shows the spatial distribution of BPUE for sharks (Figures 5A, B) and batoids per 100 km^2 (Figures 5C, D); location of all observer sets are shown by the presence of shaded hex cells in Figure 5, and the number of observed fishing operations per hex cell in Supplementary Figure 5 (Observed sets panel). High-diversity areas of elasmobranch catch were at the eastern (Karpaz (Karpas)) and western (Koruçam (Kormakitis)) capes, and in Famagusta and Güzelyurt (Morphou) Bays, although, onboard observer effort was higher in Famagusta Bay due to favourable weather conditions in this area (Supplementary Figure 5). *Dasyatis* spp. were the most abundant genus caught across the study (n = 401), followed by *Squalus* spp. (n = 299) and *Raja* spp. (n = 115). *Dasyatis* spp. was one of the most abundant genus caught in all five of the coastal areas across the three zones, and *Squalus* spp. in areas 1, 2, 3a and 3c (Supplementary Figure 5). If the mass bycatch event that occurred in 2019 in Zone 3a were removed, *Squalus* spp. would no longer be one of the top three genus in this area; the order would be *Dasyatis* spp., *Torpedo* spp. and *Raja* spp.

4 Discussion

Using novel methods in an under-studied, yet regionally important fleet, this study helps further our understanding of elasmobranch diversity and distribution in Cyprus, and the
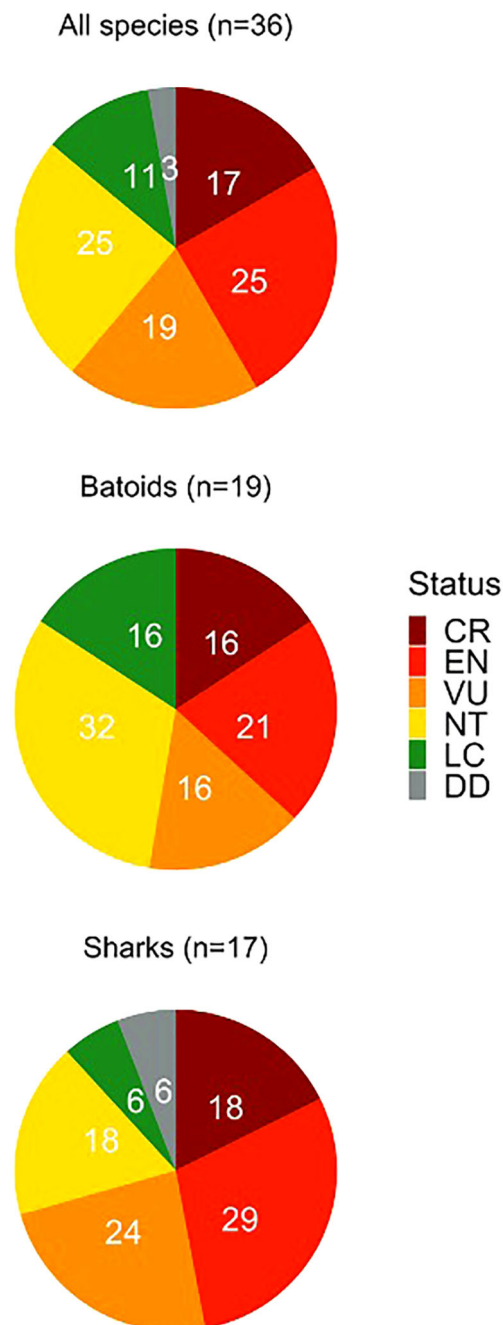
FIGURE 3
IUCN global Red List status for all species caught as bycatch across study period, subdivided into batoid and shark species. The categories for classifying a species risk of extinction: CR, critically endangered; EN, endangered; VU, vulnerable; NT, near threatened; LC, least concern; and DD, data deficient.

contribution of this coast to Mediterranean biodiversity. It also provides detailed insight into the interaction of elasmobranchs with small-scale fisheries and their wider ecology in the study area. We recorded 36 elasmobranch species in the coastal waters of Northern Cyprus, 61% of which are considered globally threatened and 50% regionally threatened in the Mediterranean (IUCN, 2022). Of these species, two of the three Mediterranean endemic species (*R. polystigma* and *R. radula*) were recorded and 24 species (65%) were previously unpublished records for Northern Cyprus, bringing the total number

of species recorded from 14 (Çoker and Akyol 2014; Akbora et al., 2019) to 39. Nearly half (47%) of the 36 species we report are priority species under the GFCM or listed on Appendix II of CITES (Figure 2), meaning that international trade should be controlled.

The most abundant threatened elasmobranch species (Figure 2) caught in our study were *D. pastinaca* (VU), *Mustelus mustelus* (Linnaeus 1758, common smoothhound EN) and *T. marmorata* (VU), differing from the top three previously reported by Carpentieri et al. (2021) for small-scale fisheries in the eastern

TABLE 1 Batoid species caught across this study.

Order:	Latin name	Common name	N	Global status	Med. status	AVM (%) (n)
Torpediniformes	*Tetronarce nobiliana*	Great torpedo ray†	7	LC	NE	0 (6)
	Torpedo marmorata	Marbled torpedo ray†	33	VU	LC	5 (21)
Rhinopristiformes	*Glaucostegus cemiculus*	Blackchin guitarfish†	5	CR	NE	NA
	Rhinobatos rhinobatos	Common guitarfish†	1	CR	EN	NA
Rajiformes	*Dipturus oxyrinchus*	Longnosed skate†	2	NT	NT	0 (2)
	Raja asterias	Starry skate	9	NT	NT	11 (9)
	Raja brachyura	Blonde skate†	1	NT	NT	0 (1)
	Raja clavata	Thornback skate	2	NT	NT	0 (2)
	Raja polystigma	Speckled skate*	33	LC	LC	0 (26)
	Raja radula	Rough skate*	4	EN	EN	0 (3)
	Rostroraja alba	White skate†	2	EN	EN	100 (1)
Myliobatiformes	*Aetomylaeus bovinus*	Duckbill eagle ray	6	CR	CR	0 (1)
	Bathytoshia lata	Brown stingray †	21	VU	NE	0 (2)
	Dasyatis marmorata	Marbled stingray†	19	NT	DD	0 (15)
	Dasyatis pastinaca	Common stingray	350	VU	VU	2 (178)
	Gymnura altavela	Spiny butterfly ray	9	EN	CR	0 (2)
	Mobula mobular	Spinetail devil ray†	1	EN	EN	0 (1)
	Pteroplatytrygon violacea	Pelagic stingray†	8	LC	LC	0 (4)
	Taeniurops grabatus	Round stingray†	5	NT	DD	0 (1)

The IUCN categories: CR, critically endangered; EN, endangered; VU, vulnerable; NT, near threatened; LC, least concern; DD, data deficient; and NE, not evaluated. *Endemic to the Mediterranean, global Redlist status included also for Mediterranean. †Species not previously recorded in Northern Cyprus. Common names and status for both global and Mediterranean are from the IUCN Red List (IUCN, 2022). At vessel mortality (AVM) as a percentage of those where mortality was recorded at hauling.

Mediterranean (*C. plumbeus*, *M. mustelus* and *M. mobular*). They also differ from the top three most abundant threatened species in both the western Mediterranean (*Alopias* spp., *P. glauca* and *I. oxyrinchus*) and central Mediterranean (*C. plumbeus*, *M. mustelus* and *G. cemiculus*). This regional variation in elasmobranch species composition may be driven by habitat or prey availability, or intensity of threats such as coastal development or fisheries activity, especially the lack of trawling activity in Northern Cyprus.

Three species that had previously been reported in Northern Cyprus, but were not found in this study were: *Etmopterus spinax* (Linnaeus 1758, velvet belly lanternshark)*, Galeus melastomus* (Rafinesque 1810, blackmouth catshark) and *Mustelus punctulatus* (Risso 1827, blackspotted smoothhound; Oray and Karakulak, 2005; Çoker and Akyol, 2014). *E. spinax* and *G. melastomus* have typically been recorded in deep water trawls (Bengil and Basusta, 2018), and thus may be unlikely to have been caught in this SSF fleet where trawling is prohibited. *M. punctulatus* has apparently been shifting periodically in the Mediterranean since the 1920s (Colloca et al., 2017), and although it had previously been reported in the area of Cyprus under the effective control of the RoC Authorities (Hadjichrisophorou, 2006) there have been no reports since 1984. It is not surprising that our study did not find all 58 species considered to be present in waters of the whole island, because, compared to Northern Cyprus, the fisheries in the RoC controlled area are more diverse (with active trawl and pelagic

longline fleets), because data availability for the latter region is far greater (partly due to the isolation of Northern Cyprus from many data collection frameworks), and because our study was restricted to include systematic methods and opportunistic observations during only recent years. In addition, it is worthy of note that fishermen from the Karpaz (Zone 3) region talk about the rare presence and capture of *Sphyrna* spp. (hammerhead sharks) but there have been no recent records in the area.

