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New observations on the behavior, ecology, and biology of sharks and rays

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New observations on the behavior, ecology, and biology of sharks and rays

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Renewed occurrence of schooling scalloped hammerhead (*Sphyrna lewini*) and of great hammerhead (*S. mokarran*) sharks in the Cayman Islands

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The scalloped hammerhead shark *(Sphyrna lewini)*, a critically endangered species with a decreasing global population, is characterised by its occurrence in large schools. Such schools are still observed today in the Pacific Ocean, but this is generally not the case in the Atlantic Ocean, and in the Cayman Islands not since the 1970s. Here we report a recent record of a school of *S. lewini* in deep water off Grand Cayman, and describe a recent, concomitant increase in numbers of the species, and its critically endangered congener, the great hammerhead (*Sphyrna mokarran*), around the Cayman Islands. Relative population trends and seasonal patterns were assessed using data from shallow and deep-water BRUVS, scientific longlining, citizen science projects including the Sharklogger Network and REEF, and social media reports. It appears that *S. lewini* may be slowly re-occupying the area, selecting and using deeper waters to school, while *S. mokarran* has also become less scarce than hitherto.

KEYWORDS

hammerhead sharks, deep sea, Caribbean, BRUVS, citizen science, schooling

1 Introduction

Large hammerhead sharks are among the most critically endangered shark species globally (Rigby et al., 2019a, b). The scalloped hammerhead shark, *Sphyrna lewini*, has a maximum size of 370–420 cm total length (TL) (Ebert et al., 2013); females mature at 200–250 cm TL and males at 180–200 cm TL (Branstetter, 1987; Hazin et al., 2001). It is considered a coastal and semi-oceanic pelagic species (Moore and Gates, 2015). The species is currently classed in the IUCN Red List as critically endangered (CR A2bd), since the global population has undergone a steep decline, likely by >80%, and is severely fragmented (Rigby et al., 2019a). The decline is principally the consequence of being caught globally as both target and bycatch in pelagic commercial and small-scale longline, purse seine, and gillnet fisheries, in which it may be retained for both meat and fins (Rigby et al., 2019a). In the Northwest Atlantic and Gulf of Mexico *S. lewini* appears to have been overfished between 1983 and 2005, and in particular between 1983 and 1995 (Jiao et al., 2011). Since then, the population is showing signs of an increase in this area (Rigby et al., 2019a).

No stock assessment has been undertaken specifically for the Caribbean. However, in the western Atlantic Ocean, Chapman et al. (2009) found that breeding females remain close to or return to their natal area for parturition. Also from genetic work, Pinhal et al. (2020) suggested that population subdivision of *S. lewini* within the western Atlantic was a product of reproductive philopatry, rather than related to oceanographic or geophysical barriers. Alarmingly, they also estimated an effective population size of only 299 (215-412 CI) for this region and suggested the population's low genetic diversity may be partly related to the sharks' philopatric behaviour, in addition to overfishing (Pinhal et al., 2020).

The great hammerhead shark, *Sphyrna mokarran*, is larger than *S. lewini*, reaching 610 cm TL, and is also considered a coastal and semi-oceanic pelagic species (Rigby et al., 2019b). It is thought to mature at 224 cm for females and 187 cm for males (Piercy et al., 2010). It occurs globally in tropical and warm temperate seas to depths of 300 m and is currently classed as critically endangered (CR A2bd), having experienced steep population decline, again most likely by >80% through most of its range (Rigby et al., 2019b). Like *S. lewini* it has been caught both as target and bycatch in coastal and pelagic fisheries, but is more often retained for its fins, which are larger than those of *S. lewini* (Rigby et al., 2019b). This species appears more sensitive to the stress of capture with a high post-release mortality rate (Gallagher et al., 2014a).

While *S. mokarran* is almost always encountered as solitary individuals (Miller et al., 2014), *S. lewini* can be encountered as solitary individuals, in pairs or in schools (Miller et al., 2013). *S. lewini* is also characterised by occurring in large aggregations (Harned et al., 2022), particularly at oceanic seamounts (Klimley, 1993). Large aggregations continue to be documented in the Pacific Ocean (Ketchum et al., 2014; Aldana-Moreno et al., 2019; Bravo-Ormaza et al., 2023), including in shallow waters (Lòpez et al., 2022) and near known nursery areas (Brown et al., 2016). In the Atlantic, however, there have been no recent records of aggregations except for an oblique reference to an aggregation in the Gulf of Mexico by Hoffmayer et al. (2013). Notably, there have been no recent sightings of schools of *S. lewini* in the Cayman Islands (western Caribbean) where until the 1970s large schools were regularly encountered by scuba divers at particular sites (MG, pers. comm.).

Given the recent paucity of records of schools of *S. lewini* or of *S. mokarran* in the Atlantic Ocean, we took advantage of a recent empirical video survey recording of a school of *S. lewini* off Grand Cayman made during our ongoing monitoring of sharks in the Cayman Islands. We combined this with other sources of data, including ongoing monitoring of sharks using BRUVS and citizen science reporting (Gore et al., 2020; Kohler, 2022), to examine whether in recent years there has been any evidence of a recovery in numbers of either species. This question was pertinent because, following detailed studies of the more common shark species in the Cayman Islands since 2009 (Ormond et al., 2017; Kohler, 2022), in 2015 all elasmobranchs were given full protection throughout Cayman waters [National Conservation Act, 2013], effectively establishing Cayman Islands territorial waters as a shark sanctuary. In this context marine environment managers were concerned to establish whether species were benefitting from this measure.

2 Materials and methods

2.1 Study area

The study area covered the three Cayman Islands - Grand Cayman (19.344°N, 81.252°W), Little Cayman (19.688°N, 80.044° W) and Cayman Brac (19.721°N, 79.796°W) - which are located on the Cayman Ridge in the centre of the western Caribbean Sea, only 37km to the north of the 7km deep Cayman Trough (Figure 1). For further description of the reefs and coastal zone see Ormond et al. (2017). The isolated location of the islands within the Caribbean and the close proximity and thus connectivity of shallow and very deep coastal waters, provides an unusual habitat for large marine vertebrates (see Gore et al., 2020).

FIGURE 1
Map showing the location of the Cayman Islands, Cayman Trough and Cayman Ridge within the wider Caribbean region. The white rectangle shows the position of the Cayman Islands, with the inset showing the relative positions of Grand Cayman and the Sister Islands (Little Cayman and Cayman Brac). Main map: reproduced with permission from Esri, USGS | Esri, TomTom, FAO, NOAA, USGS | Sources: Esri; Garmin International, Inc.; U.S. Central Intelligence Agency (The World Factbook); National Geographic Society. Map of the Cayman Islands: reproduced with permission from Department of Environment, Cayman Islands Government.

2.2 Baited remote underwater video system

Between 05 Nov 2009 and 23 Nov 2018, 1473 shallow-water BRUVS were deployed at a series of standard stations approx. 0.5 - 2km apart and up to 20m deep on reefs around large parts of all three islands, though most regularly around Grand Cayman and Little Cayman (see Kohler, 2022). The earliest surveys employed Sony Handycam video cameras enclosed in waterproof housings attached to a heavy metal frame that also carried a bait arm to the end of which was attached a mesh bag containing bait. The bait bag was also used to estimate total length of individuals, which was recorded along with the species and sex. Ebert et al. (2013) was used to determine maturity for all measurements used in this study. When GoPro cameras (gopro.com; Hero3 and Hero4) became available these replaced the Handycams and were set to run for 2 hours, while a lighter weight frame replaced the previous heavy one (see Gore et al., 2020).

In addition, between 08 Mar 2022 and 17 May 2023, as part of a deep-water survey of shark and fish biodiversity, 154 deep-water BRUVS were deployed to record species occurrence and abundance at depths of 50, 100 and 200m around the north-west, west and south-west of both Grand Cayman and Little Cayman. These BRUVS used GoPro Hero4 cameras placed inside GroupB housings (www.GroupBinc.com) attached to a lightweight frame to which a bait arm with bait-bag was also fixed. As with the shallow deployments, cameras for the deep-water surveys were set to run for 2 hours. Following retrieval for both sets of BRUVS, the video recordings were annotated by a first reviewer, with at least 25% of recordings being re-examined by a second reviewer. Identification of hammerhead species was independently checked by at least three authors.

2.3 Scientific longlining

Scientific longlines, consisting of 30 baited circle hooks hung 2m below the surface from a 500m long buoyed line were deployed on 275 occasions at suitable sites around the whole of both Grand and Little Cayman, to catch sharks for scientific tagging. The line was monitored every 30min and any fish caught removed promptly. Sharks were quickly identified and measured and any *Sphyrna* sp. quickly released without tagging (see Ormond et al., 2017).

2.4 Citizen-science

Data from two citizen-science programmes, that run by the Reef Environmental Education Foundation (REEF, a recreational diver-observation programme, www.reef.org/programs/volunteer-fish-survey-project) and the Cayman-based Sharklogger Network (a local observer programme, see Kohler, 2022) were examined for any records of *Sphyrna* species between 01 Jan 1993 and 31 Mar 2023 (REEF) and 01 Jan 2017 to 31 Dec 2018 (Sharklogger) for each of the three Cayman Islands. The 365 official recreational dive sites used by the citizen science reporters are distributed around all three islands, their locations being shown in Figure 4.1 in Kohler (2022). The numbers of both *Sphyrna* species observed were extracted and the number of hours of survey also recorded to provide a measure of the effort involved. Observers in both programmes are trained to identify and distinguish between species and sex and to estimate total length.

2.5 Social media and government database

Data from our project's Facebook site "Sharks and Cetaceans: the Cayman Islands" and from the Cayman Islands Department of Environment's (DoE) Sightings Reporting Scheme for large marine vertebrates were reviewed. Data were also gleaned from our "#SpotThatFish" where photos of fish, including sharks, could be uploaded by contributing divers and photographers. Such social media tools not only assist researchers by extending their data gathering, but as we have experienced, also provide an opportunity to raise awareness of related conservation issues with the interested public. Duplicate sightings from the same area or dive and the same time of day were discarded. Since data collected through citizen science does rely on the skill of the observer, a "certainty index" was employed to categorise the reliability of a report, ranging from 1 (very unlikely) to 5 (very likely). Usually the *Sphyrna* genus is easily recognised as such given their characteristic cephalofoils, however the species can be more difficult to distinguish without relevant experience or observational skills. No effort data were reported by members of the public posting on these social media sites, the observations being largely opportunistic.

3 Results

3.1 Baited remote underwater video systems

Data from the shallow-water BRUVS surveys undertaken between 05 Nov 2009 and 23 Nov 2018 off Grand and Little Cayman, representing 2164.5h of seabed time, included a total of eight *S. mokarran* but no *S. lewini* (Table 1). Observations of *S. lewini* on deep-water BRUVS were made on 6 occasions, with a total of 18 individuals recorded. Of these, one was of a group of at least 11 *S. lewini* swimming at a depth of >200m adjacent to a vertical wall off North Sound, Grand Cayman on 26 Mar 2022. While individual's total lengths were difficult to estimate, all were identified as juveniles. These individuals swam close together, moving horizontally across the face of the wall. No other fish were observed while these sharks were visible. There was also one *S. mokarran* recorded on a deep-water BRUVS on a separate occasion.

3.2 Scientific longlines

Scientific longline surveys (Table 1) were undertaken between 05 Nov 2009 and 12 Dec 2016 off Grand and Little Cayman, with a total soak time of 875h, resulting in two *S. mokarran* briefly

TABLE 1 Hammerhead sharks recorded within the various datasets from the Cayman Islands.

Method	Period Start & End	Surveys	Hours	Numbers & Events Recorded		
		/Reports		S. lewini Number Event	S. mokarran Number Event	Sphyrna sp. Number Event
Shallow-water BRUVS	05/Nov/2009	1,473	2,164.5	0	8	0
	23/Nov/2018			0	8	0
Deep-water BRUVS	08/Mar/2022	154	467	27	3	0
	17/May/2023			13	13	0
Scientific longlines	05/Nov/2009	275	875	0	2	0
	12/Dec/2016			0	2	0
REEF Reports	01/Jan/1993	10,807	11,376	2	4	0
	31/Mar/2023			2	4	0
Social Media	29/Jun/1996	364	not available	9	52	20
	12/Mar/2023			9	53	20
Sharklogger Network	01/Jan/2017	24,442	20,536	0	0	103
	31/Dec/2018			0	0	103

The table shows the start (top row) and end (bottom row) dates of each survey period, the number of surveys, the total observation times in hours, and the numbers of individual sharks (top of number pair) and events (bottom of number pair) for each of the datasets: shallow and deep-water BRUVS, scientific longlines, citizen science reports (REEF, 2023), social media (Facebook and DOE sites) and Sharklogger Network reports.

captured, measured and released (in addition to sharks from other genera).

3.3 Citizen science

Data from REEF (Table 1) indicated that in 10,807 surveys over 11,376 hours were conducted in the three Cayman Islands between 1993 and the end of March 2023 (29 years), resulting in two *S. lewini* and four *S. mokarran* sightings (Figure 2). The local Sharklogger Network recorded observation of *Sphyrna* sp. in all three Cayman Islands in 2017 and 2018 (Kohler, 2022). They were observed largely below 40m off the coastal wall and were relatively abundant. Analyses showed that divers reported seeing 0.004 sharks per dive, with 66% of the individuals observed considered mature (n=24,442 dives) (Table 1).

3.4 Social media data

From the social media platforms (see Section 2.5) analysed (Table 1), nine *S. lewini*, 53 *S. mokarran* and 20 undetermined *Sphyrna* sp. were reported between the end of 2009 and mid 2023 (Figure 2). All reports were of single sharks, except for one sighting of two *S. mokarran* swimming together.

3.5 Temporal trends

The numbers *S. lewini* and *S. mokarran* recorded annually from all sources between 1993 and 2023 (except Sharklogger) are plotted in Figures 2A, B respectively. There were no records of *S. lewini* between 1993 and 2008, after which a few were sighted in most years with slowly increasing frequency (Figure 2A) and significant

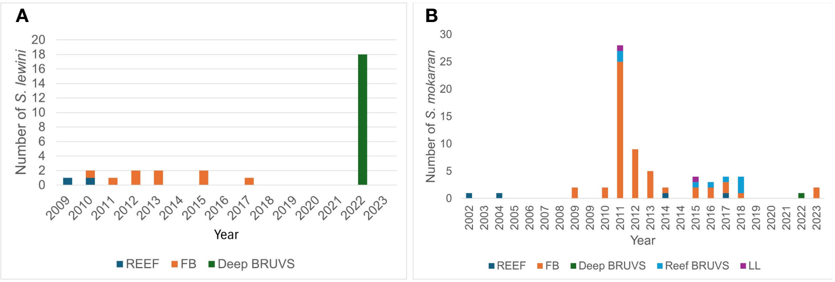

FIGURE 2
The total numbers of *S. lewini* **(A)** and *S. mokarran* **(B)** recorded by all data sources for each year from 1993 – 2023. The colour coding represents the survey methods.

variation across years from 2001 (χ^2 = 253.7 <0.001; df = 22)). The large school reported here was detected in 2022. For *S. mokarran*, there were single sightings in both 2002 and 2004, and occasional sightings each year from 2009 to 2018 (n=62 with a peak in 2011) and in 2022 (n=3). There was no upward trend in sightings of *S. mokarran* from all sources (Figure 2B), but there was a marked peak in 2011. and significant variation in numbers of sightings across years (χ^2 = 270.1; p<0.001; df = 22). The increases in number were not related to any increase in the number of BRUVS deployments or dives reported by citizen science participants.

Variation in the numbers of *Sphyrna* species recorded with month of the year is shown in Figure 3 (no data for Sharklogger). Number of *S. lewini* recorded varied from zero to 13 per month, the numbers varying significantly across the year (χ^2 = 58.3; p <0.001); there were possible peaks in spring (March/April) and autumn (September/October), but none recorded in August, December or January (Figure 3A). For *S. mokarran*, numbers were higher in late winter (January/February) and lowest in the period August to November, with none recorded in August (Figure 3B), but any variation between months was not statistically significant (χ^2 = 18.8; p >0.05).

3.6 Spatial trends

Reports included the island on which *Sphyrna* sp. were recorded, as well as occasionally information on maturity, size and sex, noting that these variables were used with parsimony. These data are shown in Table 2. The majority of *S. lewini*, *S. mokarran* and unidentified *Sphyrna* sp. were reported from Grand Cayman, where the human population and hence the numbers of participating observers was much higher. 78% (n=9) of *S. lewini* sexed and 33% of *S. mokarran* sexed (n=9) were identified as female. One 1.2m juvenile was reported for *S. mokarran* and also notably a neonate as *Sphyrna* sp. (likely *S. mokarran*), both in June.

4 Discussion

The data from a number of sources presented here reveal broadly consistent patterns during this study period for the occurrence of both scalloped (*S. lewini*) and great hammerhead (*S. mokarran*) sharks throughout the territorial waters of the Cayman Islands. There was one reported sighting of *S. zygaena* (Facebook site, see Section 2.5), but we consider this identification uncertain. Through the study period there were less than half as many records of *S. lewini* as of *S. mokarran*. This difference is greater if our recent records on the deep-water BRUVS are excluded, since otherwise only two certain sightings of *S. lewini* were recorded by REEF and six on social media. *S. mokarran* were recorded comparatively frequently by divers, recorded on BRUVS or caught on longlines in shallower water. In contrast, our deep-water BRUVS recorded a number of *S. lewini* but only three *S. mokarran*.

4.1 Depth range

The literature indicates that *S. lewini* exploit a wide range of depths, mostly between 0 and 275 m (Moore and Gates, 2015), with the maximum recorded depth being 1240 m (Anderson et al., 2022). For example Hoffmayer et al. (2013) satellite tagged a 240 cm (TL) female *S. lewini* off the Mississippi River and monitored her for 27 d, during which time she was consistently between 0 and 228 m during the day, but from 0 to 964 m at night, when most dives were to >700 m. In the western Gulf of Mexico, Wells et al. (2018) found that 33 satellite tagged *S. lewini* preferred mid to outer continental shelf within a 200 m isobath. The school of *S. lewini* off Grand Cayman were observed during the day at >200 m and occasional individuals were also recorded in shallower water by day. The sharks were swimming in a direct manner suggesting potential foraging behaviour, but these individuals may also forage at greater depths by night.

4.2 Spatial range

In general, female *S. lewini* appear to move regionally, but not between discontinuous continental coastlines (Gallagher and Klimley, 2018), whereas males will cross deep ocean (Duncan, 2006; Daly-Engel et al., 2012). Wells et al. (2018) found that *S. lewini* in the western Gulf of Mexico did not disperse over long

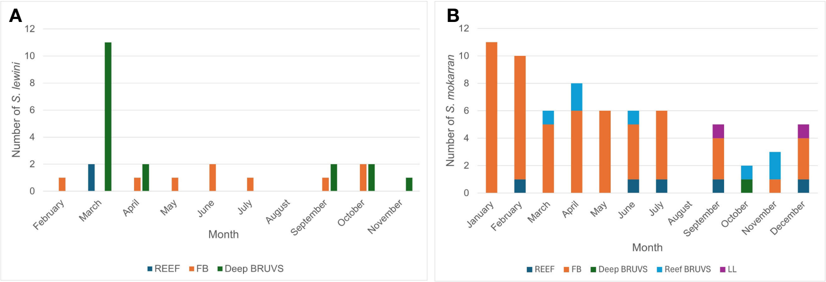

FIGURE 3
The number of *S. lewini* **(A)** and of *S. mokarran* **(B)** recorded in each month of the year across all years and from all data sources. The colour coding represents the survey methods.

TABLE 2 Location, maturity, size (TL m) and sex (where determined) for *S. lewini*, *S. mokarran* and *Sphyrna* sp. recorded in the Cayman Islands from all data sources.

	Adult	Adult-Sub-adult	Juvenile	Female	Male	Size range m	GC	LC	CB
S. lewini	2	5	11	7	2	2.4-2.7	19	2	2
S. mokarran	17	8	1	3	6	1.2-4.3	38	3	1
Sphyrna sp.	105	67	18	5	0	0.2-4.6	82	23	0

(GC, Grand Cayman; LC, Little Cayman and CB, Cayman Brac). Additionally, there was a neonate 0.2m *Sphyrna* caught and released on Grand Cayman.

distances, females tending to associate with shelf edge and males with mid-shelf areas. Nalesso et al. (2019) tagged 84 *S. lewini* at Cocos Island, on the Pacific coast of Costa Rica, and established that these sharks generally showed strong residency. However, Estupiñán-Montaño et al. (2017) reported that *S. lewini* used the area of Malpelo Island, Columbia, for resting and cleaning, but fed far away from that site. This raises the question of whether the Cayman Islands' *S. lewini* are from a distinct stock, or whether they move more widely around the western Caribbean.

The Cayman Islands are placed in a relatively isolated location in the centre of the western Caribbean and largely surrounded by very deep water, with the Yucatan Basin to the north and the Cayman Trough to the south. If the conclusion that females do not normally move between discontinuous continental coastlines (Duncan, 2006; Daly-Engel et al., 2012) is accurate, then this location makes it plausible that the 1970s schools were likely reproducing in the Cayman Islands, as well as feeding there. However, a shallower ocean ridge (~500-1500m deep) runs east-northeast from the Cayman Islands towards south-west Cuba and its adjacent small islands and this might provide a route via which sharks breeding in the Greater Antilles could reach Cayman without swimming over very deep water.

4.3 Cayman Islands *S. lewini* population

The pattern of sightings of *S. lewini* since the 1990s (Figure 3A) suggests that the species went unrecorded in the Cayman Islands during the first half of that period. Our data apart, no schools of *S. lewini* were reported by technical divers (pers. comm. Jo Mikutowicz, DiveTech), or from dives in submersibles run by Atlantic Adventures to 30m depth from 1985, or in dives in submarines run by Atlantis Deep Explorers to 100m between ca. 1983 to 2003. However, a small number of *S. lewini* appear to have been visiting the area (or possibly to have become semi-resident) since about 2009. It should however be noted that the platforms for public reporting only became available in 2009 and the public response may have been slow to respond until the platform became better known. Equally, a lull in public reports from about 2018 may reflect the fact that the platforms, though still functional, were not being so actively promoted after they became established. Nevertheless, the absence of *S. lewini* before about 2009, and the small numbers observed since, contrast with historical accounts of diving in the Cayman Islands in the 1970s, when divers described being surrounded by large schools of over 100 *S. lewini* (MG, pers. comm.).

The loss of the schools of *S. lewini* observed by divers in Cayman through the 1980s is consistent with the overexploitation of this species elsewhere, since *Sphyrna* species seem highly vulnerable to anthropogenic exploitation, partly due to their highly specialised ecology and behaviour (Gallagher et al., 2014b). As noted by Lòpez et al. (2022), large aggregations of *S. lewini* may be targeted by fishers, resulting in severe overfishing. They are also sensitive to other fisheries impacts, Zhang et al. (2022) for example reporting the significant numbers of *S. lewini* discarded as bycatch by a bottom-longline fishery in the southern US Atlantic and Gulf of Mexico between 2005 and 2019. Further, Morgan and Burgess (2007) found that the annual survival rate for tagged *S. lewini*, caught on bottom longlines set for sharks between New Jersey and Louisiana, USA, was only 8.6% for all stages of maturity. There was no known targeted exploitation of hammerhead sharks in the Cayman Islands, even though the Cayman Islands supported a traditional shark fishery that is understood to have mainly fished for sharks along the coasts of central America from the 1930s (Zeller and Harper, 2009). However, *S. lewini* could easily have been subject to capture in targeted fisheries or as bycatch in other fisheries elsewhere in the Caribbean, assuming individuals were crossing into neighbouring regions.

This newly recorded *S lewini* school in Cayman could either represent a recently protected population that is recovering from a few individuals that survived previous exploitation, or it could represent individuals from a neighbouring stock that have expanded into now vacant Cayman habitat. Re-occupation of a species range following conservation measures has been reported in some other large marine vertebrates, for example with humpback whale (*Megaptera novaeangliae*) in NE Brazil (Rossi-Santos et al., 2008) and in the Chukotka Peninsula (Melnikov, 2019).

It would be useful to determine whether the *S. lewini* now observed in Cayman are breeding there, or only feeding. The numbers of observations of *S. lewini* in the Cayman Islands are distributed through much of the year, but most frequent in spring and autumn, supporting potential seasonal visits by the species. While the timing of parturition has not been recorded in the Caribbean, it has been noted that off Cape Canaveral, Florida, USA, neonates are found in May and June, generally in water <11 m deep (Adams & Paperno, 2007). The higher number of sightings in Cayman from March/April could be related to reproductive behaviour. Alternatively, the greater numbers in spring could be related to the occurrence the spawning aggregations (SPAGs) of grouper and snapper that occur at that time of year at traditional sites off the east or west end of the islands (Whaylen et al., 2004).

4.4 Cayman Islands *S. mokarran* population

In contrast, *S. mokarran* appears to be present in Cayman throughout the year, except perhaps in August (Figure 3B). This strongly suggests that the species may be resident on the islands, even though *S. mokarran* is more usually described as a nomadic, seasonally migratory species (Miller et al., 2014). While a generalist feeder on fishes and crustacea, it is also considered a specialist at feeding on other sharks and rays, especially stingrays (Raoult et al., 2019). It is thus possible that the abundance of stingrays (*Hypanus americanus*) around the western part of Grand Cayman, particularly around the popular tourist location known as "Stingray City", (see e.g. https://en.wikipedia.org/wiki/Stingray_City,_Grand_Cayman) may support their year-round presence. Perhaps our most dramatic record of *S. mokarran* in the Cayman Islands is an image taken from the air by a local helicopter operator (J. Begot) of a *S. mokarran* chasing down and capturing a large stingray, not far from the Stingray City site. These *S. mokarran* appear to ingest the stingrays over a period of time, biting one wing on one occasion, another later on and finally the remaining body (pers. comm. J. Begot).

The observation of a neonate *Sphyrna* sp. (probably *S. mokarran*) also suggests the species breeds there. *S. mokarran* females are thought to breed once every two years, giving birth from late spring to summer in the northern hemisphere and from December to January in Australia (Rigby et al., 2019b). If the gestation period is 11 months, as generally assumed (Bester, 2008), the apparent absence of the species in August could perhaps be due to movement of individuals away from the reefs to preferred mating grounds, although this would imply pupping of *S. mokarran* in Cayman would take place in mid-summer, rather than spring. A report by a local resident (from outside the present dataset) described a hammerhead shark coming into a sound on Little Cayman on a seasonal basis, and apparently pupping; most likely this individual would have been a *S. mokarran*.

4.5 Protection and management

The return of a school of *S. lewini* to the Cayman Islands, and the evidence for the persistence locally of *S. mokarran*, are both significant for the conservation of these two critically endangered species. As noted above, there are indications that, following the introduction of management measures in Cayman and notably the inclusion of hammerhead sharks under the US Endangered Species Act, the population of *S. lewini* in the Northwest Atlantic and Gulf of Mexico region may be showing signs of stablisation (Rigby et al., 2019a). Likewise, it has been concluded that the population of *S. mokarran* in the same region may be slowly increasing (Rigby et al., 2019b), even though this trend does not have appear to in continued within the Cayman Islands over the least 5-10 years. In the Cayman Islands, not only do Marine Protected Areas (MPAs) now cover about half of the coastal shelf (to a depth of 46m) of the three islands, but all Cayman waters are in principle protected from fishing for sharks. The coastal MPAs should have afforded protection to any female *Sphyrna* pupping in the sounds, but it is not known with certainty how effective has been the prohibition on catching sharks elsewhere. Occasionally, a shark is caught as bycatch by inshore fishers; sharks are required to be released unharmed, although not all may survive the stress of capture. In addition, a small number of boats from Honduras were known to fish offshore Cayman waters for sharks prior to the prohibition, although they have not been recorded doing so since sharks were given protection. Nevertheless it seems likely that marine conservation measures in the Cayman Islands have assisted in the tentative recovery of these two hammerhead species.

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 Heriot-Watt University Ethics Committee. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

MG: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. JK: Data curation, Investigation, Methodology, Writing – review & editing. RO: Conceptualization, Funding acquisition, Visualization, Writing – original draft, Writing – review & editing. AG: Visualization, Writing – review & editing. TF: Funding acquisition, Writing – review & editing. TA: Funding acquisition, Writing – review & editing. CP-S: Data curation, Formal analysis, Funding acquisition, Writing – review & editing.

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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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Novel behavioral observations and body scarring for the bluntnose sixgill shark (*Hexanchus griseus*) offer clues to reproductive patterns and potential mating events

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Introduction

There are significant calls to scale efforts to advance our understanding of fundamental biological, ecological, and taxonomic information in the deep-sea to inform conservation and decision-making (Howell et al., 2020). There is growing interest into ocean depths between 200-1,000 m, building on recent research which suggested this depth band may contain up to 10 times the global fish biomass in all other marine habitats combined (Irigoien et al., 2014). A rich diversity of large marine predators is commonly found throughout this portion of the vertical water column, inspiring questions about the functional and ecological significance of this habitat (Braun et al., 2022).

The bluntnose sixgill shark (*Hexchanchus griseus*) is one of the largest species of deep-sea elasmobranchs (Barnett et al., 2012), reaching a confirmed total length of 482 cm (Bolivar, 1907), but with an unconfirmed report of 550 cm total length (Ebert and Compagno, 2012). The species is found circumglobally within tropical, temperate, and boreal latitudes (Finucci et al., 2020). While the species is known to have extremely large litters (47-108 pups, Ebert and Compagno, 2012), research also suggests a late age-at-maturity (females = 26.5 years; COSEWIC, 2007) and size at maturity (females at or near 400 cm TL; Ebert, 2002). The combination of these life history traits limits their population rebound potential, thus reducing their ability to sustain high levels of exploitation (Finucci et al., 2020). Due to its size and catchability, *H. griseus* is one of the better-studied deep-sea elasmobranchs, with a growing body of knowledge into its habitat use (Brooks et al., 2015; Comfort and Weng, 2015; Coffey et al., 2020) and trophic ecology (Reum et al., 2020). However, many aspects of *H. griseus* biology remain cryptic. For example, there is limited information on mating events, and thus the potential locations of reproductive grounds remain mostly speculative across its range (e.g., Ebert, 2002; Amor et al., 2017). The recent emergence of compact, innovative deep-sea observation platforms, such as video-equipped landers, is now rapidly improving our ability to detect rare deep-sea shark species' biodiversity and new record behaviors performed in the deep ocean (e.g., Phillips et al., 2019; Gallagher et al., 2023).

Here we present rare observations of *H. griseus* from The Bahamas, including reports of body scarring, which provides some of the first clues to the spatial and temporal basis for mating behavior in a deep-sea elasmobranch. Given the challenges of determining where potential reproductive behaviors take place for deep-sea vertebrates, these observations also highlight how non-invasive remote lander video systems can provide timely information on critical habitat for poorly known species of deep-sea elasmobranchs.

Materials and methods

Compliance with ethical standard

The care and use of experimental animals complied with the Bahamas animal welfare laws, guidelines and policies as approved by the local government agencies. The authors declare no conflicts of interest. Research was conducted under permits from the Dept. Environment Protection and Planning and Dept. Marine Fisheries, Bahamas Government. This study used non-invasive visual observations of *H. griseus*, therefore animals were not experimentally manipulated in any way.

From 4 – 6 November 2021, two different types of custom, free-falling, deep baited remote underwater video systems (hereafter dBRUVs) were deployed 5 - 20 km northeast of Highbourne Cay, northwest Exuma Sound, The Bahamas (24.73405, -76.81480; Figure 1). The Exuma Sound is a deep-sea inlet of the Atlantic Ocean, where highly productive, neritic systems rapidly transition into deeper slopes that extend to ~2,000 m in the central region of the Sound (Buchan, 2000).

The first dBRUV system (System A) (Giddens et al., 2021) consisted of a single Ultra-High Definition (HD) camera (Sony Handycam FDR-AX33) housed in a borosilicate glass pressure sphere (Vitrovex, NautilusMarine, GmbH). The sphere was situated above a stainless-steel detachable bottom shaft (41 cm long) that held the bait canister, and burnwire release mechanism attached to a temporary anchor. The pressure housing (33 cm diameter, 1.2 cm thick) housed the Ultra-HD (3840 x 2160) 4K video camera with 20.6 megapixel still image capability, fixed at a 45 declination from the horizontal plane. External high efficiency LED lighting (Cree XLamp CMT1930 LED), with two lights each placed 318 mm from the center of the sphere and angled downward at 45 degrees from the horizontal plane, provided an illumination of 1530 lm and a color temperature of 4000 K. A bank of 14.4 V, 2.6 Ah lithium-ion batteries provided 337 Wh for the camera and lighting system. A single waterproof bulkhead (BH10FTI, SubConn), externally located on top of the glass sphere, provided an electrical connection for battery charging and data retrieval. Depth was monitored using a boat-based echosounder.

The second dBRUV system (System B) consisted of a vertically oriented, carbon-fiber frame with pressure-tolerant flotation and an acoustic weight-release system (Phillips et al., 2019; Gallagher et al., 2023). A single GoPro Hero6 camera (GoPro), set to record 1080p video at 60 frames per second, was secured within a deep sea housing (GoBenthic, GroupB Incorporated, USA) and attached to the frame ~1.5 m above the bottom of the unit. Two potted LED lights were used to illuminate the seafloor (SiteLite, Juice Robotics, USA), and the cameras and lights were powered with a custom lithium-ion battery pack. Temperature and depth were monitored using a calibrated Starmon TD stand-alone logger (Star Oddi, Iceland).

A small amount of bait (bonito, *Sarda* spp.; 500 g) was used in both dBRUV systems to attract large marine predators and other marine life: in system B the bait was attached to a pole put in clear view of the camera; in System A the bait was placed into a perforated, 10 cm PVC tube that was out of the view of the camera. Each day, up to four dBRUVs (a maximum of two units per system) were deployed simultaneously every 1.5-2.0 km at depths between 700 – 1,100 meters, using an expendable drop weight of 20 kg. In System A, a burn wire was triggered to release the expendable weight at a pre-programmed time (5 hours). In System B, an acoustic release system (PORT LF-SD, EdgeTech, USA) was used to release the drop weight, allowing for the entire system to return to the surface upon command, resulting in deployments of 6-7 hours (as they were deployed and retrieved sequentially after System A). All dBRUVs were then located and retrieved at the surface using boat-based GPS unit and a VFH radio receiver unit (R410, 150-160 MHz; Advanced Telemetry Systems, USA). Upon retrieval, media cards from the cameras were downloaded and the footage was scanned. The presence of any large sharks was noted, as was their total time on camera, the number of passes they performed (*sensu* Shea et al., 2020), while noting biological and morphological details.

To further contextualize these findings, we undertook an extensive review of the published peer-reviewed scientific literature to quantify the relative number of studies that have used remote video camera systems to study *H. griseus* biology. Studies published prior to February 14th 2022 were included, and categorized as either behavioral, demographic, methodological, or locality record.

Results

Video data were collected from 11 deployments along the continental shelf break of the northwest Exuma Sound (Table 1), at depths up to 1110m. This resulted in a total of 3151.1 minutes (52.5 hours) of footage (286.5 ± 16.5 min, mean ± SD). dBRUVs

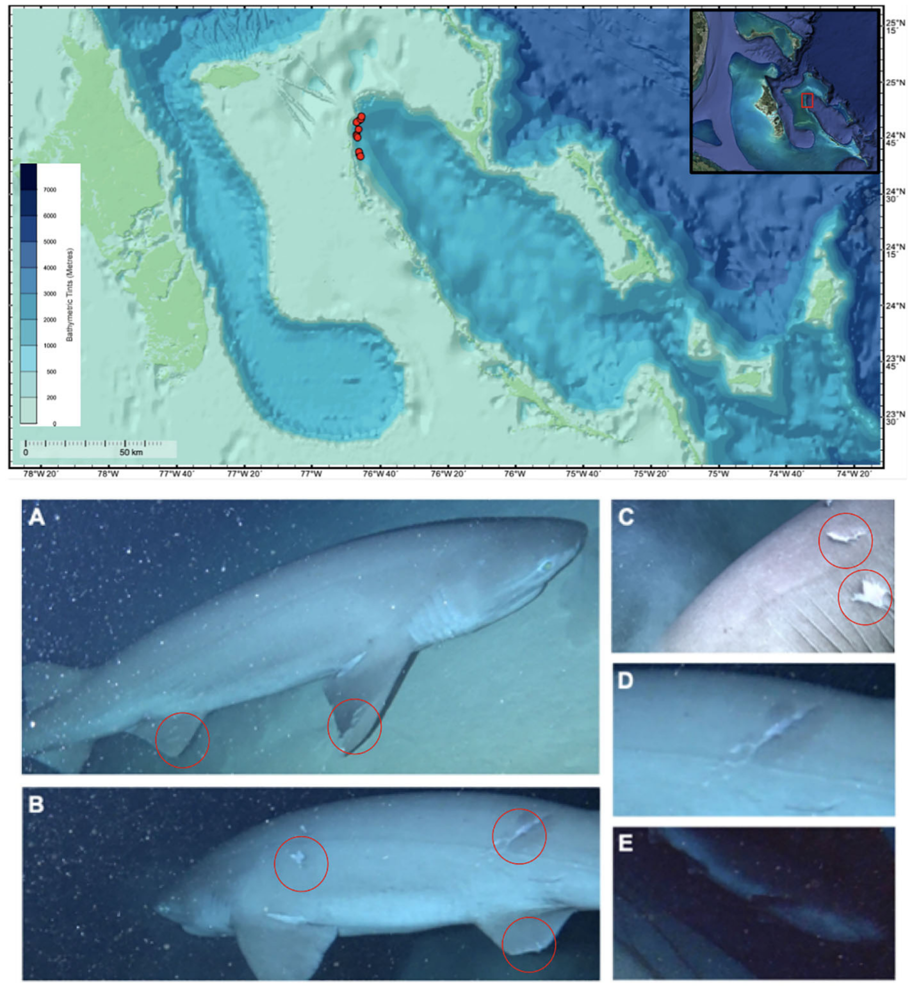

FIGURE 1
Top panel: Map of dBRUV deployments (red circles) in the Exuma Sound, The Bahamas, from the present study (with The Bahamas inset). Bottom panels: Hexanchus griseus recordings obtained from dBRUVs in the present study: **(A, B)** Large female sixgill shark bearing evidence of potential mating attempts on pectoral and pelvic fins (red circles), as well as on the body. **(C)** Tooth puncture marks from the same individual, indicative of distinctive H. griseus dentition (Corn et al., 2016), with **(D)** deep lacerations and bruising around the puncture site suggestive of recent (i.e., weeks) biting activity. **(E)** Unique individual male six gill shark. All individuals were visually estimated to be between 3.0 and 4.0 meters in total length sensu Gallagher et al. (2023).

recorded footage from 09:14 AM to 12:09 PM, at depths ranging from 876 – 1017 m (981.6 ± 61.0 m, mean ± SD). Of the 11 total dBRUV deployments, three individual *H. griseus* (2 females, 1 male) were observed independently on three deployments (27% of deployments). The size of the sharks was estimated to be between 3.0 and 4.0 m in total length (Figure 1), using the dBRUV as a reference as done in Gallagher et al. (2023). *Hexanchus griseus* were recorded for a total of 10.84 minutes, making a total of 30 unique passes at the camera, ranging between 1 – 26 passes per individual.

Two female *H. griseus* were observed on 5th November 2021, one on each camera system. These two individuals appeared to be in different life history stages and exhibited divergent behaviors. Mating scars were observed prominently on one female shark (estimated to be ~3.5 m in total length). Scars were attributed to relatively recent (i.e., within months of observation), coercive mating (and/or attempts) based on previous criteria outlined by Ritter and Amin (2019). These included subcutaneous bite marks around the gills (Figure 1C) and pectoral and pelvic fin areas (Figures 1A, B, D), which are unlikely to be reflective of competitive and predator bouts (Ritter and Amin, 2019). The mating scars on this individual bore a large bite radius, bruising around the bite area, and clear indentations suggestive of *H. griseus* teeth (Corn et al., 2016, Figures 1C, D). This individual female shark was observed for the longest period of time (9.55 minutes), and made contact with the lander system 30 times, biting the apparatus and moving the entire unit with the drop weight >5 meters. Another individual female *H. griseus*, uniquely identified by pectoral fin markings, was observed for one minute, but did not make any contact with the dBRUV system. This individual was larger, and exhibited lateral bloating, bulging near its pelvic fins, and inability to turn when swimming (this individual instead used its fins to reposition itself). A sole male *H. griseus* was seen on 6th November 2021 in the same area (on camera system B, Figure 1E), although it was only seen once for less than one minute.

A total of 18 published studies were identified that used remote deep sea camera systems and reported various aspects of *H. griseus*

TABLE 1 Deep baited remote underwater video (dBRUV) deployments during the study period, with information on location, time of day, duration, shark occurrence, behavior, and notes.

Date	Camera System	Latitude	Longitude	Total Recording Time (mins)	Depth (m)	Temp. at Depth (°C)	Sixgill Detected (Y/N)	Sex	Time Observed (min)	No. Passes
4-Nov-21	A	24.682	-76.770	276.4	1000	unavailable	N	–	–	–
4-Nov-21	A	24.691	-76.772	281.7	1100	unavailable	N	–	–	–
4-Nov-21	B	24.670	-76.764	279.6	974	6.1	N	–	–	–
5-Nov-21	A	24.831	-76.773	283.3	1000	unavailable	Y	F*	9.55	26
5-Nov-21	A	24.823	-76.784	334.7	1000	unavailable	N	–	–	–
5-Nov-21	B	24.838	-76.762	274.5	876	6.8	N	–	–	–
5-Nov-21	B	24.849	-76.759	287.9	906	6.9	Y	F	1	3
6-Nov-21	A	24.769	-76.784	286	1000	unavailable	N	–	–	–
6-Nov-21	A	24.762	-76.784	282.5	1000	unavailable	N	–	–	–
6-Nov-21	B	24.755	-76.779	284.2	924.5	6.9	Y	M	0.28	1
6-Nov-21	B	24.792	-76.773	280.3	1018	5.3	N		–	–

*presence of body scars on the individual shark.

biology. Most studies were undertaken in the Pacific Ocean and reported aspects of shark behavior (*i.e.*, feeding behaviors, 38%) and demographic information (*i.e.*, population trends, 38%). Notably, no published studies reported observations related to the observation of mating scars or notable reproductive behaviors (Supplementary Table S1).

Discussion

Knowledge of the habitats associated with reproductive behaviors of sharks remains both a challenge and priority for their conservation, and this is especially the case for deep-sea sharks which remain challenging to access and survey. In the present study, we describe several novel, twilight zone observations of live bluntnose sixgill sharks - the largest predatory shark living in the deep-sea in global oceans. Unique to our study is the documentation of large individuals and the presence of teeth marks on at least one individual, suggestive of potential mating events.

While we believe the observed scarring is indeed a result of mating behavior, we cannot validate this claim, and there are several other potential explanations for scarring. For example, sixgill sharks are known to aggregate at depth to scavenge on carcasses, and competition for access to scavenging resources could result in agonistic bites between individuals. Furthermore, predatory behavior between sympatric sixgill sharks and other large species (i.e., tiger sharks, *Galeocerdo cuvier*) cannot be ruled out. However, group mating events have been suggested for the species, supported by observations of extreme polyandry (Larson et al., 2011). Indeed, a single female bluntnose sixgill shark contained 71 pups sired by nine fathers (Larson et al., 2011). This aspect of their biology provides support for the increased prevalence of mating scars when individuals of reproductive size overlap in space and time, as reported and corroborated in our study. If the observed scars were indeed related to mating, it is plausible that the mating attempts could have occurred in months prior to the observation. Evidence from white sharks (*Carcharodon carcharias*) observed at Guadalupe Island, Mexico, suggest that scars and body lacerations may be visible for up to several months (Domeier and Nasby-Lucas, 2007).

The proposed reproductive grounds of *H. griseus* were previously inferred from the physical capture of multiple life-history stages. For example, Ebert (2002) reported a potential pupping ground of *H. griseus* in South African waters owing to the capture of both mature adults and small pups, while King and Surry (2017) reported the capture of pregnant females in the strait of Georgia. Though evidence for geographically distinct maternal lineages supports the existence of regional reproductive grounds (Vella and Vella, 2017), key information on the habitat-use of individuals prior to, and during gestation are yet to be detailed. Our systematic review of the scientific literature highlighted that previous video-based research on *H. griseus* has been historically restricted to the northeast Pacific Ocean. Many of these studies have reported demographic information, such as temporal changes in population structure through time (e.g., Dunbrack and Zielinski, 2003; 2005 Dunbrack, 2008;). It was notable, however that there was an absence of studies reporting the location of habitats associated with potential reproductive events, presenting a key conservation challenge for *H. griseus* populations, and deep-sea sharks more broadly.

Considering the cold-water temperatures, slow growth, and likely reduced metabolism of a large-bodied deep sea chondrichthyan, we also cannot fully discount potential mating behaviors occurring away from the study location. However, the unique oceanographic properties of the Exuma Sound would certainly explain why this region may be of notable biological significance for *H. griseus* – for which multiple life-history stages have been observed (Brooks et al., 2015). Northern regions of the Sound are known to support high predator diversity

(Brooks et al., 2015), which may be driven by high allochthonous neritic energetic inputs from adjacent coastal ecosystems. For example, Shipley et al. (2017a) predicted that neritic subsidies from extensive coastal habitats flanking the Exuma Sound may be modulated by strong onshore-offshore physical transport in addition to the movement and trophic interactions of deep-diving predators, such as Caribbean reef sharks (*Carcharhinus perezi*, Shipley et al., 2017b). Recent work also suggests that the bluntnose sixgill shark is likely an important vector of blue carbon (Shipley et al., 2023), which corroborates the notion that large sharks may form ecological associations with carbon sinks in the deep ocean (Dixon and Gallagher, 2023). These observations suggest this region could be an ecological hotspot that could support many important components of organismal life-histories, including that of *H. griseus*. Further observations of multiple females bearing similar scarring would further support this region as a potential mating ground for this species, although information on shark movement would be needed.

The conservation potential of deep-sea ecosystems is intrinsically tied to knowledge of key habitats supporting critical stages of organismal life-histories, such as reproduction. Our study provides new clues into the highly cryptic reproductive behavior of a large deep-sea shark, detailing the potential location of potential reproductive grounds in the elusive *H. griseus*. Such information is critical for implementing contemporary management frameworks, such as marine protected areas, to conserve biodiversity and functionality in the deep-sea. The use of innovative technologies to survey the deep-sea, as done and reported here, hold great promise for identifying future priority habitats for the conservation of deep-sea sharks.

Data availability statement

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

Author contributions

AG: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing, Data curation, Supervision, Visualization. CDS: Investigation, Writing – review & editing. DD: Funding acquisition, Investigation, Resources, Writing – review & editing. SDH: Investigation, Resources, Writing – review & editing. BP: Resources, Supervision, Validation, Writing – review & editing. OS: Conceptualization, Methodology, Writing – review & editing. JS: Methodology, Validation, Writing – review & editing. CMD: Supervision, Validation, Writing – review & editing. JG: Investigation, Resources, Validation, Writing – review & editing.

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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.

AG declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2024.1305487/full#supplementary-material

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Community science informs movement and reproductive ecology of sand tigers *Carcharias taurus* off North Carolina, United States of America

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Sand tigers are frequently observed at shipwrecks and other artificial reef habitats off North Carolina (USA), but data about occupancy, movement ecology, and site fidelity are lacking. Spot A Shark USA researchers used Wildbook© photoidentification software to spot map sand tigers in images provided by recreational SCUBA divers, or collected from remotely operated vehicles, and an offshore live-streaming camera. We uniquely identified 1837 sand tigers, 101 of which were resighted on more than one date between 2005–2021. Sand tigers of both sexes and various ages were found year-round along the northern, central and southern coast. We identified shipwrecks or artificial reef sites with consistently high numbers of shark encounters reported, sometimes with seasonal occupancy patterns. Resighted sharks were often encountered at the same or nearby locations, confirming high levels of residency and site fidelity to specific locations. Together, the mating scars seen on 121 female sand tigers and 202 females documented with rotund abdomens consistent with pregnancy highlight the importance of NC waters for reproduction. We also quantified other characteristics of the sand tigers visible in the photographs including wounds, parasitic copepods, and attached fishing gear. Our results reflect the importance of habitats off the NC coast to the movement and reproductive ecology of sand tigers at multiple life history stages. Sand tiger populations have declined in the Northwest Atlantic, so information about residence at specific locations, seasonal patterns of occupancy, and sex-dependent behaviors associated with migration and reproduction are important to future management and conservation of the species.

KEYWORDS

movement ecology, site fidelity, shipwreck habitat, shark photo identification, reproductive ecology, *Carcharias taurus*

1 Introduction

The sand tiger *Carcharias taurus* Rafinesque, 1810, is a large shark found in the neritic zone in the Northwest Atlantic (NWA) from the Gulf of Maine to Florida and the northern Gulf of Mexico (Campagno, 1984; Gilmore, 1993). Though previously described in the United States (US) by the National Oceanic and Atmospheric Administration, National Marine Fisheries Service (NOAA, NMFS; Carlson et al., 2009) as a species of concern, this designation is not a legal classification under the Endangered Species Act and is no longer in use. However, since 2000 NMFS has prohibited the take of sand tiger sharks (NOAA and NMFS, 2000). Life history characteristics, including slow growth, delayed maturity, and low productivity, make sand tigers susceptible to population decline (Goldman et al., 2006; Ha, 2006; Rigby et al., 2021) and ongoing mortality as bycatch and in targeted catch-and-release fisheries in the US (Kneebone et al., 2013; Kilfoil et al., 2017) and elsewhere within the global range (Dicken et al., 2006; Lucifora et al., 2009) contributes to their continued vulnerability (Carlson et al., 2009). Globally, the species is designated as Critically Endangered on the IUCN Red List (Rigby et al., 2021). In parts of its range, including the southwest Atlantic and Mediterranean, populations decreased by over 80% in the last century and few or no individuals are now encountered there. In east Australia and South Africa, populations with similar declines may be recovering following management measures to reduce fishing mortality. The NWA population is estimated to have declined 30–49% (Musick et al., 1993; Ha, 2006; Rigby et al., 2021). Management strategies in the US to reverse this decline include prohibition on harvest and designation of Essential Fish Habitat (EFH) and Habitat Areas of Particular Concern (HAPC) in portions of its range (NMFS, 2009, 2017).