Although there were few species for which we had multiple measurements, clearly both adult and juvenile life stages of many species are present in coastal waters of Northern Cyprus. Many coastal elasmobranch species are known to use inshore nursery grounds with high productivity that offer protection from predators (Heupel and Simpfendorfer, 2002; Tiralongo et al., 2018b), and the large bycatch event of *S. blainville* of both juvenile and adult pregnant females, suggests there is a nursery ground for this species at least in the Karpaz region, with previous records supporting this (Bengil, 2022). From the *Squatina* size classes and birthing events reported, and the presence of new-born *I. oxyrinchus* it appears that Northern Cyprus may also be an important breeding and nursery area for these Critically Endangered and Endangered species.

A large proportion of the species found in Northern Cyprus are coastal – *A. superciliosus*, *Bathytoshia lata* (Garman 1880, brown stingray), *Dasyatis marmorata (*Steindachner 1892, marbled stingray),

TABLE 2 Shark species caught across this study.

Order	Latin name	Common name	N	Global status	Med. status	AVM (%) (n)
Hexanchiformes	*Heptranchias perlo*	Sharpnose sevengill	5	NT	DD	100 (4)
	Hexanchus griseus	Bluntnose sixgill†	7	NT	NE	NA
Lamniformes	*Alopias superciliosus*	Bigeye thresher†	2	VU	EN	0 (1)
	Carcharodon carcharias	White shark†	1	VU	CR	NA
	Isurus oxyrinchus	Shortfin mako†	24	EN	CR	100 (2)
	Odontaspis ferox	Smalltooth sand tiger	2	VU	CR	NA
Carcharhiniformes	*Carcharhinus brachyurus*	Copper shark†	1	VU	DD	NA
	Carcharhinus plumbeus	Sandbar shark†	10	EN	EN	NA
	Mustelus mustelus	Common smoothhound	36	EN	VU	32 (19)
	Prionace glauca	Blue shark†	1	NT	CR	NA
	Scyliorhinus canicula	Smallspotted catshark	48	LC	NE	5 (21)
Squaliformes	*Centrophorus uyato*	Little gulper shark†	1	EN	NE	NA
	Oxynotus centrina	Angular roughshark†	3	EN	CR	NA
	Squalus blainville	Longnose spurdog	299	DD	DD	0 (61)
Squatiniformes	*Squatina aculeata*	Sawback angelshark†	2	CR	CR	0 (1)
	Squatina oculata	Smoothback angelshark†	9	CR	CR	0 (3)
	Squatina squatina	Angelshark†	19	CR	CR	14 (7)

The IUCN categories: CR, critically endangered; EN, endangered; VU, vulnerable; NT, near threatened; LC, least concern; DD, data deficient; and NE, not evaluated. †Species not previously recorded in Northern Cyprus. Common names and status for both global and Mediterranean are from the IUCN Red List (IUCN, 2022). At vessel mortality (AVM) as a percentage of those where mortality was recorded at hauling.

Taeniurops grabatus (Geoffroy St. Hilaire 1817, round stingray), *D. pastinaca*, *A. bovinus* and *Raja asterias* (Delaroche 1809, starry skate) – or found on the continental shelf and upper slopes, making them vulnerable to interactions with small-scale fisheries that are concentrated along the coast (Carpentieri et al., 2021). The majority of elasmobranch species caught by small-scale fisheries in the Mediterranean are demersal species (Carpentieri et al., 2021) caught in trammel nets or trawls, however, in Northern Cyprus trawling is not permitted, and the majority of fishers use trammel nets. Gear modifications or bycatch reduction technology could be considered to mitigate the impact of this fishery, however, a more detailed analysis of the drivers of bycatch is required to allow for greater planning and prioritisation of mitigation measures to more discrete métiers. Greater temporal analysis of catches could also help focus conservation efforts to seasonal métiers. With only Türkiye recognizing Northern Cyprus, it often falls outside international conventions, research and conservation networks which creates a challenging environment for funding the monitoring and management of the fishery.

Since the decision of fishers to retain (for trade or bait) or release was influenced by the presence of onboard observers and interaction with the authors, we have not presented data on survivorship and release rates after hauling. However, there is currently little incentive to release specimens. The relatively low vessel mortality rates (% dead on hauling) for many species illustrate the opportunity to drastically reduce bycatch mortality rates through a successful release programme in this fishery. While momentum is being gained from an ongoing education and awareness raising programme in

collaboration with local authorities, the current legal trade in endangered elasmobranch species must be tackled. Recent legislative changes are welcomed but enforcement remains a challenge. Meanwhile, it is commendable and highly positive for marine conservation, that the authorities have been able to eliminate bottom trawling. However, small-scale fishers in Northern Cyprus are currently permitted to use drift nets which are banned across the rest of the Mediterranean because of their high impacts on large pelagic species including elasmobranchs. To our knowledge, drift nets are not in use and have never been noted on any vessels or in ports, yet, their use should be prohibited to avoid their possible future use.

A larger number of individuals were caught in coastal Zone 3, indicative of the higher number of active vessels and greatest observer coverage compared to other zones (Figure 1). When looking at BPUE however, several hotspots around Mağusa (Famagusta Bay), west of Cape Koruçam and the eastern Karpaz region can be seen (Figure 5). Existing MPAs (Figure 1) appear to overlap with these areas of high elasmobranch BPUE and so may be well-placed for continued work to designate strictly protected areas within them. Indeed, two candidate Important Shark and Ray Areas have been identified in Northern Cyprus which also overlap with these hotspots and the Akdeniz MPA on the west coast, and the two Karpaz MPAs (Figure 1; https://sharkrayareas.org/e-atlas/). In the management plans for these MPAs, that were primarily put in place to protect marine turtles, monk seals, seabirds and seagrass beds, fishing with set nets is prohibited to 30 m deep and to 1.5 km offshore, although this has never been enforced. Further analysis with larger observed effort will be useful in confirming
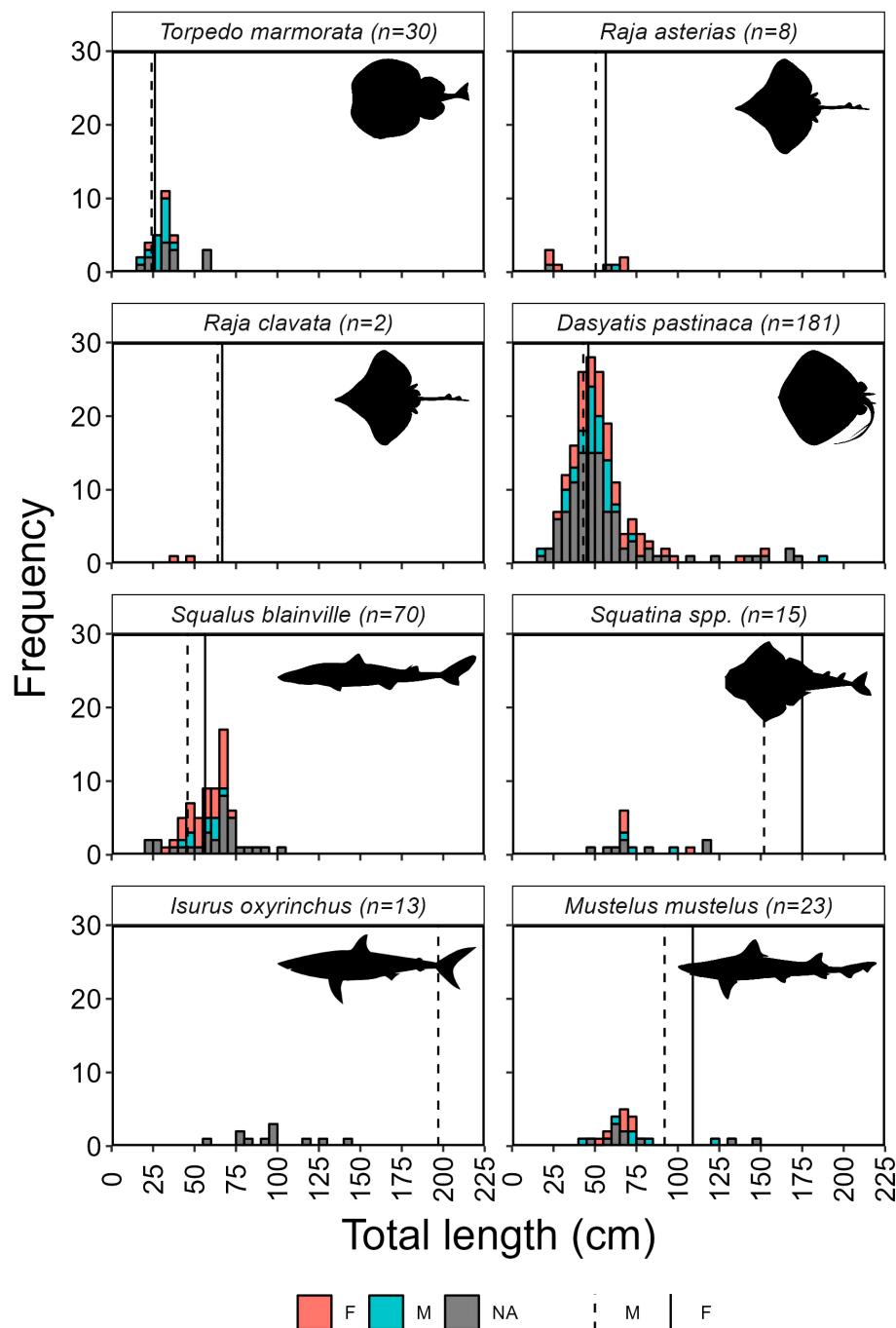
FIGURE 4
Size distribution of bycaught ray (*T. marmorata*, *R. asterias*; *Raja clavata* thornback skate; *D. pastinaca*) and shark species (*S. blainville*, *Squatina* spp.*, *I. oxyrinchus* and *M. mustelus*) recorded during onboard observations and self-reported trips between 2018 and 2022. **Squatina* sizes are derived from all three species recorded in this study as well as any unidentified *Squatina* individuals. Total length at 50% maturity (L_{50}) are given for females (solid line) and males (dashed line) of each species; L_{50} for *Squatina* is taken from *S. aculeata* estimates; L_{50} of female *I. oxyrinchus* (282.0 cm) exceeded the range of data. Illustrations from https://www.phylopic.org/.

such hotspots, refining MPA management plans, effectively integrating no-take zones to protect multiple taxa and developing new MPAs. Further studies should aim to increase onboard observation and fill the spatial gaps in our survey coverage, especially along the west and central north coasts.