Sand tigers make long seasonal migrations correlated with their reproductive cycles (Gilmore, 1993; Lucifora et al., 2002; Dicken et al., 2007; Teter et al., 2015; Haulsee et al., 2018; Dwyer et al., 2023) and often display high site fidelity (Olbers and Smith, 2019; Paxton et al., 2019; Marens, 2021; Hoschke et al., 2023). Along the US Atlantic coast, their generalized movement pattern includes annual north-south migrations. They move out of northern waters (i.e., coastal waters off New England) southward at the end of summer and early fall towards mating grounds off the southeastern United States (SEUS) coast, including North Carolina (NC; Gilmore, 1993; Teter et al., 2015). After mating in late winter and early spring (Feb–May), continued migrations as far south as Florida occur before northward movements in summer (Gilmore, 1993; Teter et al., 2015; Kohler and Turner, 2019; Marens, 2021). These NWA migratory patterns vary based on sex, age, and reproductive status, making it difficult to assign a single consistent migratory behavior. Some sand tigers, including females pregnant after mating in spring, may remain in NC waters through winter gestation and only migrate out of NC post-partum in spring or remain resident year-round (Marens, 2021). In contrast, sand tigers migrating through Gray's Reef National Marine Sanctuary (<200 nm south of the NC border) suggested short residencies at that location since most of the sharks passed through the area within 1–4 days (Williams et al., 2019). Teter et al. (2015) found seven tagged male sand tigers (168–232 cm total length) followed expected migration trends and moved out of Delaware Bay and southward along the continental shelf margin between August and October, often pausing at "rest-stops" before reaching the Cape Hatteras area by December. Once in NC waters, all males continued to occupy neritic habitats between Cape Hatteras and Cape Fear until the last tags detached in February. In contrast, three females (197–228 cm total length) leaving Delaware Bay moved in a different direction than the males, swimming east to the edge of the continental slope off New Jersey where the tags popped off after 76–151 days. Tracks for all three females were spatially and temporally similar, but such eastward movement by females had not been documented previously. The authors suggested these females were recently matured and perhaps had not yet mated. They hypothesized the offshore movements could be to avoid or delay reproduction or to take advantage of high productivity in warmer offshore waters.

Several areas on the US east coast are consistently important for sand tiger life history stages, with some individuals showing strong site fidelity to these sites. Kneebone et al. (2012) identified summer nursery grounds for juvenile sand tigers in Plymouth, Kingston, Duxbury Bay, Massachusetts. In a 2008–2011 telemetry study, juveniles were caught there as late in the year as October (Kneebone et al., 2013, 2014). Kneebone et al. (2014) reported detections for tagged sharks from the Gulf of Maine to Cape Canaveral, Florida, during all months. While summer (May–Oct) residency in New England was ubiquitous among the tagged sharks, juveniles could be found off both NC and Florida during winter (Nov–Apr). Some juvenile and adult sand tigers of both sexes consistently returned to Delaware Bay (Teter et al., 2015; Haulsee et al., 2016; Kilfoil et al., 2017), with some forming aggregations biased toward association with conspecifics of similar maturity and showing evidence of habitat preference within the bay and adjacent coastal ocean (Haulsee et al., 2018; Roose et al., 2022).

Sand tigers are found in many coastal benthic and pelagic habitats in the NWA — estuaries, nearshore (<5 miles from shore) environments, natural hard bottoms, artificial reefs including shipwrecks, and offshore to the continental shelf. There appear to be differential dependencies on specific habitats based on life or reproductive stage, sex, and time of year. Such intraspecific variability in habitat usage and movement ecology, makes it imperative to understand the role each habitat plays. Little is known about fine-scale habitat selection, occupancy, and site fidelity off the SEUS.

The NC coast has long been reported to be important habitat for this shark (Smith, 1907; Radcliffe, 1916). Gilmore et al. (1983) and NOAA NMFS (2017) suggested sand tigers give birth along the SEUS coast and recent research suggests an important role for continental shelf waters of NC for mating, gestation and possibly pupping (Marens, 2021; Wyffels et al., 2022). Sand tigers are reported year-round in North and South Carolina coastal waters, often associated with artificial reefs and shipwrecks (Farmer, 2004; Schwartz et al., 2013; Paxton et al., 2019, 2020a, Marens, 2021). Since the 1960s (Claud Hull, personal communication; Supplementary Figure 1), SCUBA divers have documented sand tigers at shipwrecks in the "Graveyard of the Atlantic," as the NC coast is known, due to the over 5000 wrecks in this area (Figure 1;

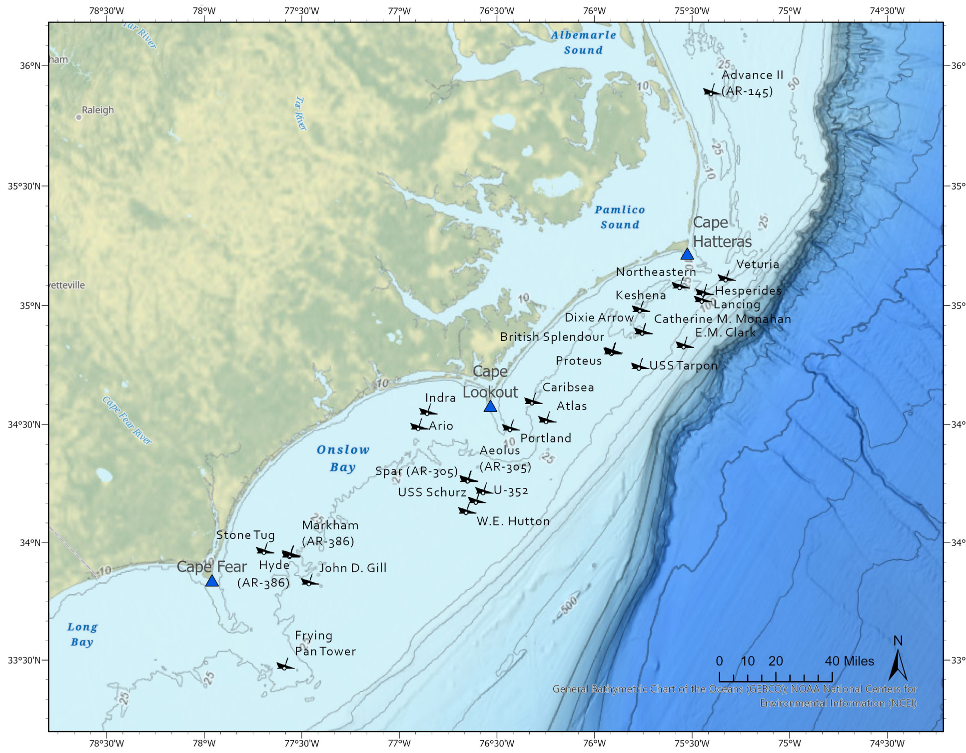


FIGURE 1
Locations of artificial reefs and shipwrecks off North Carolina, USA, where sand tiger sharks were encountered by community divers contributing to Spot A Shark USA.

Babits, 2002; Hoyt et al., 2014). SCUBA charter operators offer highly popular trips for divers to observe individual and aggregating sand tigers at shipwrecks off the coast and popular dive magazines feature stories about diving with sand tigers (e.g., https://www.scubadiving.com/drive-and-dive-sand-tiger-sharks-galore-in-morehead-city-nc; visited 26 Feb 2024). Sand tigers tend to be highly tolerant of diver presence (Campagno, 1984; Pollard et al., 1996; Bennett and Bansemer, 2004; Barker et al., 2011), making it both feasible and safe to photograph them at close range.

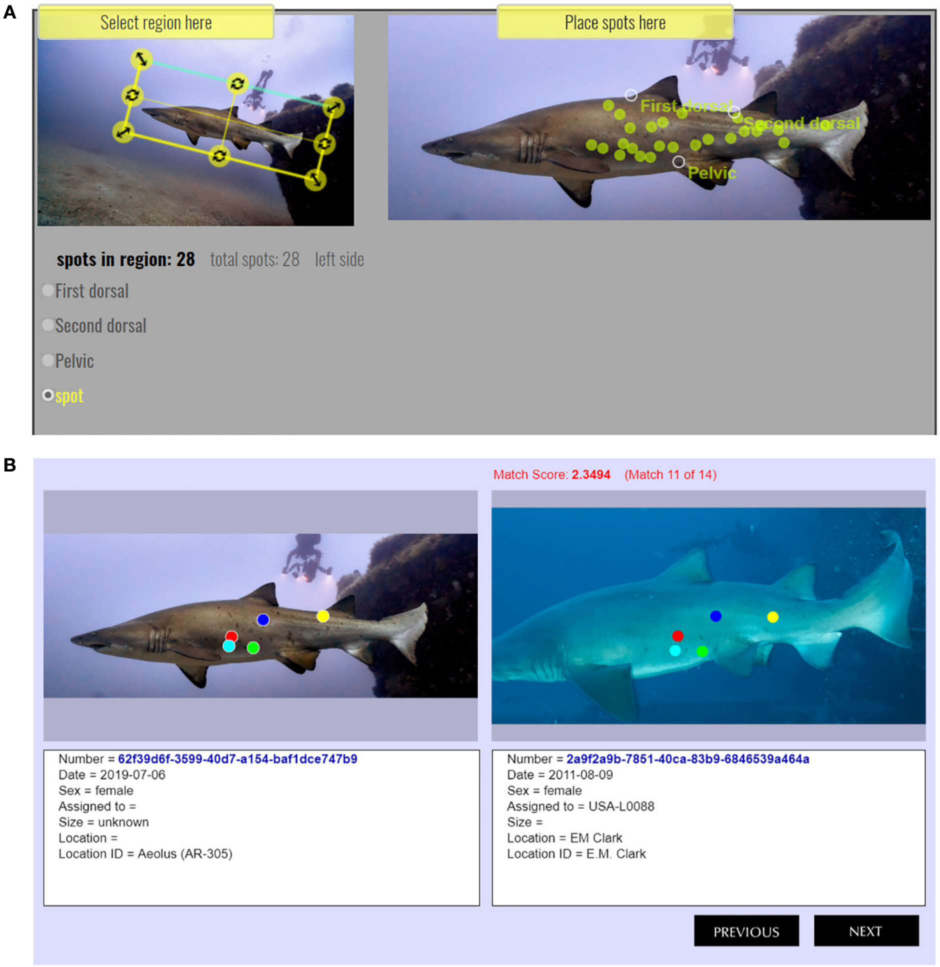
To take advantage of the serendipitous co-occurrences of individually-identifiable sand tigers, shipwrecks, and SCUBA divers in coastal NC, Spot A Shark USA (SAS; www.spotashark.com) provides a public online platform where recreational divers can upload images of sand tigers they encounter. SAS uses Wildbook© software (Arzoumanian et al., 2005; Berger-Wolf et al., 2017) to map a spot pattern (spot map; Figure 2) for each shark and stores it in a photo library database. Individual sharks are identifiable because of reddish or brownish spots (Campagno, 1984) irregularly scattered laterally along their bodies. Spots are both persistent and individually unique (Van Tienhoven et al., 2007; Bansemer and Bennett, 2008).

Wildbook© is an autonomous computational system that applies deep convolutional neural networks to identify individuals of species that are striped, spotted, wrinkled, or notched (Berger-Wolf et al., 2017). Wildbook's machine learning processes apply both modified Groth (Groth, 1986) and Interactive Individual Identification System (I^{3}S) algorithms (Van Tienhoven et al., 2007) to find possible matches for novel individuals to existing records within the SAS photographic dataset based upon spot maps. Additional biological, ecological, spatial, and temporal data may be added to individual records within SAS, providing a non-invasive method for studying sand tigers (Bennett and Bansemer, 2004; Bansemer and Bennett, 2008).

Based upon earlier records of sand tigers submitted to SAS, Paxton et al. (2019) reported site fidelity in six females to three shipwreck sites off NC. They were observed at the shipwrecks *Aeolus*, *Atlas*, and *Spar*, with encounters separated by 1–72 months and 0–46 km. Expanded participation in SAS allows for broader analysis of how sand tigers are using artificial reef habitats, most notably shipwrecks. Specifically, we used SAS data to address the following: 1) are sand tigers consistently found at NC artificial reefs in enough numbers that photographic data can be used to inform movement and reproductive ecology, 2) are there artificial reef sites that are especially important for sand tigers, 3) are there temporal patterns in habitat use or site fidelity, 4) are there difference in site use between males and females, and 5) is NC important for reproductive ecology of sand tigers?

2 Materials and methods

In 2017, the North Carolina Aquariums and WildMe© (www.wildme.org) developed SAS by building upon an existing Wildbook© research platform used to photo-document and track individual sand tigers in Australia (Barker and Williamson, 2010). SAS was made available to the US public in summer 2018 and has



spots in region: 28 total spots: 28 left side

○ First dorsal
○ Second dorsal
○ Pelvic
○ spot

Match Score: **2.3494** (Match 11 of 14)

Number = **62f39d6f-3599-40d7-a154-baf1dce747b9**
Date = 2019-07-06
Sex = female
Assigned to =
Size = unknown
Location =
Location ID = Aeolus (AR-305)

Number = **2a9f2a9b-7851-40ca-83b9-6846539a464a**
Date = 2011-08-09
Sex = female
Assigned to = USA-L0088
Size =
Location = EM Clark
Location ID = E.M. Clark

FIGURE 2
Examples from a left-sided sand tiger shark showing: **(A)** spot mapping results with location of dorsal and pectoral fins as anatomical landmarks and dots placed over spots and, **(B)** a possible match (right) for a spot mapped shark left, which is not a match.

been in operation since. We do not include any Australian records in our SAS dataset or the results reported here.

2.1 Image collection

Sand tiger images were collected from multiple sources. SCUBA divers uploaded photos directly through the SAS website. The NCA routinely conducted outreach campaigns to local dive clubs, shops, and guides to raise awareness about this community science project. In peak visitor seasons (1–3 days per week depending on weather between Jun-Aug beginning in 2021), we engaged directly with divers to collect photos as they got off dive charter boats at two SCUBA diving charter operators near Morehead City, NC. Divers interacted with SAS technicians to download their photos. Technicians collected and uploaded all pertinent data and images to the SAS platform. Some divers also submitted images from their previous years of diving prior to SAS being launched. In addition to still photos, technicians downloaded video data with permission, typically from GoPro® action cameras (GoPro, Inc., San Mateo, CA) from which still sand tiger images were extracted.

Sand tiger images for SAS were also extracted from video obtained from a remotely operated vehicle (ROV; Teledyne Benthos Stingray) footage collected from researchers conducting observations at shipwrecks *Caribsea* (16 Jul 2018), *U-352* (07 Aug 2018), and *W.E. Hutton* (07 Aug 2018).

Finally, SAS images were retrieved from video recordings and still images taken at Frying Pan Tower (Figure 1) as part of SharkCam, a live-streaming, publicly-viewable camera hosted and maintained by Explore.org (https://explore.org/livecams/frying-pan/shark-cam; visited 26 Feb 2024). The Frying Pan Tower structure (33°29′ N, 77°35′ W) is a privately-owned (https://fptower.org; visited 26 Feb 2024), decommissioned US Coast Guard Light Station located 51 km off Cape Fear, NC. Frying Pan Tower is situated above natural hard bottom habitat (Figure 1; NC Division of Marine Fisheries, 2022) in approximately 17 m of water at the seaward edge of Frying Pan Shoals. Additional details about the camera and setting are found in Burge and Harris (2021) and Coleman and Burge (2021). Still images from SharkCam were collected from a gallery of snapshots submitted by viewers, by extracting them from recorded video files, and as screenshots taken in real-time while technicians watched the live feed.

2.2 Image processing

All images were uploaded with metadata that included the submitter or SAS technician, image date of picture(s), and the location (typically at a known shipwreck). Each record of an individual shark on a given day at a specific location was considered an encounter. Each encounter submission received a unique alphanumeric identifier automatically generated by Wildbook©, which remains with that encounter for its lifetime. Ancillary information about sex, relative size, environmental conditions, shark behavior, observed injuries, or other observations could also be entered for each encounter. Multiple images of a shark are included in a single encounter when images were taken from the same date and location. If multiple sharks were visible in a submitted photo, technicians copied the photo(s) and uploaded them as separate encounter(s), with notes added to each encounter to direct data managers to which shark is associated with each encounter.

2.3 Spot mapping

Wildbook© software was used by trained technicians to spot map the shark in each encounter. This software is used for photoidentification of terrestrial (Parham et al., 2018; Verschueren et al., 2023) and marine species (Blount et al., 2022, Weideman et al., 2020), including other elasmobranchs (Rohner et al., 2013; Norman et al., 2017). To spot map a shark's photograph, the technician first rotated the photo to align the image horizontally (Figure 2A). Next, reference marks were placed on the image at the anterior insertion of the first and second dorsal fins, and the pelvic fin, as anatomical landmarks. Finally, between 3–30 additional marks were placed over discernable spots on the left or right flank of the shark. Images which did not contain the full body of the shark were usable if the three anatomical landmarks were visible. Once all spot marks were placed, the spot map was uploaded to Wildbook© for comparison to all other spot maps in the SAS database. The spot map comparisons yielded a list of possible matches (typically between 5–50) from the existing database of all SAS encounters for the newly uploaded spot map (Figure 2B). Technicians then compared the new encounter to matching encounters to verify matches or establish a lack of match, in which case the encounter represented a new shark.

New encounters received a unique SAS alphanumeric identification sequence (SAS ID) following the convention of USA, plus L or R to designate side of the shark that was mapped, plus a sequential number; for example, USA-L0003 and USA-R0458. For encounters that returned matches with an existing shark in the database, a second technician confirmed that it was a match. Once confirmed, the new encounter was assigned the same SAS ID. Thus, a unique SAS ID may include multiple encounters with the same shark on different dates and possibly at different locations. These records are referred to as resighted sharks.

Some submitted images were not suitable for spot mapping due to a blurry or dark photo, spots being obscured by other fish, the shark having no discernable spots, or missing reference landmarks. For unusable images, the encounter was labeled as UNID and given a sequential numerical identifier (e.g., UNID-16). Data from these encounters were included in our analysis of temporal occupancy but were not useable to track movement of resighted individuals over time.

2.4 Keywords

As each new encounter image was processed, technicians collected additional data and assigned a sex to the animal. Adult males were readily identifiable by the presence of fully calcified claspers extending from between the pelvic fins (Lucifora et al., 2002; Smale, 2002). Subadult males have smaller claspers (Supplementary Figure 2) that do not project at all or only slightly beyond the pelvic fin, but, if visible, were also used for sex determination. A clear view of the cloacal area made assignment of female straightforward. If not, other features of the shark were used to assign a sex of female, including the absence of claspers on a shark that appeared to be above sub-adult size in length and girth. Younger, smaller sharks tend to be longer relative to body height (slimmer), while adults are stouter. In some photos, relative size could be estimated by comparison to other sharks or fishes present, human divers in the photo or shipwreck features. Using these clues about size, large sharks with no claspers visible were assigned as females. Additionally, presence of scars at the base of the pectoral fins consistent with wounds acquired during mating helped with assigning sharks as females (Supplementary Figure 3). Finally, because females give live birth to young around 1 m in length, they become very rotund in late fall and winter months in later stages of gestation (Gilmore, 1993; Bansemer and Bennett, 2008; Wyffels et al., 2020). Sharks with prominently rounded abdomens consistent with pregnancy (Supplementary Figure 4) were assigned as females. If there were no clear sex markers, then the sex of the shark was assigned as unknown.

Technicians also added informational keywords to encounters (Table 1). An encounter may have no or several keywords. Primary keywords were used to maintain consistency in assignment, while secondary keywords further described what was observed. For example, a shark with a part of its caudal fin missing was assigned keywords of "fin damage" plus "caudal." More detailed information about an encounter was sometimes recorded in a comment area by either the submitter or the technician. These comments were reviewed for keyword attribution as well.

2.5 Statistical analyses

The dataset was downloaded from the SAS website on 22 Jan 2022. We recognize the limitations of the Spot A Shark dataset with regards to statistical hypothesis testing because 1) as a community science project sampling was not standardized, 2) data were collected in multiple formats, and 3) sampling effort was not balanced spatially or temporally. Therefore, we limited our statistical analyses to descriptive metrics using SAS 9.4 or Microsoft Excel.

TABLE 1 Descriptive keywords assigned to Spot A Shark encounters (n=1932).

Primary Keyword	Primary Description	Secondary Keyword(s)	Females	Males	Unknown	Total
Bites	Wounds consistent with bites (not mating bites)	location of bite	4	1	2	7
Copepods	Attached parasitic copepods	location of attachment	25	12	9	46
Fish	Other fish species are visible in the photo	fish species	508	77	149	734
Fishing Gear	Fishing gear visibly attached to the shark	type of gear, location of attachment	12	–	7	19
Fouled teeth	Presence of hydroids on teeth		18	2	10	30
Mating Scars	Wounds consistent with mating visible around pectoral fin		121	–	17	138
Pregnant	Sharks with enlarged rounded abdomen consistent with pregnancy		202	0	0	202
Small Claspers	Small claspers extending only slightly beyond cloaca or pelvic fin		0	41	0	41
Tagged	Presence of external tag	type of tag, location of attachment	28	7	5	40
Fin Damage	Permanent damage to one of the 7 fins; ranging from notches to missing	specific fin, severity of damage	119	16	24	159
Wound or Injury	Fresh or healing wounds	type (gash, puncture), location of wound	29	6	6	41
Scars or Scratches	Evidence of healed wounds that are fully closed over=Scar Superficial scrapes=Scratches	location of scars or scratches	163	36	59	258
TOTAL			**1392**	**233**	**307**	**1932**

Counts and frequency tables were constructed for shark occurrence by site, region, month, year, and season and combinations thereof. Regional categories for sites and seasonal categories for dates were designated consistently with those reported by Marens (2021; Table 2; Supplementary Table 1). Seasons were set as Winter (Dec, Jan, Feb), Spring (Mar, Apr, May), Summer (Jun, Jul, Aug) and Fall (Sep, Oct, Nov), corresponding to the meteorological seasons for NC. We calculated counts and frequencies for the keywords listed in Table 1.

TABLE 2 Number of sand tiger encounters in the Spot A Shark USA database compiled across months and seasons for all years (2005–2021).

Season	Month	Number of Sharks
Winter	Dec	130
	Jan	97
	Feb	32
Spring	Mar	175
	Apr	236
	May	193
Summer	Jun	195
	Jul	318
	Aug	245
Fall	Sep	111
	Oct	126
	Nov	170
TOTAL		**2028**

For resighted sharks, we calculated the days at liberty (DAL) between all sightings. To calculate distances travelled between sites by resighted sharks, we compiled a matrix of coordinates for all sites (Comer and Love-Adrick, 2016; NOAA, 2022) and computed the distances in nautical miles (nm) between all possible combinations of sites using the formula:

$$\text{Distance (nm)} = \text{ACOS}[(\sin(Lat\_place\_1 * PI()/180) * \sin(Lat\_place\_2 * PI()/180) + \cos(Lat\_place\_1 * PI()/180) * \cos(Lat\_place\_2 * PI()/180) * \cos(Lon\_place\_2 * PI()/180 - Lon\_place\_1 * PI()/180))] * 3443.8985;$$

where: Lat_place_1 = latitude of location 1, Lat_place_2 = Latitude of location 2, Lon_place_1 = longitude of location 1, Lon_place_2 = longitude of location 2 and PI() = pi

Distance calculations were converted to km using the formula:

$$\text{Distance km} = \text{distance (nm)} * 1.852$$

3 Results

In total, 2028 unique sand tiger encounters representing 1837 individual sharks with encounter dates ranging between 2005–2021 were included in this dataset. Of these, 824 were spot mapped on the right side, 924 on the left and 89 were UNID and did not get spot mapped. We note many of our results, especially those related to temporal and spatial patterns, reflect total available images (diver effort, SharkCam/Explore.org footage availability). To emphasize this, we reference our findings as “sand tiger encounters” throughout this section to acknowledge the data summarize diver interactions with sharks and availability of footage from Explore.org/SharkCam. We recognize these images are not representative of the sand tiger population as a whole.

Females made up 56.8% of the sharks, males 13.5%, and unknown sex 29.7%. All three categories were observed in all months, with females outnumbering males in all months (Table 3; Supplementary Figure 5). We received images from 30 sites, with Frying Pan Tower and Artificial Reef 255 (AR-255) Bridge Frame being the only sites that are not shipwrecks (see Supplementary Table 1 for additional details on all sites including links to archaeological and historical details). The sites are in water 6–73 m deep and lie 0.2–51 km offshore. One encounter submitted from the South Carolina shipwreck *Tauracavor*, and 16 submitted

TABLE 3 Sites of sand tiger *Carcharias taurus* images cataloged in the Spot A Shark USA database (www.spotashark.com) by sex and female: male ratio.

Site	Region	Female	Male	Unknown	Total sharks	Female/Male
Advance II (AR-145)	north	1	1	2	4	1.0
Aeolus (AR-305)	central	141	38	44	223	3.7
Ario	central	0	0	1	1	-
Atlas	central	61	6	37	104	10.2
Bridge Frame (AR-255)	north	5	2	0	7	2.5
British Splendour	north	18	3	2	23	6.0
Caribsea	central	196	37	69	302	5.3
Catherine M. Monahan	north	2	0	0	2	-
Dixie Arrow	north	50	2	5	57	25.0
E.M. Clark	north	3	2	4	9	1.5
Frying Pan Tower	south	210	62	211	483	3.4
Hesperides	north	43	0	0	43	-
Hyde (AR-386)	south	11	5	7	23	2.2
Indra (AR-330)	central	6	1	3	10	6.0
John D. Gill	south	1	0	0	1	-
Keshena	north	1	4	4	9	0.3
Lancing	north	3	15	4	22	0.2
Markham (AR-386)	south	0	0	1	1	-
Northeastern	north	1	0	0	1	-
Portland	central	4	0	2	6	-
Proteus	north	94	0	5	99	-
Schurz	central	12	9	3	24	1.3
Spar (AR-305)	central	167	58	104	329	2.9
Stone Tug (AR-382)	south	1	0	1	2	-
Tarpon	north	38	2	7	47	19.0
Tauracavor	south	1	0	0	1	-
Triangle Wrecks	north	1	0	3	4	-
U-352	central	17	7	0	24	2.4
Veturia	north	0	1	1	2	-
W.E. Hutton	central	91	25	31	147	3.6

AR, Artificial Reef site. See https://deq.nc.gov/about/divisions/marine-fisheries/public-information-and-education/coastal-fishing-information/artificial-reefs for more information.
18 additional sand tigers were sited at locations off the central coast, but shipwreck locations were not recorded.

from unknown locations on the central NC coast, were included in the SAS dataset, but were eliminated from some analyses. Fifty-one community divers submitted photographs.

3.1 Spatial and temporal occupancy

Though sharks were photographed at 30 sites, 90% of images came from just 10 sites, with Frying Pan Tower accounting for 24% of all submissions (Figures 3, 4). The other most frequent locations were *Spar* (16%), *Caribsea* (15%), *Aeolus* (11%), *W.E. Hutton* (7%), and *Atlas*, *Proteus*, *Dixie Arrow, Tarpon*, and *Hesperides* (each 2–5%). The remainder of the sites had fewer than 25 shark encounters (range=1–24). For the shipwrecks where sand tigers were photographed, 14 were designated as northern, 10 central and 6 southern (Table 3; Supplementary Table 1). Nearly 60% of encounters were from shipwrecks off the central coast, with 23% from southern and 17% from northern locations (Figure 3).

3.1.1 Site by sex

At all sites with at least 10 submitted encounters, a mixture of female, male and sex unknown sharks were photographed, with two exceptions. Only female or unknown sex sharks were reported at the *Proteus* (n=99) and *Hesperides* (n=44) wrecks. Some locations had proportionally high numbers of females present across all years. For example, *Dixie Arrow* had 25 times more females (n=50) than males (n=2) reported. The *Tarpon* had 19 times as many females (n=38) as males (n=2) reported. *Atlas* had 10 times as many females (n=61) reported as males (n=6). Only at the *Lancing* did males exceed females with 15 males reported compared to only 3 females.

3.1.2 Temporal patterns

We examined the frequency of occurrence by month and season (Table 2). Sharks were photographed in all months of the year, with 59% encountered between Apr and Aug, corresponding to the months with highest recreational diver activity (Supplementary Figure 5). In northern sites, data gaps exist in late fall and winter months. None of the sites in the central region have encounters from all months. However, a few sites have 9–11 months coverage: *Aeolus* (Mar–Dec), *Caribsea* (Feb–Oct), *Spar* (no Sept records). At FPT, where the live camera was deployed and diver comfort is removed as a bias to image gathering, shark encounters were most frequent in late fall through spring, but there were encounters for all months. When grouped by season, 20% of all encounters are from fall, 30% from spring, 37% from summer and 13% from winter.

To examine differences in seasonal occupancy by sex, we tabulated encounters of 1052 female and 224 male sharks at ten sites along the north-south latitudinal gradient. Included sites had 40 or greater total encounters (Table 3; Figure 4) and represented 63% of our total 2028 encounters. In the northern region, *Hesperides*, the northern-most shipwreck had only females (n=41) reported on only two dates (04 and 05 Jul 2020). *Dixie Arrow* had 50 female sharks reported in spring, summer and fall, but only 1 male in spring and 1 male in summer, with 76% of those females in the summer months. *Proteus* only had female sharks (n=93) in spring, summer and fall, with 83% of encounters reported in fall. *Tarpon* had 38 females in spring, summer and fall, with 63% from summer. There were only 2 males reported, one in spring and one in fall. *Caribsea* had a total of 195 female sharks with highest numbers in summer (70%). Males (n=35) were reported mostly in spring and summer with only 2 reported in fall. *Atlas* had females in spring and summer (n=59) and

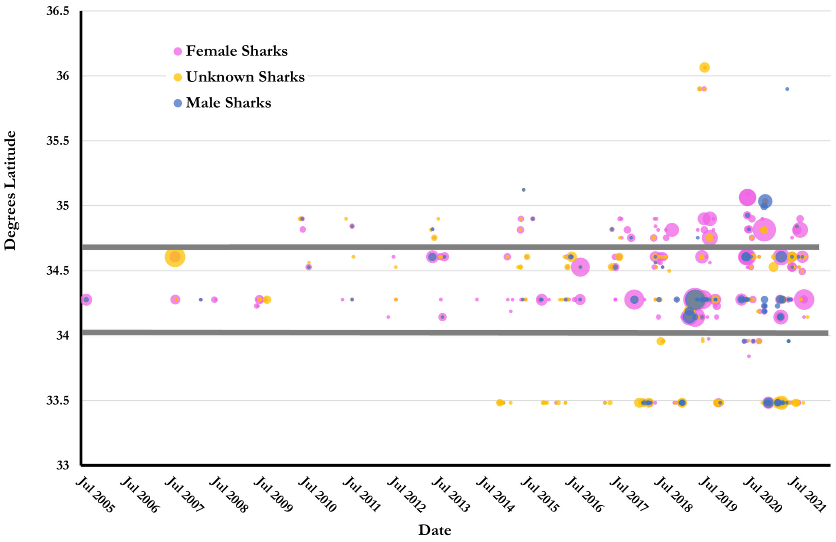


FIGURE 3
Latitudinal distribution of Spot A Shark sand tiger shark encounters in North Carolina, USA, from 2004-2021. Each dot represents a single encounter. Large dots are scaled to represent multiple encounters at the same location. Grey horizontal lines demark boundaries between the northern, central and southern regions.

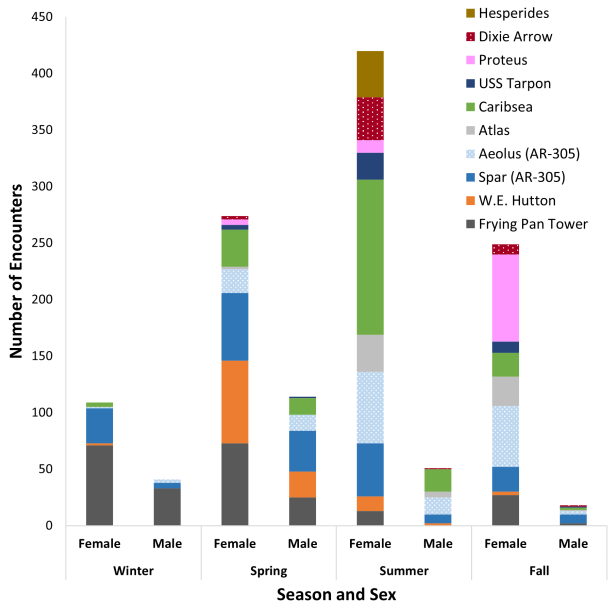


FIGURE 4
The number of sand tiger shark encounters in the Spot A Shark database by sex and season at the 10 most-frequented locations. Only locations which had at least 40 sand tiger shark encounters recorded are included. The locations are oriented from north (*Hesperides*) to south (*Frying Pan Tower*) on each bar.

fall (n=2), and males (n=6) in summer and fall. *Aeolus* had females (n=139) and males (n=35). Highest numbers of females were reported in summer and fall (84%), with most males reported in spring and summer (83%). *Spar* had females (n=160) and males (n=57). Highest numbers of females were reported in spring (38%) and summer (29%), with most males reported in spring (63%). *W.E. Hutton* had females (n=91) and males (n=25) in spring and summer. About 80% of females and 92% of males were reported in spring. In the southern region, Frying Pan Tower had females (n=184) year-round and males (n=60) in fall, winter and summer. Both females and males were reported in highest numbers in winter (39% females, 55% males) and spring (40% females, 42% males).

3.2 Photo-recaptured sharks

Most sharks were encountered only once, but 101 sharks (5.5% resighted) were observed on more than one date. Of these, 74 were seen twice, 13 were seen 3 times, 8 were seen 4 times, 3 were seen 5 times, 1 was seen 8 times, and 2 were seen 14 times. DAL between sightings ranged from 1–2176 days (mean=171.5 ± 356.4 days; median=16 days). Typically, sharks were observed at the same location during a relatively short window for all encounters, but 28 were resighted at a different location from the previous encounter. The distance between sightings ranged from 0 (i.e., seen at the same location on different dates) to 213.2 km. For sharks observed at a different location in the subsequent encounter, the distance between sightings averaged 21.1 ± 48.2 km. Below, we detail resightings of selected sharks arranged by location where they were first encountered.

3.2.1 Frying Pan Tower

Sharks first seen at Frying Pan Tower accounted for over a third of resighted sharks (n=37). All sharks first encountered at Frying Pan Tower were only resighted at the same location. The most observed shark was female USA-R0272, seen on 11 days between 28 Oct–12 Nov 2019 and next seen over a year later on 21 Dec 2020. The last dates she was seen were 12 and 15 Mar 2021. These 14 sightings spanned 504 DAL. In all photos, this shark is missing a large portion of the upper lobe of her caudal fin. In the Oct–Nov 2019 images she displayed a large abdomen and appeared pregnant (Figures 5A, B). In photos from the following winter of 2020–2021, she did not appear pregnant (Figure 5C).

Male shark USA-R0536 was seen 8 times at Frying Pan Tower. He was first encountered on 23 Nov 2019 with a nick on the lower lobe of his caudal fin. The following winter he was seen on 7 dates between 27 Nov 2020 and 18 Mar 2021, this time with additional injuries including a nick on the trailing edge of the left pectoral fin, and numerous scrapes on his snout, head and body. There were three sharksuckers *Echeneis* sp. Linnaeus, 1758 attached on his left and right flanks. On 28 Nov 2019, a size comparison with other sharks was possible, indicating that this shark was relatively smaller than several large females also visible on the live camera that day. Most of the scrapes were no longer visible on the last day the shark was photographed (18 Mar 2021).

The remainder of the resighted FPT sharks were encountered on 2—5 separate dates. The average time between sightings was 105 ± 248 days (median=6 days), ranging from 1 to 1033 DAL. Of these, 5 sharks had greater than one year between encounters. Though resightings were reported at FPT in all seasons, most occurred fall through winter. Most sharks with multiple encounters were female (61.1%), with 19.4% males and 19.4% unknown sex.

3.2.2 Aeolus

A small male, USA-R0128 (Figure 6) was photographed at the wrecks of *Aeolus* and nearby *Spar* (0.26 km apart) on 14 days between 14 May 2019 and 26 June 2021. His estimated length at the first encounter at *Aeolus* was approximately 1 m (T. Houppermans, personal observation), so it is possible this was a young-of-year shark. This small shark was photographed 7 more times on *Aeolus* that year on 19 May, 27 June, 01, 05 and 07 Aug, 15 and 19 Oct. In 2020, he was encountered at *Spar* on 08 and 09 June and at *Aeolus* on 30 Jun. In 2021 he was seen twice at *Aeolus* on 07 and 25 Jun. This shark was typically found in and around the stern section of *Aeolus*, in sheltered areas (around 27 m depth). When first encountered, the shark had a deep, wide gash on his right flank extending from the base of the second dorsal to the lateral line. The lower posterior edge of the second dorsal had been severed. Photos showed healing through summer and fall, with fully closed scars present by Oct 2019. He was last observed on 23 June 2022, swimming just above the wreck.

USA-L0199 (Supplementary Figure 2) was first seen at *Aeolus* on 01 Jun 2016 with many scrapes on his head and left flank, then again on 27 Sep 2016 with wound healing evident. On these first two dates, he was assigned as sex unknown. On 28 Jun 2019, small claspers were visible. In the last photograph taken 17 Jul 2020 at *British Splendor* (90.7 *km* northeast of *Aeolus*), claspers appeared

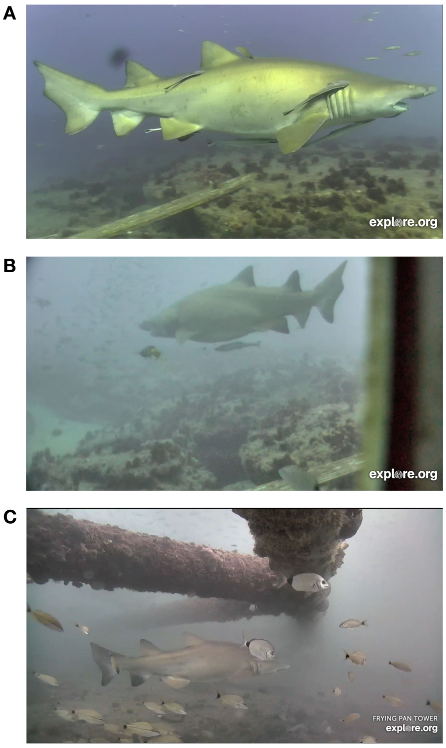


FIGURE 5
Female Spot A Shark sand tiger shark USA-R0272 encountered at Frying Pan Tower: **(A)** first observed, rotund abdomen consistent with pregnancy, portion of upper lobe of caudal fin missing, 28 Oct 2019; **(B)** rotund abdomen consistent with pregnancy, 12 Nov 2019; **(C)** abdomen no longer rotund the following winter, 21 Dec 2020. Photo credit: All extracted from explore.org video files.

fully developed. The tip of his caudal fin was missing, a healing cut (estimated 10–15 cm long) was noted anterior of the left eye, and parasitic copepods were attached on the upper left jaw.

Twelve additional sharks first observed at *Aeolus* were later resighted. One female with most of the first dorsal fin severed, but fully healed, was first seen on 06 Jul 2019 and then 4 more times Apr–Jul 2021 on either *Aeolus* or *Spar* (730 DAL) with scrapes on her head that healed over those months. Another three sharks, two females and one male, were resighted four times at either *Aeolus* only (n=2) or *Spar* (=1). There were eight sharks (n=6 females) sighted on two dates, at the *Aeolus* or *Spar* both times. The other two were seen at *W.E. Hutton* (female; 14.8 km south; 1506 DAL) and *Advance* (male; 213 km northeast; 2176 DAL).

3.2.3 Spar

Of the 23 sharks first observed at *Spar*, most were female (n=19) and seen only twice (n=18). Additionally, 1 male and 3 unknown sex sharks were resighted. DAL ranged from 8-2059. Resighted sharks were most frequently seen again at either *Spar* (n=12) or *Aeolus* (n=8). Total DAL ranged from 8–2059 (mean=438.2 ± 470.5 SD; median). Female USA-R0184 was first seen on *Spar* on 16 Nov 2015 and appeared pregnant. Another 24 sand tigers are visible (but too indistinct for spot mapping) in this submitted photograph including several more with large abdomens. She was next encountered over 5.5 years later on 06 July 2021 at *Atlas* (46.3 km northeast), representing the longest DAL in our dataset. Female USA-R0222 was seen twice on *Spar* in Aug 2019 and noted to have notches on the trailing edge of first dorsal fin (Supplementary Figure 6). She was subsequently encountered at *Aeolus* on 16 May 2020. A portion of her right pectoral fin was missing, and the wound appeared sealed and edged in white scar tissue. She was seen on 14 Jul 2020 and again on 12 Aug 2020 with the wound appearing completely healed over.

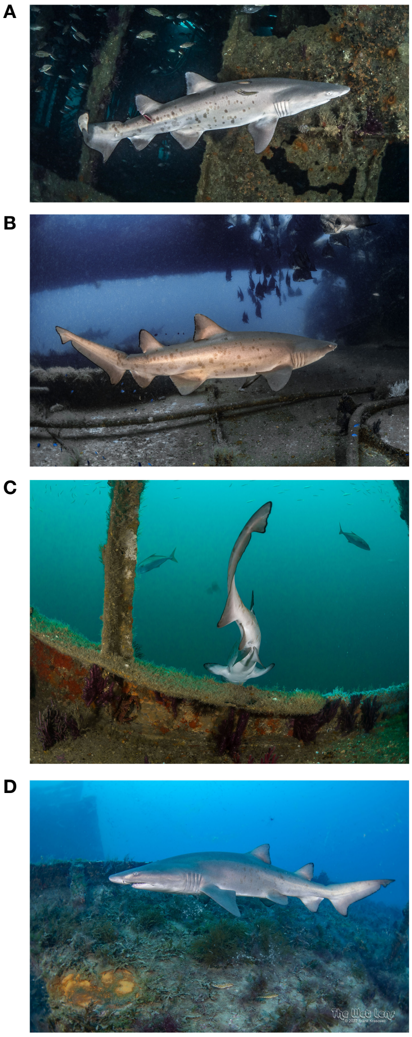


FIGURE 6
Small Spot A Shark male sand tiger shark USA-R0128 encountered: **(A)** first siting, bottom of second dorsal fin missing and fresh wound on side below second dorsal, length estimate <1m by diver, *Aeolus*, 14 May 2019; **(B)** wound appears healed, *Aeolus*, 07 Aug 2019; **(C)** subadult claspers visible, Spar, 07 Jun 2021; **(D)** last siting, *Aeolus*,23 Jun 2022. Photo credits: 3. **(B)** Tanya Houppermans; **(D)** Frank Krasovec.

Other sharks with notable DAL intervals include female USA-L0275 observed above the sea floor adjacent to *Spar* on 05 July 2019. She was next observed at *Aeolus* on 12 and 14 Oct 2021 (832 DAL). Female USA-L0195 was seen at *Spar* on 01 May and 26 Jun 2019. When resighted on 06 Jun 2021 at *Spar* (777 DAL) she exhibited large, red scrapes on and below her second dorsal fin and extending over half of her caudal fin, as well as less significant scrapes dorsally above the gills. USA-L0120 is an unknown sex shark seen first at *Spar* on 09 Sept as a juvenile based on morphology and size relative to other fishes in the image. The shark was resighted on 09 Aug 2011 at *Aeolus* (699 DAL). Female USA-L0109 was at *Spar* on 15 Aug 2007 amid a large school of tomtate *Haemulon aurolineatum* Cuvier in Cuvier & Valenciennes, 1830 and then encountered again on *Aeolus* on 08 Jul 2009 (693 DAL). Female USA-R0181 was observed at *Spar* 01 May 2019 and then at Frying Pan Tower (124 km southeast) after 681 DAL on 12 Mar 2021. Adult male USA-L0378 was resighted at the *U-352* (9.3 km southeast of *Spar*) after 569 DAL, with two sharksuckers attached ventrally.

3.2.4 Caribsea

Of 10 resighted sharks first encountered at *Caribsea*, only two were seen more than twice and only one was resighted at another location. This was USA-R0294, an unknown sex shark first seen on 10 Jun 2016 with several small scrapes on its head and snout. He was photographed again on 03 Aug 2020 (1515 DAL) at *Atlas* (11.1 km southeast) also with scrapes near the right eye. Sex could be determined in this second encounter. Of the remaining 9 sharks, five were females and four were males. Female shark USA-R0154 was seen at *Caribsea* on 01 Sept 2018 with healing mating scars on the left pectoral fin. She was seen again on 22 and 25 June 2020 (663 DAL) also with mating scars on right pectoral fin and additional wounds along the right flank. Another female, USA-L0522, was also reported at *Caribsea* with mating scars on her left pectoral fin and other wounds on her flank on 25 Jun and 05 Jul 2020. Two sub-adult males were resighted at *Caribsea*. First, USA-L0514 was observed on 25 Jun and 03 Aug 2020 with visible small claspers, the second dorsal fin folded over and parasitic copepods attached at the upper front jaw and tip of the snout. USA-L0819 was photographed at *Caribsea* on 06 Jul, 28 Aug, and 29 Sep 2021, with small claspers visible each date.

3.2.5 W.E. Hutton

Female USA-R0219 was seen at *W.E. Hutton* on 27 Jun 2019 and described by the submitting diver as a small sub-adult. She was resighted twice on *Aeolus* (14.8 km south) on 06 Jul and 03 Aug 2019 (37 DAL). A female appearing pregnant, USA-L0161, was encountered at *W.E. Hutton* on 30 April 2019 (Supplementary Figure 7). She was seen again at the same location on 27 June (58 DAL), but no longer displayed a large abdomen.

3.2.6 Hesperides

On 04 Jul 2020, a diver photographed 20 female sand tigers at *Hesperides*, 9 of which exhibited mating scars. The following day the same diver encountered 21 females, five of which were resightings.

3.3 Keywords

Over 1900 keywords were assigned to sharks during image processing (Table 1). We observed 19 sharks (12 female, 7 unknown sex) with attached fishing gear. Of these, six were at *Aeolus*, three at Frying Pan Tower, two at *Hyde* and the rest distributed among eight other sites. Sharks with attached fishing gear were mostly photographed in summer and early fall months. For 17 of these sharks, fishing hooks and sometimes trailing line were visible. Hooks were in the jaw for all but one shark, which had the hook embedded in its head. One shark had a section of gillnet tangled in its teeth and one had a length of rope (estimated 1.5 m long) around its caudal peduncle.

External tags were observed on 28 females, seven males, and five unknown sex sharks. Most were photographed at Frying Pan Tower, *Aeolus*, *Caribsea* and *W.E. Hutton*. Most tag numbers could not be discerned because the tags were fouled, numbers were not visible, or the resolution was too poor. However, we were able to confirm USA-R0756 carried a tag numbered #399299 when encountered on 30 March 2021 at *Aeolus*. The tag was reported to the NOAA Cooperative Tagging Project, which confirmed this male shark measuring 155 cm fork length had been tagged in Delaware Bay on 12 August 2019. It was visually estimated by the SAS contributing diver to be 170 cm long.

Parasitic copepods (Supplementary Figure 8) were observed attached to 25 female, 12 male and 9 unknown sex sharks, with most of those encounters submitted in Jun and Jul. Copepods were generally attached on the sharks' heads, often around the nostrils and mouth. Most sharks with copepods attached were photographed at *Caribsea* (n=25), *Spar* (n=8) and *Aeolus* (n=4). It was not possible to determine the species of copepod from any images. We observed 30 sharks with hydroids on their teeth (Supplementary Figure 9), of which 18 were female, 2 male and 10 unknown sex. Most of these were photographed at *Caribsea* (n=9), *Spar* (n=8) and *Aeolus* (n=6) in summer months.

Small claspers were noted for 41 male sharks. These were observed in encounters from all months except Feb, with the most reported in May (n=6) and Jun (n=8). Most of these sub-adult males were photographed at Frying Pan Tower (n=12), *Spar* (n=9) and *Caribsea* (n=9) with the remainder at the wrecks of *Aeolus*, *Hyde*, *Atlas*, *W.E. Hutton* and *British Splendor*.

Wounds or scars consistent with mating injuries (Supplementary Figure 3) were observed on 121 female and 17 unknown sex sharks. Of these, 41% were photographed on *Caribsea*, 14% on *Hesperides*, and 9% on *British Splendor*. These wounds were most often reported in Jun (30%) and Jul (44%), though scars were visible on sharks in various stages of healing throughout the year.

We found 202 female sharks at 18 sites displaying morphology consistent with pregnancy (Figure 7; Supplementary Figure 4). While 87% of these encounters were submissions from Jun–Dec, this keyword was assigned to sharks throughout the year. The two sites with the most females appearing pregnant are Frying Pan Tower and *Spar*, each with 41 encounters and collectively accounting for 41% of these observations. Other sites with possible pregnant females reported include *Caribsea* and *Dixie Arrow*, each with 20; *Proteus* and *Aeolus*, each with 18; *Tarpon* with 11; and the remaining sites, including all those from the northern region, which each had 1–6 reported.

Fin damage was observed on 119 females, 16 males and 24 unknown sharks (Supplementary Figure 10). Of these, 25 were missing all or a portion of the fin. Most often damage was to pectoral (n=55), caudal (n=52) and dorsal (n=46) fins, with less damage to pelvic (n=16) or anal (n=2) fins. It was not possible to discern the etiologies of these injuries. More severe injuries were

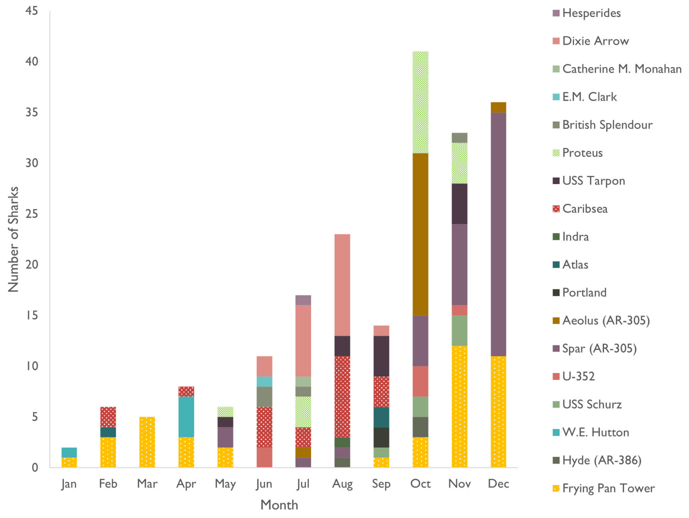


FIGURE 7
Number of female sand tiger sharks in the Spot A Shark database assigned the keyword "pregnant" in all years combined by month and location. Locations are ordered from north (*Hesperides*) to south (*Frying Pan Tower*) in all bars.

observed ranging from fresh wounds to healing injuries. Injuries included puncture wounds, and deeper cuts or gashes with incomplete healing or scarring (Supplementary Figure 11). Such wounds were observed on 29 females (not assigned as mating injuries), 6 males and 6 unknown sex sharks. For these wounds, the location(s) of the injuries were recorded. Three of these injuries appeared to be consistent with rope damage. We found an additional 7 sharks with significant bite wounds (Supplementary Figure 12). Two of these were females that also had some mating wounds at the base of the pectoral fin. Because scars and scratches could not always be differentiated, they were grouped. We observed 258 sharks (n=163 female, 36 male and 59 unknown sex) with scars or scratches visible. For these, the body location was assigned as head, body, caudal or any combination of these three areas (Supplementary Figure 13). Many sharks (n=81) had scars or scratches only on their bodies, often on dorsal or lateral areas. Scratches on the head (n=45) were typically on the snout and top of the head. The most common combination of scratch or scar locations was the head and body (n=62), and 20 sharks had scars or sctatches present on all three body locations.

4 Discussion

This study adds to our knowledge of sand tiger ecology and provides further evidence for the importance of the NC coast, and specifically shipwreck and artificial reef habitats, throughout their lives. Community science projects, including SAS, can have limitations (Berger-Wolf et al., 2017; Earp and Liconti, 2020) but are a proven research tools for elasmobranchs (Holmberg et al., 2008; Barker and Williamson, 2010; Norman et al., 2017; Giovos et al., 2019; Pottie et al., 2021), and other marine (Dudgeon et al., 2019; Dunbar et al., 2021; Blount et al., 2022) and terrestrial species (Bradsworth et al., 2017; Parham et al., 2018; Mason and Arathi, 2019; Gould et al., 2021). Globally, sand tigers exhibit variable patterns of migration based upon reproductive status, sex and age, and our data supports the notion that the NWA population does as well.

The high number of encounters in the SAS database (n=2028) allows us to examine broad patterns in STS habitat use and opens pathways of investigation into other aspects of their ecology. Additional data gathered from photographs offers insight into the prevalence of injuries, such as those from fishing activities. Fishing related injuries in our study were less than what was observed in Australia by Bansemer and Bennett (2010) who found up to 29% of females and 52% of males with attached gear or injuries attributed to fishing. Many SAS sharks were observed with wounds and injuries of varying severity, but aside from mating scars it is rarely possible to determine the cause. Healing was observed in several resighted sharks, and recovery and functionality was further evidenced by sharks with significant wounds being resighted and even pregnant. Hydroids have been observed in the teeth of sand tigers elsewhere in their range and may indicate cessation of feeding (Pollard et al., 1996). We also collected information about associations with parasites, commensals and other fish species. Data about sand tigers and intraspecific fish associations are currently being assessed (A. McClanahan, unpublished) and will add to work on sand tiger, round scad, and mesopredator associations reported by Coleman & Burge (2021).

Below we evaluate our findings relative to the five questions posed for this project by combining temporal and spatial data from encounters, including from resighted sharks, and keyword data.

4.1 Are sand tigers consistently found around NC artificial structures, including shipwrecks, in enough numbers that photographic data can be used to inform movement and reproductive ecology?

The SAS database of 1837 sand tigers reflects what has previously been reported for sand tiger distribution in NC waters. Since STS are federally protected from harvest (NMFS, 2009, 2017) anecdotal reports of captures in recreational and commercial fisheries are rare (Mcclellan Press et al., 2016; Kilfoil et al., 2017). A 27-year systematic longline survey in Virginia suggest sand tigers are a minor constituent of the total abundance of sharks with only 135 sand tigers caught among 4830 total individuals during 577 longline sets (Latour and Gartland, 2020). Similarly, sand tigers were rarely reported in a decades-old scientific survey of South Carolina waters with 2 of 297 sharks captured on bottom longlines during 74 sets (Low and Ulrich, 1984). The most comprehensive and recent information available on sand tiger populations in the NWA (Carlson et al., 2009) synthesized scientific catch data from 1974–2004, concluding that trends in abundance of sand tigers showed only modest declines between 0.2% and 6.2% and that there was not growth overfishing as would be expected for such a long-lived and very low productivity species. Despite these encouraging signs for abundance and demographics in this species the authors note that “exceptionally low productivity of sand tigers and the relatively low sample sizes on which we based our trend analyses” argued for a continued listing by NMFS as a species of special concern (Carlson et al., 2009).

We received submissions from locations coastwide in NC and throughout the year. Some sites are more heavily represented, such as the complex of wrecks in the central region that includes *Aeolus, Spar, Caribsea* and *Atlas* (n=958 of 2028 encounters), as these are frequently visited SCUBA destinations (Gerken, 2013). Because of the live camera at Frying Pan Tower, we have a high proportion of observations from that location as well. Collectively, our observations are consistent with Paxton et al. (2020a) who found up to four times as many sand tigers at artificial habitats compared to natural hard bottom or natural ledge habitats. Others also document sand tigers at artificial reef habitats in the northern and central NC coasts (Whitfield et al., 2011; Brown et al., 2020). Using acoustic telemetry, Marens (2021) reported extended sand tiger residency at Frying Pan Tower; multiple shipwreck sites including *Caribsea, Proteus, Tarpon, Atlas, Papoose, Schurz*; and multiple artificial reef sites (e.g., AR-285, 275, 255) that are not popular dive sites (Dottie Benjamin, pers. comm.).

Valuable information about the occurrence of sand tigers comes from anecdotal and photographic evidence from the dive community about the sites frequented by SCUBA divers where sand tigers are most consistently observed. Conducting field research on sharks often requires labor-intensive and expensive methods to deploy divers, angle specimens and launch telemetry equipment, resulting in relatively fewer locations surveyed, fewer sampling days and lower numbers of sharks documented overall compared to the nearly 1900 sharks in SAS. Thus, because of its crowd-sourcing approach, SAS provided a cost-effective and non-invasive research tool. Resighting sharks within and between years will continue to illuminate finer-scale patterns of behavior.

4.2 Are there artificial reef sites that are especially important for sand tigers?

Despite the non-random sampling inherent to SAS, we can draw conclusions about which sites seem to be especially important. In the southern region, Frying Pan Tower has sand tigers year-round (Figure 4), and it is the potential role it may be playing for females during winter that is of particular interest as detailed below. The complex of shipwrecks in the central region is occupied by sand tigers year-round. Likely due to accessibility for dive operators and consistently good visibility, shipwrecks in this region are highly represented in the SAS database. Unbalanced sampling makes it difficult to draw rigorous conclusions about which sites may be most important for sand tigers, but the resighting data from 101 sharks indicate sand tigers display site fidelity within and between years and residency to shipwrecks in this region. Most sharks were resighted at the same or a nearby location, typically within a relatively short time span, indicating some sand tigers may be residential at certain sites for days to weeks in a given year. However, a few sand tigers were observed at the same location with months or even years between sightings. Given the high proportion of sand tigers in the SAS database recorded at Frying Pan Tower (24%), it is noteworthy that, of the 37 resighted sharks first recorded there, none were ever photographed at any other NC locations. In contrast, 28 of the resighted sharks were observed at more than one location, averaging more than 20 km away and up to 2176 DAL, indicating that individuals move between sites within and across years.

We have the least data from the northern shipwrecks (n=329 of 2028), perhaps due to the many days when marine conditions preclude diving. While this makes it difficult to ascribe more importance to any one site in this region, *Dixie Arrow*, *Tarpon* and *Proteus*, are noteworthy as possibly being important seasonally as aggregation sites for females. Marens (2021) found overall lower average residency times for sand tigers in the northern region (2 days) compared to southern (4 days) and central (6 days) sites. Though no explanation for this is evident, it is possible that environmental, physiological, or social drivers of occupancy and movement patterns may differ between these coastal regions in NC.

Our data showed most resightings occurred at the same or nearby locations, confirming previous findings that sand tiger sharks show high levels of residency and site fidelity to specific NC artificial reef sites (Paxton et al., 2019; Marens, 2021). This was especially evident at Frying Pan Tower where all the sharks first encountered there were resighted only there, including female USA-0272 who was seen there on 14 dates in 3 years. At several other locations, sharks were often resighted at the same site on sequential days or weeks and were also shown to return to the same site after presumably migrating out of NC waters for months or even years. Given the proximity of *Aeolus* and *Spar* and the high number of sharks resighted between those two sites, this area could be functionally perceived by the sharks as a single site as illustrated by many resighted sharks moving frequently between these two locations.

Our findings are consistent with telemetry data from Marens (2021) showing *Atlas*, *Caribsea*, *Aeolus*, and *Papoose* in the central region had the longest residency of male as well as immature and pregnant female sand tiger sharks (ranging from hours to 75+ days) compared to the other two regions. Coleman and Burge (2021) quantified frequency of sand tiger shark occurrence at Frying Pan

Tower in more than 1000 video clips from Nov 2014–Jan 2019, reliably finding them every year fall through winter.

4.3 Are there temporal patterns in habitat use or site fidelity?

SAS data suggest sand tiger occupancy patterns show differential use of sites based upon season. This was true of *Dixie Arrow* and *Tarpon* where female occupancy was highest in summer, while at *Proteus* it was highest in fall. Interestingly, the occupancy patterns of sharks at Frying Pan Tower appear to be shifting. Prior to 2020, high numbers of sand tigers appeared in Oct, remained abundant through the winter, and left in April, with summer months having few or no records. In the last three years, live camera data suggest sand tigers are increasingly abundant year-round, though winter residency remains the time of peak abundance (E. Burge and A. McClanahan, unpublished data).

SAS has the most shark encounters in summer months. Consistent with generalized migratory patterns for this species driven by reproductive cycles, we begin to see higher numbers of shark encounters in spring and through summer. This is also the time of year when mixed groups of sand tigers are known to be migrating to and inhabiting summer grounds further north (Haulsee et al., 2018). Marens (2021) noted variability in movement of females in summer with some going north, while 7 were never observed outside of NC during her 3-year study. It is unknown if the sharks observed in NC in summer months are migratory, residential or a combination of these, and more research into their movement ecology during this period is needed. Because SCUBA divers who are SAS submitters are more active in summer months, our data cannot be relied upon to provide unbiased detection, and a more rigorous sampling protocol to investigate this is required.

4.4 Are there differences in site use between male and females?

Overall, we see about four times as many females as males in the SAS dataset, with nearly a third of the sharks' sex not assignable. There is not a clear explanation for the unbalanced sex ratio, as we can find no published accounts that the population as a whole demonstrates a skewed sex ratio. Except for the two locations noted previously, sharks of both sexes were encountered at all locations. However, there is some indication that males and females may be utilizing habitats differently and at times may be sexually segregated. For example, at *Atlas, Proteus, Tarpon* and *Dixie Arrow*, females far outnumbered males. In contrast, other sites had male to female ratios more consistent with that of the SAS database, including *Spar, Aeolus* and *W.E. Hutton*. Studies in other populations have also found skewed sex ratios for this species during portions of the year, attributing this to sexual differences in movement patterns and differences in habitat requirements between juveniles and adults (Parker and Bucher, 2000; Lucifora et al., 2002; Smale, 2002; Lynch et al., 2013; Klein et al., 2019). Frying Pan Tower was one site were a higher proportion of males, including sub-adult males, were present contemporaneously with females, including in winter months. This was a different dynamic from any other location both in terms of timing of peak abundances in winter and sex ratios closer to parity.

Differences in female and male SAS encounter numbers could be attributed to males, especially adults, being more transient once mating season has passed. This is supported by most male encounters being in spring and summer months. If this is the case, then males may be more likely to be moving between shipwrecks and artificial reef sites and may display shorter residency times, thus having overall lower probability of being encountered by divers. Similar disparity in male versus female residency times, though not occupancy, which was similar, was found by Marens (2021) particularly in the central region. Those data also demonstrated that males that had migrated to NC waters after being fitted with acoustic telemetry tags in Delaware were absent in summer, unlike females that showed greater variability in summer residency patterns (Marens, 2021).

4.5 Is NC important for reproductive ecology of sand tigers?

The number of female sharks exhibiting mating scars indicate mating takes place in NC after males and females arrived following winter migrations. Given that females with healed mating scars continued to be observed through summer and fall, it appears at least a portion of pregnant females are remaining in NC waters for all or part of gestation. Some female sharks overwintered at Frying Pan Tower and many females observed there and elsewhere in winter appeared to be pregnant. Two videos from Frying Pan Tower (Erin Burge/Explore.org; 9 Sep 2021 https://www.youtube.com/watch?v=sZr3Jk45hF0, 11 Mar 2021 https://www.youtube.com/watch?v=tg6i79iZVgI) and one from Aeolus (12 Oct 2021; Ethan Simmons; https://youtu.be/E2_KmZb256I) show seemingly pregnant females with distinctive movement inside their abdomens likely to be live gestating pups. While this cannot be confirmed with photographic data alone, it is consistent with ultrasonography that confirmed 21 ovulatory and gravid females in NC between Jun-Jan (Marens, 2021; Wyffels et al., 2022; James Sulikowski, pers. comm.). Two of these sharks were part of Marens (2021) telemetry study and displayed extended winter residency at Frying Pan Tower before moving north in spring, presumably after parturition. SAS data suggest other locations that possibly serve as gestation aggregation sites in winter months included *Spar*, *Aeolus*, *Schurz*, and *Tarpon*, in the central region, and *Proteus* in the northern region. Marens (2021) similarly found pregnant tagged females off all regions of NC during winter months. This reproductive philopatry and aggregatory behavior of pregnant females is consistent with observations for this species in Australia (Bansemer and Bennett, 2009; Barker and Williamson, 2010; Lynch et al., 2013), South Africa (Smale, 2002; Dicken et al., 2007) and South America (Lucifora et al., 2002, 2009).

Mating and birth have not been observed *in situ* for this species in NC, and exact timing and location of these events remains unknown. SAS encounters of females both with mating scars and appearing pregnant are reported over spans of several months. Marens (2021) reported fresh mating scars were evident on females as late as early Aug. An extended mating timespan from Mar-Jun was described (Gilmore et al., 1983; Gilmore, 1993) as it may be occurring over a wide geographic range from NC to Florida. Although sand tigers are not thought to store sperm (Gilmore et al., 1983; Gilmore, 1993; Wyffels et al., 2022), one study did find they exhibit both behavioral and genetic polyandry in South Africa (Chapman et al., 2013). Females are investing significant energetic resources to produce only one or two offspring each reproductive cycle. If females in NWA are copulating with multiple males to enhance offspring fitness, they may be extending individual mating windows to increase the likelihood of encountering fit males. Gestation lasts between 9-12 months and may be determined by water temperature for this species (Bennett and Bansemer, 2004; Tokunaga et al., 2022). Dispersal of pregnant females along the NC coast in varying depths likely means they may be experiencing a range of winter temperature regimes. Thus, mating, gestation and parturition may be occurring over protracted and overlapping periods of time, rather than in tight synchrony. While these reproductive life history events may not all be taking place in NC for all sand tigers in the NWA population, SAS data reflects the importance of this area of the SEUS for this shark, particularly for mature females.

Based upon their size at encounter, USA-0128 and USA-L0817 were likely young of year (YOY) sharks, suggesting parturition may be occurring in NC waters. We have additional photographs not useable for SAS and anecdotes from divers of very small sand tigers, less than 1.2 m in length (the maximum size at birth for sand tigers (Gilmore, 1993; Gilmore et al., 2005) that support this possibility (C. Price, unpublished data). The exact locations of NWA pupping grounds have not been established for sand tigers. Our data contain no records of possible YOY sand tigers at Frying Pan Tower (E. Burge, unpublished data), nor have we observed small sharks there to suggest pupping is occurring at or near this location, despite its importance for overwintering pregnant females. One possibility is that females give birth closer inshore at natural hard bottom sites. This habitat type is widespread, but highly patchy, in NC (NCDEQ, 2016; Steward et al., 2022), not often frequented by dive charter operators, and often exhibits highly turbid conditions not conducive to underwater photography. In Australia, females do not give birth in estuaries, but are believed to prefer rocky reef habitats on the coast (Bennett and Bansemer, 2004). In South Africa, pupping grounds are also thought to be nearshore (Smale, 2002; Dicken et al., 2007).

5 Conclusions

SAS data indicated coastal NC may serve as a connective hotspot (Lowerre-Barbieri et al., 2021) linking sharks throughout their life stages at large and small spatial resolutions. Adult males and females, and sub-adults, are consistently present, even in months when conspecifics are residing further north or south. More than 25 SAS sharks appeared to be juveniles based upon slimmer body shape and size relative to other fishes or wreck features of known size. The presence of males with small claspers, suggested that young sharks may leave as-yet-undiscovered pupping areas to inhabit artificial reef sites which may be areas of high prey fish abundance (Rosemond et al., 2018). It is unknown how many of these juveniles may be out-migrating seasonally to established summer nursery areas further north (Kneebone et al., 2012, 2014) or using artificial reefs in NC as alternative nursery areas. Many shipwrecks off NC exhibit high rugosity relative to their surroundings (Paxton et al., 2017), and such three-dimensional structure offers considerable physical and ecological complexity in otherwise homogenous seascapes, simultaneously providing access to prey and protection from predators (Paxton et al., 2020a, b; Gámez and Harris, 2022). These data will aid future evaluation by NMFS of sand tiger management status, stock assessments, consideration of additional Habitat Areas of Particular Concern (HAPC) designations, and conservation efforts. New EFH in coastal Massachusetts for YOY and juveniles and HAPC in Delaware Bay for all life stages were only recently designated following research identifying the importance of these areas (NMFS, 2017). Locating sand tiger pupping grounds and nursery areas (Heupel et al., 2007) in NC are a high priority for a more complete understanding of the role NC waters play for sand tigers throughout their lives.

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 approved by NC Aquariums internal research review board. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

CP: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. AMc: Conceptualization, Data curation, Investigation, Visualization, Writing – review & editing. EB: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – review & editing. TH: Conceptualization, Investigation, Writing – review & editing. JH: Software, Writing – review & editing.

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Conflict of interest

Author TH was employed by company Blue Elements Imaging & Exploration.

The remaining 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.2024.1362703/full#supplementary-material

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Orca (*Orcinus orca)* and shark predator-prey interactions within Cabo Pulmo National Park in the Gulf of California, Mexico

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Orcas (*Orcinus orca*) have a worldwide distribution and as apex predators feed on a wide variety of prey, including marine mammals, seabirds, sea turtles, cephalopods, teleost fish and elasmobranchs. Historically, there have been few observations of orca predation on large elasmobranch species nor are they commonly identified in the stomach contents of deceased orcas. In recent years there has been an increase in observational records of orca predation, such as on white sharks (*Carcharodon carcharias*) in South Africa, which could indicate that elasmobranchs serve a greater importance in their diet than previously considered. These events are often documented by members of the public due to the development and affordability of innovative cameras, including the use of the drones (unoccupied aerial vehicles). Here we describe new predator-prey interactions between orcas and large shark species in Cabo Pulmo National Park (CPNP), a small no-take marine protected area located in the Gulf of California. In this paper, we were able to successfully identify individuals from the described orca pods in the region; specifically, the same individuals hunting sharks over a period of nearly two years. We discuss the potential implications of these new ecological interactions, given the importance of tourism to sustain the management of CPNP, as cascading impacts have been documented elsewhere in light of orca predations on sharks.

KEYWORDS

UAV, Orca, predation, elasmobranch, marine protected area

Introduction

Orcas (*Orcinus orca*) are found worldwide in temperature, tropical, and polar waters (Forney et al., 2006), and throughout their entire range they serve as true apex predators and thus strongly influence the structure and function of marine food webs through direct (e.g., consumptive) and indirect (e.g., predation risk) effects. Orcas are flexible consumers and exhibit a suite of complex cognitive abilities that aid in foraging (Paulos et al., 2010; Hill et al., 2022). As a result, orcas have "predatory access" to virtually every marine species with which they overlap, including those species which pose the greatest energetic challenges due to their speed/mobility, size, defense mechanisms, or cognitive capabilities. Large elasmobranch fishes, such sharks (but also many species of ray), are likely underestimated in the diet of orcas (Fertl and Darby, 1996), although records are now beginning to increase in the scientific literature (e.g., Jorgensen et al., 2019; Towner et al., 2022; Higuera-Rivas et al., 2023), prompting new questions into the scale and impact of predator-prey interactions between orcas and large elasmobranchs, especially sharks which also serve as apex predators in many ecosystems (Towner et al., 2023, 2024, Dixon et al., 2023).

Orcas have been studied extensively in coastal areas of the northeastern Pacific Ocean; however, populations in tropical pelagic regions have been little studied. Knowledge of their ecology, population structure, and other characteristics is limited. Three ecotypes of orcas have been identified in the northeastern Pacific differentiated by their external morphology, behavior, and feeding ecology: 'resident' (prey on fish), 'transient' (prey on marine mammals), and 'offshore' (prey on fish) (Baird and Stacey, 1988; Ford et al., 1998; Baird, 2000; Dahlheim et al., 2008). Genetic and acoustic attributes also define the ecotypes (Barrett-Lennard et al., 1996; Hoelzel et al., 1998). Multiple populations of these ecotypes are known to exist along the west coast of North America and there are 52 groups documented in the Eastern Tropical Pacific (ETP) region extending southwest from California to Hawaii and to Peru (Olson and Gerrodette, 2008). The orcas distributed in regions of the ETP show different morphological characteristics and a generalist feeding habit compared to other ecotypes (Ortega-Ortiz et al., 2023). Orcas in the Gulf of California belong to the ETP community and have been observed feeding on both marine mammals and elasmobranchs, but they have yet to be assigned as a separate ecotype (Guerrero-Ruiz et al., 1998; Higuera-Rivas et al., 2023).

Cabo Pulmo National Park (CPNP) is a small (71 km^2) marine protected area located on the south-east coast of the Baja California Peninsula, Mexico at the entrance of the Gulf of California. It has become a world-renowned scuba-dive destination in recent years due to the high rates of marine biodiversity and abundant fish populations, including sharks, resulting from the closure of fishing activities in 1995. Shark ecotourism is highly popular within CPNP (Gallagher and Hammerschlag, 2011), with bull sharks (*Carcharhinus leucas*) serving as one of the main attractions and which can be observed across multiple dive sites (Lara-Lizardi et al., 2022).

Seasonal aggregations of adult blacktip sharks (*Carcharhinus limbatus*) occur throughout the park each winter between December and April, a phenomenon that has been noticed by locals since 2008 (Ayres et al., 2021a) and by visual censuses carried out from a vantage point close to the shore since 2013 (Asúnsolo-Rivera, 2016; El-Saleh, 2016). Using unoccupied aerial vehicles (UAVs), sharks can be clearly observed occupying coastal shallow (< 5m) waters, with largest aggregations (over 1000 individuals) recorded in the northern sector of the park before the afternoon crepuscular period (Ayres et al., 2021a). This aggregating behavior has been linked to thermoregulation, as the shallow water sea surface temperature reaches its peak (~ 23.5°C) during this time between 1500 – 1600hrs. Another driver of shallow water habitat use may be predator avoidance, which has been demonstrated in adult *C. limbatus* in Florida, USA, that seek refuge from patrolling great hammerhead sharks (*Sphyrna mokkaran*) (Doan and Kajiura, 2020).

Here, we present new empirical observations of orcas hunting and predating on sharks in the Gulf of California, specifically within the boundaries of CPNP, using a combination of observation techniques. Specifically, we describe three discrete orca and shark predator-prey interaction events spanning two years, to profile the contemporary landscape of trophic interactions occurring at the top of the food chain in this ecologically significant region. We discuss our results as they relate to similar orca and shark interactions occurring in other locations globally, while highlighting the importance of citizen science and the value of expert naturalists in forecasting ecological change at the local level.

Method and results

A set of three discrete events were documented within the boundaries of Cabo Pulmo National Park (CPNP) Gulf of California, Mexico between 2022 and 2023, all of which describe the predatory behavior of orcas interacting with sharks: (I) an orca pod patrolling the well-known aggregation site of *C. limbatus* in the north of the CPNP; (II) an orca pod attempting to kill *C. leucas* inside the CPNP; and (III) orcas (of the same pod I), successfully predating on *C. leucas* in the south of the CPNP (Figure 1).

Event I: orca pod patrolling aggregation site of *C. limbatus* in the north of CPNP

On January 9th 2022, in the north of CPNP (23°27.441'N 109° 25.555'W) at 13:35hrs, a total of six orca (one adult male, four adult females and one calf) from a previously described pod of individuals (Higuera-Rivas et al., 2023) were filmed via an unoccupied aerial vehicle (DJI Mavic 2 Pro) deployed from a 26-foot fiberglass boat with a 4-stroke outboard motor (115 hp). Two adult female orcas split off from the rest of the group at 13:40hrs and approached the shore in a directed fashion, with one individual orca moving to within ~ 100 meters from the coast (Figures 2A, B) into the known location site of the seasonal blacktip shark (*C. limbatus*) aggregation described in Ayres et al. (2021a). At the time of the filming, no aggregations of *C. limbatus* aggregations were observed, possible due to orca presence. However, during the 7-minute occurrence within the shark aggregation site, one shark was located by the two female orca, but the shark immediately swam into deeper water, presumable after detecting the orcas. The two orcas did not chase the individual shark (Figure 2C). The orcas then reunited with the rest of the group. This separation of only some of the adult or semi-adult females from the rest of the group is a common strategy when searching for potential prey. High-quality aerial video frame shots were extracted for photoidentification and topside imagery

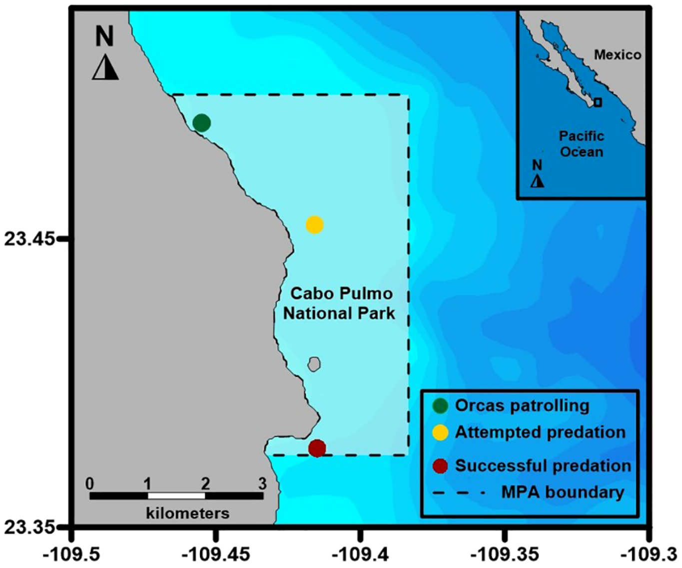

FIGURE 1
Map of Cabo Pulmo National Park and location of all three (I-III) events described in the present study.

from a Canon 1DXMK2 and 100-400mm Canon lens were taken, using distinctive features, including the eye patch, scars and nicks on dorsal fins (Bigg et al., 1987). One adult female orca, could be identified as "Quetzalli" from a distinctive notch near the base of the dorsal fin (Figure 2D), thus confirming that this group is part of the elasmobranch-eating pod of orca in the region. The photoidentification and progression of dorsal notch on "Quetzalli" is shown in Supplementary S1, Supplementary Material. Another adult

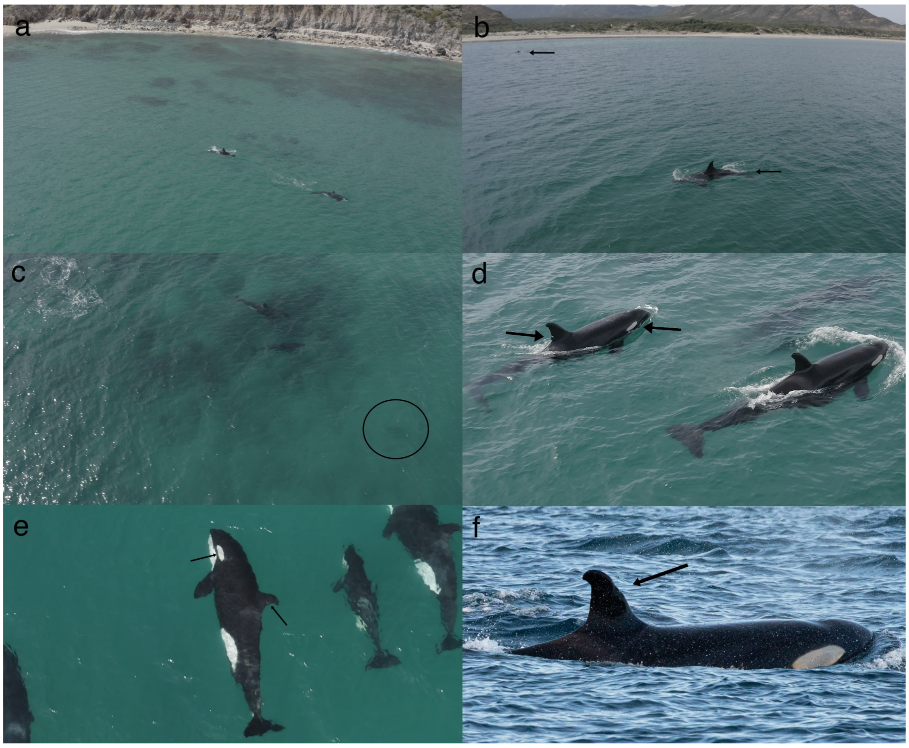

FIGURE 2
Aerial drone imagery of orcas patrolling in north of CPNP in known blacktip shark (*C. limbatus*) aggregation site on January 9th 2022 **(A)** The orcas swim along the coast. **(B)** One orca moved close to the coast (the far arrow) while "Quetzalli" (the near arrow) remained far from the coast. **(C)** The orcas locate a sole blacktip shark (the circle). **(D)** The unique eye patch and distinctive notch on the dorsal fin of "Quetzalli" are visible (black arrows). **(E)** The unique eye patch and distinctive round dorsal fin of "Waay" (black arrows). **(F)** Distinctive dorsal fin of "Waay". Photo credits: Jesús Erick Higuera-Rivas.

female orca was identified as "Waay" from the shape of the dorsal fin (Figures 2E, F), that had previously been observed preying on rays and other shark species in the Gulf of California in 2018 (Higuera-Rivas, unpublished field observations).

Event II: attempted predation of *C. leucas* at aggregation site in CPNP

On January 21st 2022, in the middle sector of CPNP (23° 27'17.712'N, -109°24'56.736'W) at 10:25hrs, a total of seven orcas (six females, one calf) were filmed by local dive guide (Carlos Lozano Hernandez) with an unoccupied aerial vehicle (DJI Mavic Air 2) deployed from a nearby beach. What followed was a coordinated attack by three of the adult female orcas attempting to predate on a bull shark (*C. leucas*) close to the dive site known as 'El Vencedor' which is approximately 15 m in depth. The shark could be seen on surface when one orca charged the shark on its side (Figure 3A), missing the shark only to return to try again immediately, then turning upside down and attempting to hit the shark with her tail, a hunting technique known as 'lob-tailing' or 'tail-slapping' (Figure 3B). The shark avoided the hit by swimming erratically and escaping. Following this, the second orca entered the frame and repeated the same behavior, first charging the shark head on, but missing, and then returning with a lob-tailing technique. Two more lob-tailing attempts by the second orca were made before the shark honed towards a local dive boat (26-foot fiberglass boat with a 4-stroke outboard motor, 115 hp), the engine was off, and tourists onboard observed the event take place (Figure 3C). The shark directly hit the starboard side of stern, splashing frantically and erratically circling the vessel before hitting the port side of the stern. One of the adult female orcas approached the vessel, making circles around and underneath the hull for approximately 30 seconds. The shark is not seen on the footage and is possibly underneath the dive boat, the orcas then leave the area, as the boat obstructed their ability to clearly hit the shark. The orcas were not seen again on the drone footage or by the passengers onboard. It was not possible to photo-identify the orcas involved in this attack, based on the available footage.

Event III: successful predation of *C. leucas* in CPNP

On December 6th 2023, in the south of CPNP (23°22'28'N, 109° 25'1'W) at 13:30hrs a total of five orcas (four adult females and one

FIGURE 3
Aerial drone imagery of orcas hunting a bull shark (*C. leucas*) in CPNP **(A)** The first orca charged the bull shark head on, and after missing employed **(B)** lob-tailing behavior to attempt to hit the shark with its tail. **(C)** Bull shark honed to local dive vessel to seek refuge from the orca predation. Photo credits: Carlos Lozano Hernandez.

calf) were sighted and filmed by a dive operator (Luis Mario Castro Arvizu from local dive operation 'Cabo Pulmo Sport Centre') using an unoccupied aerial vehicle (DJ Mavic Air 3) deployed from a 26-foot fiberglass dive vessel (115hp). Four adult female orcas were observed close to the surface in the same frame, one of which was seen with a shark in its mouth and two others appear to be sharing another shark below the surface (Figure 4A). The two orcas sharing the shark can then be observed closer to the surface where it is more apparent that it is a large shark species (Figure 4B). The sharks were identified as bull sharks (*C. leucas*) due to their morphology and their size relative to the orcas. One of the orcas then released the shark, followed by the second orca and it was left motionless just below surface, when the drone footage ends. In the same event, underwater footage with a GoPro was obtained in which one orca has carrion of the shark in its mouth (Figure 4F). The initial attack on the sharks was not witnessed but the predation event was successful as shown by the drone footage and underwater footage. From the underwater footage, two of the orcas can be identified as 'Niich' and 'Waay' as part of the elasmobranch-eating pod (that includes Quetzalli). 'Niich" has a wide notch just above the center of her dorsal fin (Figure 4D) and this image can be matched to an image from a previous predation event in the Gulf of California in 2018 (Figure 4C) where she was observed hunting pelagic stingrays (Higuera-Rivas et al., 2023). The orca "Waay" was photo-identified among the individuals in the group patrolling the zone of the blacktip shark aggregation of CPNP in Event I (Figure 4E), and can be seen in this post-kill social interaction with the bull shark (Figure 4F). She can be identified from the shape of her dorsal fin which is more rounded at the top (Figures 4E, F).

Discussion

Orcas have been documented feeding on elasmobranchs within the Gulf of California for over 50 years (Guerrero-Ruiz et al., 2007). The Gulf of California provides critical habitat for a wide range of marine mammal species such as pinnipeds which are resident year-round, as well as many species of dolphin and other large cetaceans which migrate seasonally such as blue whales (*Balaenoptera musculus*), grey whales (*Eschrichtius robustus*), and humpback whales (*Megaptera novaeangliae*). All these species are known to occur within the diet of orcas throughout their global distribution. The suite of prey options, combined with the semi-enclosed nature of the Gulf itself makes this region a highly profitable foraging

FIGURE 4
(A) Adult female orcas involved in bull shark predation event in CPNP a) Arrow points to shark in the mouth of adult female orcas. **(B)** Two adult female orcas share bull shark (*C. leucas*) closer to the surface. **(C)** Image of "Niich" in San Jose del Cabo, Los Cabos, Mexico in May 2018, during predation event on pelagic stingrays. **(D)** Image of "Niich" in CPNP during bull shark predation (Event III) **(E)** "Waay" in CPNP in blacktip shark aggregation site (Event I). **(F)** "Waay" in CPNP with bull shark carrion in mouth (Event III). Photo credits: Jesús Erick Higuera-Rivas **(C, E)** Luis Mario Castro Arvizu **(A, B, D, F)**.

ground for orcas. The events described here represent the first documented predator-prey interactions between orca and sharks within the boundaries of the marine protected area of CPNP.

The increase in use, availability and affordability of camera equipment including UAVs and underwater cameras is contributing to the ability to record these sightings and predation events. Two of the three events described in this paper were filmed by members of the public and uploaded to social media, which globally as a platform is helping advance and increase our scientific knowledge and understanding of the marine environment. In this sense members of the public become 'citizen scientists'. Based on the footage, we were able to identify the three orcas in this predator-prey interaction as "Quetzali," "Niich" and "Waay." One limitation of obtaining videos from social media and using citizens for science can be a lack of data available, in particular, suitable imagery (clear and high resolution) that can be used to identify individuals involved in predation events.

The abundance of sharks in CPNP has markedly increased in recent years. Within ten years of the park's creation (1995), local fish biomass was estimated to have increased by over 400% (Aburto-Oropeza et al., 2011), and in turn the increase in abundance and diversity of shark species (Reyes-Bonilla et al., 2016; Ayres et al., 2021b), that was not expected. This increase in the presence of sharks may have facilitated recent interactions with orca, especially because of the decline in shark populations that has occurred in the surrounding area that are not protected throughout the Gulf of California and Mexican Pacific due to shark fishing (Bizzarro et al., 2007).

A long-term monitoring project of the *C. limbatus* aggregations in CPNP was initiated in 2019 using a UAV to complete aerial surveys (Ayres et al., 2021a, b). The north beach of CPNP is surveyed every two weeks during the *C. limbatus* season to document abundance and their arrival to the park after their northerly migration in late spring into the Gulf of California. In the 2021 – 2022 (December – April) season, aggregations of *C. limbatus* were seen in the north beach of the park in December and at the start of January, however, they were not seen again in large aggregations until mid- March, approximately two months after Event I and have not been seen in this area on the aerial surveys during the 2022 – 2023 season. This clear absence of sharks coincides with the presence of orcas in the park. The site-specific absence resulting from orca predation risk is further supported by the fact that aggregations of *C. limbatus* (~100 individuals) were seen on surveys completed further south, in the center of CPNP over the season. This second survey area is protected by a reef break which would restrict access by orcas (especially at low tide), unlike the north area which has a sandy slope into deeper water in which orcas can easily navigate. The *C. limbatus* could therefore be seeking refuge in these more protected areas although in smaller aggregation sizes, as in previous years over 1000 individuals were recorded along the aggregation site in the north. Bull sharks were not seen at the dive site 'El Vencedor' where the attempted predation (Event II) occurred until three weeks after the event (personal communication from local dive guides). Our results therefore suggest that orcas could be in the process of creating an ecological "landscape of fear" (e.g., Gallagher et al., 2017) for these sharks that have only in recent years been documented in such abundances. Further evidence is required to support if the landscape of fear phenomenon is taking place in CPNP which will be provided by the on-going aerial monitoring of the park and the reported occurrences of bull sharks by divers at these sites.

The risk of predation orcas impose on sharks in other areas such as off South Africa, has caused an apparent shift in the presence of white sharks (*Carcharodon carcharias*), in which the majority of the population has disappeared entirely from several known aggregation sites along the coastline of the Western Cape of South Africa (Towner et al., 2022). These ecological shifts have dramatically affected local ecotourism operators and the socioeconomics of the industry which relied on white sharks (Gallagher and Hammerschlag, 2011). As the local ecotourism industry in CPNP relies heavily on sharks as a non-consumptive resource in diving operations, similar effects stemming from orca displacement of sharks would be dramatic, as a study recently reported an average of $694 USD is spent per visiting person per day at CPNP with an average of a 4-day stay (Pasos-Acuña et al., 2020). The continued aerial monitoring of CPNP and collaborations with local members of the public will determine the potential long-term effects of orca presence in the region and what effects of their predation on shark populations may be for local marine ecosystems and the communities that depend on them.

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

Ethical approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because The aerial imagery from the UAVs was already obtained and contributed by local members of the public in which no animals were harmed.

Author contributions

KA: Conceptualization, Writing – original draft, Writing – review & editing. AG: Supervision, Writing – original draft, Writing – review & editing. JH: Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing.

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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.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2024.1407379/full#supplementary-material

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Advancing the ecological narrative: documentation of broadnose sevengill sharks (*Notorynchus cepedianus*) in South Puget Sound, Washington, USA

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The broadnose sevengill shark (*Notorynchus cepedianus*) is a large, apex predator found in temperate waters around the world. Yet data on their distribution are limited, with reports of species occurrence typically restricted to specific bays or estuaries where they have been historically observed in high seasonal abundances. The Puget Sound is located in the southern portion of the Salish Sea, a large estuary spanning the border between northwestern Washington state, USA and southwestern British Columbia, Canada, and serves as an economic, cultural, and ecological hub. Until recently, there was only one verified record of broadnose sevengill sharks in the Salish Sea and none in the Puget Sound. However, our recent multi-agency collaborative effort revealed the presence of adult and sub-adult broadnose sevengill sharks in South Puget Sound, extending their previously known range hundreds of kilometers and into a new ecosystem. This work represents the first evidence of a significant presence of these apex sharks within the Salish Sea.

KEYWORDS

elasmobranch, marine ecology, movement, occurrence, predator

1 Introduction

Documenting spatial patterns of species occurrences is essential in explaining ecosystem dynamics and the processes that underlie them (Rangel et al., 2007; Cagnacci et al., 2010; Sims, 2010). As such, movement and distribution data have broad implications in research, conservation, and management, offering numerous insights to not only the species directly but also broader ecosystems and populations (Renshaw et al., 2023). This is particularly true for apex predators, as changes in their abundances can have significant impacts on ecosystem structure and function (*e.g.*, Elton and Nicholson, 1942; Paine, 1969; Beschta and Ripple, 2009). Thus, improving our understanding of where predators are found, and how they are affected by human activity and environmental change, is critical for managing species and maintaining ecosystem health.

In marine ecosystems, sharks often occupy key ecological positions as higher-order predators, contributing to ecosystem structure and stability (Heithaus et al., 2008; Ferretti et al., 2010; Bizzarro et al., 2017). As such, there have been efforts to determine the spatial patterns and occurrences of many shark species through various means, such as the use of fisheries-dependent data (Holts et al., 1998; Punt and Walker, 1998; Kai et al., 2017), genetics (Van Houtan et al., 2020), and electronic tagging technologies (Block et al., 2011; Queiroz et al., 2016). However, given the cost, technological complexity, and spatial extent of such studies, the movements of many shark species remain unknown or are oversimplified from lack of data (Van Houtan et al., 2020). Specific nuances such as shark habitat type use, pupping grounds, migrations, and broader spatial and temporal patterns of distribution are often generalized or inferred (Renshaw et al., 2023).

Broadnose sevengill sharks (*Notorynchus cepedianus*; BSS) are large (up to ~3 m), generalist predators that consume a wide variety of prey items, including crustaceans, teleosts, chondrichthyans, and marine mammals (Ebert, 2002; Williams et al., 2012; Funes et al., 2024). They have been reported in temperate coastal oceans around the world (Ebert, 1996), playing significant roles in maintaining marine ecosystems by virtue of their highly diverse diets (Last and Stevens, 2009). However, information on their occurrence and spatial extent, and therefore inference about their ecosystem impact, is limited to a subset of specific regions or bays within their presumed distributions – notably South Africa (Ebert, 1996), Argentina (Lucifora et al., 2005), and within the western United States in San Francisco Bay (Ebert, 1989) and Willapa Bay (Williams et al., 2012).

In August 2021, unverified anecdotal reports indicated that multiple (>10) BSS had been caught outside of their known distribution – in Hammersley Inlet, a small inlet located in the southern Puget Sound (Washington state, USA). The Puget Sound (6,200 km^2), part of the larger Salish Sea that spans the Canadian-U.S. border, hosts over 7,000 species (Center for Biological Diversity 2005) and lucrative commercial and recreational fisheries (*e.g.*, salmon, Dungeness crab, oysters; shrimp; McLeod et al., 2009; Wargo et al., 2013; Chasco et al., 2017; Morzaria-Luna, 2022), yet BSS are not known to be part of its marine ecosystems. Prior to 2021, the only verified record of BSS in the Salish Sea was located at Point Roberts, WA, near the Canadian border (Pietsch and Orr, 2019) (Figure 1). Unverified records include a single acoustic detection in the south Puget Sound of a tag attached to a BSS in Willapa Bay, WA (Williams et al., 2012).

BSS have been shown to be ecologically important in the habitats where they live (Lucifora et al., 2005; Funes et al., 2024). However, because they are not thought to reside in Puget Sound waters, BSS are conspicuously absent from ecological models there. Updated information on their range could be relevant to both BSS species management as well as the ongoing management of other species in the Salish Sea (McLeod et al., 2009; Chasco et al., 2017; Morzaria-Luna, 2022). Therefore, in this study, we aimed to collect such data by verifying the presence of BSS in the Puget Sound.

2 Methods

Field work took place in Hammersley Inlet (Figure 1) in spring and summer of 2022 and summer through winter of 2023. Hammersley Inlet is a narrow, shallow (<15 m) body of water which connects Oakland Bay to the rest of South Puget Sound. Sampling periods were chosen opportunistically around slack tides, given that Hammersley Inlet has fast currents that can prevent fishing lines from staying in place. Fishing took place by research vessel or from shore. Fishing was done using heavy tackle rods or with droplines, which consisted of a floating surface buoy attached to a vertical nylon line (test strength 1600 psi), lightly weighted at the bottom to allow free swimming of sharks if/when hooked. Each dropline was fitted with 2-3 wire leaders with 14/0 or 16/0 baited circle hooks along the vertical line, arranged so at least one hook was close to the bottom and others were suspended in the water column. Bait consisted of farmed Atlantic salmon (*Salmo salar)*, Pacific herring (*Clupea pallasii*), or opportunistically collected salmon (*Oncorhynchus* spp.) carcasses. Methods were approved under Oregon State University Institutional Animal Care and Use Committee protocol number 2020-0092 and Washington Department of Fisheries and Wildlife permit number 21-273.

Baited droplines were checked every 30 minutes for presence of sharks (either directly or for bite marks on bait) and/or to replace bait. GPS locations were noted using the boat's on-board electronics system (Garmin) when fishing from boat. When caught from the research vessel, sharks were restrained alongside the boat, securing the animal for measurements while allowing it to remain in the water and obtain aeration over the gills (Figure 2A). When captured from shore, animals were brought into the shallows and secured on top of a custom-made cradle, allowing head and gills to remain submerged while morphometric measurements were taken (Figure 2B). For each shark, we measured lengths in centimeters (cm; pre-caudal, fork, and total length in natural position), sex, as well as clasper length and rigidity (i.e., degree of calcification) in males to determine sexual maturity. Sharks were externally marked with individually numbered conventional tags (Floy Tag & Manufacturing, Inc., Seattle, WA) to identify individuals if recaptured. Hooks were removed before all sharks were released. Photos were taken (Figures 2A, B) for verification purposes by the University of Washington's Ichthyology Collection at the Burke Museum (Seattle, WA).

3 Results

We caught 9 BSS over 10 days of field sampling (Table 1). Species were verified as BSS by the University of Washington Burke Museum Ichthyology Collection using data and photos obtained while in the field (catalog number UW202515; Figure 2). They were

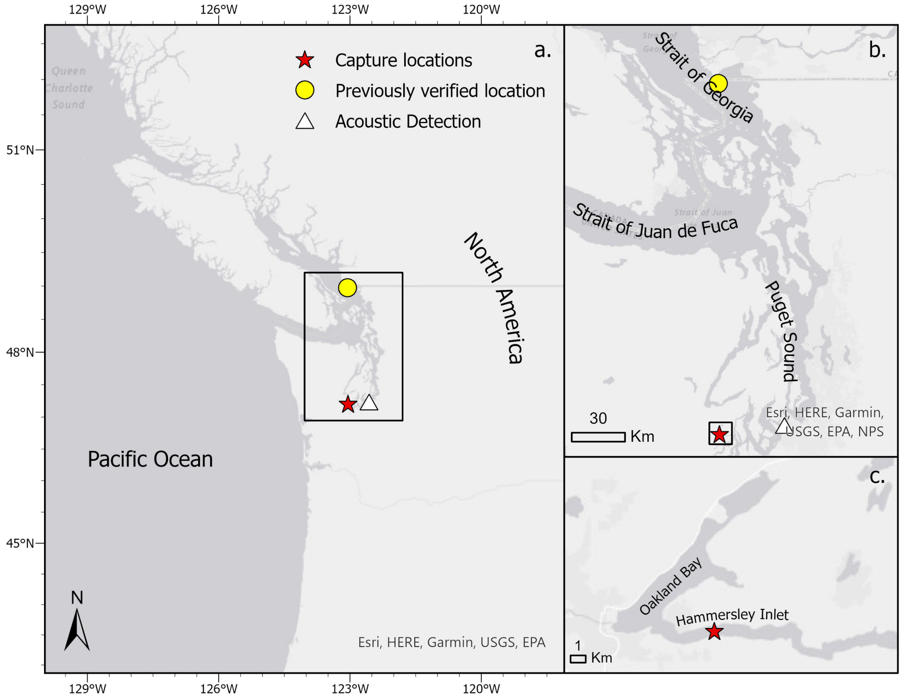

FIGURE 1
Map and locations of historical and recently identified broadnose sevengill shark locations in the **(A)** the Salish Sea between the northwest region of Washington state, USA and southern Canada, **(B)**. a zoomed view of the previously reported locations of BSS in the Salish Sea and **(C)** current evidence in Hammersley Inlet and Oakland Bay. The yellow circle indicates the only verified record in the region prior to this study, located at Point Roberts, WA. The white triangle represents an acoustic detection of a tag placed on a BSS in Willapa Bay, WA (Williams et al., 2012). The red star shows new capture locations documented within this study. The black boxes indicate the area in the subsequent tile.