The results of this study have been shared with the Northern Cyprus authorities and a proposal to expand the list of protected species and introduce the first no-take zones was accepted and policy came into effect in May 2023. Meanwhile, the strong reaction of some fishers participating in the project, sharing videos of themselves releasing threatened elasmobranch species, are an encouraging reaction to a programme of education delivered during this project. The recognition of the value and diversity of elasmobranchs among fishers is critical, so that retention moves from being the norm, to

FIGURE 5
Spatial distribution of mean bycatch per unit effort (BPUE) derived from onboard observations with location data (n=664 fishing operations) of sharks **(A, B)** and batoids **(C, D)** in set nets **(A, C)** and long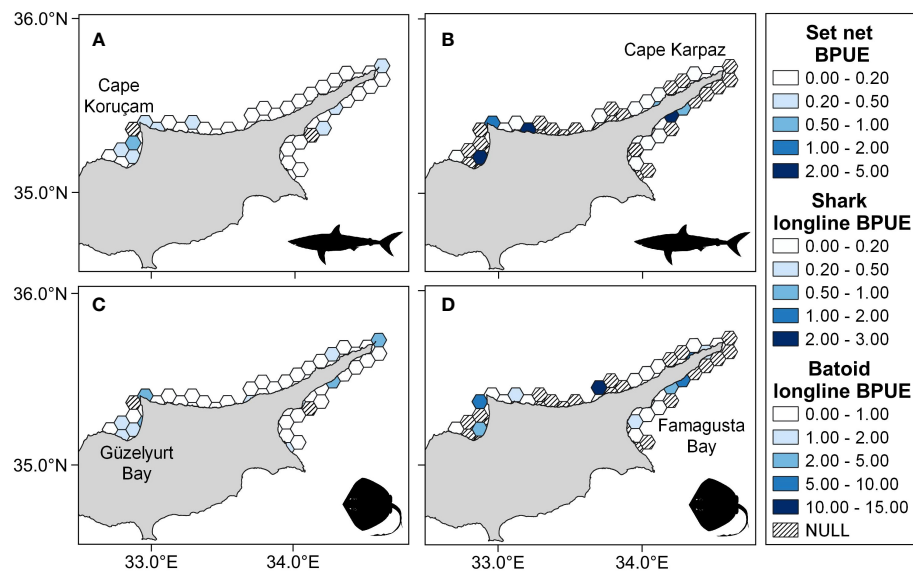lines **(B, D)** per 100 km². Cells shown indicate those where observer trips occurred; NULL cells are those where BPUE could not be calculated with the available data. Greek names for coastal areas indicated are **(A)** Cape Kormakitis, **(B)** Cape Karpas, **(C)** Morphou Bay, **(D)** Famagusta Bay.

becoming a taboo, since enforcement of elasmobranch trade bans also has inherent challenges. Bycatch mitigation gear for dolphins (Snape et al., 2018b) and turtles has been trialled in this fishery, and could be expanded to elasmobranchs in certain métiers, or at least for fishers operating in MPAs.

This study has underlined the value of collaborating with SSFs to identify marine biodiversity. Results support the existence of important elasmobranch species which should be protected from expanding fishing pressure and trade, through the development of strictly protected areas/no-take zones, development of mitigation, education, and most importantly, implementing and policing the recent ban on elasmobranch trade and no-take zones. Although management of small-scale fisheries is extremely difficult, high community engagement, education and engaging fishers through SSF co-management, can effect change (Piovano et al., 2012). As data in this location continues to grow, species-specific analysis of critically endangered elasmobranchs must be undertaken to further inform conservation planning and management of fisheries. More fine-scale distribution data would also be of benefit, to improve the accuracy and identification of species richness hotspots, and reliability of management actions. With nearly half (44%) of Mediterranean elasmobranch diversity across less than 1% of its coastline, Northern Cyprus should be considered a regional conservation priority for this taxonomic group.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was approved by University of Exeter Ethics Committee. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

RTES, DB, BJG and ACB contributed to the conception and design of the study. MaO, EGTB, JLP, DB, CC, MeO, RTES, collected data. MaO, EGTB, JLP, RTES and ACB developed the first draft of the manuscript and all authors contributed to further drafts. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1181437/full#supplementary-material

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Observations of biennial reproduction in Caribbean reef sharks 'Carcharhinus perezi'

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Effective management and conservation of threatened species biodiversity requires knowledge of reproductive biology, such as cyclicity, mode, and age at maturity. We combined reproductive endocrinology and *in-situ* ultrasonography to examine reproductive characteristics of female Caribbean reef sharks *Carcharhinus perezi*, a widely distributed, threatened marine predator which remains largely understudied throughout its range. Unique to this study was the opportunity to conduct longitudinal assessments of two individuals, recaptured across multiple seasons during sampling in The Bahamas. Within-individual, paired hormone analyses and *in-situ* ultrasounds of female sharks that were confirmed as either pregnant, non-pregnant, or reproductively active, suggest a biennial reproductive cycle for *Carcharhinus perezi*. This unique opportunity to assess the reproductive biology of the same individuals over time underscore the importance of repeated sampling for elucidating population reproductive cyclicity of highly mobile sharks in the wild.

KEYWORDS

estradiol and progesterone, ultrasound, endangered, non-lethal, elasmobranch

1 Introduction

Elasmobranch fishes exhibit conservative life-history traits including slow growth, late maturity, and low reproductive output, rendering them highly vulnerable to overfishing (Stevens et al., 2000; Dulvy et al., 2021). As a result, approximately one-third of all living elasmobranch species are listed as "Vulnerable" to "Critically Endangered" and 14% considered as "Data Deficient" by the International Union for the Conservation of Nature (IUCN) 2022 Red List (IUCN, 2022). Understanding components of a species' reproductive biology (i.e., age-at-maturity, gestation period, reproductive mode, reproductive cyclicity) and life-history strategies related to reproduction are essential for

effective management of wild populations (Hammerschlag and Sulikowski, 2011; Natanson et al., 2019). For example, such information can assist stock assessments thereby informing management decisions related to protected area designation (Awruch, 2013). This is particularly true for elasmobranch fishes who employ a suite of reproductive strategies, thereby challenging management without species-specific, population-specific, or even region-specific data (Musick et al., 2005; Hamlett et al., 2011; Natanson et al., 2019).

The Caribbean reef shark (*Carcharhinus perezi*, Poey, 1876) is a medium-bodied requiem shark found throughout the sub-tropical latitude band of the western Atlantic Ocean, with a range extended from the southern North America to South America (Castro et al., 1999; Compagno, 2002; Tavares, 2009; Carlson et al., 2021). This species exhibits a conservative life history including an estimated slow growth of 23.5 cm yr^{-1} in Venezuela (Tavares, 2009) and 8.8 cm yr^{-1} in Belize (Bond et al., 2017), late maturation around 14.8 year for males and 16.4 years for females (Tavares, 2009; Talwar et al., 2022), and a small litter size of approximately 4 pups (Talwar et al., 2022). Coupled with fisheries exploitation throughout parts of their distribution (i.e., Belize, Brazil; Carlson et al., 2021) these traits have exposed Caribbean reef sharks to population declines in parts of their range (~50-80%), thus elevating the risk assessment of this species as globally "Endangered" by the IUCN Red List (Carlson et al., 2021; Gallagher et al., 2021). Information linking the reproductive biology, spatial movement, and habitat use in Caribbean reef sharks remains poor, thereby precluding species-specific management efforts (Carlson et al., 2021).

Caribbean reef sharks exhibit a placental viviparous reproductive strategy (i.e., placental connection formed between mother and offspring; live-bearing) with an assumed biennial reproductive cycle (Carrier et al., 2004). This species is thought to exhibit low reproductive output (3-6 pups) and a relatively long gestational period (~1 year; Rangel et al., 2022). Yet, the general understanding of Caribbean reef shark reproductive biology remains extremely poor and, to date, no published studies have specifically addressed the reproductive physiology or cyclicity of the species (see Brooks et al., 2013; Talwar et al., 2022). Here, the first ever empirical reproductive hormone concentrations and *in-situ* ultrasonography for female Caribbean reef sharks was investigated by presenting data collected from The Bahamas. This analysis included multiple reproductive assessments from two individuals recaptured across various seasons, a unique opportunity given the low recapture rate of this species (~6.97%, Talwar et al., 2022).