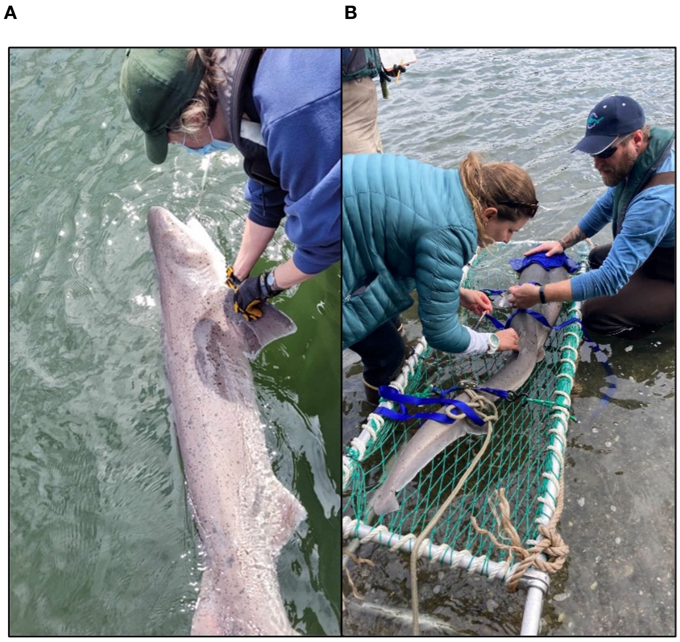

FIGURE 2
Broadnose sevengill sharks caught in Oakland Bay from boat **(A)** and from shore **(B)** during field efforts. Species identification was confirmed using the number of gills and the spotting pattern, and verified by the University of Washington Ichthyology Collection at the Burke Museum (catalog number UW202515).

positively identified by their seven paired gill slits, broad head shape, and unique spotting pattern covering the dorsal surface of the body (Barnett et al., 2012). All were males except for one female caught on the first sampling day. Male total lengths ranged from 136 cm to 212 cm (n=8; mean=166; SD=28.09); the female caught was 138 cm TL. Based on Ebert (2003) and calcification of claspers, all individuals were considered adult or subadult; no neonates or young-of-the-year (<120 cm) were caught. All sharks were captured from May to August, while none were caught during sampling efforts between September and November 2023 (Table 1). All animals were released and swam without difficulty from the capture site.

4 Discussion

We present verified and repeated evidence of BSS in South Puget Sound, the southernmost portion of the Salish Sea, more than 320 km beyond their previously documented range along the outer Washington coast. Despite the heavy anthropogenic activity in the Puget Sound, prior to our work, there was only one verified report of BSS in the entirety of the Salish Sea (over 200 km away), which occurred over two decades ago (Figure 1). Thus, BSS were not thought to occur in the Salish Sea with regularity nor are they currently considered in management efforts there (Harvey et al., 2012; Preikshot and Bobbi Cheney, 2015). However, our data suggest a seasonally consistent presence far into South Puget Sound, which has only one outlet to the north Pacific Ocean, meaning the species must occupy waters of the central Salish Sea on at least a transitory basis. With their high trophic status and ecosystem impact in other locations, these findings may suggest a need to revise our understanding of ecological dynamics in the Salish Sea. Moreover, our work has broader implications for our understanding of BSS movements globally, highlighting the value of further exploration into the range of this species.

The lack of observations of BSS in the Salish Sea prior to this study makes it difficult to determine whether BSS presence is a new development, or if they have been simply missed from surveys and fishing efforts in this area. Previous efforts to observe sharks in the Puget Sound noted other elasmobranch species, but not BSS. For instance, thousands of trawl surveys spanning decades, completed by the Washington Department of Fisheries and Wildlife, found other shark species but never a single BSS (Quinnell, 1991; Palsson et al., 2002, Palsson et al., 2003; Blaine et al., 2020). Additionally, the nearby Seattle Aquarium aimed to specifically assess shark presence in the area using baited remote underwater video cameras (BRUV), deployed at night every other month for over 12 years (2002-2005, 2008-2015) from under the aquarium in downtown Seattle (Griffing et al., 2014). This work successfully recorded >250 observations of bluntnose sixgill sharks (*Hexanchus griseus*), but no BSS. Both bluntnose sixgill sharks and BSS scavenge for carrion (Ebert, 1991, Ebert, 1994) and can co-occur at the depth range of these surveys since both species move into shallower waters at night (Andrews et al., 2009; Barnett et al., 2010); therefore, it is reasonable that extensive nighttime BRUV efforts capturing bluntnose sixgill sharks would have also observed BSS had they been in the area. No bluntnose sixgill sharks were taken as bycatch during BSS target fishing for this

TABLE 1 Dates of targeted broadnose sevengill shark (BSS) fishing, hook soak times (field hours x # of hooks), and total number of BSS caught each day.

Date		Hook soak times	# BSS caught	Sex	PCL (cm)	TL (cm)	Est. maturity
2022	5/23	12.75 hrs	2	M	152	212	A
				F	94	138	I
	7/7	40.25 hrs	2	M	140	195	I
				M	131	185	I
2023	6/8	13 hrs	1	M	115	165	I
	6/14	32 hrs	2	M	103	151	I
				M	99	145	I
	8/11	28 hrs	1	M	100	139	I
	8/15	8.5 hrs	1	M	94	136	I
	9/15	5 hrs	0	No individuals captured			
	10/6	50 hrs	0	No individuals captured			
	11/29	32.5 hrs	0	No individuals captured			
	11/30	2.5 hrs	0	No individuals captured			

Information on individual BSS caught include sex (M, male; F, female), pre-caudal length (PCL) and total length (TL) in centimeters, and estimated sexual maturity. Sexual maturity estimates (A, sexually mature 446 adult; I, immature sub-adult) are based on previous work by Ebert (2003) and degree of clasper calcification for males (mature = rigid, immature = non-rigid) and total length for females (mature 447 = >250 cm TL, immature = 120-250 cm). No neonates or young-of-the-year individuals (<120 cm TL) were captured.

study, likely due to their deeper depth preferences during the day compared with broadnose sevengill sharks (Andrews et al., 2009; Barnett et al., 2010). However, annual presence of pinnipeds, a key prey species for BSS (Lucifora et al., 2005; Funes et al., 2024), has steadily increased in this region since the 1990s. Carretta et al. (2016) hypothesized that natural pinniped predators, such as orcas, may increase their presence in this region with this increasing prey abundance. It is therefore possible that BSS presence in this region may be the result of a recent distributional shift in response to changes in local prey resources.

Regardless of the persistence or novelty of BSS in South Puget Sound, our findings emerge at a timely junction in the management of the Puget Sound's resources. Given the importance of apex predators in ecosystem regulation, studies of ecosystem and resource management should expand to include sharks such as BSS in the Puget Sound. To support this management, future research efforts should focus on better defining the ecological role of BSS here specifically. By assessing BSS population abundance, identifying their fine- and large-scale movements, exploring patterns of seasonal presence, connectivity with other populations, and examining BSS foraging ecology within Puget Sound and the Salish Sea at large, we can better quantify the impact they are likely having in this productive region.

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 Institutional Animal Care and Use Committee (IACUC). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

JS: Conceptualization, Investigation, Methodology, Supervision, Visualization, Writing – original draft, Writing – review & editing. EP: Conceptualization, Investigation, Methodology, Writing – review & editing, Visualization. DL: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. LH: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – review & editing. AM: Writing – review & editing. TC: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

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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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Observational data on the reproductive condition of female Oceania fantail rays, *Taeniura lessoni*, from Drawaqa Island, Fiji

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Data on the reproductive biology of elasmobranchs are essential for understanding their life history. Published studies on batoid ray reproductive biology are comparatively scarce, leading to limited understanding and data gaps. The Oceania fantail ray, *Taeniura lessoni*, is a good example. This Data Deficient nearshore stingray is restricted to Melanesia, with lacking biological and ecological data, including reproduction. To expand upon the limited life-history data for this species, this short paper provides observational data on the reproductive condition in female *T. lessoni*, at Drawaqa Island, Fiji. Field work involved direct observations and ocean temperature measurements. Over 40 days spanning three months, 105 surveys were conducted across five sites, resulting in 71 sightings of the species. Based on spot patterns and body markings, four female individuals were identified. Between January and March 2024, these females exhibited convex dorsa indicating advanced gestation, transitioning to concave dorsa suggesting parturition. The presence of neonates from early March onwards coincided with the estimated parturition period inferred from the rays' condition. Furthermore, a female previously pregnant was photographed with a dermal abrasion around her pectoral fin, possibly indicating pre-copulatory biting, suggesting a continuous reproductive cycle. The average monthly water temperature at the surveyed sites remained relatively stable throughout the study. Collectively, our findings suggest that Drawaqa Island provides suitable habitat niches for reproductive activities in female *T. lessoni*. Repeated and long-term data is certainly needed to confirm either a continuous reproductive cycle or seasonal peaks. While preliminary, our observational data represents the first documentation on female reproductive condition in a stingray in Fiji.

KEYWORDS

batoids, reproductive biology, Melanesia, data deficient species, range restricted, *Dasyatidae*

1 Introduction

Elasmobranchs (sharks, skates, and rays) are an evolutionarily conserved, diverse, and threatened vertebrate group (Dulvy et al., 2021; Sherman et al., 2023). Threats encompass direct and indirect fisheries activities (Worm et al., 2024), ocean warming (Rosa et al., 2017; Osgood et al., 2021), and habitat degradation, including disturbances to coastal breeding and pupping grounds (Sherman et al., 2020; Simpfendorfer et al., 2023).

Elasmobranchs have developed nine distinct reproductive strategies (Awruch, 2015) over their 400-million-year evolutionary history (Kriwet et al., 2008). Batoids, the most diverse group of cartilaginous fishes (Aschliman et al., 2012), comprise four orders, 23 families and 663 species, with many more yet to be described (Last et al., 2016a). While the largest order, Rajiformes (skates), is strictly oviparous, the remaining orders (*Myliobatiformes, Rhinopristiformes*, and *Torpediniformes*) are viviparous, with lecithotrophic and matrotrophic modes of reproduction (Conrath and Musick, 2012; Awruch, 2015). Myliobatiformes, including the family *Dasyatidae* (stingrays), rely on lipid histotrophy via trophonemata. Stingrays contain 19 genera, 86 extant species (Last et al., 2016a), and represent the most abundant group of rays occurring in tropical and subtropical coastal waters (White and Dharmadi, 2007). Stingrays produce broods of one to 10 with gestation periods up to 11 months, while smaller tropical species have shorter gestation periods of three to six months (Fahy et al., 2007; Pierce et al., 2009; Mull et al., 2010; Furumitsu et al., 2019). The duration of reproductive cycles varies among stingrays (Walker, 2020), and annual and biannual cycles have been confirmed in wild populations (Ramírez-Mosqueda et al., 2012; Schieber et al., 2023). However, batoid ecology and life history are comparably less understood than in sharks (Bräutigam et al., 2016; Martins et al., 2018; Jorgensen et al., 2022), with more than 250 Data Deficient species in the International Union for Conservation of Nature (IUCN) Red List (IUCN, 2023).

The Oceania fantail ray, *Taeniura lessoni*, (Last, White & Naylor, 2016) was described in 2016 (Last et al., 2016b), marking the second species within the genus alongside the widely-distributed bluespotted lagoon ray, *T. lymma*, (Forsskål, 1775). The disc width (DW), defined as the maximum distance between the wingtips (Serra-Pereira et al., 2010), ranges between 18 cm to 22 cm in female *T. lessoni* paratypes (Last et al., 2016b). The mature male holotype has a 20.9 cm DW, with large claspers measuring 21.2% of this width, while the immature paratype male measured 18.5 cm (Last et al., 2016b). *T. lessoni* is smaller than *T. lymma* and lacks the pair of vivid blue longitudinal stripes found along the tail. Also, the species appears to be restricted to Melanesia, including Papua New Guinea, Solomon Islands, Vanuatu, and Fiji (Last et al., 2016a; Hylton et al., 2017). *T. lessoni* inhabits shallow-water coral reefs, usually at depths of 20 m and less, typically shelters in caves during the day, and forages at night (Last et al., 2016a). In *T. lymma*, the embryos are initially nourished by the yolk sac and subsequently feed on uterine secretions (Hamlett et al., 2005; Musick et al., 2005; Abel and Grubbs, 2020). Fecundity has been reported with brood sizes of one to seven in wild individuals (Ferreira, 2013; Pereira et al., 2017). However, reproductive biology data for *T. lessoni* is lacking, and this species is classified as Data Deficient by the IUCN (Kyne and Finucci, 2018). In Fiji, *T. lessoni* is regularly observed during snorkeling trips and dives. Sightings are reported from across the archipelago (Glaus et al., 2024a), and the species is caught in small-scale fishing activities (Glaus et al., 2024b). To expand upon the limited life-history data for this range-restricted species, this study represents preliminary observational data focusing on the distribution and reproductive condition of female *T. lessoni*.

2 Materials and methods

2.1 Study area

The study area was located approximately between 17° South latitude and 177° East longitude (Figure 1) and focused on the coastal waters off Drawaqa Island within the Yasawa Island Group in western Fiji. Besides a single tourism operator (Barefoot Manta Island Resort), Drawaqa Island is uninhabited and belongs to the traditional landowners of Mua-ira on the nearby island of Naviti (Murphy et al., 2018). The northern point of Drawaqa Island includes five beaches, which were surveyed: Goat, Lagoon, Manta, Sunrise, and Sunset Beach (Figure 1).

2.2 Field work

Since January 2024, research on Drawaqa Island has been exploring environmental factors influencing spatiotemporal variation in ray species abundance and distribution, with the data presented here being part of a larger dataset. Fieldwork involved direct observations and ocean temperature measurements using three HOBO water temperature Pro V2 (U22) data loggers deployed on-site. Over 40 days across January to March, 105 surveys were conducted to observe rays. Each survey lasted 45 min for standardization, and were conducted as roving explorations, including 53 snorkel surveys, 32 beach walks, and 20 SCUBA dives: Sunrise Beach (68 surveys: 39 snorkels, 20 dives, 9 walks), Lagoon Beach (11 surveys: 10 walks, 1 snorkel), Goat Beach (10 snorkels), Manta Beach (9 surveys: 2 snorkels, 7 walks), and Sunset Beach (7 surveys: 6 walks, 1 snorkel). Snorkel surveys were conducted at all sites along 100 m line transects parallel to the shoreline. Maximum depth was 12 m, with visibility extending to the seafloor. SCUBA dives, performed only at Sunrise Beach due to sufficient depth, followed predetermined 100 m line transects perpendicular to the shore, from the shallows to a maximum depth of 21 m, with visibility ranging from 8 to 20m. For each line transect, the surrounding visibility was scanned for the presence of *T. lessoni* within the maximum visible range on either side of the line. The line transects followed the reef structure along the same route for each snorkel and dive survey. Beach walks were carried out at four of the five sites.

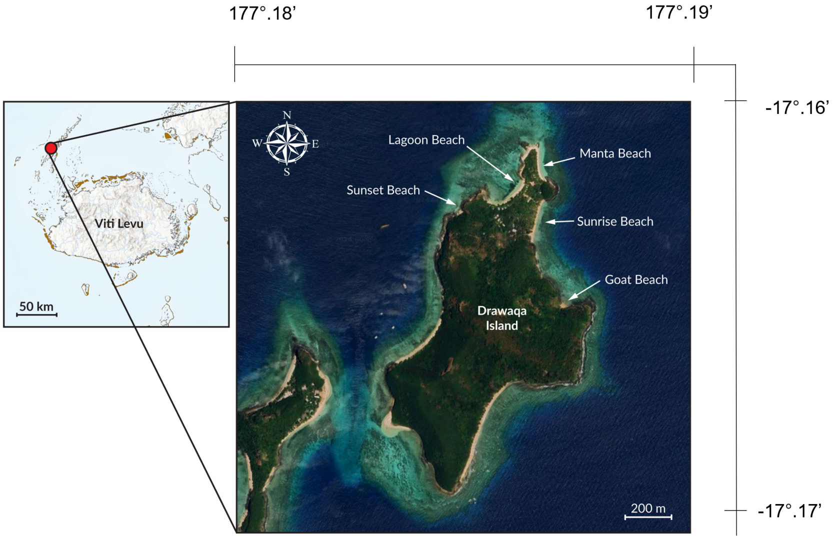

FIGURE 1
Location of the five beaches surveyed in Drawaqa Island, in Western Fiji.

Whenever possible, sighted *T. lessoni* were photographed using either a GoPro12 or Olympus TG7. Images of *T. lessoni* were recorded to identify distinct spotting patterns and body markings (McIvor et al., 2023). Photographs were also inspected for signs of advanced gestation (i.e., convex dorsum), recent parturition (i.e., concave dorsum) (Henningsen, 2000; Spieler et al., 2013), and evidence of mating behaviour (i.e., fresh bite wounds) (Kajiura et al., 2000). The sex of rays was determined by the presence or absence of claspers (Awruch et al., 2008), and the DW was estimated *in situ* and from images (Supplementary Figure 1). *T. lessoni* with a DW greater than 20 cm were classified as mature, and rays less than 20 cm DW were considered immature (Last et al., 2016b). Size at birth for *T. lymma* is 13 cm to 14 cm DW (Last et al., 2016b). As *T. lessoni* is smaller and due to the uncertainty associated with *in situ* estimates, only the smallest individuals, with an estimated DW of 10 cm (roughly palm-sized), were considered as neonates.

2.3 Data handling

The data were recorded, with individuals whose sex could not be determined denoted as “nA”. Plots were generated using the ggplot2 package (Wickham, 2011; Wickham and Bryan, 2023) in R (R Development Core Team, 2005). Catch per unit effort (CPUE) was calculated by dividing total *T. lessoni* sightings at a site by the number of surveys conducted there.

3 Results

T. lessoni were observed in 71 surveys (67.6% of the total number surveys). Number of sightings by location varied: Sunrise Beach (*n*=47), Goat Beach (*n*=9), Lagoon Beach (*n*=6), Manta Beach (*n*=6), and Sunset Beach (*n*=3). At Sunrise Beach, mature females were sighted 38 times, six times at Goat Beach, and once each at Manta Beach and Sunset Beach. Mature males were observed three times each at Sunrise Beach and Goat Beach, and once at Sunset Beach. Neonates were sighted from early March onwards: six times at Lagoon Beach, two times at Manta Beach, and one time each at Sunrise and Sunset Beach (Supplementary Figure 2). CPUE was highest at Goat Beach (0.9 *T. lessoni* observed per survey), followed by Sunrise Beach (0.7 *T. lessoni* observed per survey), Manta Beach (0.7 *T. lessoni* observed per survey), Lagoon Beach (0.6 *T. lessoni* observed per survey), and Sunset Beach (0.4 *T. lessoni* observed per survey).

Snorkelling recorded the highest number of sightings (*n*=48), followed by beach walks (*n*=15), and dives (*n*=8) (Table 1). Mature females and males were mostly observed during snorkelling surveys, while immature specimens were predominantly sighted during beach walks (Table 1).

At Sunrise Beach, four females in advanced gestation with conspicuously convex dorsa (Figure 2) were recorded, occurring in shallow waters down to eight m depth. These four females were collectively recorded 24 times at the same site from the beginning of January until the end of March 2024. Individual 1 was recorded 13 times, Individual 2 six times, Individual 3 four times, and Individual 4 once, accounting for 63.2% of all female *T. lessoni* sightings at Sunrise Beach (*n*=38, Supplementary Figure 2). The DW of Individual 1 was estimated to be 24 cm to 25 cm, DW of the remaining three individuals in advance gestation was estimated between 21 to 25 cm.

The first indication of parturition was noted on February 29, 2024, when Individual 1 was photographed with a concave dorsum (Figures 3A, B). Over the following two weeks, Individual 3 (March 4) and Individual 2 (March 12) followed suit (Figures 3C-F). Additionally, Individual 1 exhibited a visible dermal abrasion on the left pectoral fin (Figure 3B; Supplementary Figure 3).

TABLE 1 Sightings of *T. lessoni* across different survey methods, including the number of sightings per sex, and likely maturity level based on *in situ* DW estimates.

Survey type	#Sightings	#Mature F > 20 cm	#Immature F 15-20 cm	#Neonate F 10 cm	#Mature M >20 cm	#Neonate M 10 cm	#Mature nA >20 cm	#Immature nA 15-20 cm	#Neonate nA 10 cm
Snorkel	48	36	0	1	6	1	3	0	1
Beach walk	15	4	1	2	1	0	1	1	5
Dive	8	6	0	0	0	0	2	0	0

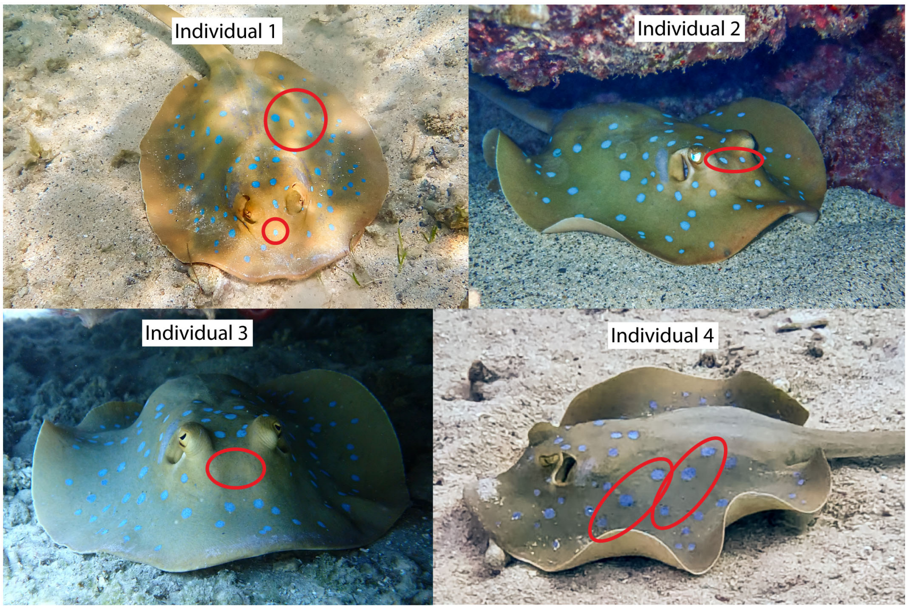

FIGURE 2
Photographs of individual *T. lessoni* in advanced gestation observed at Sunrise Beach, Drawaqa Island, with convex dorsa, indicative for advanced gestation. Encircled in red are selected differentiating features: Individual 1 displays single antorbital blue spots, and a row of three prominent vertical blue spots followed by a row of horizontal blue spots posterolateral; Individual 2 has two antorbital blue spots; Individual 3 features a plain antorbital area, including a tail partly cut (shown in Figure 3F), and Individual 4 has scapular and posterolateral rows of three blue spots.

During the study period, the water temperature ranged from 28.22°C to 33.08°C. The lowest temperature was recorded at Sunrise Beach in January with an average of 29.94°C, while the highest temperature was measured at Sunset Beach with an average of 30.52°C in March.

4 Discussion

This study represents the first documentation of advanced gestation in a stingray species in Fiji. We recorded four pregnant *T. lessoni* (Figure 2), of which three gave birth between the end of February and mid-March, based upon photographic evidence (Figure 3). The presence of neonates from early March onwards coincided with the estimated parturition period inferred from the rays' condition. We were unable to determine the periodicity of the reproductive cycle and the gestation period, but *T. lessoni* may reproduce asynchronously and aseasonally. In a captive stingray species (formerly known as *Dasyatis kuhlii* and *Neotrygon kuhlii*) mating was observed immediately after parturition, suggesting the absence of a specific breeding season (Janse and Schrama, 2010). Based on bycatch data from eastern Indonesia, neither distinct seasonal reproductive cycles nor synchronicity in three stingray species could be determined, indicating a continuous reproductive cycle (White and Dharmadi, 2007). The dermal wound around the pectoral fin in Individual 1 (Figure 3B; Supplementary Figure 3) suggests a bite or abrasion from a pectoral grip or pre-copulatory biting, indicating that copulation may occur soon or immediately after parturition, as observed in captive *T. lymma* (Smith et al., 2017). Therefore, a continuous cycle with the potential for multiple pregnancies annually seems likely. However, long-term data, ideally combined with ultrasound diagnostics (Murakumo et al., 2020), are required to document gestation and to determine whether *T. lessoni* follows a continuous reproductive cycle or exhibits seasonal peaks around Drawaqa Island. To ensure practicality and minimal invasiveness, we determined maturity levels based solely on *in situ* size estimates. Assaying sex steroid hormones instead, enables accurate determination of maturity levels and depiction of cumulative proportions across different developmental stages (Mull et al., 2010). The surveyed sites on Drawaqa Island exhibit similar average temperatures ranging from 29.94°C to 30.52°C. Neonates and presumably immature *T. lessoni* were mostly observed during beach walks (Table 1), and at Manta Beach and Lagoon Beach

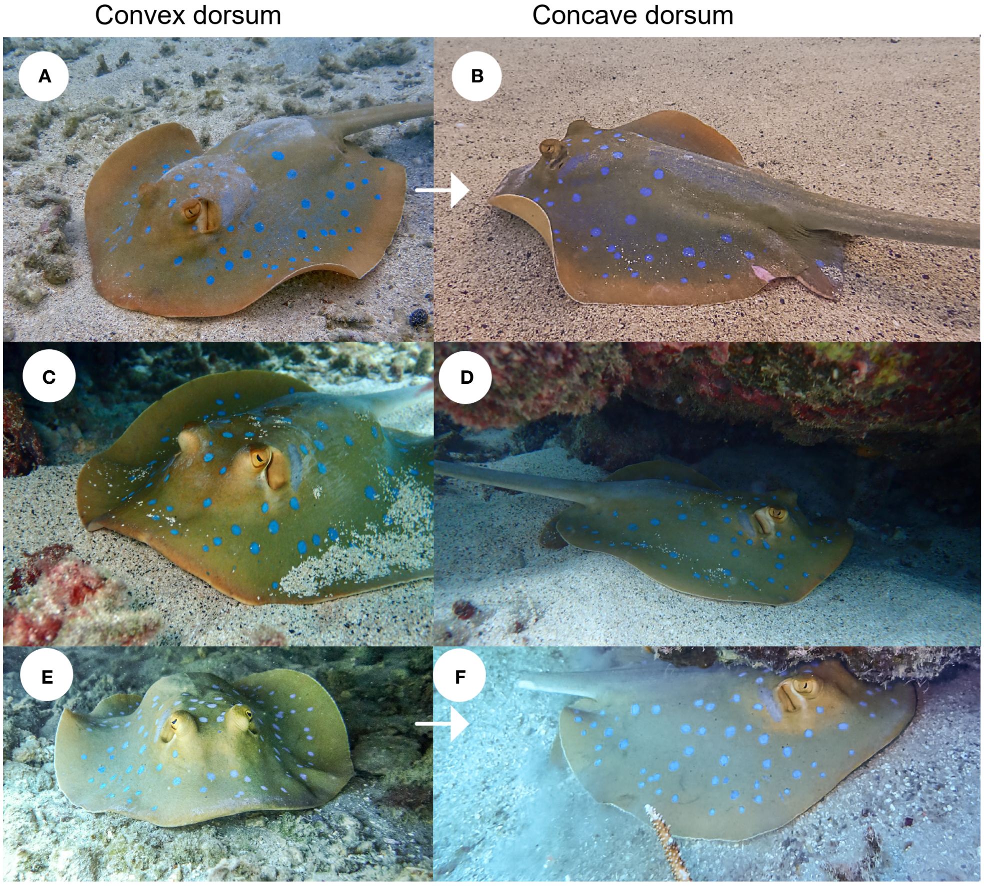

FIGURE 3
Visual comparison of Individuals 1, 2, and 3 during advanced gestation **(A, C, E)** and post-partum **(B, D, F)** stages. Image **(A)** of individual 1 was taken on February 3, 2024, during advanced gestation, while **(B)** was captured on February 29, 2024, post-partum. Image **(C)** shows the individual 2 on January 13, 2024, during advanced gestation, and **(D)** on March 12, 2024, post-partum. Image **(E)** shows the individual 3 on February 11, 2024, during pregnancy, and **(F)** on March 4, 2024, post-partum.

(Supplementary Figure 2). Manta Beach and Lagoon Beach have a similar habitat composition with areas of sandy patches, rubbles, and seagrass assemblages, which could provide shelter for neonate *T. lessoni* (Dabruzzi et al., 2013). Sunrise Beach, predominantly frequented by mature females, has narrower expanses of sandy patches. Except for Individual 4, which was sighted only once, the other three females remained at Sunrise Beach, indicating repeated use of the area. Interestingly, mature males were only encountered seven times (Table 1). One possibility to explain the skewed sex ratio towards females could be attributed to the increased energetic requirements of females during gestation (Jirik and Lowe, 2012), and thus the selection of areas that offer favourable conditions, including prey availability (Delpiani et al., 2013) and protection from predators (Martins et al., 2018).

Moving forward, a small-scale passive acoustic telemetry study, combined with ongoing environmental data collection, could help better understand presence, activity patterns, sexual segregation (Simpson et al., 2021), and seasonal or long-term site fidelity (Schlaff et al., 2014; Elston et al., 2021; Kraft et al., 2023). The waters surrounding Drawaqa Island are home to at least nine batoid species: *T. lessoni* (Data Deficient), *Mobula birostris* (Endangered), *Aetobatus ocellatus*, *M. alfredi*, *Pateobatis fai*, *Taeniurops meyeni* and *Urogymnus asperrimus* (all Vulnerable), *Rhynchobatus australiae* (Critically Endangered), and *Neotrygon* sp (Gordon and Vierus, 2022; Glaus et al., 2024a). Our preliminary data indicate that Drawaqa's nearshore waters likely serve as pupping grounds for *T. lessoni*. Considering this, along with the diversity of species, the easy accessibility, and the continued monitoring setup, this location is ideal for further studies to deepen our understanding of ray ecology and biology.

Data availability statement

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

Ethics statement

Ethical approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because the study was non-invasive and did not include any handling of living animals. The findings solely derive from visual observations.

Author contributions

KG: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Visualization, Writing – original draft, Writing – review & editing. TV: Conceptualization, Data curation, Validation, Visualization, Writing – original draft, Writing – review & editing. RM: Data curation, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing.

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Conflict of interest

RM is a board member of the Drawaqa Marine Conservation Trust.

The remaining 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.2024.1401258/full#supplementary-material

SUPPLEMENTARY FIGURE 1
In situ DW estimate of Individual1.

SUPPLEMENTARY FIGURE 2
Number of T. lessoni sightings and estimated maturity levels across the surveyed sites.

SUPPLEMENTARY FIGURE 3
Individual1 feeding, showing visible dermal abrasion on the left side.

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Observation of the critically endangered soupfin shark (*Galeorhinus galeus*) in the Changing Salish Sea

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1 Introduction

High trophic-order predators play a critical role in ecosystems, exerting top-down control on prey species populations through direct predation (Terborgh et al., 1999; Pinnegar et al., 2000; Feit et al., 2019) and modify the behavior and habitat utilization of lower trophic level species through their presence in these systems (Ripple and Beschta, 2004; Heithaus et al., 2007). Therefore, shifts in the abundance and distribution of top predator species within an ecosystem can have cascading effects on lower trophic levels, changing trophic dynamics and ecosystem function (Heithaus et al., 2008; Baum and Worm, 2009; Newsome and Ripple, 2015). Thus, identifying shifts in the spatiotemporal habitat utilization of predators can be critical to the management of ecologically and economically valuable systems.

As a highly migratory marine predator, the soupfin shark (*Galeorhinus galeus*; aka school shark, tope) occupies a high trophic position (Bizzarro et al., 2017) and can exert top-down effects through direct predation upon forage fish, commercially valuable fish species, and smaller sharks (Nakatsu, 1957), significantly influencing trophic dynamics and biodiversity of their system. This coastal-pelagic species possesses an amphitropical distribution, with several geographically and genetically distinct populations throughout temperate waters (Chabot and Allen, 2009; Chabot, 2015) and seasonally inhabits a diverse array of habitats, spanning from shallow estuarine systems to the mid-continental slope (Thorburn et al., 2019; COSEWIC, 2021).

The northeast Pacific (from British Columbia, Canada, to Baja California, Mexico) once supported an extensive but brief fishery for soupfin sharks in the 1930s-40s (COSEWIC, 2021), and this species is still regularly encountered in fisheries and fishery surveys throughout these coastal waters. However, while common in estuarine systems in other regions (Walker et al., 2020; Nosal et al., 2021), soupfin sharks were not thought to inhabit the Salish Sea (Pietsch and Orr, 2015; Blaine et al., 2020; Lowry et al., 2022). The Salish Sea is an expansive inland waterway covering 16,925 km^2 across Northwestern Washington State and Southern British Columbia (Figure 1) that serves as a nexus of economic, environmental, and cultural interests (Gaydos et al., 2008; Jones et al., 2021). Despite consistent and extensive research and fishing efforts, apart from two recent strandings and a reported commercial catch in its northernmost extent in 2016 (Figure 1), soupfin sharks have not been previously described in the Salish Sea. However, in the last few years, there have been a growing number of anecdotal reports, by recreational fishers, of soupfin sharks and the largely sympatric broadnose sevengill shark (*Notorynchus cepedianus*) in the southernmost extent of the Salish Sea (aka South Puget Sound ~about 300km from previous strandings; Schulte et al., 2024).

At sufficient population levels, the presence of a novel marine predator species in the Salish Sea may influence system trophic dynamics and could necessitate consideration in ecosystem-based fisheries models currently utilized in the region (Morzaria-Luna et al., 2022). In order to address the anecdotal reports of soupfin sharks in South Puget Sound, we launched a multi-agency collaborative project aimed to: 1) confirm the presence of this species at a single site; and 2) evaluate whether reported encounters were merely stray occurrences (akin to the sporadic observations of juvenile gray whales in the Mediterranean Sea; Manfrini et al., 2023) or likely to be the result of a range extension driven by favorable climatic conditions and prey species abundance.

2 Methods

2.1 Site description — Hammersley Inlet

Hammersley Inlet is a narrow channel at the western edge of the South Salish Sea, near Shelton, WA, spanning approximately 10 kilometers in length with an average depth of 7 meters. Hammersley Inlet serves as the only connection between Oakland Bay — a shallow, highly productive, fjord-like estuary — and the greater Salish Sea (Figure 1C). Hammersley Inlet is characterized by significant current exchange, with peak flow velocities reaching 2.2 m/s, resulting in river-like hydrology throughout much of the inlet (Albertson et al., 2012). By water course distance, the eastern mouth of Hammersley Inlet is > 300 km from the mouth of the Strait of Juan de Fuca, providing extremely limited connection to open ocean waters, and the inlet is characterized by prolonged water

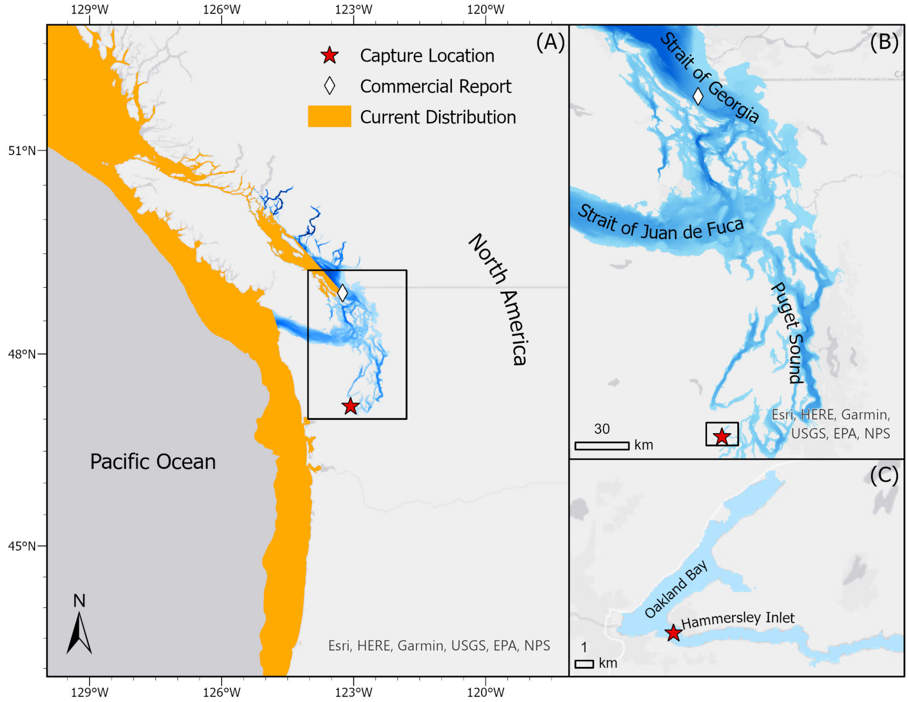

FIGURE 1
(A) Map showing the previously reported distribution of the soupfin shark (*Galeorhinus galeus*) in orange, the location of the reported commercial capture (diamond) (COSEWIC, 2021), and the capture location of the specimen reported here (star). The extent of the Salish Sea, encompassing the Strait of Juan de Fuca, Strait of Georgia, and Puget Sound, is represented in blue (https://wp.wwu.edu/salishseaatlas/). **(B)** Map showing the 3 major sub-basins of the Salish Sea; Strait of Juan de Fuca, Strait of Georgia, and Puget Sound. **(C)** Map showing the sampling site; Hammersley Inlet, Oakland Bay, and the capture location of the specimen described. Each black square represents the extent of the subsequent map.

residency times due to a combination of complex topography and intrabasin sills at several locations along this pathway (Khangaonkar et al., 2011). The west end of Hammersley Inlet is occupied by an industrial lumber mill, a marina, and other urban infrastructure.

2.2 Sampling

In response to anecdotal reports from recreational fishers, we employed a systematic approach to confirm the presence of soupfin sharks in Hammersley Inlet. We integrated fishing effort with opportunistic sampling of salinity and temperature within the inlet to identify environmental covariates associated with their occurrence outside of their previously known distribution. We used two primary fishing methods; multi-hook droplines deployed via research vessel and shore-based fishing using heavy tackle fishing rods. Hooks were baited with farmed Atlantic salmon (*Salmo salar*) and striped bonito (*Sarda orientalis*). These species were chosen because they are assumed prey in other systems but do not occur naturally within the study region (Klemetsen et al., 2003; Viñas et al., 2010); they can be excluded from any dietary analyses if observed in recovered stomach contents.

Each dropline consisted of a 8mm 3-strand nylon line, and from 1-3 PVC-coated wire leaders with baited circle hooks. This configuration was anchored with a 5kg weight then suspended from an inflatable PVC buoy and is designed to oscillate vertically signaling the presence of an animal on the line when deployed in calm inland waters. The baited hook was positioned between one and three meters above the anchor weight. This placement allowed water currents to impart a more lifelike movement to the bait, enhancing its attractiveness, and serving to minimize interactions with the benthic scavengers that also inhabit the inlet. For each sampling trip, the dropline sets were initially limited to a 30-minute duration and then were adjusted based on daily shark activity. This minimized the risk of predation on captured animals.

From shore, we utilized 3m casting rods rigged with 45kg breaking-strength monofilament fishing line, a 100g sinker, and leaders specially designed to target medium shark species. These leaders consisted of a 1m strand of braided steel wire covered in a translucent green nylon coating and a single barbless circle hook. The nylon coating's semi-transparent nature helps reduce the visibility of the leader underwater and protect the sharks from wire abrasions.

2.3 Data collection

Captured sharks were brought to the surface, restrained alongside the research vessel, and positioned into the direction of the tidal current flow to better irrigate seawater over the gills. Once properly positioned, we collected morphometric data (length, sex, clasper state, and biological tissue samples) from each individual. Sharks were measured (precaudal length, fork length, and total length [TL]). For male sharks, claspers were measured from their insertion to tip, and sexual maturity was determined based on the degree of ossification in the inner structure and clasper length in relation to the pelvic fins (ICES, 2018). A small sample of tissue from the second dorsal fin was collected for genetic cataloging. Directly prior to release sharks were then tagged with uniquely numbered ID tags to aid in the identification of recaptured individuals.

Environmental covariates measured during sampling consisted of salinity (ppt) and temperature (°C), which were recorded using a YSI Pro30 (Yellow Spring Instruments Inc., USA). These measurements were taken at both the sea surface and sea floor to address potential vertical stratification and the effect of solar surface warming. Additionally, these data were collected opportunistically during periods within the inlet without soupfin shark catches. Tidal stage data was also recorded for each captured shark.

3 Results

During this exploratory study, we conducted 146 hook hours of effort over 8 field excursions to Hammersley Inlet, resulting in the capture of one soupfin shark, confirming the presence of this species outside of their known distribution. Despite the effectiveness of shore-based fishing efforts in capturing other elasmobranch species within the inlet, this method did not result in the capture of any soupfin sharks. Research vessel-based techniques were solely responsible for the capture of this species during our sampling efforts. The captured individual was a mature male measuring 153 cm in TL and was identified as *G. galeus* based on species-specific distinct morphological features. These include the specimen's large body size, small second dorsal fin which closely resembled the size and shape of the anal fin, and long terminal caudal lobe which markedly distinguishes the species from other members of Triakidae (Figure 2). Capture occurred at a depth of 8 m, ~2 m above the seafloor, with a sea surface temperature and sea surface salinity of 17.6°C and 22.5 ppt, respectively, and a seafloor temperature and seafloor salinity of 16.8°C and 22.8 ppt, respectively. The individual was encountered during a rising tide, 2.88 hours before high water. Genetic material, photos, capture location coordinates, and other encounter metadata were submitted to the University of Washington's Burke Museum fish collection (accession number 2023-003, catalog number UW202516).

4 Discussion

We present the first scientifically confirmed observation and capture of a soupfin shark in South Puget Sound, the southernmost extent of the Salish Sea, well outside of their previously described distribution (~300 km). These findings suggest that soupfin sharks are likely more pervasive in the Salish Sea than previously considered. There are two likely scenarios why the soupfin shark was not previously described in the Salish Sea: 1) this observation represents the first documentation of a species that has thus far evaded scientific surveys and significant fishing effort in a highly urbanized waterway, or, 2) this observation represents a potential emergence of the soupfin shark as a novel marine predator species in the Salish Sea.

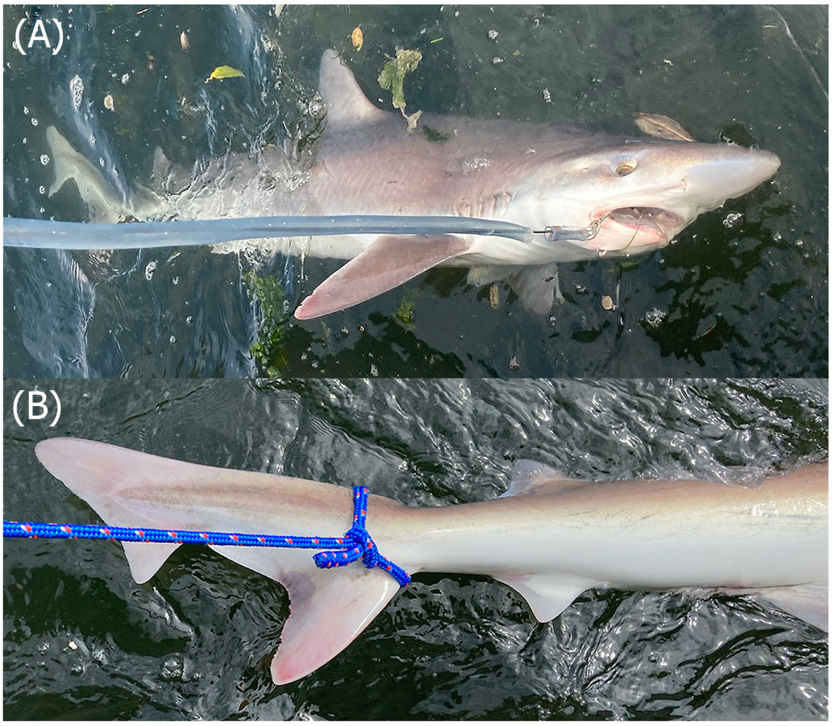

FIGURE 2
Photos of the captured 153 cm TL male soupfin shark (*Galeorhinus galeus*) in Hammersley Inlet, highlighting distinctive morphological characteristics of the **(A)** head, **(B)** tail, and second dorsal fin. Photos: **(A)** D. Lowry & **(B)** M. English.

It is conceivable that our observation of the soupfin shark in South Puget Sound represents the initial detection of an established but cryptic population of this highly mobile species within the Salish Sea (Scenario 1). However, we find this scenario unlikely. In this case, soupfin sharks would have to utilize habitats or exhibit behaviors that make them inaccessible to fishing gear (e.g., recreational, trawl, gillnet, longline gear), though these methods have been employed in the Salish Sea for over a century and are effective at catching soupfin in other regions. Further, soupfin sharks have not been observed on underwater video surveys within the Salish Sea, despite the efficacy of these methods in recording other shark species inhabiting the system (Lowry et al., 2022).

Instead, we suggest the presence of soupfin sharks likely represents an expansion of the seasonally occupied foraging habitat of this species (Scenario 2), potentially mediated by ongoing shifts in thermal conditions and prey species community composition within the Salish Sea. As a result of significant local anthropogenic pressure and influenced by broader climatological regimes and human-caused climate change, the Salish Sea has experienced pervasive shifts in trophic structure and species composition (Harvey et al., 2010; Greene et al., 2015; Ruggerone et al., 2019). For example, over the past century, the Salish sea forage fish community has been dominated by Pacific herring (*Clupea pallasii*), Pacific sand lance (*Ammodytes personatus*), surf smelt (*Hypomesus pretiosus*) and other smelt species (Osmeridae) (Kincaid, 1919; Therriault et al., 2009; Greene et al., 2015; Frick et al., 2022), while earlier accounts indicate periodic spikes in the abundance of Northern anchovy (*Engraulis mordax*) that were closely correlated with increased sea surface temperature and likely primary productivity throughout the Salish Sea (Morin et al., 2023). Forage fish play a critical role in marine systems, facilitating the flow of energy from basal trophic levels to higher-order predators, while simultaneously exerting top-down effects through predation upon planktonic species and the early life stages of their predators (Cury et al., 2000; Hallfredsson and Pedersen, 2009; Minto and Worm, 2012; Pikitch et al., 2014). The most recent significant spike in anchovy abundance in the Salish Sea began around 2014, aligning with a period of elevated sea surface temperatures in the region (Amos et al., 2014; Duguid et al., 2019), simultaneous with the increase in anecdotal reports of soupfin sharks by recreational fishers, which prompted this investigation.

Though we only report one capture, it is important to note that our catch per unit effort (CPUE) may be understated; we balanced our sampling between soupfin sharks and broadnose sevengill sharks, which were also recently discovered to inhabit the inlet (Schulte et al., 2024). For example, the 14/0 and 16/0 hooks, which are sufficiently large to minimize bycatch, likely discriminated against smaller soupfin sharks which are more selective feeders. Bait quality and fishing gear conspicuity may have been limiting factors as well. Expanding upon this initial exploration, future work should use smaller size 5/0 and 10/0 hooks which have proven effective in capturing an increased number of soupfin sharks across numerous size and age classes in other regions (Elías et al., 2005). Additionally, long-term acoustic tagging and monitoring has been utilized to identify highly migratory behavior and the periodicity of interannual site fidelity in soupfin sharks in other regions (Nosal et al., 2021). Therefore, we recommend additional sampling efforts for this species, incorporating both satellite and acoustic tagging, to understand the residency and habitat utilization of soupfin sharks within the Salish Sea and connectivity to the broader Northeast Pacific.

Our work contributes to a preliminary understanding of soupfin shark distribution and habitat preferences within the Salish Sea ecosystem that can both inform our understanding of changing regional ecosystem dynamics and the management of this critically endangered shark species.

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 Oregon State University IACUC-2023-0373. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

EP: Conceptualization, Investigation, Visualization, Writing – original draft, Writing – review & editing, Data curation, Project administration. JS: Conceptualization, Investigation, Writing – review & editing, Data curation, Project administration, Writing – original draft. DL: Conceptualization, Investigation, Writing – review & editing, Data curation, Funding acquisition, Project administration, Resources, Writing – original draft. LH: Investigation, Writing – review & editing, Funding acquisition, Project administration, Resources, Writing – original draft. ME: Investigation, Writing – original draft, Writing – review & editing, Data curation, Visualization. TC: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing, Data curation, Funding acquisition, Project administration, Resources, Supervision.