2 Materials and methods

All protocols for capture and sampling were approved by the Arizona State University Institutional Animal Care & Use Committee (IACUC; #20-1745) as well as the Government of the Bahamas annual fishing permits granted to Beneath the Waves Non-profit Research Organization (BTW; BS-2021-991344 and BS-2022-348632).

Female Caribbean reef sharks were opportunistically captured between August 2021 and July 2022 using scientific drumlines (see Gallagher et al., 2014) in coastal waters of Great Exuma, The Bahamas (research permit BS-2021-765539). Individuals were measured (total length, cm), tagged with conventional dart tags, and a 10 mL blood sampled was taken via caudal venipuncture and stored in sodium heparin-lined vacutainers. Reproductive state of females was identified *in-situ* using an Ibex EVO II portable ultrasound (E.I. Medical Imaging) with a 60 mm curved linear array 5-2.5 MHz transducer (model 290470) capable of a 30 cm scan depth. Briefly, *in-situ* ultrasound scanning was performed on the ventral surface from the pectoral to the pelvic fin in both a transverse and longitudinal orientation to obtain cross sectional and lengthwise images of the reproductive tract, ovaries and follicles (Sulikowski et al., 2016). On return to the lab, blood was separated into primary constituents (red blood cells, plasma, and platelets) and ~2mL plasma was stored at -20°C. Samples were shipped to Arizona State University (Glendale, AZ) for processing of reproductive hormones.

Following Sulikowski et al. (2004) 17β-estradiol (E_2) and progesterone (P_4) concentrations were quantified using standard radioimmunoassay techniques. The average hormone extraction recovery was 72.5% for E_2 and 55.7% for P_4. The mean inter-assay coefficients of variation for E_2 and P_4 were 3.44% and 24.4%, and the mean intra-assay coefficients of variation were 9.61% and 7.95%, respectively. Samples that fell below the detectable limits of the assay were concentrated and re-assayed. Final concentrations were corrected for procedural loss during the extraction using individual sample recoveries.

3 Results

Two mature females (IDs 00546, 00543) were captured on August 13th, 2021, in Great Exuma, The Bahamas (Table 1). *In-situ* ultrasonography indicated that neither female was pregnant based on the appearance of an empty uterus (Figure 1B; Sulikowski et al., 2016). Hormone concentrations were measured (Sulikowski et al., 2004) for one female (ID 00543) which revealed E_2 and P_4 concentrations of ~36.5 pg ml^{-1} and ~306.1 pg ml^{-1}, respectively (Table 1). Both females (IDs 00546, 00543) were subsequently recaptured on December 14th, 2021 (Table 1). *In-situ* ultrasonography confirmed for a second time that neither female was pregnant at time of recapture. E_2 and P_4 concentrations for the same female (ID 00543) were ~64.6 pg ml^{-1} and ~72.8 pg ml^{-1} P_4, respectively; Table 1). On July 19th and 21st, 2022, two additional females (IDs 00521, 00767; Table 1) were sampled in Great Exuma, The Bahamas with obvious, healed but recent mating scars (~1 month old) along the axis of the body (Figure 1A). These scars, coupled with candle-like structures in the uterus detected via *in-situ* ultrasonography (ID 00521; Figure 1C) allowed us to confirm pregnancy at capture for both females (IDs 00767, 00521). However, due to technical difficulties with equipment in the field, the ultrasound footage of one female (ID 00767) was not preserved. Hence, in Table 1, we refer to this female as "presumably pregnant"

TABLE 1 Summary data of female *C. perezi* sampled in Great Exuma, The Bahamas from August 2021 through July 2022 including blood plasma estradiol (E_2) and progesterone (P_4) concentrations as well as field ultrasonography evaluations.

Sampling Date	Conventional Tag ID	Total Length (cm)	Latitude	Longitude	E_2 Concentration pg mL^{-1}	P_4 Concentration pg mL^{-1}	Ultrasonography Evaluation
08/13/2021	00546	188	23.482992	-75.703163	–	–	Not Pregnant
08/13/2021	00543	194	23.482992	-75.703163	36.5	306.1	Not Pregnant
12/14/2021	00546	–	23.49791	-75.72291	–	–	Not Pregnant
12/14/2021	00543	–	23.49791	-75.72291	64.6	72.8	Not Pregnant
07/19/2022	00767	182	23.771292	-76.1105302	241.6	1349.0	Presumably Pregnant
07/21/2022	00521	201	23.771292	-76.1105302	33.4	146.6	Pregnant

Total length taken in December 2021 was not used due to sampling discrepancies.

instead of "pregnant". Further, E_2 and P_4 concentrations for one female (ID 00521) were ~33.4 pg ml^{-1} and ~146.6 pg ml^{-1}, respectively, and for the second female (ID 00767) were ~241.6 pg ml^{-1} and ~1349.0 pg ml^{-1}, respectively (Table 1).

4 Discussion

Interpretation of hormone concentrations (i.e., estradiol (E_2), progesterone (P_4)) in the context of elasmobranch reproduction has been investigated since the mid-1900s (Becerril-García et al., 2020).

Generally, it has been shown that high levels of E_2 are associated with preparing the female reproductive tract for ovulation through stimulating the production of vitellogenin by the maternal liver (Awruch, 2013). Conversely, high levels of P_4 have been hypothesized to play an antagonistic role toward E_2, downregulating the production of vitellogenin and prompting ovulation (Verkamp et al., 2022). In this sense, the increase in E_2 and decrease in P_4 concentrations from August to December demonstrated by individual #00543 is presumed to be indicative of vitellogenesis (Awruch, 2013), suggesting that this female was entering a pre-ovulatory state and preparing for the next mating

FIGURE 1
(A) An enlarged photo example of mating scars seen on both females caught in July 2022 (IDs 00767, 00521), **(B)** *in-situ* ultrasounds performed on non-pregnant 2021 female (ID 00543) with empty uterus and **(C)** pregnant female (ID 00521; bottom) captured in 2022. Candle-like structures are highlighted in red. These structures are thin, pleated egg envelopes seen in placental sharks (see Hamlett et al., 2011). The presence of these envelopes indicate that this female had completed ovulation.

season (Awruch, 2013). This observation is supported by previous work on the placental viviparous blue shark, where high concentrations of circulating E_2 were directly linked to the synthesis of vitellogenin by the maternal liver during the follicular phase (Fujinami and Semba, 2020). Given that Caribbean reef sharks are thought to follow a biennial cycle with a long gestation (~11-12 months; Rangel et al., 2022), it is presumed that this female (ID 00543) had pupped earlier in the year before the first time she was sampled. Furthermore, the absence of soft tissue structure inside the uterus of this female, observed via *in-situ* ultrasonography (Figure 1B) further supports the presence of this pre-ovulatory state suggested by hormone concentrations. Because these observations occurred during an odd year, this could suggest a biennial reproductive cycle for Caribbean reef sharks, at least for The Bahamas population. The subsequent capture of two reproductively active females (IDs 00521, 00767), with evidence of pregnancy in both (ID 00767, 00521; Figure 1C), during an even year provides additional support for a biennial reproductive cycle for this sub-population.

High individual-level variation in hormone concentrations was observed across individuals sampled in this study (Table 1). The levels of E_2 in female 00767 were 4-7 times greater than those observed in the other females. In addition, the P_4 levels of this female were very high (Table 1) which is similar to levels observed in other ovulated/early post-ovulatory females of different species (Sulikowski et al., 2016). These hormonal observations coupled with recent mating scars and early-stage pregnancy assessments via ultrasound support the notion that this was a recent pregnancy. The female from August 2021 (ID 00543) that was not pregnant had similar levels of E_2 and P_4 as the pregnant female sampled in July 2022 (ID 00521). This has been observed in other species such as tiger sharks (*Galeocerdo cuvier*; Sulikowski et al., 2016; Hammerschlag et al., 2018) where hormone concentrations were found to be of similar levels between females in different reproductive stages. The low levels of E_2 concentrations exhibited by the pregnant female in this study (ID 00521) is consistent with the completion of ovulation (Awruch, 2013). However, P_4 concentrations for this individual were lower than expected, especially when compared to the other reproductively active female from July 2022 (ID 00767). Since the sampling efforts represent a temporal snapshot of the hormonal profiles of these sharks, this variation in this small window of time is expected based on previous studies and can most likely be attributed to individual differences in the exact timing of the reproductive cycle (Verkamp et al., 2022). To provide population-level resolution across seasons and major reproductive events, we recognize the need to obtain a larger sample size (e.g., 10 or more individuals).