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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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Behavioral response of megafauna to boat collision measured via animal-borne camera and IMU

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Overlap between marine megafauna and maritime activities is a topic of global concern. Basking sharks (*Cetorhinus maximus*; CM) are listed as Globally Endangered under the IUCN, though reported sightings appear to be increasing in Ireland. While such trends in the region are welcome, increasing spatiotemporal overlap between CM and numerous water users poses an increased risk of boat strikes to the animals. To demonstrate the risk and impact of boat strikes on marine megafauna, we present camera-enabled animal-borne inertial measurement unit (IMU) data from a non-lethal boat strike on a CM within a proposed National Marine Park in Ireland. We tagged a ~7-m female CM in County Kerry, Ireland, which was struck by a boat ~6 h after tag deployment. Comparison of pre-strike data with 4 h of video and ~7.5 h of IMU data following the boat strike provides critical insight into the animal's response. While the CM reacted momentarily with an increase in activity and swam to the seafloor, it quickly reduced its overall activity (i.e., overall dynamic body acceleration, tailbeat cycles, tailbeat amplitude, and vertical velocity) for the remainder of the deployment. Notably, the animal also ceased feeding for the duration of the video and headed towards deep offshore waters, which is in stark contrast to the pre-strike period where the animal was consistently observed feeding along the surface in shallow coastal water. This work provides insight into a CM's response to acute injury and highlights the need for appropriate protections to mitigate risks for marine megafauna.

KEYWORDS

boat strike, basking shark, inertial measurement unit, non-lethal injury, marine megafauna, behavior

Introduction

Overlap between marine megafauna and maritime activities has long been, and is increasingly, a topic of concern, especially given the increase in maritime traffic and the continued declines of some species. It has been demonstrated that phenomena such as the formation of "marine roads" (i.e., shipping routes) pose significant risk to large surface-active marine megafauna (Pirotta et al., 2019). For example, Womersley et al. (2022) found that >90% of whale shark (*Rhincodon typus*) horizontal space use overlaps with persistent large vessel traffic and presents a significant mortality risk to these endangered sharks and Kite-Powell et al. (2007) estimated that collisions with large ships likely caused ~75% of known anthropogenic mortalities of critically endangered North Atlantic Right Whale (*Eubalaena glacialis*). While many studies focus on direct mortality caused by vessels, not all injuries cause direct mortality and non-lethal injuries can have significant short- and long-term consequences for animal physiology and behavior (Rennolds and Bely, 2023). For example, an initial flight response can negatively affect energy budgets if it either requires increased muscle recruitment or reduces energy intake (i.e., if the behavior results in the loss of foraging opportunities; Rennolds and Bely, 2023). Additionally, the injury itself could have longer-term negative impacts through a sustained altered behavioral state to compensate for the injury or due to tissue recovery and/or immune responses [see Rennolds and Bely (2023) for a review of sublethal mechanical injury biology]. There is also the possibility that injuries that appear non-lethal initially can eventually lead to mortality long after the event. It is therefore important to understand the responses and recovery periods of animals impacted by non-lethal strikes, especially given the high probability of strikes to marine megafauna that spend significant amounts of time near the surface. To date, most research efforts have relied on scarring quantity and recovery over time to understand these impacts (e.g., Bradford et al., 2009; Lester et al., 2020; Penketh et al., 2020), as behavioral observations directly surrounding a strike are incredibly rare. Here, we describe the immediate impacts of a non-lethal vessel strike on the behavior of a female basking shark (*Cetorhinus maximus*; CM) that was captured with a camera-enabled inertial measurement unit (IMU).

The basking shark is the world's second largest fish and was historically abundant worldwide. Overfishing and culling efforts up until the late 20th century led to global declines (e.g., McFarlane et al., 2009; Finucci et al., 2021; McInturf et al., 2022) from which CMs have been slow to recover, leading to listing as Globally Endangered by the IUCN (Rigby et al., 2019). In Ireland, however, reported sightings appear to be increasing (Irish Basking Shark Group, personal communication); during certain months, individuals aggregate in shallow coastal hotspots, feeding at the surface on calanoid copepods (*Calanus* spp.; Baduini, 1995; Sims and Quayle, 1998; Miller et al., 2015) and potentially mating (Sims et al., 2022). Following public support, CM became the first fish to be listed under Section 23 of Ireland's Wildlife Act of 1976 in 2022, rendering it illegal to injure or willfully interfere with the breeding or resting place of CM. Simultaneously, the Irish Basking Shark Group (IBSG), a local organization of researchers, conservationists, and educators, drafted a voluntary Code of Conduct for CM to limit anthropogenic impacts from human activities (i.e., swimming, drones, and vessel strikes), with the intention to guide binding protective legislation. Similar science-based plans for other megafauna such as whale sharks (Jones, 2021) and manta rays (*Mobula alfredi*; Murray et al., 2020) have been proposed and successfully adopted in other regions. In April 2024, Ireland announced the creation of its first National Marine Park, protecting 70,000 acres of land and sea, including seasonal CM habitat. However, though these collective actions represent a promising opportunity to protect the species in one of the few locations where CM sightings persist, specific management plans have yet to be developed let alone implemented; a lack of deliberate and enforced guidelines to ensure the intended protections can result in negative consequences for marine megafauna (for example, Legaspi et al., 2020). Thus, while an increase in both sightings and public interest are encouraging signs for the recovery of the species as a whole, the increasing spatiotemporal overlap between CM and numerous water users (e.g., fishing and commerce)—even the ecotourism industry itself—poses an increased risk to the animals without appropriate protections in place.

To demonstrate the risk and impact of boat strikes on marine megafauna, we present camera-enabled animal-borne IMU data from a non-lethal boat strike on a CM within the proposed National Marine Park in Ireland. We describe the immediate response and the first few hours of the subsequent recovery period of the animal. To our knowledge, these are the first published IMU data and direct observation of such an event on any marine species and has important implications for our understanding of the non-lethal impact of anthropogenic interactions with CM and marine megafauna more broadly.

Methods

In April 2024, we deployed a Customized Animal Tracking Solutions (CATS; Australia) camera-enabled IMU package on a CM within Ireland's newly proposed National Marine Park near the Blasket Islands, County Kerry (Supplementary Figure 1). The IMU consisted of inertial sensors (12 channels of data: tri-axial accelerometers, magnetometers and gyroscopes, depth, temperature, and light; see Chapple et al., 2015) with a towed camera. The package was affixed to the body using two 12-cm monofilament leaders inserted into the dorsal musculature with a tagging pole and was set to release from the animal after ~12–18 h. The animal was sexed using underwater video and length was estimated based on comparison with the tagging vessel.

Data processing

Tag accelerometers were sampled at 200 Hz, gyroscopes and magnetometers at 50 Hz, and depth, pressure, light, and temperature at 10 Hz. We used custom-written scripts in MATLAB, 2023 following Cade et al. (2021) to decimate all data to 10 Hz, correct tag orientation for animal orientation (pitch, roll, and heading), and synchronize the video data from a towed float with the inertial sensors. Animal location was estimated for the duration of each deployment by interpolating pseudotracks of the animal derived from animal heading and speed, estimated from the amplitude of tag vibrations (Cade et al., 2018), between the tagging location, with four resight locations over the first 5 h of the deployment and the tag pop-off position.

Following Andrzejaczek et al. (2018); Andrzejaczek et al. (2020), we calculated vertical velocity (VV; the rate of change in depth over a 1-s period) and split the depth record into vertical swimming phases (ascending, descending, and level swimming). The depth data were smoothed using a 10-s running mean, and we calculated the average VV by taking the difference of this smoothed depth between successive points at 1-s intervals. Swimming phases were defined as $VV > 0.05$ m s^{-1} (ascents), $VV<-0.05$ m s^{-1} (descents) or 0.05 m s^{-1} $>VV>$ -0.05 m s^{-1} (level). Note VV is presented below as the absolute value of VV but labeled as ascent or descent.

As a proxy of relative energetic expenditure, overall dynamic body acceleration (ODBA) was calculated by summing the absolute value of dynamic acceleration from all three axes (Wilson et al., 2006). The dynamic component of acceleration was calculated by subtracting the gravitational component from the raw acceleration for each axis. The gravitational component of acceleration (static acceleration) was determined using a 3-s box smoothing window on the raw acceleration data (Shepard et al., 2008).

Finally, we used a continuous wavelet transformation of the dynamic component of angular acceleration (i.e., sway) in Ethographer (Igor Pro 9, WaveMetrics, USA) to calculate tailbeat cycle (TBC; the time to complete a full tailbeat) and tailbeat amplitude (TBA; a proxy for the acceleration of the tailbeat wave) following Sakamoto et al. (2009). Note that the amplitude here is not a direct measurement of the intensity of the CM's dynamic movement, but rather a proxy for the relative intensity using a Morlet wavelet function to decompose the acceleration signal. This allows a relative measure of TBA to be compared across the deployment.

We grouped the calculated metrics into 30-min bins to compare response and recovery following the boat strike to baseline behavior (similar to Whitney et al., 2016). Because the distributions were not normal, we also assessed parameter values before and after the strike using a Wilcoxon non-parametric comparison of means.

Video was reviewed for evidence of feeding (i.e., gills open, Figure 1A) before and after the strike as a proxy of resumption to pre-strike behavior.

Results

We tagged a ~7-m female CM at 08:02 local time (UTC+1) on 24 April 2024 while it was feeding on the surface near the Blasket Islands, Ireland (52.089°N, 10.420°W). Immediately following tagging, the shark dove to ~40 m for 7 min and then returned to the surface (Figures 2A, B) and began actively feeding. It swam in tortuous routes east of the Blasket Sound, mostly on the surface and feeding (Figures 1A, 2A, C), with an occasional dive to ~40 m for the next 5 h 50 min. At 13:53:30, the shark was actively feeding at <1 m depth when it attempted to make a large and quick evasive movement (Figure 1B). Within a second, a large boat keel cut across the back of the shark, just behind the dorsal fin (Figures 1C, D), and the shark was tumbled through the water (Figure 1E). Once righted, the shark immediately increased tailbeat frequency and powered down to the seafloor for 30 s (Figures 1F, 2D; Supplementary Videos 1, 2). There was immediate damage to the dermis, anti-fouling paint, and a red abrasion, posterior to the dorsal fin where the keel struck the shark, but no apparent bleeding or open wound (Figure 1G). The shark remained deeper than 10 m, consistently along the seafloor (Figure 1H), as it swam in a more directed route, interspersed with periods of near motionless movement near the sea bottom (Figure 2E; Supplementary Video 3) for the next 7 h 27 min without feeding, until the tag released at 21:22 (Figure 2).

Inertial sensors revealed a response in the behavior of the shark immediately following the strike and for the remainder of the deployment. Once the animal righted itself, there was an immediate burst of speed to 5.6 m s^{-1} with an average of 3.1 m s^{-1} ± 0.52 (mean ± SD) during the 30-s descent. This coincided with an increase in downward vertical velocity (2.3 m s^{-1} ± 0.26) as it swam toward the seafloor, but once the animal reached the seafloor, the vertical velocity (0.12 m s^{-1} ± 0.085) stayed below pre-strike level (0.22 m s^{-1} ± 0.21; $p < 0.0001$) with little variability (Table 1; Supplementary Figure 2). Positive vertical velocity (i.e., ascent; 0.088 m s^{-1} ± 0.038) also remained below pre-strike levels (0.16 m s^{-1} ± 0.098; $p < 0.0001$), suggesting an overall decrease in vertical activity (Table 1; Supplementary Figure 3). Similarly, ODBA initially spiked (0.37 g s^{-1} ± 0.20) during the 10 s following the boat strike, which was ~8× greater than the mean pre-strike value (0.047 g s^{-1} ± 0.02). Then, ODBA fell below the pre-strike value for the remainder of the deployment (0.027 g s^{-1} ± 0.016; $p < 0.0001$; Table 1; Supplementary Figure 4). Similar to ODBA, TBC and TBA initially peaked in activity (i.e., lower TBC and higher TBA) immediately after the strike (1.9 s ± 0.63 and 5.7 ± 0.82), but then decreased (5.2 s ± 1.1 and 1.6 ± 0.76) below pre-strike levels (4.4 s ± 0.42 and 2.5 ± 0.95; $p < 0.0001$ for both) for the remainder of the deployment (Table 1; Supplementary Figures 5, 6, respectively).

Discussion

We present data showing an immediate response by a CM to a boat strike in the newly delineated National Marine Park. While the CM reacted to the strike with highly energetic movement away from the event, it quickly reduced its overall activity (ODBA, TBC, TBA, and vertical velocity) for the remainder of the deployment. Notably, the animal also ceased to feed at any point across the remaining ~7.5 h and headed towards deep offshore waters. This is in stark contrast to the pre-strike period where the animal was observed feeding along the surface in shallow coastal water for 84% of the first ~6 h. It is common for animals to increase

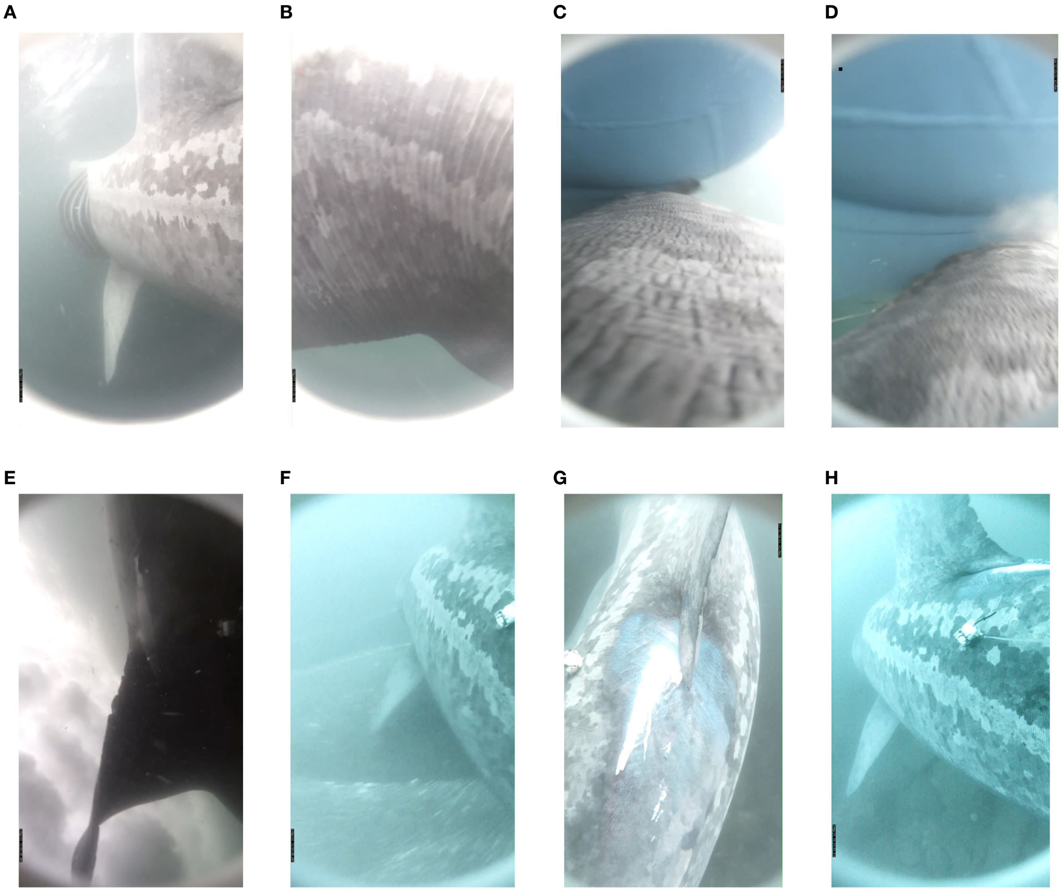

FIGURE 1
Still photos taken from the animal-borne camera show the basking shark **(A)** feeding at the surface prior to the boat strike. At 13:53:30, **(B)** the shark attempted to make a large and quick evasive movement. However, **(C, D)** within a second, a large boat keel cut across the back of the shark, just behind the dorsal fin and **(E)** the shark was tumbled through the prop wake. **(F)** The shark immediately increased tailbeat frequency and powered down to the seafloor for 30 s. **(G)** Anti-fouling paint (blue), damage to the dermis, and a red abrasion were evident posterior to the dorsal fin where the keel struck the shark. **(H)** The shark remained associated with the seafloor as it swam in a more directed route into deeper waters for the next 7 h 27 min without feeding.

activity during recovery periods after handling (e.g., fish, Dolton et al., 2022; sharks, Iosilevskii et al., 2022; and mammals, Shuert et al., 2021). However, the CM exhibited flight behavior and a reduction in activity, such that it appeared to be resting on the bottom at times (Figure 2E; Supplementary Video 3). This difference likely stems from the difference in the stressor and required response; capture stress (i.e., fight time, handling) initiates changes in blood chemistry (e.g., buildup of lactate; Gallagher et al., 2014) and increased activity can aid in the removal of lactate via oxidation (Iosilevskii et al., 2022). Because the CM was responding to a brief acute stressor instead of capture, it likely did not require increased activity to drive oxidation, but instead responded to traumatic event/injury with reduced activity (Mercier et al., 2003). We note that species- and individual-specific differences do occur in acute stress response; thus, we cannot make a broader prediction of post-strike behavior across CM.

Animals in studies of post-release mortality and recovery likely employ very different behavioral and physiological pathways to cope with capture stress than an acute event/injury such as observed here. As these are, to our knowledge, the first direct data collected during a boat strike and the extent of any internal injury is unknown, the expected time to mortality or recovery of the CM is uncertain. While the initial strike was apparently non-lethal and CM did remain active for the 7.5 h following the event, the deployment duration was not sufficient to confirm a recovery back to pre-strike behaviors or determine any long-term consequences (e.g., effects of missed feeding opportunity; Rennolds and Bely, 2023) or eventual mortality. More work is needed to understand the long-term implications and recovery from such events.

It is important to note that while this interaction induced significant behavioral changes and cost to the animal and apparent abrasions, it did not leave obvious external injury definitively indicative of a boat strike (e.g., deep gashes or deformations). Thus, while physical evidence of boat strikes has been opportunistically observed in the area (Massett, personal observation; Supplementary Figure 7), observations of physical damage likely underestimate the actual prevalence of interactions. This finding is also informative for work in other areas where

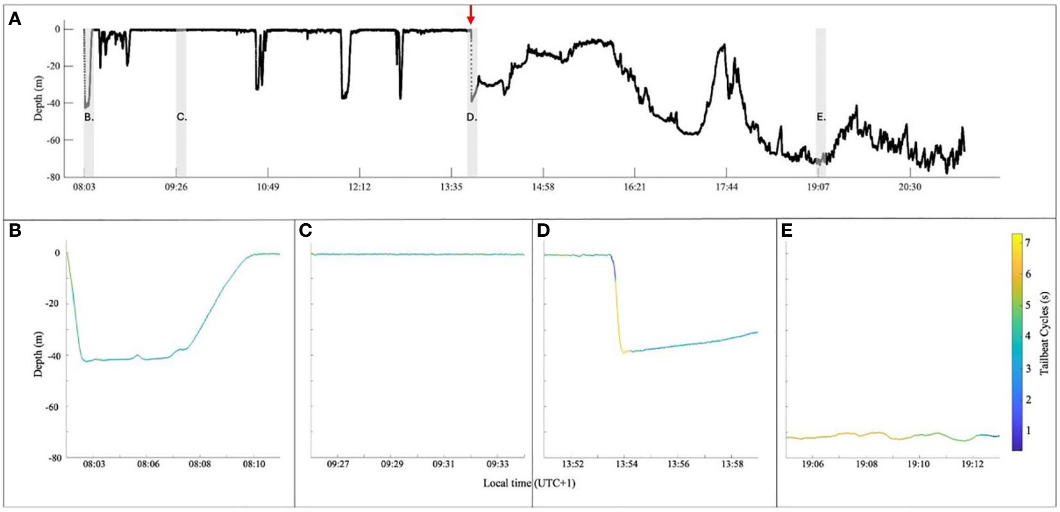

FIGURE 2
(A) Depth data (black) over the course of the deployment show the surface-associated foraging prior to the strike event (red arrow). Each point is a depth s^{-1}. The spacing of the points immediately following the event indicates relatively how quickly the basking shark swam down, which can be compared to the response to tagging (first descent). The shark did not return to the surface after the boat strike but stayed along the bottom for the duration of the videos. **(B–E)** present 8-min sections of the entire depth plot (indicated by the shaded sections in **(A)**) with the line colored by Tailbeat Cycle, including **(B)** immediately post-tagging, **(C)** feeding at the surface, **(D)** the boat strike, and **(E)** the period where the animal was near motionless on the bottom.

research efforts have relied on scarring quantity and recovery over time to understand the risks and impacts of boat strikes (e.g., Bradford et al., 2009; Lester et al., 2020; Strike et al., 2022).

These data emerge at a critical moment in the protection of CM, with the opportunity to justify and inform imminent management efforts for the species. With the increasing numbers of sightings in the region, and throughout Ireland (IBSG, personal communication), it is very likely that CM–vessel interactions will continue to increase with the recovery of the population. While observations of physical damage are imperfect, as mentioned above, a systematic recording of apparent physical damage could nonetheless provide a relative or minimum measure of interaction rates. Thus, we recommend prioritizing a systematic catalog of physical injuries to provide a conservative baseline of boat strike interactions and inform management actions. Additionally, with CM's recent listing under Ireland's Wildlife Act of 1976, which increased levels of protection from injury and disturbance in critical breeding or resting habitats, the newly delineated National Marine Park in County Kerry represents an opportunity to establish stronger protection for CM and other marine species. These protections further justify a systematic catalog of injuries and could mitigate threats, not only of these boat strikes, but also other anthropogenic risks encountered by marine species (e.g., ground lines connecting lobster traps; Supplementary Figure 8) within a defined marine area.

TABLE 1 Results from a Wilcoxon non-parametric comparison of means before and after the strike for the six different metrics analyzed from the IMU.

Response	χ^2	d.f.	p
Depth	30,145	1	<0.0001
ODBA	14,480	1	<0.0001
Descent vertical velocity	242	1	<0.0001
Ascent vertical velocity	928	1	<0.0001
Tailbeat cycle	12,152	1	<0.0001
Tailbeat amplitude	11,532	1	<0.0001

There have also been community-led efforts to mitigate such harmful human–wildlife encounters, such as through the Code of Conduct proposed by the IBSG. This document (available in English and Irish; https://www.baskingshark.ie/downloads) specifically identifies vessel strikes as a potential CM threat and offers guidelines for reducing these risks. However, neither this nor any other version of the Code of Conduct has been formally adopted as enforceable policy by any regulatory body. Within this conservation context, the data we present therefore offer further support for both a legally binding Code of Conduct and a formal management plan within the marine park, and throughout Ireland, to enhance protection measures for the species here.

Boat strikes have been reported as an ongoing global threat to CM (e.g., McInturf et al., 2022) and other marine megafauna (e.g., Kite-Powell et al., 2007; Womersley et al., 2022), but defining their scope and impact is challenging given the lack of reporting and opportunities to record behavioral responses immediately before and after events. Though overlap with vessel activity is likely to be highest in locations such as Ireland, where CM are still sighted regularly at the surface, we recommend that research and conservation efforts throughout the species' range consider the likelihood that boat strikes, and their subsequent non-lethal effects, may be more common than previously considered. More work needs to be done to assess their impact on the short- and long-term recovery of this 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 approved by Oregon State University-Institutional Animal Care and Use Committee #IACUC-2023-0373 Health Products Regulatory Authority of Ireland (#AE19136/P127). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

TC: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – original draft. DC: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Writing – review & editing, Data curation, Formal analysis, Supervision, Visualization. NM: Investigation, Methodology, Resources, Writing – review & editing. JG: Conceptualization, Funding acquisition, Supervision, Writing – review & editing. NP: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – review & editing, Resources. AM: Conceptualization, Funding acquisition, Supervision, Writing – review & editing, Investigation, Methodology, Project administration, Resources, Data curation, Visualization.

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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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2024.1430961/full#supplementary-material

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First evidence of white sharks, *Carcharodon carcharias*, in the tongue of the ocean, central Bahamas

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The white shark, *Carcharodon carcharias*, is an iconic apex predator, playing an important ecological role across its range. Persistent bycatch and overfishing led to white shark declines, but recent studies in the North Western Atlantic (NWA) revealed evidence for regional recovery, and highlighted the importance of Southeastern Florida and the Gulf of Mexico as overwintering grounds for maturing white sharks. However, despite its proximity to Florida and comparably productive habitats, records of white sharks in The Bahamas are extremely rare, with a comprehensive survey of sightings and captures describing only one white shark between 1800 - 2010. Here, we reveal acoustic tracking detections of ten white sharks from 2020 - 2024 along the western edge of the Tongue of the Ocean off Central Andros Island, The Bahamas. White sharks were originally tagged off the coast of the United States and Canada, and detected off Andros Island, The Bahamas from November-May. White sharks were detected along the drop-off zone of the reef at ca. 25 m, exclusively between dusk and dawn, with the number of detections suggesting transient behavior. These findings expand our knowledge of white shark distribution in the NWA, highlighting data gaps in The Bahamas and underlining the importance of collaborative protective measures for species recovery.

KEYWORDS

white shark, acoustic tracking, Andros, range expansion, *Carcharodon carcharias*, Bahamas

Introduction

The white shark *Carcharodon carcharias* is a large-bodied, wide-ranging species with broadly distributed populations in temperate and subtropical waters worldwide (Compagno, 2001). As upper-trophic-level predators, they play an important ecological role (Carrier et al., 2010), primarily feeding on small sharks and rays, squid, and benthic fishes as small juveniles (< 2.5 m, Estrada et al., 2006; Clark et al., 2023) and incorporating marine mammals into their diet as they grow (Tricas and McCosker, 1984; Hussey et al., 2012). Large juvenile, subadult, and adult white sharks seasonally aggregate near pinniped colonies often when water temperatures correspond to their preferred range (Klimley et al., 2001; Bruce and Bradford, 2015; Hewitt et al., 2018; Kock et al., 2022; Winton et al., 2023). Much of our understanding of white shark movement ecology comes from studies where animals are tracked with acoustic or satellite tags at these sites (e.g., Neptune Islands, South Australia; Guadalupe Island, Mexico; California and Cape Cod, U.S.A), which have documented philopatric behaviors, such as high site fidelity, seasonal residency as well as long-distance return migrations (Bonfil et al., 2005; Jorgensen et al., 2010; Bruce and Bradford, 2015; Skomal et al., 2017; Huveneers et al., 2018; Bastien et al., 2020).

White sharks also occur in the tropics (Tirard et al., 2010), but information on their distribution and occurrence in these regions are limited, particularly for populations that experienced dramatic declines, including the population in the North Western Atlantic (NWA; Curtis et al., 2014). White sharks were never targeted commercially in the region, but bycatch and capture in commercial and recreational fisheries were sufficient to reduce abundance by as much as 73% in the 1970s and 1980s (Curtis et al., 2014). Consequently, harvest was prohibited in the US Atlantic in 1997 (NMFS 1997). As a result of this protective measure, alongside other international measures (*i.e.*, in 2002 they were listed on Appendix I and II of the Convention on the Conservation of Migratory Species of Wild Animals (CMS), in 2004 they were added to the Convention on International Trade in Endangered Species (CITES) Appendix II), the NWA population appears to be recovering (Curtis et al., 2014; Rigby et al., 2019).

Despite these measures and evidence of recovery, only recently have we begun to learn about their horizontal and vertical space use in the NWA, through various biotelemetry studies (Skomal et al., 2017; Bastien et al., 2020; Lowerre-Barbieri et al., 2021; Franks et al., 2021; Winton et al., 2021; Bowlby et al., 2022). Adult white sharks have been shown to exhibit an ontogenetic shift in their space use from near-coastal, shelf-oriented waters to pelagic habitat, with frequent excursions to mesopelagic depths (Skomal et al., 2017). White sharks migrate to the southeast shelf waters of North Carolina to Florida during the late fall when water temperatures generally drop below 12°C in the NWA (Casey and Pratt, 1985; Curtis et al., 2014; Skomal et al., 2017; Bowlby et al., 2022), with some individuals traveling as far as the Gulf of Mexico in winter and early spring (Skomal et al., 2017; Franks et al., 2021). They show strong site fidelity to the NWA, returning seasonally to specific regions (e.g., Massachusetts and Atlantic Canada), with most white sharks exhibiting an annual migratory cycle, spending the majority of their time over the continental shelf (Franks et al., 2021; Bowlby et al., 2022). Movements between regions are rapid and directed, with white sharks exhibiting no stop-over behavior, which is common for other large coastal-pelagic sharks and fishes (Skomal et al., 2017; Lowerre-Barbieri et al., 2021; Franks et al., 2021). More recently, there has been an increase in white shark sightings and detections via acoustic tracking data in the Canadian Atlantic (Bastien et al., 2020; Bowlby et al., 2022). This trend matches reports from Massachusetts and the U.S. north Atlantic (Curtis et al., 2014) and is probably a result of regional trends in recovery of the white shark population, indicative of a return to their former range where they have long been rare or absent (Winton et al., 2023). However, it is likely that other factors have played a role, including climate change, increasing prey abundance (e.g., due to pinniped recovery), and/or insufficient sampling and monitoring preventing their documentation in certain parts of their range (Bowlby et al., 2022).

Despite annual overwintering in Florida's Gulf and Atlantic shelf waters, records of white sharks in nearby Bahamian waters are very rare. In their catalog of elasmobranch fishes in the region, Bigelow and Schroeder (1948) included one record of a "*positively identified capture of a great white shark in the vicinity of the Bahamas near Nassau*". More recently, Curtis et al. (2014) compiled comprehensive capture and sightings records from the NWA from the years 1800–2010; of those 649 verified records, only one white shark was documented in Bahamian waters. This shark, estimated at ~ 4 m total length, washed ashore on the west coast of Grand Bahama, 6th June 2008. Here we report the first records of white sharks in the central Bahamas using shelf edge habitat along the reef wall of the Tongue of the Ocean (TOTO), in an area adjacent to the northern bight of eastern Andros Island. We discuss the implications of these new records within the context of the species recent recovery in the NWA, and consider whether these are a result of species' recovery, inability to observe them due to inadequate sampling and monitoring, or a combination of the above.

Materials and methods

Study area

The Bahamas are a group of about 700 islands and cays in the NWA. The Atlantic basin extends within the archipelago, forming a semi-enclosed deep-sea trench (40 km wide, 200 km long) known as the Tongue of the Ocean (TOTO), which is bordered by Andros Island to the west and Exuma cays to the east. An extensive fringing reef runs along the western edge of the TOTO, quickly dropping to a deep pelagic zone (~1500 – 3000 m depth) with gullied slopes beyond 400 m of the shelf (Buchan, 2000). The three main islands of Andros (North, Mangrove Cay and South) are separated by bights, large shallow channels 1 - 5 m deep and 3 - 6 km wide that trifurcate the island from east to west.

Receiver array

Omnidirectional acoustic receivers (VR2W; Innovasea systems®) were initially deployed in 2019 (n = 9) to monitor the space use of sharks and rays on the flats (depth: 3 m) and backreef (depth: 12 m) of the north bight of Andros Island (Figure 1). Coverage expanded annually to include the reef wall (depth: 25 m) from High Cay (24.6482°, -77.6894°) to Driggs Hill (24.2317°, -77.59986°) as well as the deep pelagic zone (depth: 300 to 500 m)

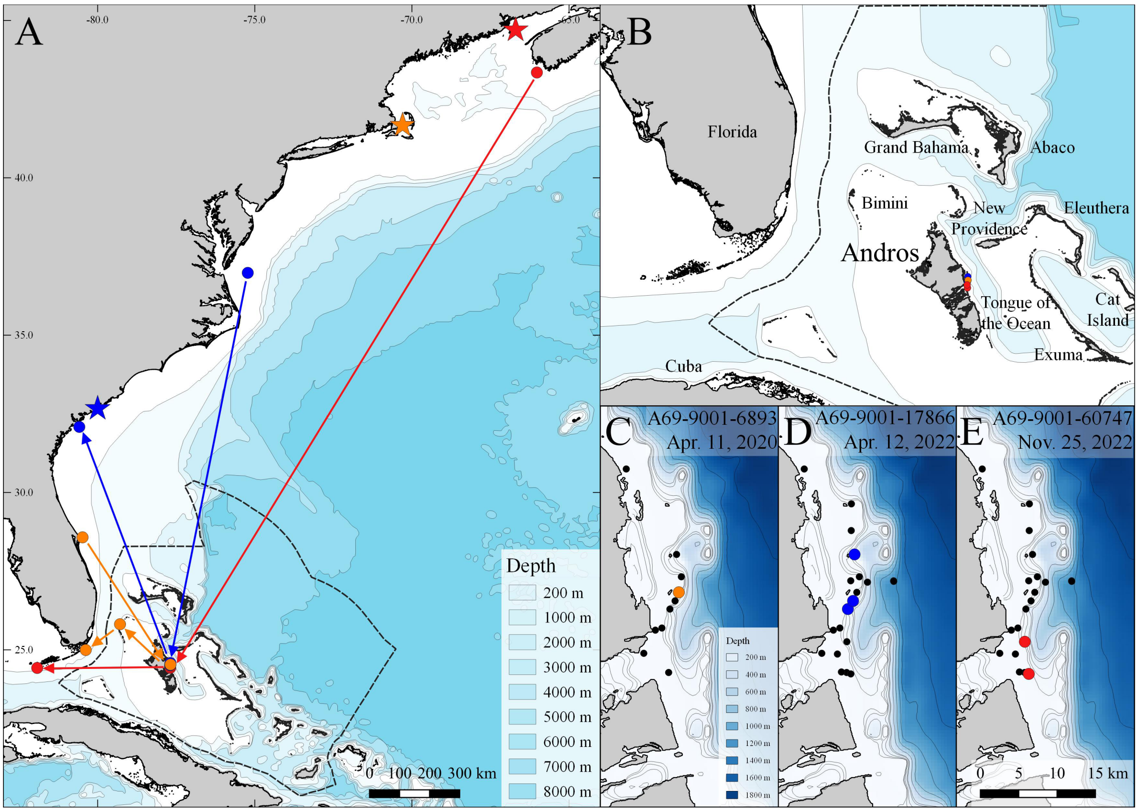

FIGURE 1
Acoustic detections highlighting regional movements of three (n = 3) white sharks, *Carcharodon carcharias* pre-and-post movement to Andros Island, The Bahamas. Colored stars, circles, and arrows represent different individuals (orange, blue, red). Stars indicate tagging locations (see Table 1) and colored circles in region-wide map **(A)** indicate detection locations in Andros Island, and the last detection of each individual prior to and after detection off Andros Island. Arrows show least cost paths based on detection sequences but do not represent travel routes based on variation in the timing of detections prior and subsequent to detections off the Andros Island array **(B)**. Colored circles in Andros-specific maps **(C–E)** indicate the detection locations for each individual; black dots indicate locations of acoustic receivers where white sharks were not detected.

adjacent the AUTEC U.S. Navy base outside of Cargill Creek (24.4862°, -77.7125°) (Table 1). The receivers were retrieved for data download and returned to the recording location in the same 24 h period every 6 - 12 months for 5 years. All receivers were either moored to augers, screwed into soft sediment, or secured to a line and float weighted by an 18 kg concrete-filled cone. Data were filtered to remove spurious detections, which were defined as any single transmitter detection occurring alone within a 24 h period. All other detections were treated as genuine and were assigned a tidal phase (low, high or mid; semi-diurnal) and a lunar illumination percentage (0 = new; 100% = full). High and low tide times were obtained from NOAA (National Oceanic and Atmospheric Administration; Fresh Creek TEC4613) and detections were assigned phases defined by: high and low tide = 2 h either side of high and low tide times, respectively, and mid-tide = the 2 h in the middle. Lunar illumination percentage was obtained from the U.S. Astronomical Applications Department (https://aa.usno.navy.mil). Participation in a data-sharing network (Ocean Tracking Network, OTN) allowed us to detect tagged animals. We calculated minimum straight-line distance between tagging location and detecting receivers to determine individual white shark movements in the region. Furthermore, we included additional information on individuals that were recorded in other arrays pre-and-post their detections in Andros.

Shark tagging

White sharks (n = 331) were equipped with acoustic transmitters (V16-4x, V16-6x, or V16TP-6x; power output 158 dB; Innovasea Systems$^{®}$) during dedicated research trips from 2010 to 2023 (for details see Skomal et al., 2017; Winton et al., 2023). These were conducted throughout the year off the east coast of North America from Jacksonville, U.S., to New Brunswick, Canada. Transmitters deployed on the white sharks included in this study all had random transmission intervals of 60 - 120s (estimated battery life 3321 days) and were tagged externally using a tagging pole (Table 1). At the time of tagging, the total length (TL) of each shark was visually estimated to the nearest 0.3 m via expert consensus using the vessel pulpit length (3.2 m) for scale (Skomal et al., 2017). Underwater video of each shark was also captured allowing for sex determination (Winton et al., 2023). All tagging was conducted under Exempted Fishing Permits (SHK-EFP-11-04, SHK-EFP-12-08, SHK-EFP-13-01, SHK-EFP-14-03) issued by the NMFS Highly

TABLE 1 Acoustic detection summary for ten white sharks, *Carcharodon carcharias*, recorded in Andros Island, The Bahamas, from 2020 - 2024.

Tag ID	Tagging Location	Tagged	Sex	Estimated Length (m)	Year Detected	Month Detected	No. Detections	No. Receivers	No. Days	Range
A69-9001-6893	Cape Cod, USA	10-Sep-19	F	3.0	2020	April	6	1	1	1
A69-9001-17848	Cape Cod, USA	10-Jul-17	M	3.7	2022	May	2	1	1	1
A69-9001-17866	South Carolina, USA	15-Mar-18	M	2.1	2022	April	8	3	1	1
A69-9001-60747	N. Brunswick, Canada	23-Aug-22	U	3.0	2022	November	6	2	1	1
A69-9002-4975	Cape Cod, USA	25-Sep-20	M	3.7	2022	April	4	2	3	13
A69-9001-3077	South Carolina, USA	10-Mar-21	M	3.5	2023	May	2	1	1	1
A69-9001-5287	Cape Cod, USA	25-Oct-19	F	3.0	2023	February	7	2	1	1
A69-9001-62514	Cape Cod, USA	7-Oct-21	M	2.7	2023	March & April	7	2	3	12
A69-9001-62516	Cape Cod, USA	31-Aug-21	M	3.4	2024	January	8	4	2	8
A69-9001-62506	Cape Cod, USA	3-Aug-22	M	3.4	2024	April	4	1	1	1

Migratory Species Management Division and permits issued by the Massachusetts Division of Marine Fisheries.

Results

Ten white sharks (size range at tagging = 2.1 - 3.7 m [estimate total length]; 2 females, 7 males, 1 unknown sex) were detected from 2020 to 2024 on acoustic receivers deployed along a 27 km stretch of fringing reef wall (Green Cay to Gibson Cay; depth 25 m) off the east coast of Andros Island (Figures 2, 3; Table 1). Detected white sharks were tagged at two sites in the U.S., Cape Cod, Massachusetts (n = 7), and Hilton Head, South Carolina (n = 2), with one individual tagged off the coast of New Brunswick, Canada. Their time at liberty since tagging ranged from 5 months to 6 years at the time of detection off Andros Island (Table 1).

No sharks were detected in Andros on receivers < 25 m deep, nor at deepwater receivers (300 - 500 m offshore of fringing reef wall; Figures 2, 3). Detections were recorded between November 25 and May 17 during all sampling years, with five white sharks detected in April and two in May. Seven of the ten sharks were detected on a single day on 1 - 4 of the receivers (Table 1). Three sharks were detected on receivers multiple days: A69-9002-4975 was detected across 13 days (3 to 15 April 2022), A69-9001-62506 was detected across 8 days (23 to 30 January 2024), and A69-9001-62514 was detected across 12 days (28 March to 4 April 2023) (Figures 2, 3). All white shark detections were between 20:00 and 07:00, across different lunar phases (range: 0 – 98% lunar illumination; median 54.5%) and throughout the tidal cycle (high 13%, low 41%, and mid-tide 46%).

Individual movements

White shark A69-9001-60747 was tagged on 26 September 2022 off New Brunswick, Canada, and was detected 60 days later off Andros Island on 25 November 2022. This represents a minimum southern movement of ~ 2300 km (38 km per day). This shark was subsequently detected 12 days later off the Florida Keys on 7 December 2022, after another ~ 490 km movement (45 km per day) (Figure 1). Female white shark A69-9001-6893 was tagged on 10 September 2019 off Cape Cod, MA, U.S., and was detected on 9 March 2020 off Cape Canaveral, FL, U.S., a movement of ~ 1700 km. Next, she was detected off Andros on 11 April 2020, a southern

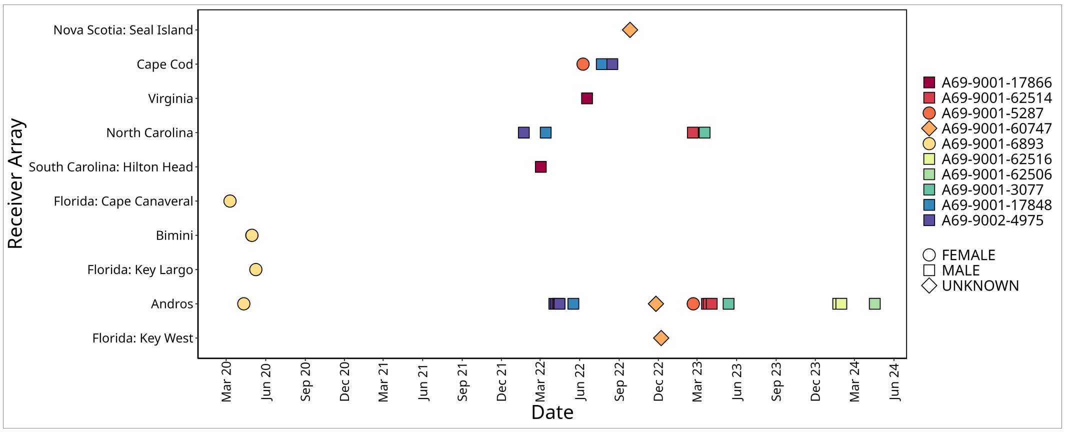

FIGURE 2
Time series of acoustic detections for all ten white sharks, *Carcharodon carcharias*, prior to, during, and after detections on Andros Island receivers. Detection data from other receiver arrays, ordered by latitude, were provided via the Ocean Tracking Network. Point shape denotes sex; point fill color denotes animal ID.

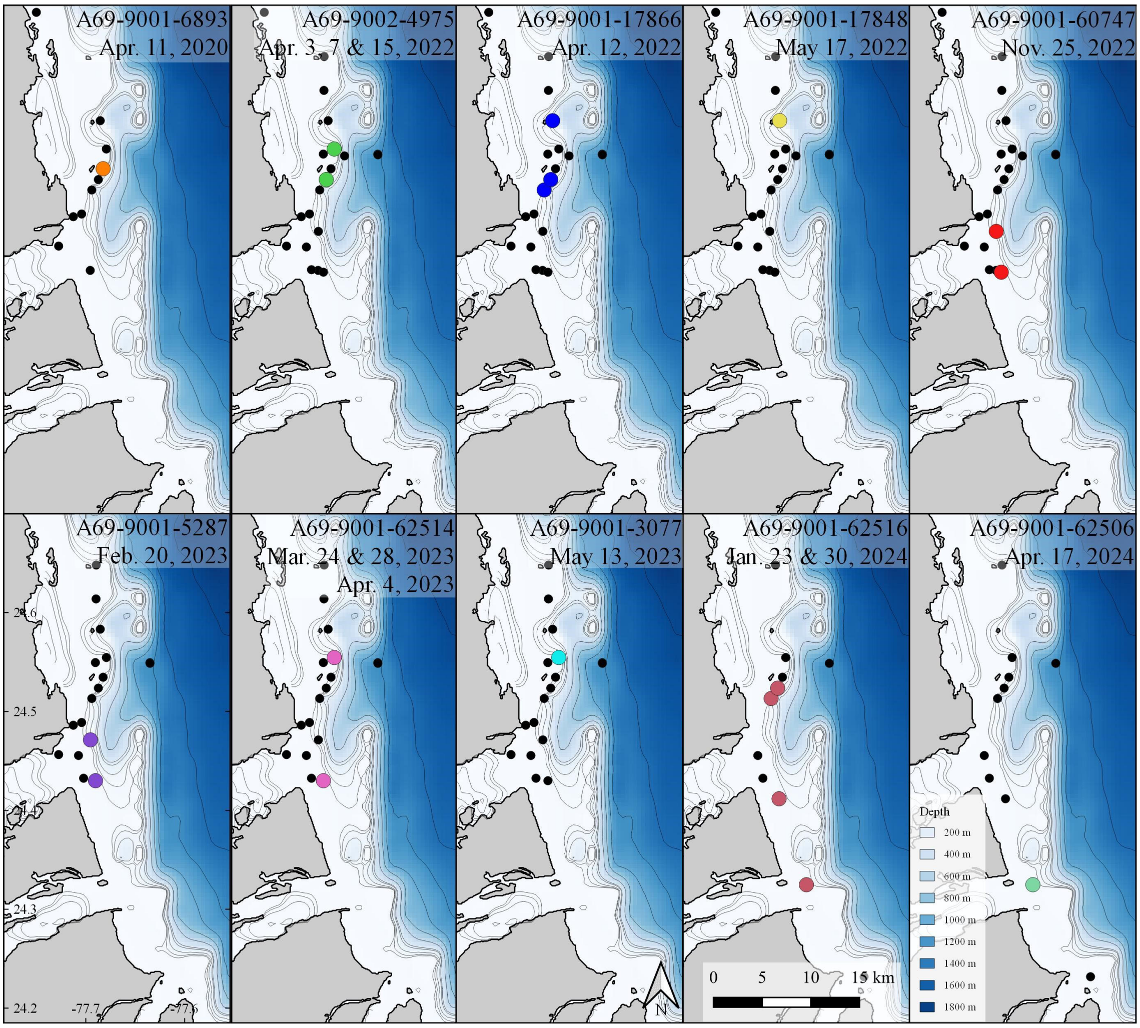

FIGURE 3
Acoustic detections of all white sharks (n=10), *Carcharodon carcharias* off Andros Island, The Bahamas. Black dots indicate locations of acoustic receivers where white sharks were not detected.

movement of ~ 520 km, and then off North Bimini, Bahamas, 18 days later on 29 April 2020, a further ~ 200 km. Finally, she was detected on 8 May 2020 off Key Largo, FL, U.S. after crossing the Gulf Stream, totaling a minimum travel distance of 900 km in 60 days (Figure 1). Male white shark A69-9001-17866 was detected on 2 March 2022 off Savannah, GA, U.S. and detected off Andros 40 days later on 12 April 2022. He was then detected on 18 June 2022 off Virginia Beach, VA, U.S. ~ 65 days later, after a northern movement of ~ 1400 km (Figure 1).

Discussion

This study provides the first evidence of white sharks using waters in the Tongue of the Ocean (TOTO), Andros Island, The Bahamas. Across the last 70 years, only two records exist of the species in this region of the NWA, with none in the central Bahamas. Our acoustic detections of ten white sharks of varying size and sex along the TOTO near-shore shelf across four years (2020-2024) represent an important finding that expands the current known distribution of white sharks in this part of the world. This also broadens our understanding of elasmobranch diversity in The Bahamas, which is a shark sanctuary where all sharks are protected through national legislation, and supports previously published and anecdotal evidence of white sharks in Bahamian waters.

White shark detections in our array were across short-time periods (1 to 13 days) during the late fall, winter, and early spring. They were recorded in other arrays (e.g., A69-9001-60747 in FL Keys and A69-9001-6893 in Bimini) soon after being detected in Andros (12 and 18 days), suggesting transitory-type behavior to/through the TOTO. Other tracking studies also found that white sharks exhibited rapid migrations in winter and spring along near-shore shelf waters of the southeastern U.S. Atlantic and Gulf of Mexico (GOM) (Skomal et al., 2017; Lowerre-Barbieri et al., 2021). Franks et al. (2021) found that nearly 40% (14 of 36) of the sharks tracked between 2013 and 2019 spent time in waters around the Florida Keys or in the GOM during the overwintering period, with nine returning to the GOM in subsequent periods. Philopatric behavior for white sharks is common across their range (Jorgensen et al., 2010; Huveneers et al., 2018), however despite four years of coverage in the TOTO, we did not detect individuals across multiple years. This, coupled with most white sharks detected in our array being larger juveniles or sub-adults (2.5 – 3.5 m), might indicate that our detections are a result of exploratory behavior exhibited by this life stage. Tagged juvenile white sharks are nearly 5 times more likely to be observed in Canadian waters than a tagged adult (Bowlby et al., 2022), which is hypothesized to be a result of younger animals dispersing more widely across suitable coastal habitats than older age classes. Consequently, immature white sharks are more likely to be detected on acoustic monitoring arrays outside of tagging areas (Bowlby et al., 2022). Given recent evidence for recovery in the NWA, our detections are also likely a result of white sharks returning to historically important parts of their range and the increased numbers of younger life stages being tagged off Cape Cod. However, it's important to consider the limited fishery monitoring capacity in The Bahamas compared to the east coast of the US and that detections were exclusively along the drop off, which could mean white sharks have always used the habitat, but due to deepwater use and no commercial longlining, have gone undetected. It will be important to expand acoustic tracking coverage within the TOTO and broader Bahamas archipelago to determine the scope and drivers of their space and habitat use in the region.

Despite most detections in our array spanning only one or two days, three individuals were recorded across almost two weeks. Given the limited receiver coverage in the TOTO (< 47 km linear range), perhaps some white sharks partially overwinter in the area, like those in the southeastern U.S. Atlantic and GOM. White shark detections were exclusively overnight on the fore-reef edge (25 m) along the interface between the reef and deepwater habitats, where the depth rapidly increases to 40 m then to 400 m. This steep slope is ideal habitat for ambush predators like white sharks (Klimley et al., 2001; Martin et al., 2005; Hammerschlag et al., 2006). One study in Guadalupe Island, Mexico, a location notorious for steep drop-offs close to shore, observed approaching, bumping, and biting of an autonomous underwater vehicle at depths of 53 to 90 m, providing direct evidence of predatory behavior at depth (Skomal et al., 2015). Although pinnipeds, which are a key prey species across its range, do not exist in the Bahamas (but see McClenachan and Cooper, 2008 for extinction of the Caribbean monk seal *Monachus tropicalis*), there is an abundance of coastal sharks and rays (e.g., Caribbean reef, *Carcharhinus perezi*, and silky sharks *Carcharhinus falciformis*; Talwar et al., 2022; Shipley et al., 2023), as well as reef (e.g., Nassau grouper *Epinephelus striatus*; Stump et al., 2017) and pelagic fishes (e.g., dolphinfish *Coryphaena hippurus*; Merten et al., 2015) that are known to use this transition zone in the TOTO, and could provide an important food source for white sharks. Caribbean reef sharks making night time excursions to > 50 m during the winter months (Shipley et al., 2017; Guttridge pers. obs.) would conceivably be targets for white sharks cruising the shelf.

The TOTO is also recognized as an important habitat for a variety of cetaceans (e.g., bottlenose dolphins, *Tursiops truncatus*, and short-finned pilot whales, *Globicephala macrorhynchus*; Claridge et al., 2015), particularly Blainsville's beaked whales, *Mesoplodon densirostris*, that are resident to the area (Hin et al., 2023). The presence of a U.S. Navy base, Atlantic Undersea Test and Evaluation Center (AUTEC) that regularly conducts sonar testing, has resulted in two decades of research on the distribution, abundance, and behavioral responses of cetaceans (Hazen et al., 2011; Claridge et al., 2015; Joyce et al., 2020; Benoit-Bird et al., 2020). Skomal et al. (2017) hypothesized that during their overwintering residency period, white sharks may feed on whales off the southeastern U.S. coast and in the GOM. Beaked whales and bottlenose dolphins have been identified as part of white sharks' diet in other regions (Long and Jones, 1996; Heithaus, 2001; Celona et al., 2006), and the population of Blainville's beaked whales in the TOTO is one of the highest densities to have been estimated (Hin et al., 2023). When examining the deep scattering layer, the western TOTO was characterized by intense, relatively thin layers compared to other parts of the region, offering high quality foraging opportunities for these whales (Hazen et al., 2011). In turn, the foraging effort of Blainville's beaked whales matched this with 2.5x more clicks in the Western TOTO compared to the Eastern TOTO (Benoit-Bird et al., 2020), with surveys revealing a higher density of squid with higher mean mantle lengths (i.e. larger size) in this zone, leading to much more profitable foraging habitat. Thus, there is horizontal overlap between where white sharks were detected and beaked whale foraging grounds. Further, after a deep dive the whales spend an extensive period (66 – 155 min) in the upper 50 m of the water column (Baird et al., 2006; Joyce et al., 2020), which is the vertical zone white sharks spent 95% of their time when overwintering in the SE U.S. and GOM (Skomal et al., 2017). Thus, these local cetaceans, or perhaps the squid beak whales are hunting, could provide an important prey source and motivate white sharks to use the TOTO.

Despite a growing body of knowledge about the movement ecology of white sharks in the NWA, we have provided the first evidence of their presence in the western TOTO in central Bahamas. The Bahamas declared its waters a Shark Sanctuary in 2011 and has banned commercial longlining and gillnetting since 1993, thus The Bahamas is an important refuge for this iconic and vulnerable species. However, despite these new records, the spatial and temporal extent to which this species utilizes these waters remains unclear. While our findings are most likely a result of regional recovery, it is possible that white sharks exploit the productive mesopelagic zone whilst in the TOTO but have remained undetected when using this deepwater habitat because of insufficient monitoring. Clearly further research is required to learn more about white shark presence in The Bahamas including continued long-term collaborative monitoring and tagging programs which allowed our team to reveal these novel records.

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 approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because tagging was conducted under Special Permits issued to GS.

Author contributions

GT: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing. MP: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing. GA: Funding acquisition, Project administration, Resources, Writing – original draft, Writing – review & editing. WM: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. DS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. SG: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

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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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2024.1451808/full#supplementary-material

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Eat-clean-repeat: reef manta rays (*Mobula alfredi*) undertake repetitive feeding-cleaning cycles at an aggregation site in Seychelles

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To maximise energy efficiency, manta ray (*Mobula alfredi*, *M. birostris*) foraging and cleaning behaviours are thought to often be mutually exclusive, whereby individuals will only forage when prey density thresholds are met and will only clean when foraging is too energetically costly (i.e., thresholds are not met). Here, snorkel surveys and remote camera cleaning station footage show reef manta rays (*M. alfredi*) undertaking repetitive and short-term movements between surface-feeding and cleaning station visits around D'Arros Island, Seychelles. These observations demonstrate that foraging and cleaning behaviours are not mutually exclusive even when prey densities are high. At D'Arros Island, the proximity of cleaning stations to highly productive foraging areas may afford individuals the opportunity to undertake non-foraging activity without incurring significant energy loss from the shifts in behaviour. These data inform a more nuanced understanding of this species' use of key habitats.

KEYWORDS

energy expenditure, behaviour, manta ray, activity regime, habitat use

1 Introduction

Manta rays (Myliobatidae, Bonaparte 1838) (*Mobula alfredi*, *M. birostris*) are planktivorous ectotherms with a circum-global distribution in tropical and sub-tropical waters (Lawson et al., 2019). As planktivorous species, manta rays must often cope with the boom-and-bust characteristic nature of oligotrophic environments (i.e., Papastamatiou and Lowe, 2005; Papastamatiou et al., 2007), whereby sporadic foraging opportunities may be followed by periods of starvation (Rohner et al., 2017). In response to their patchily distributed prey resource (Folt and Burns, 1999; Srokosz et al., 2003), manta rays often form predictable aggregations at known locations ("aggregation sites") often associated with high prey availability (Couturier et al., 2011; Armstrong et al., 2016; Harris et al., 2020; Harris and Stevens, 2021). Understanding the function of these aggregation sites is important for mobulid conservation (Palacios et al., 2023). Importantly, foraging opportunities are not wholly responsible for animal presence, with cleaning station visitation (O'Shea et al., 2010; Jaine et al., 2012), socialisation and/or mating opportunities (Stevens et al., 2018) and predator avoidance (Germanov et al., 2019) also known to be drivers.

Different behaviours have different energetic consequences to the individual (e.g., Kacelnik and Houston, 1984; Houston, 1995; Christian et al., 1997; Williams et al., 2006) and current understanding suggests that, in response to this, different behaviours (particularly foraging and cleaning) are often mutually exclusive, triggered by differing conditions and generally occurring at different times (O'Shea et al., 2010; Barr and Abelson, 2019; Weeks et al., 2015). For example, the slow swimming speed associated with filter-feeding is thought to be energetically costly (Alexander, 1990; but see Paig-Tran et al., 2011), and as a result, it is only energetically efficient for manta rays to forage when prey densities reach a threshold that outweighs the energetic costs of filter-feeding (Armstrong et al., 2016; Armstrong et al. 2021b). It even appears that different fine-scale local environmental conditions preferentially drive foraging behaviour and cleaning station visitation within aggregation sites around the world (e.g., Dewar et al., 2008; Jaine et al., 2012; Couturier et al., 2018; Harris and Stevens, 2021). While specific local conditions, such as tidal height and range, may be strongly associated with certain behaviours (e.g., foraging, cleaning), it does not restrict these behaviours from being undertaken under other conditions and cleaning and/or foraging may very well occur outside of these constraints within the same aggregation site, albeit less-frequently (e.g., Jaine et al., 2012; Harris and Stevens, 2021; Harris et al. 2021).

The way an animal uses energy in its environment and how conditions influence the energetic costs of foraging and locomotion can be reconstructed from an animals' energy landscape (Shepard and Lambertucci, 2013). Requirements modulated through energy landscapes can provide explanation to an individual's habitat use, distribution, and migration pathways (Wilson et al., 2012; Shepard and Lambertucci, 2013). Given the disproportionate risk of extinction in large elasmobranchs and shifting global conditions affecting energy balance (Lawson et al., 2019), it is therefore increasingly relevant to develop a more nuanced understanding of energetics and habitat use in these species.

Here, observations are reported of reef manta rays (*M. alfredi*) around D'Arros Island, Seychelles undertaking frequent cycles between surface foraging and cleaning behaviours. To the best of our knowledge, these data suggest definitively for the first time that foraging and cleaning behaviours are not always mutually exclusive in reef manta rays. This has implications for our understanding of reef manta energetics as well as the functioning of key reef manta ray aggregation sites.

2 Methods

D'Arros Island (5°24.9'S, 53°17.9'E) is a 2 km long by 1 km wide coral sand cay, situated within the Amirantes Island Group, Seychelles (Figure 1). D'Arros is encompassed by a recently gazetted 'Zone 1' marine protected area (MPA) that affords the site "high biodiversity protection" (Official Gazette No 34 – Ministry of Agriculture, Climate Change and Environment, 2020). The surrounding reefs provide habitat for over 400 individual reef manta rays (Grimmel, *unpublished data*) that make use of productive foraging areas and cleaning stations year-round with seasonally driven peaks in their presence around the island (Peel et al., 2019, 2020).

2.1 Cleaning stations

The coastal reefs north of D'Arros Island host several cleaning stations, two of which have been identified and are monitored with stationary cameras set at fixed locations at ~ 20 m depth ("MantaCam"). These two stations are situated to the north of D'Arros Island approximately 150 m apart along a west-east axis and are located within 10 meters from the start of the reef crest, where the reef steeply slopes from ~3 m to ~ 20 m depth. As individual manta rays can be identified from unique ventral markings (Beebe and Tee-Van, 1941; Marshall et al., 2008), cameras face upwards to capture images of the ventral surface of reef manta rays as they use the cleaning site. GoPro Hero 8 and 9 cameras (GoPro Incorporation, California, United States of America) with Voltaic 75V always-on external batteries (Voltaic Systems, New York, United States of America) are housed inside waterproof casings and take pictures on timelapse every 10 seconds during daylight hours (0630-1830 or 0600-1800, depending on season). Cameras are changed regularly to ensure near continuous coverage of the sites. Data from each station is downloaded and processed by identifying individual mantas using the station against an ID database of all known individuals within the wider Seychelles archipelago (Seychelles Manta Ray Project). To distinguish between cleaning behaviours and cleaning station visitation for socialization (or other behaviours), cleaning was identified when cleaner wrasse were observed interacting with the individual. Date and timestamps for each sighting are recorded. Any individual that stayed at the station for a prolonged period had the entire duration of that visit noted down.

2.2 Surface-feeding

Reef manta ray surface-feeding behaviour was monitored via survey, whereby a small vessel patrolled the coastal areas (within 1 km from shore) of D'Arros, completing a full circumference of the island. During these surveys, the vessel completed a distance of approximately 7 km around the island at a maximum speed of 8 knots, with the skipper and at least two snorkelers locating manta

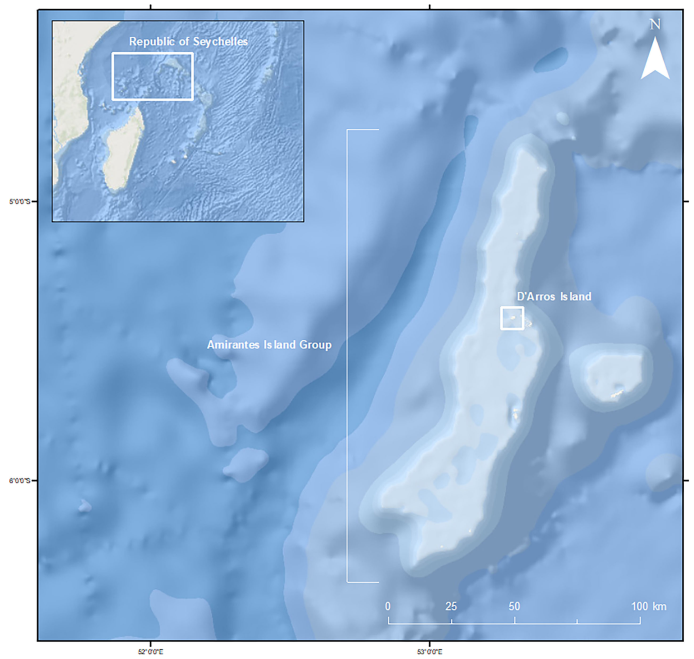

FIGURE 1
D'Arros Island (5°24.9'S, 53°17.9'E) situated within the Amirantes island group of the Seychelles archipelago. Manta survey efforts contained to shallower water depths around the perimeter of the island (within 1 km). Two cleaning stations are situated along the northern edge of the island. Exact locations are not disclosed to protect key habitats of the species. Basemap source: Esri®.

rays. On survey days described here, surveys were undertaken by three or four snorkelers (excluding the skipper) for a duration of approximately three hours. While individuals may be located along the entire survey area, during the surveys identified here individuals were predominantly located along the 2 km stretch north of D'Arros, intersecting where the two cleaning stations are located. The closest proximity of surface-feeding individuals to cleaning stations was approximately 20 m, when individuals were located directly above the cleaning stations. Manta rays located from aboard the vessel were followed via snorkel to collect photo identification of their ventral surface using GoPro Hero 9, 11 or 12. Footage was cross-referenced with the ID database to identify and pinpoint timestamps for each individual when they were surface-feeding. Surface-feeding was identified as when cephalic lobes unfurled and individuals' mouth was open.

2.3 Feeding-cleaning cycles

Repetitive movements, grouped as total numbers of behavioural transits (one-way movements from surface-feeding to cleaning station use or vice versa) as well as feeding-cleaning cycles (feeding, cleaning and back to feeding or vice versa), were quantified from survey and camera data. Behavioural transits were quantified over a maximum of 1-hour periods whereby an individual that was observed surface-feeding (or cleaning) must be observed undertaking the other behaviour within 1-hour of the initial observation. Feeding-cleaning cycles were quantified over a maximum of 2-hour periods whereby an individual had to be observed surface-feeding (or cleaning), transit to the other behaviour, and then return to the original behaviour within the 2-hour period. This timeframe was based on survey duration and the time-period over which the majority of reef manta rays were observed surface-feeding. Individuals that cycled or transitioned between behaviours but outside of these parameters were not included.

3 Results

Across four survey days during November and December 2023, 11 identified individual reef manta rays undertook repetitive behavioural transits and feeding-cleaning cycles (Table 1). A total of 27 transits ($\mu = 2.45 \pm 0.78$ S.D) and 15 cycles (1.36 ± 0.5) were undertaken, with cycles ranging from 11 to 102 min (66 ± 29) in duration (Table 1). Here, duration of cycles is the total time taken for the individual to move from one behaviour (e.g., cleaning) to another (e.g., feeding) and then return again to the original behaviour (e.g., cleaning). Median time taken for an individual to transit between behaviours was 28 minutes ($\mu = 29.48 \pm 17.75$ S.D). SC-MA-0014 undertook the quickest transits between behaviours with a mean of 8.33 ± 5.44 minutes. SC-MA-0101 undertook the longest transits with a mean of 48.5 ± 2.5 minutes (Table 1).

Cleaning-only behaviour was the most commonly seen behaviour across the four survey days. The second most common behaviour was feeding-cleaning cycles except on 18 December where feeding-only was the second most common behaviour. The 21 November (n=23) had the highest proportion of individuals undertaking both feeding and cleaning behaviours (56.5%) and

TABLE 1 Metadata table of individual reef manta rays (*Mobula alfredi*) that undertook movements between surface-feeding and cleaning behaviours.

Date	Individual ID	Sex	Maturity	Size class	Surface-feeding (timeframes)	MantaCam West (timeframes)	MantaCam East (timeframes)	Behavioural Transits	Feeding-Cleaning Cycles
14-Nov-23	SC-MA-0298	M	Immature	2	1009-1017, 1140-1144, 1232-1235	–	1104-1112, 1341	2	1
21-Nov-23	SC-MA-0006	M	Mature	3	0900-0901, 1056-1058	1009-1011	1014, 1033-1047, 1126	2	1
21-Nov-23	SC-MA-0100	F	Mature	4	0917-0921, 0928-0929, 1054	0929-0931, 1043-1047	0847-0849, 0941, 1126	4	2
21-Nov-23	SC-MA-0101	F	Mature	4	916	0824-0825, 1009-1011, 1028-1042, 1135-1139, 1306	1002-1009, 1011-1013, 1015-1023, 1141-1154, 1221-1230, 1253-1303	2	1
21-Nov-23	SC-MA-0092	M	Mature	3	0821-0843, 0903-0905, 1026	–	0642-0639, 0938, 0956—0959	2	1
21-Nov-23	SC-MA-0014	F	Mature	3	0842-0846, 0857-0905, 0914	0931-0952	0851-0853, 1427-1430	3	2
21-Nov-23	SC-MA-0321	F	Immature	2	0820-0840, 1056-1057	–	0956-0959, 1121-1125	2	1
21-Nov-23	SC-MA-0281	M	Immature	2	0920, 1055-1058	1135, 1154	0832-0837, 1008, 1154-1158, 1612-1638	4	2
21-Nov-23	SC-MA-0267	F	Immature	1	0905-0916, 1058-1106	1034, 1154, 1434, 1539	1154-1158, 1318-1322, 1434-1442, 1526-1539	2	2
18-Dec-23	SC-MA-0007	F	Mature	4	0824, 0909-0912, 0915-0916, 0947, 1046-1052	–	0916-0920	2	1
19-Dec-23	SC-MA-00461	M	*NA*	*NA*	0928-0930, 0936-0943, 1100-1112, 1130-1136, 1148-1157	1002	0955-0958	2	1

Surface-feeding timeframes are based on how long the individual is in one video. MantaCam timeframes are based on the first and last timestamp for individuals photographed at that cleaning station. Where timeframes consist of a single time, the individual was at the cleaning station or video footage from snorkel surveys were of the individual < 1 minute. This does not necessarily represent the amount of time the individual was feeding or cleaning in the area but instead reflects either the snorkeller moving to a different individual or limitations in MantaCam camera angle. To account for variability in individual wingspan (wingtip to wingtip) estimation based off stereo video, individuals were classed into binned estimates of their wingspan: (1) 2-2.5 m, (2) 2.6-3 m, (3) 3-3.5 m, (4) 3.6-4 m and (5) 4+ m.

feeding-cleaning cycles (34.8%). The 18 December (n=10) had the lowest proportion of individuals exhibiting both behaviours (14.3%) and feeding-cleaning cycles (7%), with an equal representation of cleaning-only and feeding-only behaviours (42.9% for both). Most individuals (excluding SC-MA-0298, -0092, -0321 and -0007), not only undertook repetitive movements between surface-feeding and cleaning behaviours but also repetitively moved between visiting each of the two cleaning stations (Table 1).

4 Discussion

An individual's capacity to minimise energy expenditure (i.e. cost of locomotion) and maximise energy gain through foraging has direct (e.g., body condition, mortality) (du Plessis et al., 2012; Giles et al., 2020) and indirect (e.g., reproductive success) (Ballard et al., 2010; Jeanniard-du-Dot et al., 2017; Jeanniard du Dot et al., 2018) consequences on fitness and survival. According to optimal foraging theory, animals must maximise energy acquisition by obtaining as much energy as possible through foraging in a given period of time (Schoener, 1971; Norberg, 1977, 2021). Under this assumption, when conditions favour foraging (e.g., plankton densities exceed a threshold), manta rays will exclusively forage in order to meet energetic demands and will not expend energy undertaking other behaviours elsewhere (see O'Shea et al., 2010; Barr and Abelson, 2019). Findings here, however, demonstrate short-term cycles between surface-feeding and cleaning station use in reef manta rays at a Seychelles aggregation site for the first time. At D'Arros, high-use cleaning stations adjacent to the northern reef slopes coincide with areas of preferred feeding (Peel et al., 2024). The reefs on the northern shores have a steeper topographic relief relative to the south, potentially driving greater upwellings of plankton and influencing observed spatial patterns in feeding at the site. Similarly, the steeper reef slopes around the north of D'Arros make for more pronounced deep reef patches that act as quality cleaning stations. The proximity of these cleaning stations to highly productive foraging areas may mean that cleaning activity can be undertaken without significant loss of energy or that intermittent cleaning confers other benefits to the individual. Cleaning stations play an important role in manta ray fitness, offering an important location for socialisation and potentially improving social learning, predator avoidance and contribute to foraging efficiency through collective feeding events (Perryman et al., 2019). As a result, manta rays are thought to preferentially select cleaning stations with hard coral substrate and specific cleaner wrasse species with these 'quality' cleaning stations contributing to a spatial map the individuals possess of their landscape (Armstrong et al. 2021a). Here, reef manta rays most commonly exhibited cleaning behaviours, whether these were cleaning-only or cleaning alongside feeding behaviours. While cleaning may very well be the most common behaviour over surveyed days, cleaning stations are consistently monitored by remote camera across the entire day whereas surveys are limited to the survey period (~3 hours). Therefore, it is possible that an animal that was only observed cleaning also surface-fed but was not identified during the survey. Regardless, functional cleaning and foraging habitat in close proximity may benefit not just foraging success but also sociability in this population whereby interrupted or unsuccessful cleaning events can be returned to after a brief foraging 'break'. Such quality cleaning sites occurring in concert with productive foraging areas may play an important role in selection of key high-use habitats.

Observations here offer a unique perspective into the energy landscape of D'Arros Island reef manta rays where the seascape hosts opportunities to undertake multiple behaviours simultaneously without suffering an apparent net energy loss through reduced foraging opportunities. Energy landscapes are dynamic, varying in space and time (Masello et al., 2017) and so these observations may be situational even within the D'Arros Island aggregation site as both localised and broad-scale environmental conditions influence energetic costs of behaviour for the population. It should be noted that the need to maximize foraging activity, as according to optimal foraging theory, may not be applicable to all populations of manta ray. Some populations live in highly productive environments where they do not suffer the energetic knife edge associated with oligotrophic conditions (see Papastamatiou and Lowe, 2005; Papastamatiou et al., 2007). For populations where feeding to satiation is not favoured, repetitive and simultaneous cycles between foraging and cleaning events may offer a unique opportunity to undertake a multitude of behaviours without a net energy loss. Regardless of the potential circumstantial nature of these feeding-cleaning cycles, the data presented here offers important consideration for conservation of key aggregation sites of vulnerable species.

While other aggregation sites suggest feeding and cleaning behaviours may present closer to mutual exclusivity in manta rays (O'Shea et al., 2010; Barr and Abelson, 2019), specific habitats, such as D'Arros, may provide for alternative energetic strategies. D'Arros is not the only site that may offer habitat characteristics that afford multiple behaviours occurring simultaneously. Manta rays in aggregation sites within Raja Ampat, Indonesia, use the habitat for foraging and cleaning, though repetitive feeding-cleaning cycles have not been formally identified at these sites (Setyawan et al., 2020). Areas such as these may be important for both social behaviour and energy acquisition through foraging. Continuous effort should be made into identifying habitat where individuals undertake multiple key behaviours simultaneously and can be done using non-invasive techniques (e.g., remote camera as used in this study, drones as discussed in Setyawan et al., 2020). Increasing anthropogenic pressure at manta ray aggregation sites, particularly cleaning stations (Tratalos and Austin, 2001; Murray et al., 2020), could have ongoing consequences for the capacity of individuals to meet their energy demands if these areas coincide with highly productive foraging areas. Defining the function and overlap in important cleaning, feeding or socialisation areas is therefore imperative to the long-term conservation of these species.

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 Seychelles Bureau of Standard, Seychelles Government. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

RN: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. HG: Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Writing – review & editing. DP: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. EM: Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. AA: Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. RB: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing, Supervision.

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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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First evidence of predation on an adult porbeagle equipped with a pop-off satellite archival tag in the Northwest Atlantic

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Pop-off satellite archival tags (PSATs) have been increasingly deployed on marine megafauna to remotely monitor their movements, behavior, and environmental preferences. These tags have also allowed the occasional novel documentation of ecological interactions, such as predation on tagged animals. A PSAT deployed on a pregnant porbeagle *Lamna nasus* in the Northwest Atlantic suggests the shark was predated upon in mesopelagic waters near Bermuda. Predation was evident approximately 5 months after tagging based on depth and temperature data transmitted by the PSAT. Four days prior to PSAT pop-off, depth data indicated that the tag continued to descend and ascend in the water column while the temperature remained approximately 5°C above ambient levels, even at several hundred meters in depth, indicating ingestion. Given the location of predation and elevated temperature at depth recorded by the pregnant porbeagle's tag, potential predators include endothermic shark species such as the white shark *Carcharodon carcharias* and shortfin mako *Isurus oxyrhinchus*. This is the first evidence of predation on a porbeagle globally and provides novel insight into inter-specific interactions for this large, threatened shark species.

KEYWORDS

ecology, reproduction, inter-species interaction, Bermuda, *Lamna nasus*, white shark, shortfin mako

1 Introduction

Pop-off satellite archival tags (PSATs) have been traditionally utilized to examine the three-dimensional movements of elasmobranchs over varying temporal and spatial scales (e.g., Skomal et al., 2021). PSATs have also been used to document post-release mortality (PRM) associated with capture and handling (e.g., Goldsmith et al., 2017), and have opportunistically documented predation events in species such as Atlantic salmon *Salmo salar* (Lacroix, 2014) and white marlin *Tetrapturus albidus* (Kerstetter et al., 2004). To discern between the two types of mortality, several characteristic changes can often be observed in the PSAT data to indicate tag ingestion by a predator, including the sudden absence of light (e.g., Béguer-Pon et al., 2012), temperature increases (coinciding with the stomach temperature of predatory species), and alterations in depth range or diving behavior (e.g., Kerstetter et al., 2004).

Porbeagle *Lamna nasus* are large-bodied, highly mobile sharks documented in cold-temperate waters of the Northwest Atlantic through previous PSAT studies (Skomal et al., 2021). The Northwest Atlantic populations' life history is characterized by late ages at maturity (Natanson et al., 2002), low reproductive output (Jensen et al., 2002; Natanson et al., 2019), and low estimated annual natural mortality (Cortés and Semba, 2020). We present the first documented predation of an adult female porbeagle affixed with a PSAT that migrated off the continental shelf waters of New England to the Sargasso Sea, and suggest the predators likely responsible for the observed mortality.

2 Methods

Porbeagles were caught via rod-and-reel (following Anderson et al., 2021) southeast of Cape Cod, Massachusetts, US in late October of 2020 and 2022. Captured porbeagles were brought onboard the vessel and a saltwater pump was inserted into the shark's mouth to facilitate respiration. Total time on deck averaged less than 15 minutes. Sex, fork length (FL; cm), total length (TL; cm), and capture location were recorded. Females near or above the size at maturity (210-230 cm FL; Jensen et al., 2002) were examined for pregnancy via ultrasound following Sulikowski and Hammerschlag (2023). 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 24 cm scan depth was used to obtain images of the reproductive tract. 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 uterus, respectively.

Porbeagles were tagged with both a fin-mounted satellite linked transmitter (hereafter "finmount tag") and a PSAT. The finmount tag attached to the predated porbeagle was a Sirtrack F6F FastGPS Argos transmitter (Lotek Wireless). The finmount tag was attached to the sharks' first dorsal fin following established protocols (e.g., Gibson et al., 2021). Additionally, a 365-day PSATFLEX (Lotek Wireless) was looped through a hole in the first dorsal fin of each shark following Anderson et al. (2021). PSATs were programmed to collect pressure in dbar (i.e., depth in m, hereafter referred to as depth) and ambient water temperature (°C) at 20-s intervals for the pre-programmed 365-day deployment period, after which the tags were designed to float to the sea surface and transmit archived data (via Argos satellites) in compressed 2-hour bins (i.e., one depth and temperature record every 2 hours). The PSATs are designed to report daily light-based geolocation estimates that are determined onboard the tag by manufacturer proprietary software (Ekstrom, 2004) and thus raw light-level data are not reported. PSATs were programmed to release and transmit prematurely if pressure remained constant (± 5 m) for 3 days, indicating a mortality or a shed tag floating at the sea surface or washed ashore.

For geographic location data, PSAT light-based geolocation estimates were discarded due to a low number of reliable position estimates. In particular, light-based PSAT latitude estimates ranged from −90.1 to 78.1 and longitude estimates from −177.7 to 175.9. Latitudinal error estimates ranged from 0 to 67.0° (mean ± SD = 10.4° ± 15.0°) and longitudinal error estimates from 0.2 to 51.6° (mean ± SD = 3.4° ± 6.0°). The deep diving behavior of porbeagles while in mesopelagic habitats likely contributed to a proportion of the erroneous positions in each PSAT track (Campana et al., 2010; Heard et al., 2017). Position estimates from finmount tags were downloaded from Argos satellites (CLS America, Inc.). Argos provides location accuracy using the following location classes (LC): 3, 2, 1, 0, A, B, and Z (in decreasing accuracy), with accuracy ranging from < 250 m to > 5 km for LC 3 to B (Tougaard et al., 2008). There were no transmissions categorized as the poorest accuracy LC (Z).

It is standard practice for satellite telemetry data to be regularized and interpolated with a state-space model (e.g., Jonsen et al., 2023) when assessing the habitat use of marine species. Unfortunately, due to the limited number of finmount satellite transmissions before predation and necessary discarding of PSAT geolocation estimates, tag data could not be processed with a state-space model in this study. However, given satellite telemetry data were strictly used for helping to identify the general locations of the tagged porbeagle rather than assessing habitat use patterns, the highest-accuracy finmount tag transmission for a given day were plotted using the R package 'ggmap' (Kahle and Wickham, 2013), along with the capture location and the first satellite transmission received from the PSAT after pop-off. The minimum horizontal displacement of the shark was estimated by calculating the distance (km) between the capture location and the first satellite transmission received by the PSAT following pop-off. For depth and temperature data recorded by the PSAT, the temperature-integrated diving track was plotted across the time-series using the R package 'ggplot' (Wickham, 2016). Additionally, the average ambient temperatures at depths were calculated and compared between the week before apparent tag ingestion and during the tag ingestion period.

3 Results

A 223 cm FL pregnant porbeagle was captured and tagged southeast of Cape Cod, MA on October 28, 2020 (Figure 1). The shark's PSAT transmitted its first location (LC 1) southwest of Bermuda 158 days after tagging, on April 3, 2021 (Figure 1). The minimum horizontal displacement of the shark was estimated to be 1,120 km. However, it's important to note that there was a three-and-a-half-day gap between the last transmitted depth measurement and the first satellite transmission after pop-off, during which the PSAT was likely drifting at the sea surface (e.g., Peel et al., 2020). The shark's finmount tag only transmitted one location on Georges Bank on November 12, 2020 (Figure 1). Based

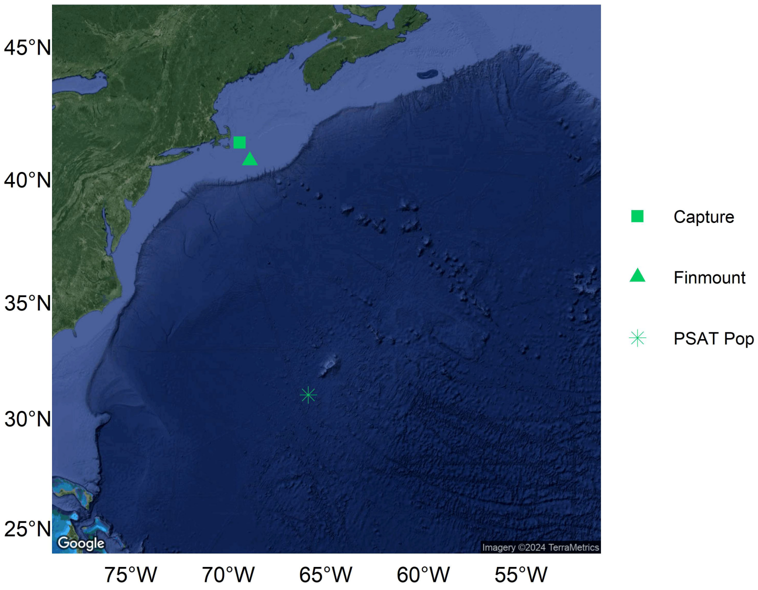

FIGURE 1
Map of the capture location, the single highest-accuracy daily finmount tag transmission, and the first transmission received from the PSAT after pop-off for a pregnant porbeagle tagged in the Northwest Atlantic. It is important to note that there is a time gap between the last depth measurement collected from the PSAT and the first transmission after pop-off.

on PSAT depth data, the pregnant porbeagle was diving between the sea surface and approximately 100 m from the day of tagging until December 26, 2020 (Figure 2). During this time, ambient temperatures recorded by the tag ranged from 10.28 to 20.54°C (Figure 2). From late December to late March, the shark transitioned diving behavior, spending nighttime at approximately 100-200 m and daytime at approximately 600-800 m, suggesting that the shark had moved off of the continental shelf (Figure 2). During this period, the shark occupied water temperatures of 6.4 to 23.52°C (Figure 2). However, on March 24, 2021, ambient temperatures recorded by the PSAT increased, ranging between 16.4 and 24.72°C despite recording a similar depth range of approximately 150-600 m (Figure 2), indicating tag ingestion. In the week prior to the apparent predation, overall average temperature recorded by the tag was 17.4°C. During that time, average temperature was 19.1°C at 100-300 m depth and 16.1°C at 500-700 m depth. In comparison, during the apparent tag ingestion period, the overall average temperature recorded by the tag was 22.2°C. Average temperature was 21.8°C at 100-300 m depth and 22.2°C at 500-700 m depth during tag ingestion.

4 Discussion

Based on previous work (e.g., Anderson et al., 2021; Bowlby et al., 2021), the post-release diving behavior for the porbeagle tagged in this study appeared characteristic for the species and suggest that capture and handling effects during tagging were not influencing the observed mortality (Bowlby et al., 2021). In addition, PRM has been shown to largely occur immediately (or within 48 hours) of release in large pelagic sharks (e.g., Campana et al., 2016), and physiological recovery of lamnid sharks post-release suggest the group are generally resilient to capture and handling methods (Musyl and Gilman, 2019; Anderson et al., 2021; Bowlby et al., 2021). Based on the collective understanding of PRM and recovery in porbeagles and other lamnids, the PSAT data described herein are indicative of natural morality caused by predation rather than from stress from the capture and handling of the shark months prior to the observed mortality. In addition, given the slight change in diving pattern and premature release of the PSAT, it is unlikely that the observation reported here was related to movement into a warmer water body.

The depth and temperature data recorded by the sharks' PSAT as well as the general location of predation can be used to identify potential predators. The sudden elevated temperatures (16.4-24.72°C) and stabilized thermal excess (5°C above ambient temperature on average) during ingestion were characteristic of gut temperatures reported for other large endothermic fish (Goldman, 1997; Sepulveda et al., 2004; Jorgensen et al., 2015). Large marine mammals, such as odontocete whales (e.g., Orcas *Orcinus orca*) maintain much higher internal temperatures (close to 40°C; Whittow et al., 1974; Strøm et al., 2019) and so were not considered as a potential predator in this instance. Two endothermic predator candidates large enough to predate upon mature porbeagles and located within the vicinity and at the time of year of the predation event include the white shark *Carcharodon carcharias* (Skomal et al., 2017) and shortfin mako *Isurus oxyrhinchus* (Santos et al., 2021). Following ontogenetic shifts in diet, prey species of adult white sharks and shortfin makos are known to include other elasmobranch species (and marine mammals) (e.g., Joyce et al., 2002). While fine-scale diving data for these potential predators are limited in the location that the predation event occurred, both white sharks (Skomal et al., 2017) and shortfin makos (Santos et al., 2021;

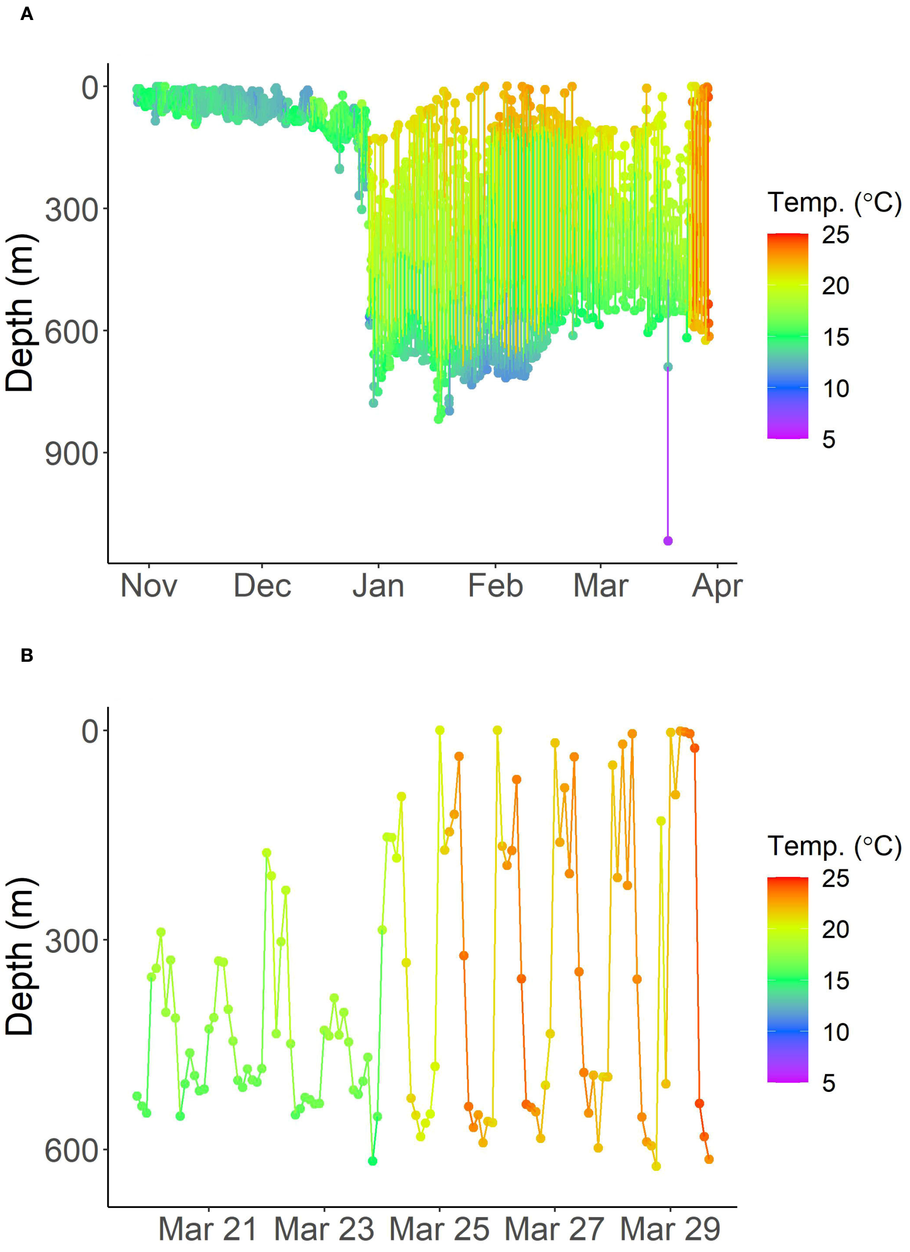

FIGURE 2
Depth and temperature time-series over the entire deployment **(A)** and the last week before pop-off **(B)** for the tagged porbeagle.

Loefer et al., 2005) have been documented to occupy broad depth distributions from the sea surface to >300 m while in offshore waters near the Sargasso Sea. However, tagged shortfin makos mainly occupied depths less than 400 m and made rapid oscillatory dives between the sea surface and deeper depths during the day while in offshore waters of the North Atlantic (Santos et al., 2021; Loefer et al., 2005), which was not characteristic of the predator's behavior in this study. In comparison to shortfin makos, large female white sharks spent more time at depths of 400-600 m while in mesopelagic waters of the North Atlantic (Skomal et al., 2017). Given this comparison, a white shark was likely the predator of our tagged porbeagle.

The data presented in this study is, to the best of our knowledge, the first evidence of predation on a porbeagle and provides novel insight into inter-specific interactions for this large, globally vulnerable (IUCN, 2023) shark species. Knowledge gaps persist regarding natural mortality rates for porbeagles, and this work suggests predation may be an underestimated source of natural mortality for the Northwest Atlantic population. For example, 11 porbeagles (excluding one immediate mortality) were tagged with year-long PSATs and transmitted data which could be used to opportunistically identify natural mortality events. Two (18.2%) natural mortality events were observed, including the predation documented in this study as well as the mortality (of unknown cause) of an adult female that also occurred near Bermuda approximately 50 days after tagging. However, it is important to consider that it has been suggested that marine animals tagged with tethered PSATs may be at increased risk of predation (e.g., tags act as lure or emits attractant signal) (Thorstad et al., 2013; Hall and James, 2021; Lennox et al., 2023). Nevertheless, continued research of mesopelagic predator-prey interactions using satellite tags is warranted to increase our knowledge of ecosystem dynamics in the open ocean and thus improve the efficacy of management strategies to maintain ecosystem health. For example, the observations presented here suggest mesopelagic waters near Bermuda are an area of important inter-species interaction and mortality for porbeagles and thus should be a location of future research efforts.

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 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

BA: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing, Project administration. LH: Writing – original draft, Writing – review & editing. KB: Writing – original draft, Writing – review & editing. JD: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – review & editing. JS: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

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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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Citizen science highlights rare behaviours by the spotted eagle ray *Aetobatus ocellatus* Kuhl 1823

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Introduction

The Spotted Eagle Ray *Aetobatus ocellatus* (Kuhl 1823) is the largest member of the Aetobatidae family reaching 300 cm disc width (DW) (Last et al., 2016), but little is known about the ecology of this species. *Aetobatus ocellatus* is distributed throughout the Indo-West Pacific and possess k-selected life history traits, such as slow growth and late maturity (Schluessel et al., 2010), which render this species susceptible to overexploitation. In parts of its range, for example in Borneo, *A. ocellatus* is commonly caught in coastal fisheries including trawlers and demersal longlines (Last et al., 2010). Such threats have led to inferred population declines of 50-79% over the last three generations (39 years) across much of its range, resulting in *A. ocellatus* being uplisted to Endangered in the recent IUCN Red List of Threatened Species assessment (Finucci et al., 2024). Given the magnitude of these threats to the species, it is necessary to identify critical habitats essential for management and conservation.

Due to their evasive nature, behavioural observations of *A. ocellatus* can be logistically challenging to obtain. To date, ten distinct behaviours of *A. ocellatus* have been described, with the predominant behaviours being swimming, foraging, chafing, cruising and escaping threats (Berthe et al., 2018). Chafing in *A. ocellatus* has been reported in French Polynesia and inferred as a behaviour to rid itself of parasites (Berthe et al., 2017), likely analogous to cleaning behaviour associated with cleaning stations that has been observed in other elasmobranch species, such as the Oceanic Manta Ray *Mobula birostris* and the Reef Manta Ray *M. alfredi* (Jaine et al., 2012; O'Shea et al., 2010; Barr and Abelson, 2019). Escaping is an avoidance behaviour in response to swimmers or close vessel approaches or loud boat engine noise (Berthe and Lecchini, 2016). In response to disturbance or a predator, eagle rays speed-up and swim away from the threat (Berthe and Lecchini, 2016), which is likely to incur an energetic cost. Recovery from these excursions may take the form of resting behaviour, as documented in *A. narinari* in Belize (Flowers and Kelley, 2020) and the Bahamas (Silliman and Gruber, 1999) where rays were recorded as being completely stationary on the substrate for prolonged periods of time. Although the drivers of this resting behaviour remains unknown, this behaviour does suggest that pelagic eagle rays utilise benthic environments for purposes beyond foraging.

Understanding the behaviour of wide-ranging and evasive elasmobranchs is not trivial and tends to be both resource and time intensive. Citizen science can complement dedicated research efforts by increasing the amount of in-water survey effort and thus data produced (Chin and Pecl, 2018; Bateman et al., 2024). For batoid species, citizen science has been used to understand the connectivity of reef manta rays *Mobula alfredi* across the Indonesian archipelago (Germanov and Marshall, 2014), to discern the range and occurrence of the rare ornate eagle ray *Aetomylaeus vespertilio* (Araujo et al., 2020a), and to understand the occurrence and abundance of 11 species in the Canary Islands (Tuya et al., 2021), amongst others. Behavioural studies have also made use of citizen science, for example, in the Philippines to understand the philopatric behaviour of whale sharks *Rhincodon typus* (McCoy et al., 2018), and in Fiji to measure the effects of provisioning on the occurrence and abundance of sharks (Ward-Paige et al., 2020). Citizen science can therefore play a key role in our understanding of threatened and rare species, including shedding light on their elusive behaviours.

Here, we present eight examples of previously unreported resting behaviour by *A. ocellatus*, six of which are from Australia, in Ningaloo Reef, Western Australia, and at Lady Elliot Island (LEI) and Mooloolaba in Queensland (Figure 1), with additional observations from both the Maldives and Japan (Table 1). Additionally, we report resting individuals also engaging in cleaning behaviour in association with the blue-streak cleaner wrasse *Labroides dimidiatus* Valenciennes 1839, the moon wrasse *Thalassoma lunare* Linnaeus 1758 and the schooling bannerfish *Heniochus diphreutes* Jordan 1903.

Methods and results

Observations of resting and cleaning behaviours by *A. ocellatus* were sourced and collated from social media posts, outreach and existing citizen science initiatives, including Fin Focus Research that invites photo submissions of elasmobranchs from recreational ocean users and ecotourism industry crew while

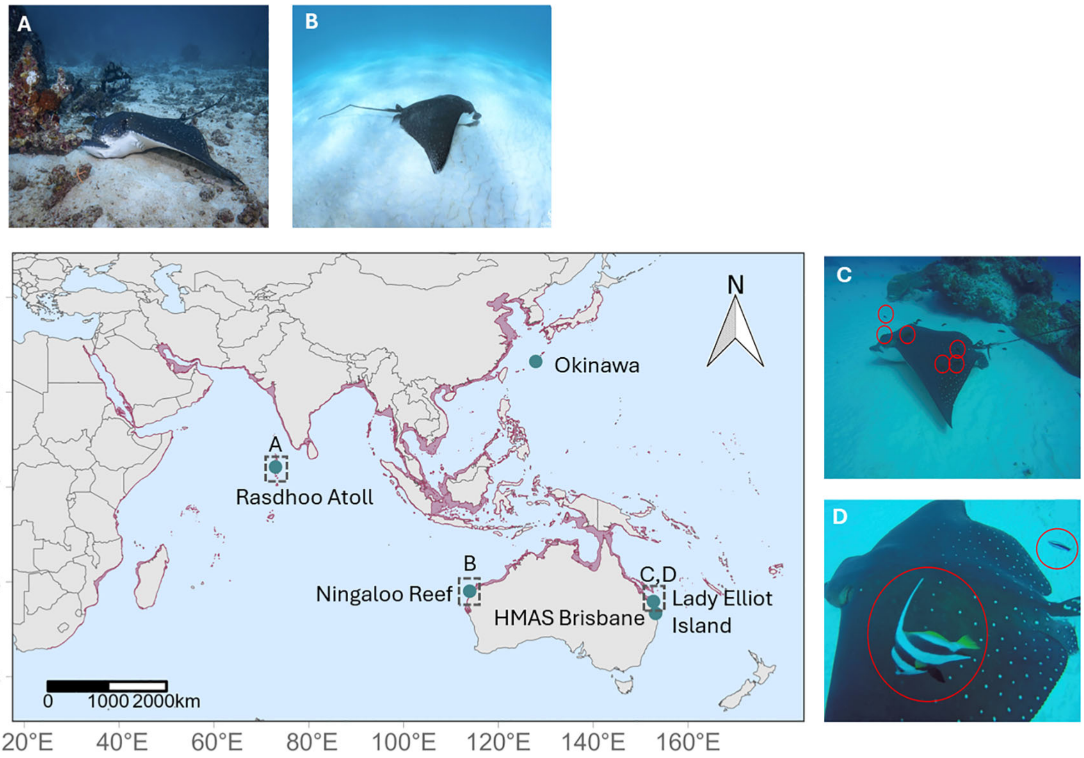

FIGURE 1
Map and examples of resting and cleaning behaviour of the Spotted Eagle Ray *Aetobatus ocellatus*. The highlighted areas indicate the known range of the species (IUCN Red List of Threatened Species, Finucci et al., 2024) and points indicate recorded observations of resting behaviour. Example photographs of resting behaviour are from **(A)** Radshoo Atoll, Maldives, **(B)** Ningaloo Reef, Australia, **(C, D)**. Lady Elliot Island, Australia. Note the red circles in **(C, D)** highlight the cleaner fish species identified in association with A. ocellatus: the blue-streak cleaner wrasse *Labroides dimidiatus*, the moon wrasse Thalassoma lunare and the schooling bannerfish *Heniochus diphreutes*.

TABLE 1 Location of resting Spotted Eagle Rays *Aetobatus ocellatus* and further information collected where available.

Date	Location	Time	Depth (m)	DW (cm)	Sex	Cleaning behaviour
07/06/2018	Lady Elliot Island, Qld, Australia	7:30	17	150	F	Y
17/06/2020	Northern Ningaloo Reef, WA, Australia	13:30	5	150	F	N
27/12/2020	Lady Elliot Island, Qld, Australia	11:30	7	130	M	Y
21/10/2021	North of Coral Bay, WA, Australia	N/A	4	N/A	M	N
17/12/2021	Mooloolaba, Qld, Australia	N/A	27	150	F	Y – no current present during this encounter
11/07/2022	Northern Ningaloo Reef, WA, Australia	14:20	6	<100	M	N
15/06/2022	Rasdhoo, Maldives	N/A	17	N/A	N/A	Cleaner wrasse present in photo.
29/06/2022	Okinawa, Japan	N/A	9	130	M	Y

DW, estimated disc width reported by observers. N/A, not available.

on tour (see https://www.facebook.com/finfocusresearch). Where possible, observers recorded estimate disc width (in m), behaviour and sex. From the collected footage, cleaner fish species were visually identified using Allen et al. (2003) and validated through FishBase (www.fishbase.org).