Although preliminary, the findings offer support for a biennial reproductive cycle in the Caribbean reef sharks captured in Exuma. However, given the high individual-level variation in hormonal concentrations, and low sample size, the need for additional non-lethal samples are needed to further support this conclusion. Despite these short comings, we believe these preliminary findings carry conservation value given the paucity of existing information for this species' overall biology as well as proof-of-concept for future research on elasmobranch reproduction.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was approved by Arizona State University Institutional Animal Care and Use Committee. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Age and growth estimates for the nurse shark (*Ginglymostoma cirratum*) over 17 years in Bimini, The Bahamas

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Age and growth estimates are essential for life history modeling in elasmobranchs and are used to inform accurate conservation and management decisions. The nurse shark (*Ginglymostoma cirratum*) is abundant in coastal waters of the Atlantic Ocean, yet many aspects of their life history remain relatively understudied, aside from their reproductive behavior. We used mark-recapture data of 91 individual *G. cirratum* from Bimini, The Bahamas, from 2003 to 2020, to calculate von Bertalanffy (vB) growth parameters, empirical growth rate, and age derived from the resulting length-at-age estimates. The Fabens method for estimating growth from mark-recapture methods was applied through a Bayesian framework using Markov chain Monte Carlo (MCMC) methods. This provided growth parameters with an asymptotic total length ($L\infty$) of 303.28 cm and a growth coefficient (*k*) of 0.04 yr^{-1}. The average growth rate for *G. cirratum* was approximately 8.68 $\pm$ 6.00 cm yr^{-1}. This study also suggests that the previous maximum age for *G. cirratum* is likely underestimated, with the oldest individual predicted to be 43 years old. Our study is the first to present vB growth parameters and a growth curve for *G. cirratum*. It indicates that this species is slow-growing and long-lived, which improves our understanding of their life history.

KEYWORDS

life history, elasmobranch, conservation, management, von Bertalanffy, mark-recapture

1 Introduction

Elasmobranchs (sharks, rays, and skates) have classically been described as relatively long-lived, slow-growing and late-maturing, with long gestation periods and low fecundity (Hoenig and Gruber, 1990; Stevens et al., 2000). Due to these life history strategies, overfishing threatens over one-third of elasmobranchs with extinction (Dulvy et al., 2014;

Dulvy et al., 2021). Ascertaining accurate information on life history traits, such as age and growth, can help to classify species' potential susceptibility to anthropogenic threats (Emmons et al., 2021). Furthermore, accurate age and growth estimates are important when assessing the vulnerability of a population and determining the risk of overexploitation (Hammerschlag and Sulikowski, 2011) because these estimates are often used directly in a variety of assessment models (Hoenig and Gruber, 1990; Baje et al., 2018; Flinn and Midway, 2021).

Extensive age and growth information can be difficult to obtain for many elasmobranchs, as several of the morphometric characteristics traditionally used for aging teleosts are lacking for elasmobranchs (Beal et al., 2022). Methods used in age and growth studies for teleosts rely on counting growth rings in hard parts such as otoliths and scales, which are not present in the cartilaginous skeleton in elasmobranchs (Das, 1994; Cailliet, 2015). Therefore, reliable information is only available for a limited number of species, with research focused primarily on those frequently caught in fisheries or of conservation concern (Cailliet, 2015). Typically, accurate aging of elasmobranchs relies upon dead specimens in order to count growth band pairs in their vertebral centra. However, this method is species and potentially regionally dependent and can result in age underestimation due to uncertainty in the frequency of band formation (Cailliet, 1990; Natanson et al., 2018; Rudd et al., 2019). For instances in which age information is difficult to obtain or not available, length-increment analysis can provide an effective alternative means for determining growth (Frazier et al., 2020). Length-increment analysis involves the collection of length measurements from the same individual over time (i.e., mark-recapture) where original age is often unknown but can be estimated through length and age relationships and known time between measurements (Harry et al., 2022). This can be a preferred method for elasmobranch research because it is not subject to some of the biases and limitations present in other aging methods (Frazier et al., 2020; Dureuil et al., 2022), however the datasets needed for this analysis are rarely available.

A limitation of length-increment analysis is that it requires a fairly large sample size, which are typically small for elasmobranch studies due to the limitation of recaptures. Due to the propensity of limited data sets, methods have been developed to work with the low sample sizes and still provide valuable insight into life history parameters of the focal population (Barker et al., 2005; Harry et al., 2022). For example, Bayesian methods overcome low samples sizes by considering prior knowledge of the species of interest (Pardo et al., 2016; Caltabellotta et al., 2021; Smart and Grammer, 2021; Dureuil et al., 2022). This preceding knowledge is used to form prior distributions of possible values to estimate growth parameters from a model (Gelman et al., 2017), such as the von Bertalanffy (vB) growth function (von Bertalanffy, 1938). Data-limited assessments are further overcome when paired with Markov chain Monte Carlo (MCMC) methods, which is an iterative procedure to obtain estimated parameter values that ensures sampling across the entire parameter space (Rudd et al., 2019). Applying these methods to growth models has become increasingly popular for overcoming the limited datasets in elasmobranch research (Smart and Grammer, 2021).

Nurse sharks, *Ginglymostoma cirratum*, are in the order Orectolobiformes (otherwise known as the carpet sharks) and are one of the most abundant shark species in shallow, coastal waters (Castro, 2000). They range from tropical West Africa and the Cape Verde islands in the eastern Atlantic, to southern Brazil and North Carolina in the western Atlantic Ocean (Castro, 2000) and display strong site fidelity (Carrier, 1985; Carrier and Luer, 1990; Chapman et al., 2005; Pratt et al., 2022; van Zinnicq Bergmann et al., 2022). *Ginglymostoma cirratum* were listed as data deficient by the International Union for Conservation of Nature (IUCN) Red List of Threatened Species, before being assessed as vulnerable (Carlson et al., 2021; Garzon et al., 2021). Despite their abundance and the recent focus on their conservation status, general data for the species is lacking aside from research on their reproductive behavior in the Dry Tortugas, Florida, USA (Carrier et al., 1994; Pratt and Carrier, 2001; Whitney et al., 2010; Pratt et al., 2022). Most of the life history data (i.e., maximum size and growth rate) available for *G. cirratum* come from the Florida Keys, USA and Brazil (Carrier and Luer, 1990; Castro, 2000; Santander-Neto et al., 2011; Ferreira et al., 2012). Castro (2000) provided some limited information for The Bahamas; however, it is the only published life history data to date for this area. Some research has assessed the demographic structure and relative abundance of this species in The Bahamas (Hansell et al., 2018; Shipley et al., 2018; Clementi et al., 2021), but significant data gaps persist. Therefore, additional research on life history traits such as age and growth are needed for the species.

Ginglymostoma cirratum are documented as abundant in the waters around Bimini, The Bahamas, with all size classes present (Hansell et al., 2018), providing an ideal study site to investigate age and growth. In this study, we use a 17-year mark-recapture dataset of *G. cirratum* from Bimini to (1) provide the first estimates for the vB growth parameters L_∞ and k for this species, (2) determine an empirical annual growth rate for the region, and (3) estimate age based on the growth parameters and known information about their length-at-birth, L_0.

2 Materials and methods

2.1 Study site

Bimini, The Bahamas, (25°73′N, -79°27′W) is a set of two islands located ~ 85 km east of Miami, Florida, USA (Figure 1). The deep waters of the Gulf Stream to the west of the island separate Bimini from Florida, while the shallow waters of the Great Bahama Bank border the east of the island. A shallow (0–3 m), tidal lagoon lies in between the North and South islands of Bimini (Trave and Sheaves, 2014). The western side of the island consists of sandy flats, reefs, and seagrass habitat (van Zinnicq Bergmann et al., 2022). Bimini's lagoon and the east side of the island consist of mangrove-fringed and seagrass habitats that serve as nurseries for various juvenile shark species (Feldheim et al., 2002; Jennings et al., 2012; Trave and Sheaves, 2014).

2.2 Capture methods

Ginglymostoma cirratum were caught within a 10 km radius of Bimini from 2003–2020 during fishery-independent surveys (Figure 1). Individuals were caught using a variety of fishing methods including shallow water longline surveys (Hansell et al., 2018; Smukall et al., 2022), hand capture, and other methods consisting mostly of drumline (Gallagher et al., 2014), polyball fishing (Guttridge et al., 2017), traditional rod and reel fishing, and gillnets (Dhellemmes et al., 2021). For safe handling, larger sharks captured using a hook were measured and tagged while secured next to the boat. Hand captures of small *G. cirratum* were conducted by snorkeling mangrove edges or rocky ledges. They were visually identified and grasped between their gills and pectoral fins with one or two hands and brought to the surface for sampling. At the surface, the shark was placed in a tub (~150 cm diameter; 500 L volume) filled with seawater for data collection and tagging. Precaudal length (PCL) and total length (TL) measurements were recorded. The TL of the sharks was obtained by stretching a measuring tape that followed the curvature, maintaining contact with the animal from the tip of the head along the dorsal side of the body to the tip of the tail. The sex of individuals was based on the

FIGURE 1

The study site of Bimini, The Bahamas, in proximity to Florida, USA. The colored symbols represent the capture and recapture locations of *Ginglymostoma cirratum* that were recaptured ≥ 90 days apart, from 2003-2020 from fishery-independent surveys using various capture methods. Captures that appear on land were in mangrove-fringed habitats.

presence of claspers. Lengths at maturity for *G. cirratum* were determined 223–231 cm TL for females and 214 cm TL for males based on Castro (2000) or were determined on the calcification of claspers in males. Sharks were fitted with a passive integrated transponder (PIT, 12.34 mm x 2.04 mm; Destron Fearing Inc.), and/or a dart tag (National Oceanic and Atmospheric Administration Cooperative Shark Tagging Program) placed into the musculature at the base of the first dorsal fin.

2.3 Data preparation

Ginglymostoma cirratum with captures ≥ 90 days apart were used for analysis to ensure sufficient time had passed between captures for notable growth to be detected and limit the influence of human measurement error (Simpfendorfer, 2000; Boggio-Pasqua et al., 2022). Individuals were represented in the analysis only once, despite if there were multiple recaptures, and the difference in TL and time between capture was determined based on first and last capture. We investigated the dataset for outliers and removed any recaptures with unrealistic observation error (e.g., negative growth). Separation of sexes was considered for data analysis, but given the already low sample size, sexual separation was avoided to prevent further reducing our dataset and due to no significant difference in the TLs between sexes (Supplementary Material).

2.4 Data analysis

The von Bertalanffy (vB) growth function using L_0 was used as the basis for estimating age and growth from the mark-recapture data:

$$L_t = L_\infty - (L_\infty - L_0)e^{-kt} \qquad (1)$$

where L_t represents length at age t, L_∞ is the asymptotic maximum length, L_0 is the length-at-birth, and k is the Brody coefficient or growth constant determining how fast L_∞ is approached as t nears ∞ (Equation 1). When age is unknown, length measurements from mark-recapture studies can be used in an approach called the Fabens (1965) method to solve for the parameters L_∞ and k:

$$\Delta L = (L_\infty - L_t)(1 - e^{-k\Delta t})$$

$$\Delta L = L_{t+\Delta t} - L_t \qquad (2)$$

with ΔL as the expected change in length over Δt for an animal with an initial length of L_t (Equation 2; Haddon, 2011). *Ginglymostoma cirratum* length data was analyzed using a Fabens model with Bayesian methods ("GrowthEstimation" GitHub script; Dureuil et al., 2022 in R version v.4.3.1). The R packages 'TMB',' 'tmbstan,' and 'rstan' were used to build the model. TMB uses a No-U-Turn Sampling (NUTS) algorithm to estimate the growth parameters, which is an advanced Markov chain Monte Carlo (MCMC) method (Hoffman and Gelman, 2014). A prior distribution was given to L_∞, k, and σ^2 (Table 1) to conduct the
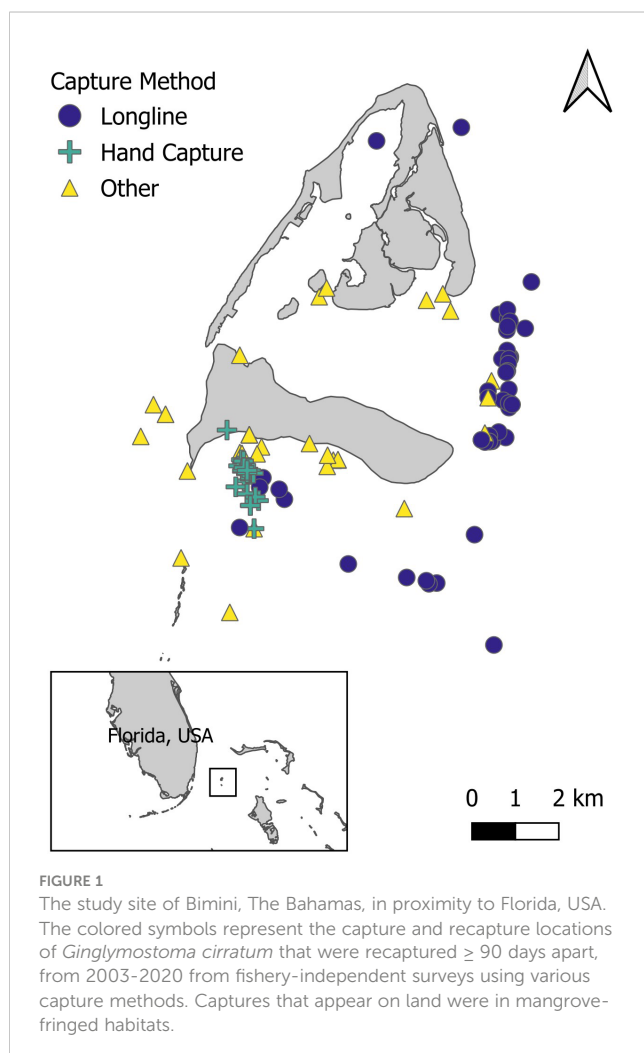
Bayesian inference (Gelman et al., 2014). Summary statistics were derived from the posterior distribution of the parameters given the data using the NUTS algorithm.

A value for L_0 was required for fitting the vB growth curve. Based on observed catch data from Bimini *G. cirratum* and published length-at-birth information, we assigned L_0 to be 24 cm TL (Castro, 2000; Carrier et al., 2003). Smaller lengths at birth are recorded in the literature for *G. cirratum* (Carrier et al., 2003), however it was suggested that these pups were potentially born prematurely. The age estimations were obtained through Equation 3 to plot the vB growth curve:

$$t = \frac{\log_e\left(\frac{(L_\infty - L_0)}{(L_\infty - L_t)}\right)}{k} \qquad (3)$$

When analyzing small sample sizes, a lognormal prior distribution can result in more stable MCMC iterations (Dureuil et al., 2022). This was applied to L_∞ and uniform prior distributions were assigned to k and σ^2. We used a lognormal prior for L_∞ because there was previous information available for this parameter. To calculate the lognormal mean and standard deviation for the lognormal prior distribution of L_∞, we first supplied the average maximum length (L_{max}) for individuals, which was the average of the three largest individuals in our population (Dureuil et al., 2022). We obtained an L_{max} of 247 cm TL, represented as the lognormal median. Next, we gave our best determination of L_∞ for *G. cirratum*. We referenced FishBase to find previous L_∞ reported for *G. cirratum* but were unable to confirm the reliability of this data. As a result, we searched the available literature to find an upper limit for L_{max} for *G. cirratum*, which was 316.8 cm TL from Brazil (Santander-Neto et al., 2011). We computed L_∞ from this using the upper limit of $L_{max} = 316.8$ cm TL and taking $\frac{Lmax}{0.99}$, which resulted in an L_∞ of 320 cm TL (Dureuil et al., 2021). The mean was obtained by taking log ($\frac{Lmax}{0.99}$) and was 248.53 cm TL. The standard deviation was computed such that the lognormal 99th percentile was 1.2 ($\frac{Lmax}{0.99}$) and was 27.75 cm TL. As there was no available data for k, we used a uniform prior distribution, which defines the lower and upper bounds for the parameter. We improved our confidence in our prior k distribution of 0.01 yr^{-1} to 1.00 yr^{-1} by using the lowest and highest published information on k for Orectolobiformes and creating a range that encompasses a realistic set of values (Chen et al., 2007; Huveneers et al., 2013; Perry et al., 2018). The uniform prior distribution used for σ^2 was narrow because we had confidence in the TL measurements and was set as 0.00 cm to 20.00 cm TL.

Posterior distributions were determined from the prior distribution of the parameters (L_∞, k, and σ^2) and the data. Three chains were run in the Fabens model with Bayesian methods applied, each with 10,000 iterations and a burn in period of 5,000 samples. Convergence of the chains was assessed by visualizing trace and pairs plots, and the R-hat and effective sample size criteria (ESS) (Supplementary Material; Vehtari et al., 2021; Dureuil et al., 2022). Autocorrelation was assessed using diagnostic plots from the 'Bayesplot' R package (Gabry, 2020).

3 Results

3.1 Capture data

A total of 851 *Ginglymostoma cirratum* individuals (378 females, 458 males, and 15 sex not recorded) were caught between 2003–2020 in Bimini, The Bahamas (Table 2). There were 137 total individuals that were recaptured at least once for a recapture rate of 16.10%. Longline surveys and hand capture were the primary capture methods, and most individuals were caught on the east and south sides of the island (Table 2, Figure 1).

3.2 Growth analysis

There were 91 *Ginglymostoma cirratum* recaptured ≥ 90 days apart used for analysis for a recapture rate of 10.69%. We removed 24 recaptures ≥ 90 days apart due to inconclusive or negative growth from human measurement error. Individuals used for analysis ranged from 48–252 cm TL at first capture (Supplementary Material). Time at liberty for the individuals caught ≥ 90 days ranged from 93–3,132 days (0.25–8.58 years, Figure 2) with an average number of days between captures of 702.30 days ± 610.18.

The MCMC chains mixed, indicating the model successfully converged (Supplementary Material). Autocorrelation was not present, so thinning was not applied to the model (Supplementary Material). The estimated vB growth parameters were $L_\infty = 303.28$ cm TL (95% credibility interval [CI]: 268.34 cm TL–348.13 cm TL) and $k = 0.04$ yr^{-1} (95% CI: 0.03 yr^{-1}–0.05 yr^{-1}) (Table 1). The 95% CI for σ^2 was 6.12 cm–8.28 cm TL (Table 1). The empirical annual growth rate for the 91 individuals was 8.68 ± 6.00 cm yr^{-1} (Figure 3). The change in length as a function of time at liberty for all 91 *Ginglymostoma cirratum* is shown in Figure 4A. Six individuals displayed growth rates between 19.21–29.07 cm yr^{-1} that ranged from 65.20 cm–215 cm TL at recapture. These recaptures

TABLE 1 Prior distributions and von Bertlanffy growth parameter estimates of *Ginglymostoma cirratum* near Bimini, The Bahamas.