Here we describe six of the eight reported examples of resting *A. ocellatus* for which further information was available (Table 1). On 07 June 2018 at 7:30 am (moon irradiance 40%, low tide *c.* 9:10 am) a female *A. ocellatus* was observed resting at 'Lighthouse Bommies' in LEI (24.11 S, 152.71 E) albeit no depth information was provided. While resting, this individual was also being cleaned by three species of fish: blue-streak cleaner wrasse *Labroides dimidiatus*, the moon wrasse *Thalassoma lunare* Linnaeus 1758 and the schooling bannerfish *Heniochus diphreutes* Jordan 1903 (Figure 1C). This individual was visually estimated at 150 cm DW and 230 cm total length. The ray departed following collection of photo-ID footage. Similarly, on 17 June 2020, at 1:30 pm (moon irradiance 14%, low tide *c.* 2:30 pm) at 'Ranger Bommies' on the Ningaloo Reef (21.93 S, 113.94 E; Figure 1B), an *A. ocellatus* female individual was observed resting on the substrate at *c.* 5 *m* depth, visually estimated at 150 cm disc width (DW) and total length of 230 cm (Figures 1A, B). There was no predator (i.e., shark or cetacean) noted within the vicinity of the resting eagle ray.

On 27 December 2020 at 11:30 am (moon irradiance 94%, low tide *c.* 12:50 pm) at 'Lighthouse Bommies' on LEI (Figures 1C, D) a male *A. ocellatus* was observed resting on the substrate at *c.* 7 *m* depth, visually estimated at 130 cm DW. Upon close inspection, this individual ray was being cleaned by a blue-streak cleaner wrasse *L. dimidiatus* Valenciennes 1839. A fourth observation was filmed and reported on 21 October 2021 at *c.* 9:30 am (moon irradiance 99.9%, low tide *c.* 4:45 am) from 'Mooch Patch' in approximately 4 m depth north of Coral Bay (22.91 S, 113.77 E) on the Ningaloo Reef. The male *A. ocellatus* was observed resting (with a remora on the substrate behind the ray) for a few minutes, and when the observer dived down adjacent to the ray it slowly rose and swam away. No other information was recorded for this encounter.

A fifth observation was made at the HMAS Brisbane dive site in Mooloolaba, Queensland, on 17 December 2021 at 9:30 am (moon irradiance 97.5%, low tide *c.* 1:30 am). The ray was resting at 27 m whilst getting cleaned by at least four blue-streak cleaner wrasse *L. dimidiatus* (Supplementary Figure 1). A sixth observation was made at 2:20 pm on 11 July 2022 (moon irradiance 92.8%, low tide *c.* 2:39 pm), again at 'Ranger Bommies' on the Ningaloo Reef during a snorkel ecotour. A male *A. ocellatus* was observed resting on the substrate at *c.* 6 m depth, visually estimated at >100 cm DW, with a large Bottlenose Wedgefish *Rhynchobatus australiae* also seen resting approximately 5 m away. The photographer making the observation dived down very close to the *A. ocellatus* for photographs, and no cleaner fish were observed. As the diver ascended and broke the surface, the ray began to move away. A female zebra shark *Stegostoma tigrinum* was also observed resting at the same site during the snorkel.

On 15 June 2022, a seventh observation was reported in Rasdhoo atoll, Maldives (moon irradiance 99.8%), of a resting *A. ocellatus*. Although at least two blue-streak cleaner wrasses can be observed in the photo, we cannot assume active cleaning was taking place. An eight observation was reported from Diamond Beach, Okinawa, Japan, on 29 June 2022, of a resting *A. oceallatus* at 9 m depth. The ray was also getting cleaned by at least two blue-streak cleaner wrasse *L. dimidiatus* (see Supplementary Video 1).

Discussion

The resting behaviour of benthopelagic rays is not fully understood, nor well documented. For which size was estimated herein were adult-sized (100-150 cm DW; Last et al., 2016; Schluessel et al., 2010). There are several plausible hypotheses for the observed resting behaviour. The first, is that resting behaviour is a result of post-copulatory or post-courtship behaviour. This explanation seems unlikely, however, given the lack of mating scars on the three confirmed female *A. ocellatus* reported here, and that courtship/copulation usually involves the male grasping or biting the female (Berthe, 2017; McCallister et al., 2020). A second theory, is that eagle rays might rest for short periods of time before foraging in the sand (Smith and Merriner, 1985) or potentially after feeding. Matern et al. (2000) suggest that feeding on deeply burrowed prey items may be energetically demanding, and that Bat Rays *Myliobatis californicus* feed in shallow, warm waters and subsequently move to rest in cooler water, potentially acquiring metabolic or reproductive benefits. Furthermore, Flowers and Kelley (2020) noted that the resting *A. narinari* had sand streaks on its dorsum, which they generate during foraging with sediment jetting through their spiracles, further highlighting the likelihood of this as post-foraging behaviour. Finally, resting behaviour may be a form of predator avoidance, as reported in French Polynesia (C. Berthe, pers. comm.). A decrease in activity can lower the probability of an animal being predated upon (Werner and Anholt, 1993; Gerritsen and Strickler, 1977), by reducing the probability of being detected or encountered (Lima, 1998); by remaining stationary, the ray may be able to exercise vigilance against potential predators by scanning its environment for the presence of predators. This may allow the animal to avoid or delay a flight response, therefore reducing the energetic costs associated with fleeing a predator (Møller and Erritzøe, 2014), albeit *A. ocellatus* have a dark dorsal colouration and tend to forage in sandy substrates. Given the high costs of escape behaviour, an alternative theory is that these observations may represent resting behaviour following an escape from a predator (Heithaus and Vaudo, 2012). Further research is needed, however, to elucidate the energetics of predator-prey interactions involving *A. ocellatus*, as suggested by Ajemian et al. (2012). Although no predators (e.g. large sharks such as hammerhead sharks) were observed in the cases presented here, their presence in these locations is not uncommon (MacNeil et al., 2020). It should be noted that humans were in-water in the vicinity of the rays during the behaviours reported herein, and short-term behavioural responses of elasmobranchs to humans have been documented (e.g. whale sharks, Araujo et al., 2017; reef sharks, Bradley et al., 2017; manta rays, Murray et al., 2020), as it has for *A. ocellatus* (e.g. Berthe and Lecchini, 2016).

In the Galápagos, Pacific eagle rays (*Aetobatus laticeps*) have also been observed resting with no obvious indication of the drivers (D. Pazmiño, pers. comm.). Our observations corroborate that observed for *A. narinari* in Belize (Flowers and Kelley, 2020) and indicate a possible association with cleaning behaviour. To confirm the drivers of these behaviours, future research needs to move beyond opportunistic observations. One tractable avenue could be to combine the use of accelerometer tags with animal-borne cameras (e.g. Huveneers et al., 2018) to help elucidate the drivers of eagle ray behaviour. For example, a complete decline in overall dynamic body acceleration (ODBA) following a rapid increase could indicate a predator avoidance, or a routine ODBA followed by a complete stop could indicate a resting period following an extended period of foraging. The camera footage could then ground truth the drivers of these behaviours for *A. ocellatus*, as has been performed on manta rays (Stewart et al., 2019).

Our observations of resting *A. ocellatus* also show cleaning behaviour on four occasions – likely five if counting the Maldives report – both from LEI, Mooloolaba and Japan. It remains unclear whether the cleaning was opportunistic, or whether the resting in association with cleaning is common behaviour. Cleaner-client associations have been documented for various species elasmobranchs, like the reef manta ray *Mobula alfredi* Krefft 1868 (Kashiwagi et al., 2011) and the pelagic thresher shark *Alopias pelagicus* (Nakamura, 1935; Oliver et al., 2011). In the Indo-West Pacific the moon wrasse *T. lunare* and blue-streaked cleaner wrasse *L. dimidiatus* are often the main cleaner species (e.g. Araujo et al., 2020b), though other species like the schooling bannerfish *H. diphreutes* are also common. Their ecological role is important for healthy communities as they remove ectoparasites, dead tissue or mucus (Côté, 2000). The relationship between *A. ocellatus* and cleaning stations has not been thoroughly assessed, yet our results herein highlight the species' association with healthy reef communities, and habitat use of reef ecosystems beyond the simple need to forage. Further research is necessary to determine their fidelity to specific cleaning stations, like that observed in reef manta rays for example (Venables et al., 2020), and whether resting behaviour is indeed closely associated with cleaning. The cleaning and resting behaviour reported from Mooloolaba, Australia, noted no current present during the encounter, which raises the question whether resting behaviour during cleaning interactions could be attributable to a lack of current (NM, pers. obs.).

The observations reported here highlight the value of citizen science for documenting elasmobranch behaviour in the wild. Although further research is required to fully understand the drivers of resting behaviour in *A. ocellatus*, we can continue to engage members of the general public to contribute rare and interesting observations that shed light on the behavioural ecology of elusive elasmobranchs.

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

Ethical approval was not required for this study involving animals in accordance with the local legislation and institutional requirements. No animal was restrained for this study and observations were non-invasive and opportunistic in nature, made by citizen scientists.

Author contributions

GA: Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation. RB: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. JS: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. CG: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. NM: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. DW: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. JR: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. AJ: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. KS: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. EL: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

Author NM is the owner of Nigel Marsh Photography. Author DW was employed by company Ningaloo Discovery. Author JR was employed by company Sail Ningaloo. Author AJ is the owner of OceanAimee Photography. Author KS was employed by the company Heppoko Taylor Inc.

The remaining 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.2024.1406803/full#supplementary-material

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Temporal niche partitioning as a potential mechanism for coexistence in two sympatric mesopredator sharks

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Global marine biodiversity declines require bold and ambitious plans to safeguard ecosystem services. Overfishing, habitat loss and projected climate impacts have yielded deleterious effects on marine predators, in particular, driving increasing threat of extinction for many shark species with implications for ecosystem health. Identifying and protecting critical habitat areas for sharks is fundamental to their protection, and may allow for species recovery. Here we use baited remote underwater video stations to investigate spatial and temporal patterns of habitat use by sharks in a Special Area of Conservation (SAC) that is centrally important to the regional blue economy in the UK, the Cardigan Bay SAC. Our results show heterogeneous spatial habitat use and temporal trends in habitat sharing between smallspotted catsharks *Scyliorhinus canicula* and nursehounds *S. stellaris*. Nearshore reef habitats are substantially more important than non-reef habitats for both species. The relative abundance of each species, however, is roughly inversely seasonally proportional, with *S. canicula* and *S. stellaris* relative abundance highest in March and September, respectively. Temporal niche partitioning may be an important mechanism in marine systems, but has not been widely investigated in sympatric shark communities. These findings are directly relevant for ongoing development of the Welsh Marine Evidence Strategy (2019-2025) and the Welsh National Marine Plan, particularly to inform spatial planning to strengthen the role of SAC management design in protecting important shark areas.

KEYWORDS

Cardigan bay, elasmobranch, marine protected area, marine spatial planning, Northeast Atlantic, Scyliorhinidae

Introduction

Marine predatory fish biomass has decreased dramatically since the onset of industrialised fishing (Myers and Worm, 2003, 2005) and global elasmobranch (shark and ray) populations are in long-term decline (Myers and Worm, 2003, 2005; Dulvy et al., 2014; MacNeil et al., 2020; Dulvy et al., 2021; Pacoureau et al., 2021; Simpfendorfer et al., 2023). The role of sharks in structuring marine communities (Ferretti et al., 2010; Heupel et al., 2014), and thus the potential for wider ecosystem impacts linked to ongoing depletion (Heithaus et al., 2008; McCauley et al., 2012), have prompted calls for conservation action (Chapman and Frisk, 2013). Popular approaches include implementing marine reserves or shark sanctuaries (Worm et al., 2013; Chapman et al., 2021), whereby spatial protection may facilitate population recovery (Speed et al., 2018). Successful outcomes, however, are linked both to anthropogenic influence (Kaplan et al., 2015; Juhel et al., 2018; Letessier et al., 2019; MacNeil et al., 2020) and ecological factors including species residency and movement ecology (Dwyer et al., 2020). A panacea for management would extend benefits to multiple species, but this highlights a prohibitive knowledge gap: the long-term mechanisms of shark coexistence (Heupel et al., 2019). Niche partitioning can facilitate species coexistence through reduced competition for resources (Chesson, 2000; Albrecht and Gotelli, 2001; Bethea et al., 2004; Heupel et al., 2018). There is evidence for this in sharks, particularly in the context of trophic (Curnick et al., 2019; Galindo et al., 2021; Chandelier et al., 2023; Weideli et al., 2023), and spatial partitioning (Gallagher et al., 2017; Matich et al., 2017; Afonso et al., 2022). Temporal niche partitioning, on the other hand, may be an important ecosystem process but has received little research attention (Lear et al., 2021) - it is unclear even how widespread it is amongst marine apex and mesopredators (Bass et al., 2021). Sharks are a diverse taxonomic group, in which ecological interactions between the same sympatric species can differ between locations (Shiffman et al., 2019). Hence, elucidating processes that structure shark communities should be a priority, to both better predict and mitigate wider effects of ecosystem decline, and address specific knowledge gaps facing regional management.

Shark research priorities include population status, movement ecology and critical habitat areas (Jorgensen et al., 2022). Attention is skewed towards large, charismatic, high conservation value and/or heavily depleted species (Shiffman et al., 2020), while temperate species are relatively under-studied (Jorgensen et al., 2022). The smallspotted catshark *Scyliorhinus canicula* is a widespread Northeast Atlantic mesopredator (trophic level 3.8). It is listed as 'Least Concern' by the International Union for Conservation of Nature (IUCN) Red List, with a stable population (Finucci et al., 2021a). Nevertheless, it should be considered for species-level management attention given food web centrality (Wieczorek et al., 2018), genetically distinct populations within the UK (Gubili et al., 2014), and mesopredator linkage between lower and upper trophic levels (Vaudo and Heithaus, 2011). Furthermore, given widespread depletion of marine predatory biomass (Myers and Worm, 2003, 2005), the relative trophic position of mesopredators in exploited systems is likely higher than in unexploited systems (Prugh et al., 2009; Ritchie and Johnson, 2009). This could elevate the importance of species such as *S. canicula* in structuring marine communities. In contrast with *S. canicula*, the nursehound *S. stellaris* is listed as 'Vulnerable' by the IUCN Red List (Finucci et al., 2021b), yet relatively under-researched (Supplementary Table S1). Studies have identified refuging behaviour in both sheltered and exposed coastal environments, with some philopatry (Sims et al., 2005), and it is prominent bycatch in Celtic Sea, Irish Sea, and Bristol Channel lobster and crab pot fisheries (Moore et al., 2023). Nevertheless, ecological evidence paucity is concerning given trophic level (4.0), sports fishing blue economy importance (Tim Harrison, personal comms.), and widespread availability in fishmongers and food takeaway establishments (Hobbs et al., 2019).

International programmes such as the Decade of Ocean Science for Sustainable Development (Ryabinin et al., 2019), the European Union (EU) Biodiversity Strategy 2030 (European Commission: Directorate-General for Environment, (2021)), and the EU Green Deal (European Commission: Directorate-General for Research and Innovation, (2021)) present opportunities for ambitious marine spatial planning targets. These should seek to strengthen species and habitat protection, and incorporate restoration and recovery objectives (Santos et al., 2019; Harris et al., 2022; Holness et al., 2022; Manea et al., 2023). In Wales, UK, a network of Special Areas of Conservation (SAC) has general objectives to safeguard species and habitats under the Habitats Directive (SACs; Habitats Directive, 1992). Additional regional biodiversity action plans include the Welsh Marine Evidence Strategy (2019-2025) and the Welsh National Marine Plan. Given that the most vulnerable species to extinction in continental shelf seas are long-lived and occupy upper trophic levels (Coulon et al., 2023), meeting these targets requires regional level species ecology knowledge. As such, there is currently an important and timely opportunity to contribute research-driven locally relevant ecological evidence to further strengthen national SAC management objectives in line with international programmes.

Baited remote underwater video stations (BRUVS) are commonly used to study elasmobranch ecology including distribution (White et al., 2013) and population trends (Flowers et al., 2022), behaviour (Sherman et al., 2020; Lester et al., 2022) and trophic interactions (Bond et al., 2019), anthropogenic influence (Clementi et al., 2021; Letessier et al., 2024), and diversity (Simpfendorfer et al., 2023) and abundance patterns (Bond et al., 2012; MacNeil et al., 2020; Cook et al., 2024). Here we undertook, to our knowledge, the first standardised BRUVS sampling of the shark community assemblage in Cardigan Bay SAC, Wales, UK. Specific objectives were to: 1) establish a baseline of shark species present; 2) elucidate spatio-temporal variations in relative abundance of shark species observed; and, 3) investigate mechanisms of species co-existence in the context of regional spatiotemporal habitat use by sharks.

Materials and methods

The Cardigan Bay SAC, west Wales, covers approximately 1000 km^2, extending from Ceibwr Bay in Pembrokeshire to Aberarth in Ceredigion (Figure 1). The SAC status is based on seven qualifying

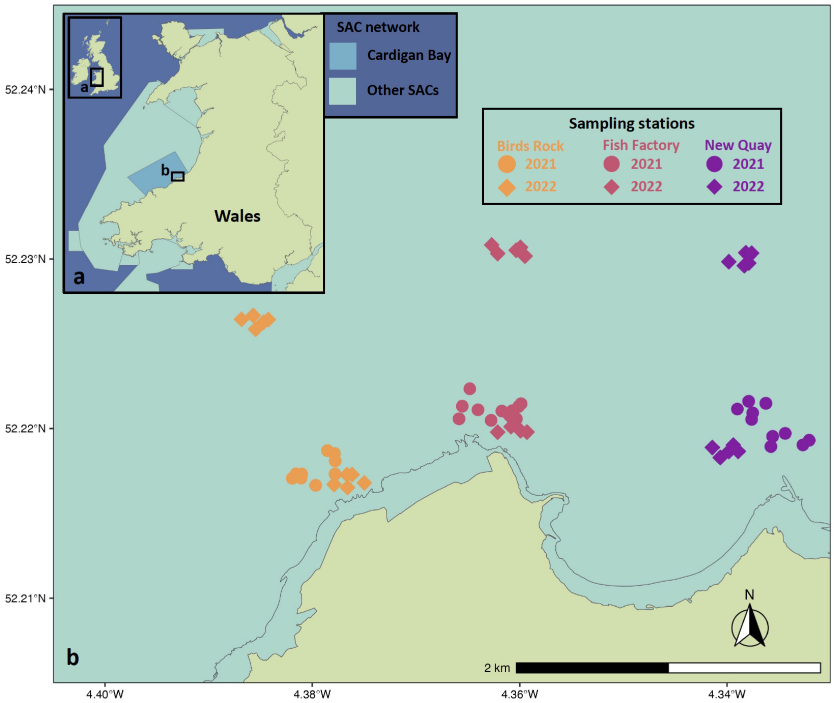

FIGURE 1
Wales, UK **(A)** location of Special Areas of Conservation (SAC), including Cardigan Bay SAC. **(B)** Cardigan Bay SAC baited remote underwater video stations sampling sites (Birds Rock, Fish Factory, New Quay) with stations therein, including spatially discrete offshore and nearshore stations sites sampled in May, July and September 2021, and March, May, July and September 2022.

features under Annex I and II of the Habitats Directive, including river lamprey *Lampetra fluviatilis*, semi-resident bottlenose dolphins *Tursiops truncatus*, and submerged or partially submerged sea caves. The SAC is important for the regional blue economy, particularly wildlife watching tours. Sports fishing charters seasonally catch and release sharks including *Scyliorhinus stellaris* and tope *Galeorhinus galeus*, while *S. canicula* can be bycatch in small scale local fisheries (Tim Harrison, personal comms.).

Three sampling sites were identified to represent different habitat types at comparable distances from the closest human population centre (New Quay; 52.21515, -4.35887), all frequently visited by wildlife watching boat trips (Sarah Perry, personal comms.). All sampling areas were approximately 1 km^2, within which GPS coordinates for sampling points (hereafter, stations) were randomly generated using the Cruise tool in Google Earth. Ten stations were generated per sampling site for 2021. For 2022, an additional three offshore sampling sites were added, both to increase spatial coverage of the study and to explore spatial connectivity between nearshore sites and wider Cardigan Bay. Five stations were randomly generated per sampling site for 2022 (Figure 1; Supplementary Table S2). Thus, each repeat sampling effort aimed to cover a total of 30 stations across 5 days, although this was not always possible due to weather conditions. Sampling was conducted in May (12th – 16th), July (5th – 9th) and September (22nd – 25th) 2021, and March (28th – 29th), May (23rd), July (11th – 16th), and September (12th – 13th) 2022.

The BRUVS setup comprised an aluminium frame (approximately 1 m x 0.6 m x 0.6 m) with a 1 m bait arm in front of the camera position and supporting a wire mesh cage in the middle of the field of view. A GoPro Hero7 Black camera set to 1440 video format at 30 frames per second was mounted in a GoPro SuperSuit housing (60 m depth rated), approximately 40 cm from the base. The mesh cage was baited with approximately 1 kg of chopped oily fish, scad *Trachurus trachurus* or herring *Clupea harengus*, to encourage bait plume dispersal (Dorman et al., 2012; Whitmarsh et al., 2017). A rope with sufficient scope to avoid toppling the BRUVS was attached to a surface buoy to mark location (Murray et al., 2019).

BRUVS were deployed between 09h00 and 17h00 to ensure sufficient light for video recording (Jones et al., 2021) and were left to record for a minimum of 60 minutes before retrieval. This both aligns with recommended protocol (Langlois et al., 2020) and is the minimum time required to sample 66% of fish species including several shark species in nearshore North Atlantic waters (Unsworth et al., 2014), and therefore conducive to meeting our research objectives. Up to 11 stations were sampled per day, and BRUVS deployed simultaneously were separated by at least 1 km to ensure independent sampling (Harvey et al., 2019). A Garmin GPSMAP 86s handheld GPS was used to navigate to each station. Depth was either estimated using a rope marked at 50 cm intervals and weighted with a dive weight (2021), or measured using a Platimo Echotest II handheld depth sounder (2022). Additional data were recorded at both drop and haul time: Beaufort wind force scale as a measure of sea state and percentage cloud cover.

Data recording began when the BRUVS settled on the substrate. Video quality was assessed using standardised criteria (Jones et al., 2021), with four rankings from excellent to unusable

TABLE 1 Baited remote underwater video stations set in the Cardigan Bay Special Area of Conservation per year, site and habitat, and the number of usable sets according to standardised criteria (Jones et al., 2021).

Sampling year	Sampling month	Deployments	Usable datasets
2021	May	30	29
	July	30	30
	September	23	22
2022	March	17	8
	May	9	2
	July	30	28
	September	13	2
Sampling site	**Year/location**		
Birds Rock	2021 nearshore	27	25
	2022 offshore	10	5
	2022 nearshore	7	5
Fish Factory	2021 nearshore	28	28
	2022 offshore	10	7
	2022 nearshore	13	6
New Quay	2021 nearshore	28	28
	2022 offshore	15	9
	2022 nearshore	14	8

(Supplementary Figure S1). This excluded a similar proportion of datasets from each site from analysis (Supplementary Figure S1), yielding a total of 121 video datasets for full annotation (Table 1; Supplementary Table S2). For those retained for full annotation, sampling effort was standardised with two independent observers watching each video file to 60 minutes using QuickTime Player software. One observer watched real-time playback to identify which stations yielded a shark observation. These observations were cross-referenced by a senior annotator watching at double playback speed, who also recorded species and the time of first arrival for each species of shark observed for each BRUVS. Next, we recorded MaxN, defined as the maximum number of individuals per species in the field of view at any given point in time throughout a BRUVS set. This prevents double counting individuals (Willis et al., 2000).

Data were analysed using R software (v4.2.2; R Core Team, 2022). We first calculated mean MaxN ± standard error (SE) as the metric for relative abundance to standardise sampling effort among study sites (Goetze et al., 2018). We then excluded *Mustelus* sp. from further analyses given only a single observation of this taxon. Given non-normal data distribution, we used generalised linear models to investigate the association between sampling year and month (temporal explanatory variables), site and nearshore/offshore location (spatial explanatory variables), depth and image quality (environmental explanatory variables), MaxN of other shark species observed (proxy for partitioning), and the relative abundance of each species (MaxN; response variable). We examined error structure using the R package 'DHARMa' (Hartig, 2020) to check residual diagnostics for all response variables, selecting a negative binomial structure for both *S. canicula* and *S. stellaris* (Supplementary Table S3). We then used the 'dredge' function in the R package 'MuMIn' (Barton, 2020) to identify all possible variable combinations, followed by an information theoretic approach (Akaike's information criteria, AIC; Akaike, 1998) to identify the best predictive model ($\Delta AIC = 0$; Supplementary Table S4).

Results

We recorded sharks on 43 of 152 of BRUVS deployed in the Cardigan Bay SAC, Wales (28%; Supplementary Figure S2). Total species richness was three: smallspotted catshark *Scyliorhinus canicula* (Figure 2), nursehound *S. stellaris* (Figure 2), and an individual smooth hound shark *Mustelus* sp. of unknown species. The most frequently sighted species, *S. canicula*, was recorded on 19% of BRUVS, compared with 16% for *S. stellaris* (Supplementary Figure S2). Furthermore, of those BRUVS that yielded sightings, 75% recorded both *S. canicula* and *S. stellaris* within the first 40 minutes of recording (Supplementary Figure S3), justifying our standardised 60 minutes sampling effort.

Mean MaxN of *S. canicula* varied between 0.78 ± 0.17 at the offshore New Quay sites, and 0 ± 0 at the nearshore Fish Factory sites (Figure 3), while mean MaxN of *S. stellaris* varied between 0.97 ± 0.16 at the nearshore New Quay sites and 0 ± 0 at both the Birds Rock and Fish Factory offshore sites (Figure 3). The results of our GLM analysis ($p < 0.0001$; Supplementary Table S5) support this finding that the New Quay sites are more important than either of the other sites for both species, although particularly for *S. stellaris* and at the nearshore rather than offshore New Quay sites (Table 2).

Regarding temporal variation, sampling month was a factor in the second top and the top model predicting MaxN of *S. canicula* and *S. stellaris*, respectively (Supplementary Table S4), with GLM analysis revealing an association between sampling month and *S. stellaris* MaxN ($p < 0.01$; Table 2; Supplementary Table S5). Temporal patterns of mean MaxN throughout the year at the New Quay sites were approximately inversely proportional for each species (Figure 4); relative abundance of *S. canicula* was highest in March (1.0 ± 0) and lowest in July (0.37 ± 0.14), while for *S. stellaris* this was highest in September (1.7 ± 0.3) and lowest in March (0.25 ± 0.25).

Discussion

Elucidating mechanisms that structure marine communities is fundamental for management, not least to predict and mitigate negative global change impacts. Sharks are imperiled predators, and long-term research bias resulting in ecological evidence paucity for less charismatic species is a concern given increased mesopredator importance in depleted systems (Prugh et al., 2009; Ritchie and

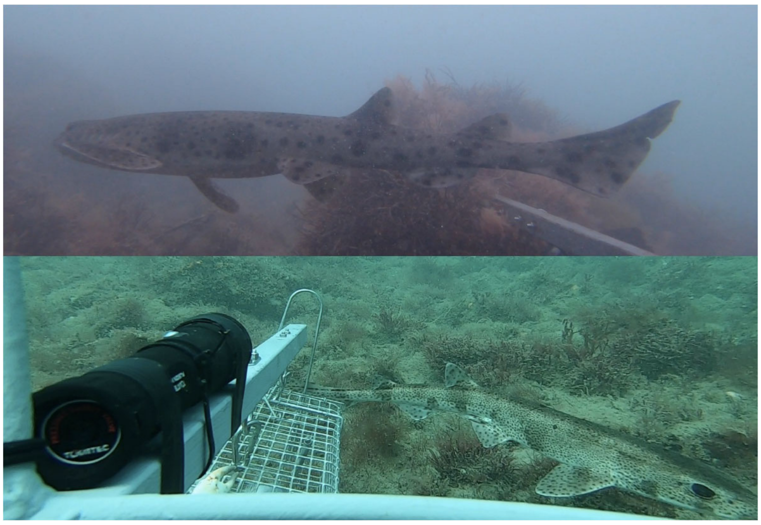

FIGURE 2
A smallspotted catshark *Scyliorhinus canicula* (below), and a nursehound *S. stellaris* (above), observed during baited remote underwater video stations sampling in the Cardigan Bay Special Area of Conservation, UK. Note *S. stellaris* second dorsal fin origin well ahead of anal fin insertion, anal fin base equal or greater than interdorsal space, and longer than anal-caudal fin space, blotches on flank.

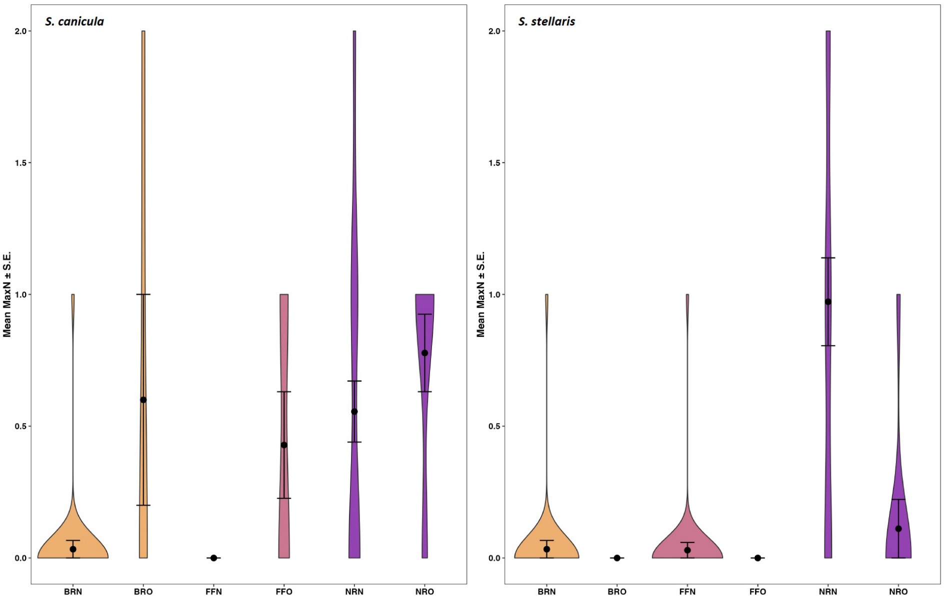

FIGURE 3
Mean MaxN (± S.E.) of smallspotted catsharks, *Scyliorhinus canicula* (left), and nursehounds, *Scyliorhinus stellaris* (right) recorded at baited remote underwater video stations sampling sites in the Cardigan Bay Special Area of Conservation, UK. Site codes: BR, Birds Rock; FF, Fish Factory; NR, New Quay; "N" suffix, Nearshore; "O" suffix, Offshore.

TABLE 2 Model-averaged coefficients from top models predicting the association between environmental variables and MaxN observations of smallspotted catsharks *Scyliorhinus canicula* and nursehounds *S. stellaris* recorded at baited remote underwater video stations sampling sites in the Cardigan Bay Special Area of Conservation, UK.

	Estimate	Std. Error	z value	Pr(>|z|)
Response = *S. canicula*				
Intercept	-3.27	0.67	-4.94	7.7 x 10^{-7}
Image rank 3	0.21	0.42	0.51	0.61
Image rank 4	1.25	0.56	2.22	0.03
Site New Quay	1.65	0.64	2.58	0.01
Site Birds Rock	0.55	0.77	0.72	0.47
Offshore	1.59	0.46	3.5	0.0004
S. stellaris maxN	0.36	0.21	1.7	0.09
Response = *S. stellaris*				
Intercept	-3.98	1.03	-3.85	0.0001
Site New Quay	3.47	1.01	3.42	0.0006
Site Birds Rock	0.18	1.41	0.13	0.9
Month March	0.08	1.09	0.07	0.94
Month May	0.19	0.47	0.42	0.68
Month September	1.11	0.39	2.82	0.005
Offshore	-1.79	1.08	-1.67	0.09

Johnson, 2009). Niche partitioning is an ecological process that may facilitate sympatry, yet remains a relative knowledge gap. Moreover, temporal niche partitioning may be an important driver but is less studied than either trophic or spatial partitioning. In this context, we use standardised BRUVS sampling to obtain novel insights into temporal niche partitioning amongst two sympatric mesopredator sharks. We identify approximately inversely proportional seasonal nearshore habitat use in a spatially discrete area within our study site, adding to an evidence base of ecologically similar marine predators that share space. These behaviours may facilitate long-term reduction of direct interactions and competition for resources, and our findings both suggest important habitat areas for multiple species and highlight opportunities for further research.

BRUVS are widely applied to investigate elasmobranch ecology but rely on individuals approaching sufficiently close to the camera to be identified, with inherent potential for species present to not be recorded. This may be the case with our dataset; species including tope *Galeorhinus galeus* and smoothhound *Mustelus* spp. are caught by recreational fishers within our study sites, but we observed only a single smoothhound. This is a shortcoming in our protocol and precludes establishing a baseline of species present in Cardigan Bay SAC, but also highlights an opportunity for alternative approaches to address this remaining knowledge gap, such as environmental DNA. Nevertheless, we have obtained evidence for spatial use by *S. canicula* and *S. stellaris*, two important mesopredators in nearshore Northeast Atlantic marine environments, with comparable trophic levels (3.8 ± 0.3 SE and 4.0 ± 0.3 SE, respectively; Froese and Pauly, 2000). This is notable given that *S. stellaris* distribution estimates have, to date,

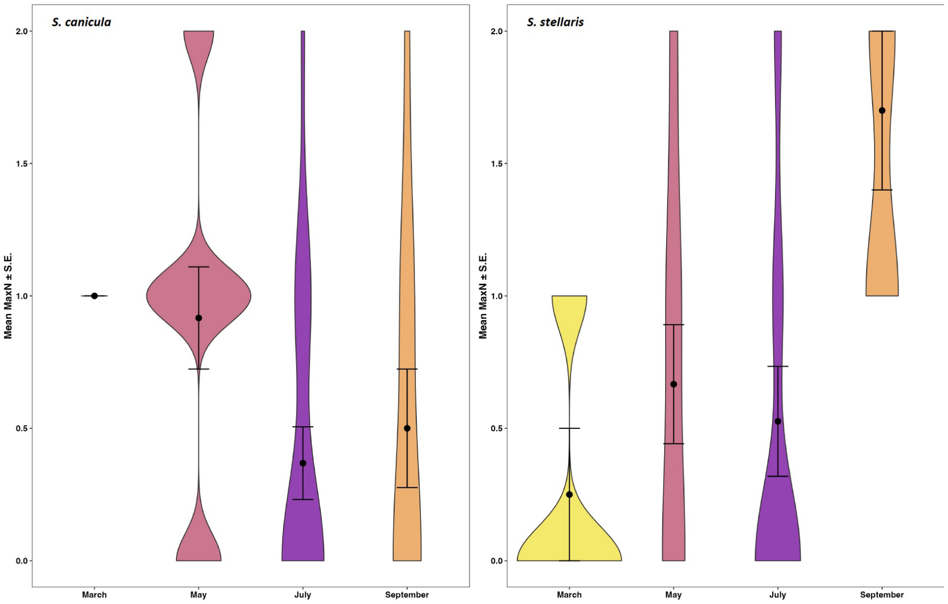

FIGURE 4
Temporal variation in mean MaxN (± S.E.) of smallspotted catsharks *Scyliorhinus canicula* (left) and nursehounds *S. stellaris* (right) recorded at baited remote underwater video stations within the New Quay sampling sites in the Cardigan Bay Special Area of Conservation, UK.

predominantly relied on invasive trawl or fisheries-dependent sampling (Martin et al., 2010; Moore et al., 2023) rather than *in situ* sampling. *S. stellaris* have also been under-reported compared to *S. canicula* for BRUVS set in the UK (Jones et al., 2021), and may be at risk of local extinction in parts of their distribution (Ragonese et al., 2013).

Our results suggest that the New Quay nearshore and offshore sites are substantially more important for both study species and should therefore be a priority for regional management, given both a higher observation frequency and higher relative abundance than the other study sites. Additionally, both *S. stellaris* and *S. canicula* were recorded during every sampling month and both sampling years at these sites. Furthermore, spatial partitioning and temporal abundance variations amongst *S. canicula* and *S. stellaris* may constitute temporal niche partitioning in these sites as distinct from the other study sites, although the influence of factors such as prey availability, reproduction or parturition remains unclear. For example, differential *S. canicula* relative abundance between March and May could reflect dispersal behaviours that drive ontogenetic and sexual segregation patterns (Cook, 2023). Notably, however, the highest seasonal relative abundance recorded for *S. stellaris* (September) correlates with bycatch peaks in Welsh lobster and crab pot fisheries (Moore et al., 2023). Resource partitioning, whether for space, time, or diet, facilitates the co-existence of sympatric species (Bethea et al., 2004; Page et al., 2005; Dale et al., 2011; Fossette et al., 2017; Weideli et al., 2023), and may be a key mechanistic driver of marine community composition (Kinney et al., 2011; Gavrilchuk et al., 2014). Trophic partitioning in sharks has been extensively studied through dietary analyses (Papastamatiou et al., 2006; Sommerville et al., 2011; Vaudo and Heithaus, 2011; Heithaus et al., 2013; Tilley et al., 2013) and, despite similar trophic levels, has been observed between *S. canicula* and *S. stellaris* (see Yemisken et al., 2019). Temporal niche partitioning is less well documented, although distinct diel activity patterns within coastal shark assemblages may facilitate temporal partitioning of foraging times by driving less dominant predators to suboptimal foraging times (Lear et al., 2021). Importantly, our BRUVS sampling was only conducted during daylight hours, therefore it was not possible to investigate temporal niche partitioning in the context of either diel or nocturnal behaviours. Although weather conditions prevented sampling from October 2021 to March 2022, we would expect continued correlation between abundance patterns and regional pot fisheries bycatch (see Moore et al., 2023). Should it remain infeasible to test this in the future using BRUVS (with either or both seasonal and nocturnal sampling using lights), acoustic telemetry could elucidate the period of partitioning behaviour, whether on a short cycle (days) or a long cycle (months/seasons).

Spatial distribution away from core habitat areas (here, New Quay nearshore) is important to determine spatial connectivity and migration corridors. Here, *S. stellaris* was only detected in the New Quay sites, while *S. canicula* was detected in both the New Quay sites as well as the offshore sites of both Birds Rock and Fish Factory. The sample size is small but suggests differential use of areas around the core habitat area that may reflect differential movement ecology, and should be further investigated. *S. canicula* show a strong tendency to repeatedly use the same entry and exit areas between a marine protected area and surrounding waters in Northwest Spain (Papadopoulo et al., 2023). Regional management requires the identification of such priority areas for protection.

Our results suggest habitats under Cardigan Bay SAC designation are potentially important areas for both *S. canicula* and *S. stellaris*. These findings are a step towards the ecological evidence base needed for regional management to confer conservation benefits on local shark populations. Standardised BRUVS sampling yields comparable datasets and conservation insights on unprecedented scales (MacNeil et al., 2020; Simpfendorfer et al., 2023). Therefore we strongly recommend engagement with other researchers to align continued BRUVS monitoring of Cardigan Bay with refined objectives and standardised protocols. For example, better speciation of visually similar species (such as *S. canicula* and *S. stellaris*, and *G. galeus* and *M. mustelus*) arising from small camera advancement encourages the inclusion of reference images in BRUVS studies. Individual identification of sharks enables novel insights into social network structure (Mourier et al., 2012; Jacoby et al., 2021; Mourier and Planes, 2021), and has been achieved with *S. canicula* in a laboratory setting (Hook et al., 2019). Verifying *in situ* individual identification of *S. canicula* and *S. stellaris* on BRUVS could strengthen population estimates and present a more affordable and non-invasive alternative to traditional tagging studies, yet yield similar insights (Flowers et al., 2017; Cerutti-Pereyra et al., 2018). Stereo-BRUVS analysis that yields size estimates could be used to determine age classes and possible ecological drivers of habitat use, such as reproduction or predator avoidance. For example, nursery areas are used by several shark species (Zanella et al., 2019; TinHan et al., 2020; LaFreniere et al., 2023), including *S. canicula* (see Cau et al., 2013, 2017). These are areas where neonate or young-of-year sharks are more commonly encountered than in other areas, have a tendency to remain or return for extended periods, and use the area repeatedly across years (Heupel et al., 2007), and are thus candidates for protection to safeguard critical life processes such as parturition and maturation.

A paucity of mesopredator ecological evidence is prohibitive for effective management design, and should not be disregarded given the ecological importance of these species in depleted systems. Sharks including *S. stellaris* and *S. canicula* should be more prominent within SAC research and management programmes, particularly in the context of identifying regionally important shark areas such as nursery habitats and migration corridors. These objectives are all relevant for conservation planning, not least the Welsh Marine Evidence Strategy (2019-2025) and Welsh National Marine Plan, but also towards the objectives of the international Decade of Ocean Science for Sustainable Development (Ryabinin et al., 2019), and could thus strengthen the evidence base for protection measures to promote regional shark recovery.

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 approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because sampling was conducted using non-invasive camera technology.

Author contributions

NC: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. AJ: Data curation, Investigation, Writing – review & editing. SLP: Data curation, Investigation, Supervision, Writing – review & editing. SEP: Conceptualization, Supervision, Writing – review & editing. JC: Conceptualization, Supervision, Writing – review & editing.

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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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2024.1443357/full#supplementary-material

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On the tracks of white sharks in the Mediterranean Sea

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White sharks (*Carcharodon carcharias*) are among the most widespread, charismatic, and studied predators in the ocean. However, their conservation status is concerning in many ocean sectors, most notably the Mediterranean Sea, which hosts one of the least known and most endangered populations globally. Though they were historically abundant and widely distributed in the region, Mediterranean white sharks have declined to dangerously low abundance levels, impacted by centuries of coastal and, more recently, industrial fishing. The IUCN lists this species as Critically Endangered in the region, but information about its current abundance and ecology is scarce, hindering effective management and conservation. Here, we describe our initial effort to find and track the remaining Mediterranean white sharks and report what we have learned from these activities. In 2021-2023, we conducted three pilot expeditions in the Sicilian Channel, covering four major sites, collecting 159 eDNA samples, and carrying out 359 hrs of pelagic mid-water baited video surveys, 43 hours of deep-water benthic baited video surveys, and 111 hrs of fishing. Baited video surveys detected 42 species of bony fishes, elasmobranchs, marine mammals, and turtles. We detected white sharks at four sites from eDNA samples. Though we did not observe white sharks directly, these activities supported the identification of one of the last strongholds of this population in the region and started a multi-institutional white shark conservation program in the Mediterranean Sea, aiming to track the last white sharks in the region, estimate their abundance and extinction risk, characterize the species' ecology and inform management and conservation.

KEYWORDS

white shark, BRUV, eDNA, field expedition, distribution models, elusive species, fishing impact, extinction risk

1 Introduction

White sharks (*Carcharodon carcharias*) have been sporadically but regularly detected in the Mediterranean Sea throughout history (Fergusson, 1996; Gubili et al., 2011; Boldrocchi et al. 2017; Tiralongo et al., 2020). They are part of a genetically distinct population for which we know very little about their ecology and biology. Recent analyses of incidental catches and sightings indicate these sharks are some of the largest on the planet, reaching sizes up to 7 meters (Moro et al., 2020; De Maddalena and Heim, 2012); however, these data also suggest that this population is one of the most endangered globally. The IUCN has classified the Mediterranean white shark as Critically Endangered (Dulvy et al., 2016). Historically abundant and widely distributed, Mediterranean white sharks have declined to dangerously low abundance levels in the region, impacted by centuries of coastal and, more recently, industrial fishing (Moro et al., 2020). Despite the pressing need to effectively protect these species, we know very little about them outside of incidental and fisheries interactions, inhibiting their effective management and conservation. Therefore, it is now crucial to facilitate more research and exploration to advance knowledge and conservation of this population. Specifically, we need to increase our understanding of the Mediterranean white sharks' population size and structure, as well as their distribution, migration patterns, and potential connectivity with the Atlantic populations. This information is crucial to plan effective conservation and recovery programs.

White sharks are present in the Mediterranean Sea, but the very low population density and the absence of conventional aggregation sites, such as pinniped colonies (Klimley and Anderson, 1996), have limited monitoring and exploration of this population and consequently hindered research and conservation. Previous monitoring studies that attempted to deploy electronic tags on Mediterranean white sharks were hampered by limited information on the species' seasonality and distribution and were not able to find live individuals (Soldo and Pierce, 2005; Micarelli et al., 2023). The limited Mediterranean white shark samples available in zoological and osteological museum collections have been leveraged in genetic and isotopic analyses to characterize the population's phylogenetics, biogeography, and connectivity and reconstruct important ecological and evolutionary aspects of this population (Leone et al., 2020; Gubili et al., 2011; Wagner et al., 2024). However, while these valuable efforts continue, detecting individuals in the wild remains a core challenge.

Given the importance of interacting with live animals in the Mediterranean to characterize the ecology of the species there and worldwide, and the established challenge this presents, we recognized the need to develop a broader, more holistic suite of approaches to interact with these animals in the wild. Here, we report on the planning and execution of three multifaceted research expeditions in the Mediterranean Sea in search of the white shark.

2 Methods

Expeditions were prepared throughout the year, and involved securing funds for field operations, developing occurrence models for predicting shark occurrence, and establishing partnerships with local researchers and operators able to provide local expertise and infrastructure and facilitate the on-site operations.

2.1 Pre-expedition work

With an integrative and historical approach, we analyzed all opportunistic white shark occurrence records in the Mediterranean Sea, building on the work of Moro et al. (2020), and produced high-resolution species occurrence predictions (HRM) (Jenrette et al., 2023; Panunzi et al., 2024) for the region. These models, Jenrette et al. (2023) for 2021-2022 and Panunzi et al. (2024) for 2023, were used to estimate the relative probability of observing white sharks in different Mediterranean sectors and seasons and informed expedition periods and locations for search and survey operations.

Our spatial analyses, updated with real-time sightings and environmental information, identified the Sicilian Channel as a seasonal white shark occurrence hot spot in the early summer. These findings corroborated anecdotal evidence that this region may be a nursery area for small sharks (Fergusson, 2002; De Maddalena and Heim, 2012), and an important feeding ground for adults following annual spawning aggregations of bluefin tuna (*Thunnus thynnus*) between May and June.

We then developed the on-site capacity and local infrastructure to support expeditions and physically prepared for the field operations (e.g., permit applications, bait acquisition, gear preparation, etc.). In each expedition, we identified a primary port as the base of our onshore operations. In 2021, we based our expedition in Marsala, Sicily, the most important longline fishing port in the Sicilian Channel (Borsellino et al., 2006; Pignalosa et al., 2019) and a major hub for the bluefin tuna fishery (Cermeño et al., 2015). Bluefin tuna are expected to be a major white shark prey source in the Mediterranean (Moro et al., 2020), and, thus, likely attract white sharks together with other shark predators during their spawning migration through the Sicilian channel. In 2022, we focused our operations on Lampedusa, a small island (20 km^2 surface) occupying a strategic position in the middle of the Sicilian Channel. In 2023, we worked out of Monastir (Tunisia), and Lampedusa. Monastir, located across the Sicilian Channel from Lampedusa, is a convenient touristic marina near an important fishing port, Teboulba, in the northern part of the Tunisian plateau.

We began on-the-ground work by first conducting interview surveys throughout the year in the above sectors to collect additional historical data on local white shark catches and sightings, focusing on the main ports of interest in the Sicilian Channel, including ports in Sicily, Lampedusa, Tunisia, and Malta. Specifically, we conducted interview surveys to collect additional historical data on local white shark catches and sightings in the Italian fishing ports of: Sciacca, Mazara del Vallo, Marsala, Trapani, and Favignana in 2021; and Lampedusa in 2022. In 2023, we began an systematic monitoring of the main Tunisian fishing ports (Monastir, Teboulba, Djerba, Zarzis, Kelibia). A detailed description of these surveys and monitoring efforts will be included in a separate contribution. For the scope of our field expeditions, we used the most immediate information about historical interaction hotspots, i.e., sectors fishers indicated as likely to result in white shark encounters, and additional photographs to update our white shark record database feeding the species occurrence models (Panunzi et al., 2024). Finally, local collaborators and our global team visited the primary ports in the weeks leading up to the expedition to further strengthen our partnerships with local fishers and their communities.

2.2 Expedition work

From June 11th through June 23rd, 2021, we spent a total of 10 days sampling. We sampled for five days around the Egadi Islands, primarily Marettimo and Favignana. We then spent one day each at Pantelleria Shoal and "Banco Murena" (Moray Eel Bank), before traveling to Pantelleria and then Lampedusa. We spent three days sampling around Lampedusa, including the area surrounding the island of Lampione, a marine protected area located 13 miles west of the island, within the Isole Pelagie Marine Reserve. During this expedition, we worked from a number of vessels-for-rent, including a 15 m catamaran and two 5 m, 40HP, zodiacs (Figure 1). From June 1st through June 8th, 2022, we focused on the area surrounding Lampedusa, including Lampione and the Secca di Levante banks to the east, executing our work from a 33-foot ARS Mare yacht with 2 inboard 110 HP Caterpillar engines. From May 15th to June 1st, 2023, we spent a total of 18 days, including three overnight longline trips, sampling in the region from a mixture of Tunisian and Italian fishing vessels. We first surveyed the waters off Monastir before moving our base of operations to Lampedusa. We also completed two eDNA transects from Monastir to Lampedusa on May 21st and from Lampedusa to Malta on June 1st. Operations were supported by a 27 m sail yacht 'Blue Titan' which acted as a floating accommodation, cruising vessel, support boat for zodiac operations, and platform to conduct eDNA sampling and analysis. Fishing operations were conducted from two professional longlining fishing boats in Tunisia and Lampedusa. BRUV deployments were carried out from a 7 m, 225HP zodiac rented in Lampedusa.