Parameter	Prior Distribution	Mean or Minimum Bound	Variance or Maximum Bound	Reference	Parameter Estimates (95% Credibility Intervals)
L_∞	Lognormal	248.53	27.75	BBFSF catch data; Santander-Neto et al., 2011; Dureuil et al., 2022	303.28 cm (268.34 cm - 348.13 cm)
k	Uniform	0.01	1.00	Chen et al., 2007; Huveneers et al., 2013; Perry et al., 2018	0.04 yr^{-1} (0.03 yr^{-1} - 0.05 yr^{-1})
σ^2	Uniform	0.00	20.00	Santander-Neto et al., 2011; Dureuil et al., 2022	6.12 cm - 8.28 cm

Total Overall Captures by Gear Type		
Gear Type	Number of Individual *G. cirratum*	Median Total Length (cm)
Longline	468	215.00 ± 42.89
Male	264	218.00 ± 41.52
Female	194	211.00 ± 44.56
Unknown	10	220.00 ± 63.15
Hand Capture	151	57.75 ± 17.60
Male	76	55.10 ± 17.11
Female	73	62.30 ± 17.87
Unknown	2	82.20 ± N/A
Other	232	139.00 ± 56.99
Male	118	172.00 ± 60.00
Female	111	129.50 ± 49.97
Unknown	3	120.00 ± 57.74
Total	851	
Total Recaptures by Gear Type		
Gear Type	Number of Individual *G. cirratum*	Median Total Length (cm)
Longline	44	171.70 ± 41.54
Male	27	170.00 ± 44.25
Female	17	173.40 ± 38.11
Hand Capture	54	66.85 ± 14.90
Male	27	65.50 ± 13.68
Female	27	68.50 ± 15.96
Other	39	126.50 ± 55.78
Male	20	107.90 ± 53.21
Female	18	130.20 ± 53.67
Unknown	1	242.00
Total	137	

Information on gear type and the number of individuals caught per sex.

occurred 95–375 days (0.26-1.03 years) after initial capture. Figure 4B displays the distribution of growth rates among the individuals. The vB growth curve estimated from the MCMC analysis is shown in Figure 5. Estimated ages for *G. cirratum* ranged from 1–43 years.
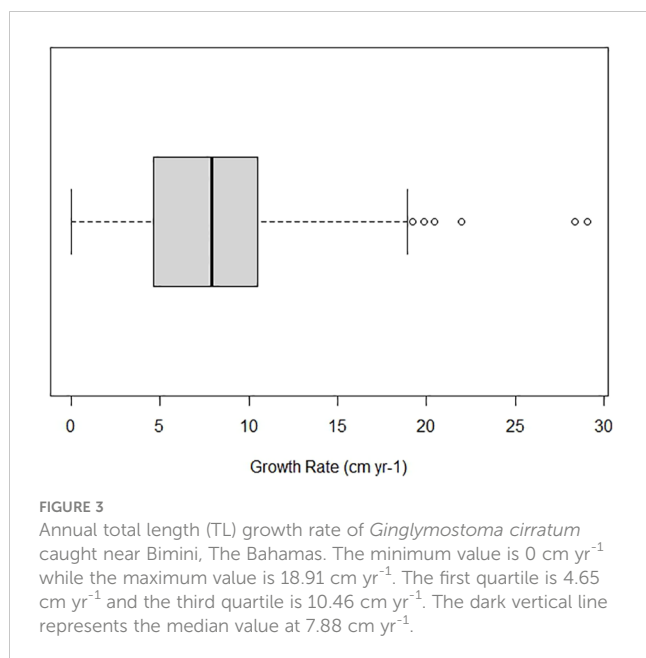
4 Discussion

This study used a relatively large sample size of recaptured *Ginglymostoma cirratum* from Bimini, The Bahamas, to provide the

FIGURE 2
Distribution of days between first and last capture for *Ginglymostoma cirratum* recaptured ≥ 90 days apart near Bimini, The Bahamas.
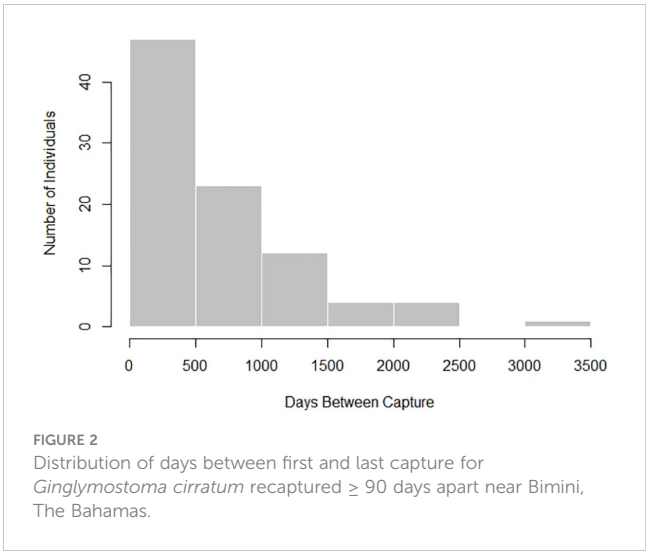
first vB estimates for this species. Previous studies looked at growth in *G. cirratum* (Carrier and Luer, 1990; Ferreira et al., 2012), but they did not obtain vB estimates and only an empirical growth rate that is not directly informative for fisheries assessment models (Flinn and Midway, 2021). Since their threat level has been reassessed from data deficient to vulnerable only recently (Carlson et al., 2021; Garzon et al., 2021), having vB estimates will provide valuable information for assessing vulnerability. Furthermore, these age and growth parameters for *G. cirratum* can be used to directly obtain other life history parameters, such as natural mortality (*M*), which are influential for stock assessments (Dureuil and Froese, 2021; Dureuil et al., 2021).

Our results are indicative of *G. cirratum* in Bimini being slow-growing and relatively long-lived like many other elasmobranch species. These life history strategies and their large body size would put them at a greater susceptibility to threats like overexploitation and habitat destruction (Dulvy et al., 2021; Wong et al., 2022). Fishing pressure may have affected *G. cirratum* in the earlier years of this study, but fishing for elasmobranchs is no longer permitted in this region with the establishment of The Bahamas Shark Sanctuary in 2011 (Sherman et al., 2018). They are not afforded these same protections across their range and are a target species in some countries (Garzon et al., 2021), potentially putting their populations at risk. More species-specific life history information is needed for *G. cirratum* from different regions to ascertain their regional susceptibility to overexploitation. Although overfishing no longer impacts *G. cirratum* in Bimini, habitat alteration could still affect their life history. Destruction of habitat known to be used by *G. cirratum* has been occurring in Bimini since 1997 (Pratt and Carrier, 2007; Carlson et al., 2021; Bettcher et al., 2023), which includes the construction of an extensive tourist complex (Gruber and Parks, 2002; Jennings et al., 2008; Trave and Sheaves, 2014). This could have detrimental impacts on *G. cirratum* in Bimini if it removes habitats that are essential to their survival since their life history strategies indicate that they may not have quick recovery potential (Cortés, 2000; Gallagher et al., 2012). The Florida Keys,

FIGURE 3
Annual total length (TL) growth rate of *Ginglymostoma cirratum* caught near Bimini, The Bahamas. The minimum value is 0 cm yr^{-1} while the maximum value is 18.91 cm yr^{-1}. The first quartile is 4.65 cm yr^{-1} and the third quartile is 10.46 cm yr^{-1}. The dark vertical line represents the median value at 7.88 cm yr^{-1}.
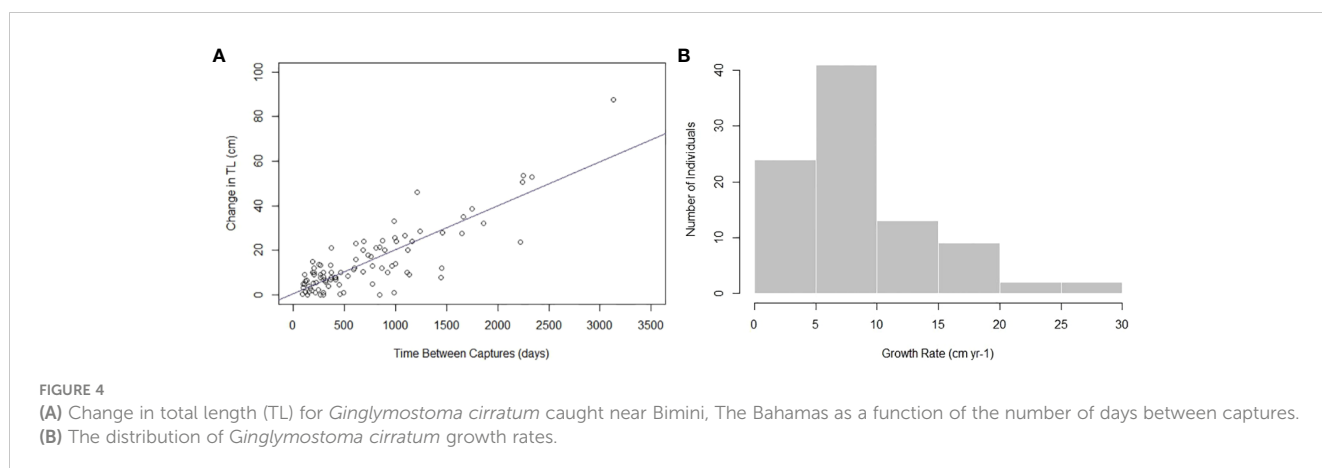
Florida, USA, where *G. cirratum* are also abundant, have a similar habitat structure and pressures to Bimini (Castro, 2000; Heithaus et al., 2007). This is notable because the Florida Keys and Bimini are close in proximity. Therefore, research on life history information from *G. cirratum* in this region could help better inform regional management and their response to anthropogenic disturbances.
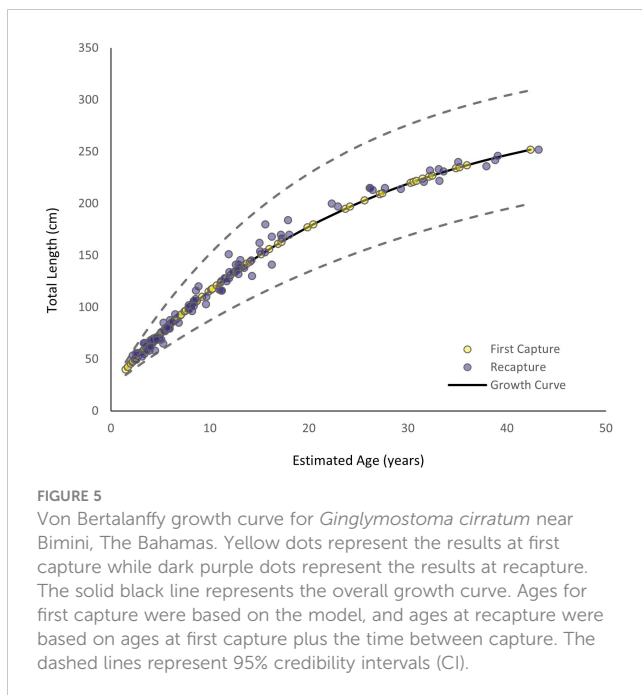
The largest *G. cirratum* recaught in this study for analysis was 252 cm TL, however, a female of 280 cm TL was caught a single time. *Ginglymostoma cirratum* have been reported at larger TLs in the Florida Keys, USA, at 312 cm and in Brazil at 316.8 cm (Castro, 2000; Santander-Neto et al., 2011). The estimate of L_∞ = 303.28 cm TL obtained for the Bimini *G. cirratum* population indicates that they may reach a smaller theoretical maximum length compared to other areas. Maximum size typically correlates with local habitat structure and latitude (Thorson et al., 2017), potentially contributing to the regional differences seen between the maximum sizes reported, especially since this species has the propensity for strong site fidelity (Carrier, 1985; Carrier and Luer, 1990; Chapman et al., 2005). However, individuals could reach larger lengths in Bimini due to the wide 95% credibility interval for L_∞ of 268.34 cm –348.13 cm TL and could have been underrepresented in our dataset.

The previously published maximum lifespan for *G. cirratum* was 25 years (Clark, 1963), however, a recent study from the Dry Tortugas in the Florida Keys, USA, reported observations of the oldest individual in their population being ~ 43 years and still reproductively active (Pratt et al., 2022). Our vB estimates predict that the oldest individual in our study was 43 years old, thus supporting the age observation from Pratt et al. (2022). Age data can be inconsistent and unreliable for many elasmobranchs, so although the ages are estimates from this study, it will help contribute to a better overall understanding of *G. cirratum* life history (Rudd et al., 2019). Accurate maximum age estimation is a key component of population modeling and important for effective management of species (Loefer and Sedberry, 2003; Brooks et al., 2016). Castro (2000)'s reported lengths-at-maturity for *G. cirratum* suggest they could be 20-30 years old at maturity based on our vB growth curve. Further research on age and age at maturity in this species is needed to support this claim.

Comparisons with other species are difficult to make for *G. cirratum*. They are the only orectolobiform that is a shallow, resident, coastal species in The Bahamas and across the Atlantic Ocean. Furthermore, species in the Orectolobiformes vary drastically in their morphology, with limited life history information available (Goto, 2001) and a great deal of variability for what data is available (Chen et al., 2007; Huveneers et al., 2013; Perry et al., 2018). General species information in their family, Ginglymostomatidae, is also severely lacking. However, based on numerous other age and growth studies, it is well-known that growth estimates can vary widely within orders and families. Because of this, future research must focus on obtaining species-specific life history information for *G. cirratum* from other regions to gain a more comprehensive understanding of their growth and to make biologically appropriate comparisons (Wong et al., 2022). Since our study is the first to provide age and growth estimates for *G. cirratum* or for the Ginglymostomatidae family, no comparisons of growth parameters are possible yet. Consequently, the empirical

FIGURE 4
(A) Change in total length (TL) for *Ginglymostoma cirratum* caught near Bimini, The Bahamas as a function of the number of days between captures. **(B)** The distribution of *Ginglymostoma cirratum* growth rates.

FIGURE 5
Von Bertalanffy growth curve for *Ginglymostoma cirratum* near Bimini, The Bahamas. Yellow dots represent the results at first capture while dark purple dots represent the results at recapture. The solid black line represents the overall growth curve. Ages for first capture were based on the model, and ages at recapture were based on ages at first capture plus the time between capture. The dashed lines represent 95% credibility intervals (CI).

growth rate from our study will be examined below to demonstrate why this measure is not as informative as vB estimates and can be problematic.

The average growth rate of 8.68 ± 6.00 cm yr^{-1} for *G. cirratum* in Bimini represents a diverse range of length classes, providing a comprehensive growth representation of the population (Haddon, 2011). Previous growth rate determination for *G. cirratum* only included sexually immature individuals (Carrier and Luer, 1990; Ferreira et al., 2012). The exclusion of adults creates disproportionate growth rates in favor of faster growth associated with early life stages (Francis and Francis, 1992). This could have been due to the different gear types used that can present bias towards certain capture lengths and impact estimates of growth rate (Gwinn et al., 2010; Emmons et al., 2021; Smart and Grammer, 2021; Smukall et al., 2021). Adults were included in our sample size, according to reported lengths-at-maturity from Castro (2000) and observations of calcified claspers from Bimini catch data. Growth rate can also fluctuate and vary regionally depending on changes in food availability, predation, and temperature (Hutchings, 2002; Thorson et al., 2017; Grimmel et al., 2020; Liu et al., 2021), even at very small scales (Dibattista et al., 2007), further complicating the validity of empirical growth rates from localized datasets being expanded for describing the overall growth in a population.

Our study included *G. cirratum* from a variety of length classes and used many gear types and tackles, likely presenting the most representative growth rate for this species to date. However, we advocate for future research to focus on determining vB growth estimates for *G. cirratum* because of the difficulties present in comparing empirical growth rates within species that was outlined in the previous paragraph. Although also influenced by size ranges and habitat influences (Cailliet and Goldman, 2004), the growth coefficient is a more informative measure than an empirical growth rate because it may be linked to longevity, fecundity, and

size at maturity (Mejia-Falla et al., 2014). Obtaining vB growth estimates using Bayesian methods additionally helps reduce biases, such as missing length classes and gear selectivity, because of the use of prior information (Pardo et al., 2016; Smart and Grammer, 2021). We were able to account for neonates in the vB estimates because of the L_0 parameter. This was missing for obtaining our empirical growth rate due to no recaptured individuals from this length class. Overall, future research should focus on obtaining vB growth estimates for *G. cirratum* to allow for more biologically appropriate comparisons.

Without previous life history information available for *G. cirratum*, their conservation and management were poorly informed before this study, demonstrated by their recent vulnerability status despite being an abundant species. Although afforded protections in The Bahamas, *G. cirratum* are subject to different anthropogenic threats across their range and accurate age and growth estimates from other regions do not yet exist. We urge future research to obtain species-specific age and growth estimates that can be used to make comparisons between populations from different regions and inform future stock assessments. This information is important since our results demonstrate that this species is slow-growing, large-bodied, and long-lived, characteristics that can make elasmobranch species more susceptible to anthropogenic threats.

4.1 Conclusions

This study presented the first vB estimates for *Ginglymostoma cirratum*, around Bimini, The Bahamas. It also determined the average growth rate for *G. cirratum* in this region and estimated ages based on length-increment data. The growth information resulting from this study indicated that *G. cirratum* are slow-growing, capable of reaching large sizes, and longer lived than previously thought. These results addressed a significant data gap for *G. cirratum*, contributing to a better understanding of their life history which can be incorporated into conservation and management decisions.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This study was over a long duration and across multiple surveys, therefore, beyond the scope of a single IACUC. However, the similar methodology for capture, handling, and data collection has been approved through IACUC protocols. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2024.1265150/full#supplementary-material

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