2.2.1 eDNA

At each survey location, we sampled water to detect white shark DNA and confirm their presence (Supplementary Figure S1). At each station, we collected water from 0-100 m depth. We used filtered mitochondrial DNA, amplified using white shark specific primers (Lafferty et al., 2018), and visualized the target gene via gel electrophoresis (Jenrette et al., 2023). This process, from water collection to visualization of white shark eDNA, could be completed in as little as 4.5 hours. This quasi-real-time knowledge of species presence allowed us to hindcast where sharks likely shed their DNA (Dagestad et al., 2018) and adapt sampling and field operations as we proceeded with the surveys. Later onshore, we performed library preparation and sequencing of positive samples to confirm white shark presence (Johri et al., 2019; Truelove et al., 2019) using the approach detailed in Jenrette et al. (2023).

2.2.2 BRUVs

After DNA sampling, we deployed a line of pelagic-baited remote underwater cameras (Pelagic-BRUVs) to look for the presence of sharks in our sampling sectors. We used a set of 4 BRUVs spaced every 200 meters and deployed at 10 meters depth. Each BRUV unit consisted of a metal frame with one or two GoPro Hero 8 cameras pointed toward a bait canister extending from the

FIGURE 1
(A) Jeremy Jenrette and Brendan Shea deploying a drop camera off Marettimo (left) from a 5 m Zodiac rented in Marsala in 2021; **(B)** 42 foot catamaran chartered from Sailing Sicily in 2021; **(C)** 33-foot ARS Mare yacht used in 2022; **(D)** Blue Titan, sailboat used as a survey support boat; **(E)** 7 m zodiac rented in Lampedusa for BRUV deployments; **(F)** Amir el Bahr, Tunisian fishing vessel used for fishing and tagging operations.

mainframe. In 2021, the BRUV longline had units with a 5° convergence stereo-camera set-up, which were tethered in-line to the research vessel as we drifted, allowing the team to deploy additional chum consistently. In 2022, we used a single camera on each BRUV and fixed the longline using an anchor tethered to the buoy associated with the most up-current BRUV. In 2023, we used a stereo-camera setup again, tethering the longline to a zodiac, with the entire line left to drift while the team on-board chummed continuously. BRUVs recorded videos for 1-5 hours per deployment (Figure 2).

In 2021, we also deployed deep-water BRUVs (or drop cams) to test for the presence of white sharks in deeper waters close to the seafloor. The deep-water system consisted of a vertical frame, 20 kg of weights, floats, and a housed, pressure-rated GoPro camera (to record up to 6 hours in HD) mounted 1 meter above the seafloor. Integrated lights illuminated the seafloor in the area, and a bait bag was attached to the main frame to attract white sharks. The units were recovered via acoustic release. All BRUVs were baited with tuna remains, sardines, or general fish scraps.

BRUV videos were viewed and annotated daily. In addition to being viewed by a team member for annotation, videos were processed with a shark detection software for automatic shark identification and classification (Jenrette et al., 2022) as a cross-check and for testing a novel autonomous BRUV postprocessing system. Videos were played back using VLC media player (Version 3.0.6) or QuickTime Player (Version 10.5). We collected information for any identifiable species observed. Annotation data consisted of the date, position of the camera in the longline, species name, the timestamps of an individual's entry and exit from the camera frame, and MaxN - a relative abundance index equal to the maximum number of conspecifics observed in any single video frame during a deployment (Langlois et al., 2020; Whitmarsh et al., 2017)

2.2.3 Chumming

While BRUVs were deployed, we continuously deployed additional attractants, including blood, oil, and fish pieces (preferentially bluefin tuna), to draw individuals close to the boat and the BRUV longline. In 2021, we almost exclusively chummed with tuna carcasses. In 2022, we used bluefin tuna, bottlenose dolphin, and a range of other pelagic and demersal fish scraps available in the local fish markets. The bottlenose dolphin was sourced from the National Research Council in Mazara del Vallo, Sicily, which systematically recovers and necropsies carcasses stranded in the Sicilian Channel. In 2023, we used a diverse array

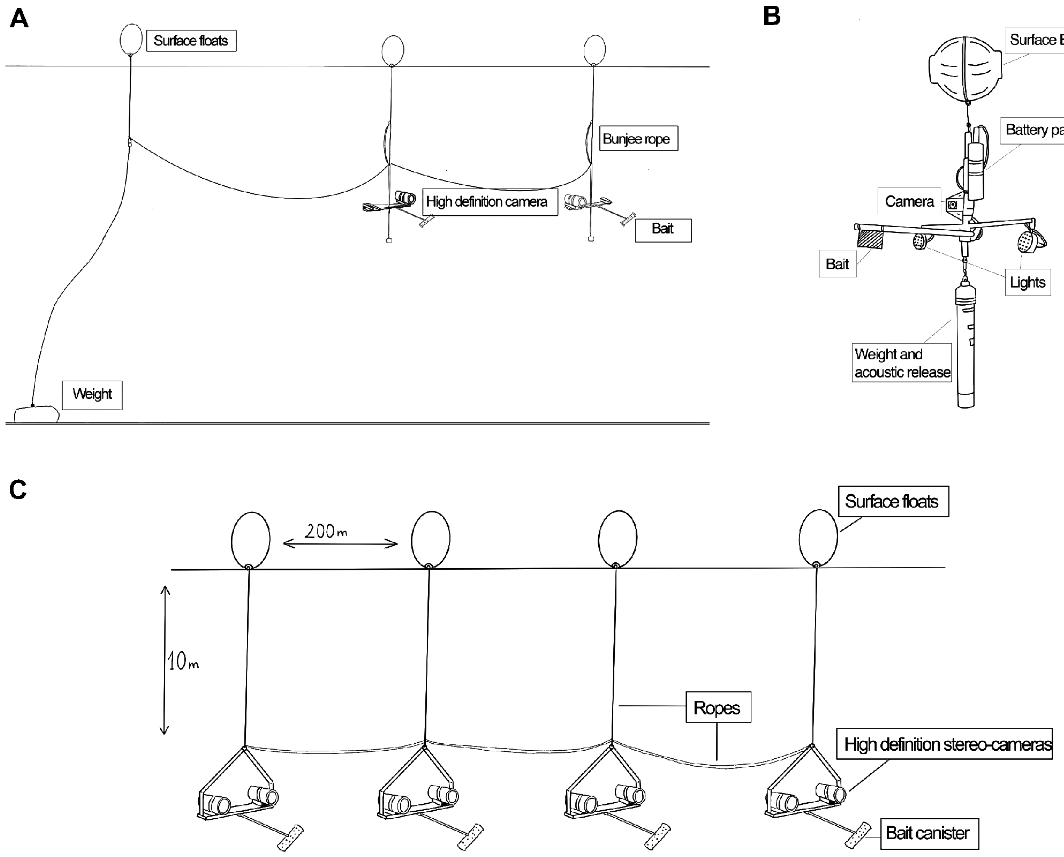

FIGURE 2
Schemes of longline BRUVs and drop-cams used during the 3 expeditions. **(A)** Longline BRUV rigging used in 2022 with mono-camera setup; **(B)** drop-cams used in 2021; and **(C)** Longline BRUV rigging used in 2021 and 2023 with stereo-camera setup.

of species, preferentially scombrids. We generally deployed between 70 and 240 kg of attractant each day.

2.2.4 Fishing

Several different fishing methods were employed over the years based on gear availability and location. Free-drifting longline deployments were deemed hazardous in the surveyed sectors, given the high marine traffic characteristic of the region and the resultant risk of losing gear or damaging vessels. Therefore, in 2021 we used baited handlines terminating at 14/0 circle hooks that were deployed from the research vessel and actively fished by team members. We spent approximately 24 hours fishing in 2021. In 2022, we used stand-up rod-and-reel set-ups, aiming to cover a greater portion of the water column while fishing. For stand-up rod-and-reel fishing, we used Penn 130ST International II reels mounted on curved rod butts, which were attached to ~150 cm rod tips and rigged with 130–250 lb test monofilament fishing line and a leader consisting of a 152-cm long, 480 lb test braided stainless steel wire terminating in either a 16/0, 18/0, or 20/0 circle hook. We fished for approximately 35 hours in 2022. In 2023, we worked with commercial longline vessels to deploy short bottom longline sets targeted to catch juvenile white sharks. We conducted three longline trips, deploying four bottom longline sets and one meso-pelagic longline set. One fishing trip was completed off Monastir and two off Lampedusa (Figure 3; Supplementary Table S1). In total, we spent 52 hours fishing in 2023, deploying 4,350 hooks.

2.2.5 Tagging

We were prepared with a variety of electronic tags in the event we encountered a white shark, including both satellite and archival tag types. Large sharks that were attracted to the chum but not physically captured were to be tagged using pop-off satellite archival tags (PSATs; Model: MiniPAT; Manufacturer: Wildlife Computers, Redmond, WA, USA) deployed from a tagging pole following Jorgensen et al. (2010). PSATs were programmed with a range of deployment times 180-270 days, with the mortality switch and depth-threshold release (set to 1800 m) both enabled. In the event we physically captured an animal, we also had non-satellite linked archival tags (model: TDR-Mk9-286F, Manufacturer: Wildlife Computers, Redmond, WA, USA) and fin-mounted, satellite-linked Smart Positioning and Temperature (SPOT) tags (model: SPOT5; Manufacturer: Wildlife Computers) ready for deployment.

3 Results

In all expeditions together, we conducted 359 hours of BRUV deployments, 43 hours of drop cam deployments, 52 hours of longline fishing, ~35 hours of rod-and-reel fishing, and ~24 hours of handline fishing, and collected 159 water samples for eDNA analysis. Using these methods, we identified multiple megafauna species, such as bluefin tunas, mako sharks, turtles, dolphins, mobulids, and other species (Supplementary Table S2).

Mid-water BRUVs detected 16 species of bony fishes, elasmobranchs, and marine mammals (Supplementary Table S2). We deployed 7 drop cams in 2021 at depths ranging from 73 to 651 meters (Figure 3). Drop cams detected 26 species, including 7 elasmobranchs and 19 bony fishes (Supplementary Table S3).

No animals were encountered while fishing with handlines or rod-and-reel. However, our 2023 longlining operations in Tunisian and Lampedusa were more productive, catching 15 species of fish, and invertebrates (Supplementary Table S1), and allowed us to tag a juvenile shortfin mako shark (*Isurus oxyrinchus*) for the first time in

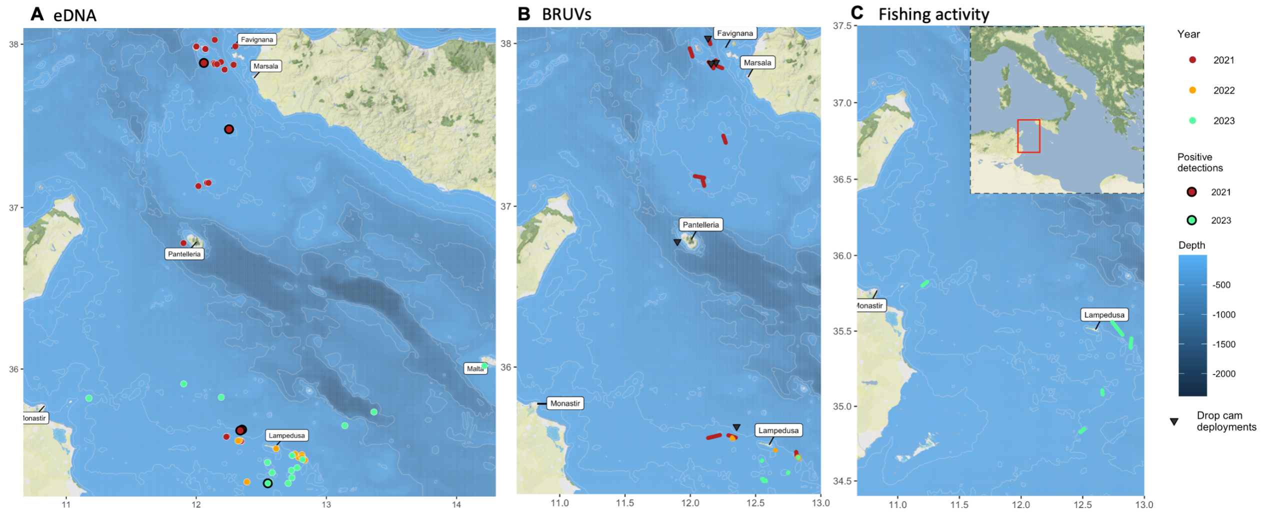

FIGURE 3
Map of the sampling stations surveyed during the expedition. **(A)** indicates locations where we sampled eDNA. Dots are color-coded by collection years. Positive detections are indicated with black borders. **(B)** shows the pelagic BRUV (colored segments) and drop-cam deployments (triangles). **(C)** indicates longline deployments.

the Mediterranean Sea (Supplementary Table S1, Shea et al. submitted manuscript[1]). The average catch rate was 18.81 individuals (any species) per thousand hooks (range: 7.5 - 54.17). The highest catch rates (54.17) were recorded when catching with mesopelagic longlines, while the bottom sets recorded an average of 10 individuals per thousand hooks. Meso-pelagic longlines were expected to have a lower chance of catching juvenile white sharks but also to increase the range of habitats to be sampled by the gear, which was slightly modified to probe pelagic and demersal environments, i.e. increasing the probability of a diverse shark by-catch.

Although no white sharks were observed from the boat at any time, we successfully detected the presence of white shark DNA in 5 samples out of 159 from 2021-2023, across the Sicilian Channel; four in 2021, which included a false-negative detection while on board (Jenrette et al., 2023), and one in 2023. Positive samples were collected at the Pantelleria Banks, northern and southern ends of Lampedusa, and the Egadi Islands (Figure 3). These results confirmed that white sharks were in the area, spatially (within 25 km) and temporally (within 48 hrs) close to at least four sampling locations during our expedition (Jenrette et al., 2023; Collins et al., 2018).

4 Discussion

We carried out three white shark research expeditions in the Mediterranean Sea, one of the world's most heavily utilized and inhabited ocean regions, where this species is rare and sparse (Moro et al., 2020; Coll et al., 2010). When we started this program, we did not know where to systematically encounter white sharks in the region and previous work (i.e., Soldo and Pierce, 2005) suggested interacting with live animals would be difficult. This led to the planning and execution of a series of pilot expeditions to expand the tools and approaches conventionally used to find these animals (Soldo and Pierce, 2005; Micarelli et al., 2023) and increase our understanding of their ecology and biology. Over these three years, we did not directly interact with live white sharks, but collected novel information on their current population density, catches, historical hotspots, and current presence and interactions with fisheries, leading us to identify the remaining stronghold of this population in the Mediterranean Sea.

Detecting white shark eDNA supported our predictions of where and when finding the animals in the region. However, our cruises, combined with year-round investigations over the last three years, suggest that the Mediterranean White shark population may be smaller and more fragmented than previously thought (Moro et al., 2020; Panunzi et al., 2024). Therefore, a more focused effort is required to document and tag these elusive animals. The eDNA assay results combined with particle drift models suggested that white sharks were likely within a radius of 13.5 nautical miles from our sampling sites within a period of two days (Jenrette et al., 2023) (Figure 3). Despite the population being seemingly sparse and elusive, the rapid onboard sampling and processing gave us an early qualitative alert to the presence of individuals. Follow-up analyses with highly sensitive detection assays refined these results, leading us toward sectors where these animals may have a systematic presence. Continued development of quasi-real-time eDNA detection approaches and the use of specific primers for the Mediterranean population can help future expeditions to more rapidly adapt sampling strategies in response to positive detections.

We did not detect white sharks on pelagic BRUVs or drop cams; this is not surprising given their scarcity and typical sighting rates at other robust white shark hotspots. In Cape Cod (Northwest Atlantic), 1-hour deployments recorded an average of 0.29 individuals hr^{-1} (Shea et al., 2020) and in Port Stephens, New South Wales (Australia), 0.07 sharks hr^{-1} were recorded from BRUV deployments (Harasti et al., 2016). These locations are small hotspots with large numbers of adults and juveniles, respectively (Harasti et al., 2016; Bruce and Bradford, 2012; Bruce et al., 2013). Additionally, pelagic BRUVs have a lower detection capability than benthic ones as they probe habitats with a sparser animal density. Yet, they are as efficient as scientific longline surveys to detect changes in relative abundance (Santana-Garcon et al., 2014). Our pelagic BRUVs recorded a MaxN hr^{-1}of 0.0057. A similar study in Western Australia recorded a combined MaxN hr^{-1} of 0.311 for nine shark species detected (Santana-Garcon et al., 2014). These estimates underscore the lack of observations in the present study, which, however, is in line with other indices detected in the Mediterranean Sea. A recent pelagic BRUV survey around the Balearic islands (northwest Mediterranean) successfully recorded 11 blue sharks (*Prionace glauca*), and a bluntnose sixgill shark (*Hexanchus griseus*), generating a comparable shark detection rate (0.0064 sharks hr^{-1}). This study employed a modified BRUV system capable of 24-hour continuous deployment for 1,884 hours (no MaxN reported) (Prat-Varela et al., 2023). Given the depleted state of the Mediterranean and the remote nature of the pelagic realm, longer deployments such as these, or marine-adapted models of camera traps widely used in terrestrial ecology (Bicknell et al., 2016), may increase the potential for observing white sharks.

In general, our BRUV results revealed a scantness of marine megafauna and ichthyofauna in one of the most biodiverse sectors of the Mediterranean Sea (Coll et al., 2010). After over a decade, large predatory sharks remain heavily depleted in the Mediterranean Sea due to intensive coastal and industrial fishing (Ferretti et al., 2008). The Mediterranean Sea is one of the most exploited large marine ecosystems globally (Kroodsma et al., 2018), and the central Sicilian Channel's offshore banks (i.e., Pantelleria shoal), were considered among the best spots for fishing in the past (Altobelli et al., 2017). In this sector, at least 13 nations fish with longlines, purse seines, bottom trawls, and small artisanal fishing gears in coastal and offshore waters (Figures 4A, B). Here, deploying our gear was sometimes challenging as there was a high risk of entanglement with other set fishing nets and interfering with the operations of other fishing boats in the surrounding waters. Remote sensing with Automatic Identification System (AIS) and Synthetic Aperture Radar (SAR) indicates that fishing is intense and often unmonitored throughout the Sicilian Channel and Tunisian plateau. In Tunisian and Libyan waters, where virtually no fishing boats are equipped with AIS transmitters, SAR reveals comparably intense fishing activities, even with its

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[1] Shea, B., Chapple, T., Echwikhi, K., Gambardella, C., Jenrette, J., Moro, S., Schallert, R., Block, B. and Ferretti, F. First satellite track of a juvenile shortfin mako (*Isurus oxyrinchus*) in the Mediterranean Sea. In review at *Frontiers of Marine Science*.

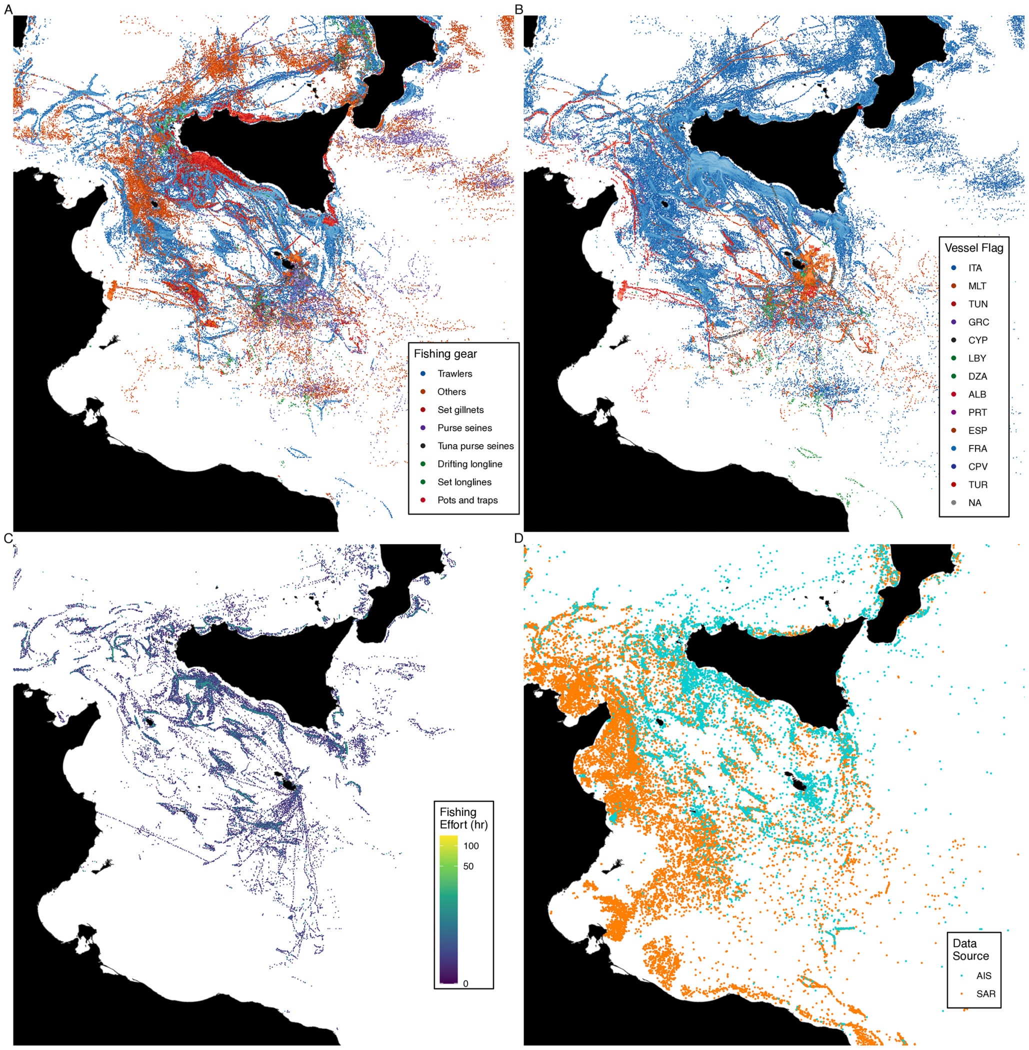

FIGURE 4
Fishing effort detected through AIS, by nation **(A)** and fishing method **(B)** recorded in the Sicilian Channel in 2019. **(C)** AIS data recorded in May 2023. **(D)** SAR detections matched (Green) and unmatched with AIS detections (Orange). Data are from the Global Fishing Watch database (globalfishingwatch.org).

limitations to efficiently capture small-scale coastal fishing (Paolo et al., 2024), which is prevalent in the region.

While we could not visually detect a white shark, these pilot expeditions were essential to gather information on the challenges, available infrastructures, and logistical problems associated with successfully detecting free-living white sharks in the Mediterranean Sea. We devised multiple solutions and established a network of collaborators, building infrastructure to support a more programmatic future effort. A major challenge was not having an adequate research vessel to host scientists, equipment, needed materials, and tenders. This affected methodology and limited the operations' geographic range. The type of vessels required for this work is expensive and rarely available through the networks of yacht owners we used for our surveys. Fishing vessels often have restrictions on their availability. Sailboats and catamarans are often available and affordable options, but these have limited cruising speed and space and inadequate auxiliary tenders or workboats. A large, refrigerated cell for bait storage (up to 240 kg day^{-1}), allowing for continuous sampling over longer periods without the need to visit shore, and a wide deck to conduct operations safely and without damaging the boat are crucial for conducting these operations seamlessly. Due to the boat's limited storage capacity, we often faced the additional challenge of needing to continuously source bait. Through agreements with local seafood wholesalers, we stored and refrigerated bluefin tuna remains in their facilities when tuna catches were processed and accessed as needed. Still, our onboard transport capacity remained a limiting factor, forcing us to adapt at times and use different baits. The fishing vessels we used in 2023 (Figure 1) increased our chances of tagging sharks and allowed us to operate more flexibly with multiple survey approaches. However, finding fishing vessels can be difficult when survey timing overlaps with peak fishery seasons.

The three pilot expeditions we completed have evolved into a multi-institutional monitoring program on White Sharks in the Mediterranean Sea, aimed at saving this population from extinction. The data and information collected during these three years of fieldwork and activities on the ground were crucial to refining our search strategy and selecting focal areas for the following years. Our ongoing monitoring of sightings and catches continues to update habitat and distribution models, increasing our chances of interacting with white sharks in upcoming expeditions. What began as a broad search in the Sicilian Channel has narrowed as we have constantly updated search strategies in response to our data, initially focusing on the southern SC (e.g., Lampedusa) and eventually moving our effort toward Tunisia in 2023. While we sporadically recorded sightings of white sharks from other Mediterranean regions and northern sectors of the Sicilian channel (e.g., Tiralongo et al., 2020), Tunisia consistently appeared on social media and other channels with reported catches, contrasting the patterns of fishing detected in the region (Figure 4). However, a deeper investigation of fishing activities visible from SAR and AIS revealed that Tunisia's fishing intensity was comparable to, if not greater than, the SC's northern sectors, leading us to deepen our investigations of the interaction between fisheries and sharks in Tunisia.

White Sharks are still in the Mediterranean Sea, but we do not know how many individuals are left and where and whether they occur year-round. Therefore, it is now imperative to leverage the infrastructure and experience from our first three trips to unfold a more articulated monitoring program with year-round activities on the ground and follow-up expeditions. We will focus our efforts on Tunisia, which appears to be one of the population's last strongholds. Here, we will intensify monitoring and sampling efforts for biological material and fisheries data. Meanwhile, we will expand our search to other Mediterranean sectors, scaling up with eDNA sampling across the region and planning explorations in other hotspots identified by our habitat and distribution models.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. DNA sequences of cytochrome b gene PCR amplicons from white shark detections are accessible through the Virginia Tech Data Repository at https://doi.org/10.7294/27048106. Further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was approved by Virginia Tech Institutional Animal Care and Use Committee (IACUC) protocol for project #21-208. The study was conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

FF: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization. BDS: Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. CG: Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis. JFJ: Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis. SM: Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis. KE: Writing – review & editing, Resources, Investigation. RJS: Writing – review & editing, Methodology, Investigation. AJG: Writing – review & editing, Resources, Investigation. BAB: Writing – review & editing, Supervision, Resources, Methodology. TKC: Writing – review & editing, Resources, Methodology, Investigation, Funding acquisition.

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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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Aerobic and anaerobic poise of white swimming muscles of the deep-diving scalloped hammerhead shark: comparison to sympatric coastal and deep-water species

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Scalloped hammerhead sharks (*Sphyrna lewini*) routinely perform rapid dives to forage on mesopelagic prey. These deep dives consist of intensive swimming followed by recovery periods in the surface mixed layer. Swimming muscle temperature profiles suggest that *S. lewini* suppresses gill function as a means to reduce convective heat loss during dives into cool water. Such intensive swimming behavior coupled with reduced respiration prompted us to test whether the aerobic and anaerobic metabolic capacities of the white swimming muscle tissue of this species are greater than those of other shark species from the same region. The activities of key enzymes used in aerobic and anaerobic metabolism provide an indirect indicator of the metabolic potential ("poise") of a tissue. Here we measured the maximal activities [international units (μmol substrate converted to product per min, U) per gram of wet tissue mass at 10°C] of the citric acid cycle enzymes citrate synthase (CS) and malate dehydrogenase (MDH) and glycolytic enzymes pyruvate kinase (PK) and lactate dehydrogenase (LDH) from white swimming muscle of *S. lewini*. Enzyme activities, and ratios of these enzyme activities that indicate relative indexes of aerobic to anaerobic capacity, were compared to those measured in three sympatric coastal carcharhinid sharks and two deep-dwelling species, *Echinorhinus cookei* and *Hexanchus griseus*. This is the first report of swimming-muscle enzyme activity for these deep-dwelling species. In comparison to the other species, *S. lewini* had significantly higher activities of both LDH and MDH in the white muscle, and a higher MDH/CS ratio. The high LDH activities suggest that the white muscle of *S. lewini* relies on relatively high rates of anaerobic ATP production, with would result in build up of high lactate levels, during deep foraging dives. High MDH activity in *S. lewini* white muscle suggests the potential for lactate levels to be rapidly reduced when aerobic conditions are restored while in the surface mixed layer between dives. These biochemical characteristics may enable *S. lewini* to swim rapidly while suppressing gill function during deep dives and thereby exploit a very different ecological niche from sympatric shark species (e.g., coastal carcharhinids) and hunt more rapidly via faster swimming for deep-water prey compared to species that permanently inhabit deep depths.

KEYWORDS

enzyme activity, lactate dehydrogenase, malate dehydrogenase, white muscle, *Sphyrna lewini*, *Hexanchus griseus*, deep-diving

Introduction

The scalloped hammerhead shark, *Sphyrna lewini*, shares overlapping daytime distributions in tropical and warm temperate coastal environments with other sympatric species (coastal carcharhinids), but exploits a very different ecological niche by foraging in deep, cold habitats. Scalloped hammerhead sharks conduct repetitive nocturnal deep dives (over 600m, sometimes exceeding 1000m) into cold water (as low as 5°C), presumably to forage on mesopelagic prey in the oxygen minimum zone (Jorgensen et al., 2009; Bessudo et al., 2011; Hoffmayer et al., 2013; Spaet et al., 2017; Anderson et al., 2022; Hutchinson et al., 2023; Royer et al., 2023). Royer et al. (2023) reported that *S. lewini* reduces convective heat loss at the gills during these deep dives, and suggested that this strategy of suppressing gill function is broadly similar to a "breath hold" dive. Biologger data of Royer et al. (2023) revealed that these deep dives are characterized by steep descents with swimming bursts when approaching maximum depth, intense activity throughout the deepest phase, with bottom times lasting an average of approximately 4 minutes, and a consistent high-frequency, high-amplitude tailbeat during ascent (Figure 1). During the ascent, at a depth of ~250m, swimming intensity decreases abruptly, sharks level out and ascend more slowly. This part of the ascent is characterized as the "inflection point." On reaching the surface mixed layer, sharks swim using a lower tailbeat frequency and amplitude until starting the next deep dive. Overall, these dives last an average of 56 minutes (from the start of the slow descent to the end of the slow ascent), with the high-activity phase of the dives (from the start of the fast descent to the end of the fast ascent at the inflection point) lasting an average of 17 minutes. The sharks stayed within the top 50 meters of the water column during their interdive intervals which lasted an average of 43 minutes and ranged from 18 minutes to >3 hours. Temperature measurements of the white swimming muscle show that *S. lewini* stays warm throughout the deepest portion of the dives and substantive cooling only occurs during the latter stages of the ascent phase and, once initiated, is rapid. These rapid changes in body temperature at different points of the dive cannot be explained by simple thermal inertia. Modeling of heat transfer coefficients (Royer et al., 2023) indicates that convective heat transfer at the gills is suppressed during the high-activity phase of the dives. Although the active mechanism for reducing heat loss at the gills is unknown (e.g., shunting blood away from the gills, reducing blood flow to the gills, reducing ram ventilation by closing the mouth, gill slits or both), any of these will inhibit the shark's ability to absorb oxygen from the environment. Video of a scalloped hammerhead shark swimming along the seabed at a depth of 1,044m showed its gill slits tightly closed, whereas similar images from surface waters show these sharks swimming with their gill slits wide open, supporting the gill-slit-closing hypothesis (Moore and Gates, 2015; Royer et al., 2023). A sudden cooling in muscle temperature as scalloped hammerhead sharks approach the surface at the end of each dive suggests that they have opened their gill slits to resume gill ventilation while still in relatively cool water.

Powering swimming during these repetitive, intense, deep dives necessitates a high energetic output by the locomotor muscles. It is possible that *S. lewini* relies on anaerobic energy production during these deep dives due to the combination of intense swimming activity and suppression of normal respiration. This highly active swimming combined with apparent "breath holding" should be reflected in the activities of key muscle enzymes involved with locomotion and energy mobilization.

Anaerobic metabolism becomes an essential process when aerobic pathways of ATP production cannot sustain cellular energetic demands (Pörtner, 2002). Burst swimming typically requires anaerobic metabolism and a greater reliance on muscle energy stores (Williams et al., 1997). In fishes, fast-twitch glycolytic white (type II) muscle is the largest tissue mass and is specialized for anaerobic high-intensity swimming when power output is needed beyond what the slow-twitch red muscle (type I) is capable of producing (Bernal et al., 2003; Seamone and Syme, 2016). High-intensity swimming events, such as the chasing of prey, typically require anaerobic pathways independent of oxygen availability and deplete muscle creatine-phosphate and glycogen stores, leading to a concomitant buildup of lactate and H^+ byproducts (Guppy and Hochachka, 1978; Bernal et al., 2003; Kane, 2014).

Generally, elasmobranchs found in warm shallow waters (above mesophotic depths) have higher metabolic rates and stronger burst-

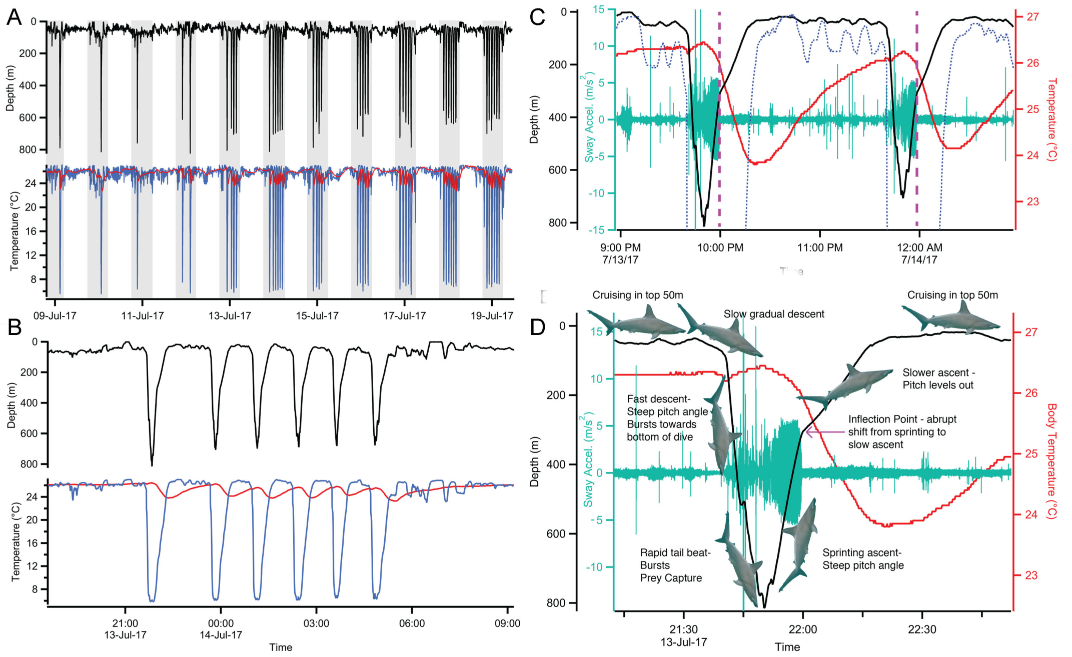

FIGURE 1
Deep-diving behavior and body temperature of *S. lewini*, reproduced from Royer et al. (2023). Ambient (blue), and intramuscular (red) temperature profiles from a scalloped hammerhead shark deep-diving during nighttime **(A)**, with repetitive deep-dives shown during a single evening **(B)**. **(C)** Depth profiles (black), body temperature (red), ambient water temperature (blue dashed), and swimming activity [tailbeat sway acceleration (teal)] during two successive deep dives. Dotted lines indicate the inflection point of the ascents when the swimming activity and pitch angle abruptly decrease. **(D)** Distinct phases of a deep dive.

locomotor capabilities relative to deep-dwelling elasmobranchs (Treberg et al., 2003; Condon et al., 2012). This is supported by electronic tagging data, with slower vertical velocities, slower swimming speeds, and slower tail beats for *H. griseus* and *E. cookei* than for epipelagic shark species (Nakamura et al., 2011, 2015; Comfort and Weng, 2015; Andrzejaczek et al., 2018; Coffey et al., 2020; Royer et al., 2023). Capacity of tissues for aerobic and anaerobic metabolism can be estimated by measuring the maximal activity of key enzymes involved in the production of ATP. The activities of the enzymes citrate synthase (CS) and malate dehydrogenase (MDH) have been widely used as indicators of tissue aerobic capacity. CS is the first catalyst in the citric acid cycle (Childress and Somero, 1979) and its activity correlates with tissue mitochondrial density (Moyes et al., 1992; Dickson et al., 1993; Dickson, 1995; Duong et al., 2006) and whole animal metabolic rate (Torres and Somero, 1988; Hochachka and Somero, 2002). MDH is used in the citric acid cycle in the shuttling of reducing equivalents between the mitochondria and cytosol. MDH is also involved in gluconeogenesis, the synthesis of glucose from smaller molecules such as pyruvate formed by oxidation of lactate accumulated during anaerobic function (Kane, 2014; Rogatzki et al., 2015). Activities of the enzymes pyruvate kinase (PK) and lactate dehydrogenase (LDH) have been used as indicators of anaerobic capacity. PK is a good indicator of the capacity for glycolysis, as it catalyzes pyruvate formation during glycolysis (Somero and Childress, 1980). LDH catalyzes the reversible conversion of pyruvate and NADH to lactate and NAD$^+$ to maintain redox balance, allowing anaerobic production of ATP to continue in the cytosol; therefore, LDH activity is considered a strong indicator of tissue anaerobic capacity (Hochachka et al., 1982). Measurements of the maximal activities of all four of these enzymes have been widely used in previous studies to assess the aerobic and anaerobic capacity of muscle in fishes (Childress and Somero, 1979; Sullivan and Somero, 1980; Somero, 1992; Dickson et al., 1993; Vetter and Lynn, 1997; Bernal et al., 2003; Treberg et al., 2003; Ombres et al., 2011; Condon et al., 2012; Drazen et al., 2015; Saavedra et al., 2016; Pinte et al., 2021).

The combination of intensive swimming and likely suppressed gill function suggests that *S. lewini* relies heavily on anaerobic metabolism during deep dives (Meekan and Gleiss, 2023). These characteristics may enable *S. lewini* to swim rapidly while suppressing gill function during dives and thereby exploit a very different ecological niche from sympatric shark species (e.g., coastal carcharhinids) and hunt more rapidly for deep-water prey compared to slow-moving shark species that permanently inhabit deep depths. Therefore, we predicted that the white muscle tissue of *S. lewini* possesses enzyme characteristics that facilitate anaerobic metabolism during deep dives, and the necessary aerobic metabolism to allow for rapid recovery (i.e., the breakdown of anaerobic end products) in well-oxygenated surface waters during intervals between dives. Furthermore, we predicted that the white muscle enzyme characteristics of *S. lewini* can be distinguished from those of both sympatric shallow-water shark species (e.g., coastal carcharhinids) that share similar daytime distributions and deep-water shark species, by having a greater capacity for both anaerobic and aerobic metabolism in the white swimming muscle. To test this hypothesis, the maximal activities of the enzymes CS, MDH, PK, and LDH were measured to assess the aerobic and anaerobic poise of the white muscle of adult *S. lewini*. Activities of these enzymes were compared to those measured in other coastal tropical/temperate shark species [sandbar shark (*Carcharhinus plumbeus*), blacktip shark (*Carcharhinus limbatus*), and tiger shark (*Galeocerdo cuvier*)] and in two deep-water sharks [bluntnose sixgill (*Hexanchus griseus*) and prickly shark (*Echinorhinus cookei*)].

Materials and methods

Tissue sampling

White muscle tissue samples were collected from sharks caught on demersal long-lines set in waters off the island of O'ahu (Hawai'i, USA). Shallow species (*S. lewini, G. cuvier, C. plumbeus, C. limbatus*) were caught at depths of 5-50 meters and deep species (*H. griseus* and *E. cookei*) were caught between depths of 250–300 meters. Muscle samples were collected from a 2-cm incision below the base of the first dorsal fin using a 5mm or 8mm biopsy punch. Samples were placed in a cryovial and immediately dropped into a Dewar flask containing liquid nitrogen. Samples were later transferred to and stored in a -80°C freezer for 3-24 months. This storage time falls within the allowable timeframe between tissue sampling and assay running without compromising (decreasing) tissue enzyme activity (Dickson et al., 1993; Condon et al., 2012).

Enzyme assays

The maximal activities of the enzymes CS, PK, LDH, and MDH in each white muscle tissue sample were measured using enzyme assay protocols based on those established by previous studies (Childress and Somero, 1979; Treberg et al., 2003; Condon et al., 2012; Friedman et al., 2012). Frozen muscle samples were weighed and homogenized in a Kontes Duall ground glass tissue grinder with ice-cold 10 mM Tris-HCL buffer (pH 7.55 at 10°C) at a ratio of 0.1 g of tissue to 1 ml of buffer. Duplicate homogenates were prepared for each sample unless the total sample was less than 0.05 g, in which case a single homogenate was prepared. CS assays were performed before centrifugation for the other enzyme assays. Homogenates were then centrifuged at 5000*g* for 5 minutes, and the supernatants were used in the assays.

All assays were run in a volume of 2 mL at 10°C in a Shimadzu UV 1601 spectrophotometer with a water-jacketed 12-cell cuvette holder attached to a water chiller (Condon et al., 2012). Activities were measured as change in absorbance over time and reported in international units (U; µmol substrate converted to product per min) per gram of wet tissue mass. PK, LDH, and MDH assays were run at 340 nm for 40 seconds and CS was run at 412 nm for 3 minutes. Enzyme assays were run under the following saturating substrate conditions: citrate synthase: 0.1 mM 5,5-dithiobis-nitrobenzoic acid (DTNB), 50 mM imidazole HCl (pH 8.0 at 10°C), 2 mM $MgCl_2$, 0.1 mM acetyl-CoA, 0.5 mM oxaloacetate. After recording background change in absorbance over time with the tissue homogenate present, the CS reaction was initiated by adding oxaloacetate substrate. Pyruvate kinase: 80 mM Tris HCl (pH 7.8 at 10°C), 100 mM KCl, 10 mM $MgSO_4$, 10 U/ml lactate dehydrogenase, 0.1 mM fructose 1-6 bisphosphate, 5.0 mM adenosine diphosphate, 150 µM NADH. The PK reaction was initiated by the addition of 1.0 mM phosphoenol pyruvate. Lactate dehydrogenase: 80 mM imidazole (pH 7.8 at 10°C), 100 mM KCl, 150 µM NADH, 2 mM sodium pyruvate. Malate dehydrogenase: 100 mM Tris HCl (pH 8.1 at 10°C), 20 mM $MgCl_2$, 150 µM NADH, 0.5 mM oxaloacetic acid. LDH and MDH reactions were initiated with the addition of the supernatant.

Data analysis

The body mass for each shark was estimated from measured lengths using length-weight relationships from Kohler et al. (1996) for *S. lewini, G. cuvier, C. plumbeus*, Ebert (1986) for *H. griseus*, and Pollack et al. (2019) for *C. limbatus*. No length-weight relationships were available for *E. cookei*. Regression analysis was used to evaluate whether muscle enzyme activity scaled with shark body size for each species. Normality of enzyme activity data was assessed by examining distribution histograms for each species and enzyme, and using Levene's test to assess homogeneity of variance. Due to the non-normal (based on histograms and NQQ plots) and heteroscedastic (Levene's test, all $P < 0.05$) nature of the data and the unequal sample sizes among species, non-parametric Welch's ANOVAs with *post-hoc* Games-Howell tests were used to assess interspecific differences in muscle enzyme activity. High activity of MDH observed in *S. lewini* prompted a *post-hoc* interspecific comparison of the ratio of the aerobic enzymes (MDH and CS). The ratio of MDH to CS activity was calculated for each individual shark, and then averages and standard deviations were calculated for each species. Based on similar data characteristics mentioned above, non-parametric Welch's ANOVA with a *post-hoc* Games-Howell test was used to assess interspecific differences in the MDH:CS ratio between species.

Results

Body size scaling effects on enzyme activity

All *S. lewini* (n = 10) and *E. cookei* (n = 5) sampled were mature adult males. All *G. cuvier* (n = 22) were immature sub-adults or juveniles. One *C. limbatus* (n = 5) and 12 *C. plumbeus* (n = 24) were mature adults. Body length measurements were missing for 2 *G. cuvier* and 3 C. *plumbeus*. Two of the male *H. griseus* (n = 9) were mature adults, 2 of the other males were large sub-adults, and all 5 of the females were large sub-adults, based on clasper calcification (for males) and estimates of age at maturity from (Ebert, 2002). Regression analyses showed no significant scaling of enzyme activity with body size for the species studied (all P values > 0.05), with the exception of LDH and MDH for *S. lewini* (P = 0.02, P = 0.01) and *H. griseus* (P = 0.03, P = 0.02). The largest individual of both species had unusually low LDH and MDH values. When those two individuals were removed as outliers, no significant body size scaling was observed (all P values > 0.05). As a result, mean enzyme activities, unadjusted for body size but with the outlier data included, are reported in Table 1 and used for interspecific comparisons.

White muscle enzyme activities

Sphyrna lewini white muscle exhibited high activities of LDH, CS, and MDH (Table 1), with the activities of LDH and MDH of *S. lewini* standing out as much higher in comparison to the sympatric coastal (*C. plumbeus*, *C. limbatus*, *G. cuvier*) and deep-water (*H. griseus*, *E. cookei*) species (Figures 2, 3A). MDH activity in *S. lewini* was significantly greater than that in all of the coastal and deep-water species (all $P \leq 0.007$, Games-Howell tests; Figure 1). LDH activity was significantly greater in *S. lewini* than in the coastal sharks *C. limbatus* ($P = 0.02$) and *C. plumbeus* ($P = 0.003$) and the deep-water shark *H. griseus* ($P = 0.006$). Average CS activity in *S. lewini* was the highest amongst the species studied, but not significantly so (all $P > 0.05$) (Figure 2). PK activity in *S. lewini* did not differ significantly from that measured in the coastal sharks *C. limbatus* and *C. plumbeus* and in both deep water sharks, but was significantly lower than that of *G. cuvier* ($P < 0.001$). MDH activity relative to CS activity was highest in *S. lewini* compared to the other coastal carcharhinids and bathyal shark species, followed by *C. plumbeus* and *G. cuvier* (Table 2, Figure 3B). Though the statistical comparisons of MDH: CS ratios between *S. lewini* and all other species except *E. cookei* were not significant (all $P>0.05$), it is noticeable in Figure 3B that *G. cuvier* and *C. plumbeus* also have high CS activity but not the correspondingly higher MDH activity observed in *S. lewini.*

Discussion

Based on its higher LDH and MDH activities (Figure 3A), and to a lesser extent the relatively high CS activity (Table 2, Figure 3B), *S. lewini* white muscle has higher anaerobic and aerobic capacities than other coastal carcharhinids and bathyal shark species that do not exhibit the repetitive nocturnal dives observed in *S. lewini.* Analyses of white muscle metabolic poise suggest that *S. lewini* possesses enzyme characteristics that facilitate high anaerobic metabolic activity during deep dives and the necessary aerobic metabolism to allow for rapid recovery, through the breakdown of anaerobic end products, when near the surface between dives. These physiological characteristics are proposed to enable the observed high activity levels of *S. lewini* even while they are suppressing gill function during dives into cold habitats. These same features would allow them to recover quickly in well-oxygenated surface waters through rapid processing of the lactate built up during anaerobic activity (Meekan and Gleiss, 2023). The intense swimming activity exhibited by *S. lewini* during deep dives requires ATP production at a high rate (Meekan and Gleiss, 2023). It is unknown to what extent anaerobic ATP production in the white muscle is required during dives, but the high intensity swimming, including frequent bursts, recorded by Royer et al. (2023) suggests that the white fast-twitch muscle is active at depth, making it likely that anaerobic metabolism and lactate production occur. This would then require the lactate to be removed from the white muscle or possibly processed within it after a dive. It is possible that red slow-twitch muscle is also recruited during the rapid but sustained swimming phases of each deep dive, reducing to some extent the amount of lactate produced in the white muscle. Regardless of the extent to which deep-diving behavior relies on aerobic versus anaerobic capacity, these behavioral and physiological characteristics likely enable *S. lewini* to exploit a very different ecological niche than other sympatric shark species (e.g., coastal carcharhinids with daytime distributions overlapping that of *S. lewini*).

The white muscle enzyme profile of *S. lewini* suggests that the deep-water hunting capacity of this species may be facilitated by rapid recovery in surface waters between dives. Higher levels of LDH allow for higher rates of glycolysis to power white muscle contraction during bursts and high-speed swimming, but can also cause a high rate of lactate buildup in the cytosol. The amount and fate of lactate produced in the white muscle of *S. lewini* during intensive swimming during dives is unknown. Previous studies indicate that teleosts and the dogfish shark, *Squalus acanthias*, retain lactate produced from strenuous exercise in their white muscle (for up to 4 hours in *S. acanthias*) (Milligan and Wood, 1986; Girard and Milligan, 1992; Richards et al., 2003), but little evidence for gluconeogenesis within fish white muscle has been found. Lactate can be converted to pyruvate and then used as a substrate for ATP synthesis or in gluconeogenesis under aerobic

TABLE 1 White muscle enzyme activity (mean ± standard deviation) reported in U/g wet mass at 10°C for each species.

Species	N	BW Range (kg)	BW Mean (kg)	CS	PK	LDH	MDH
S. lewini	10	61.0 – 156.6	79.5 ± 33.9	1.08 ± 0.59	41.03 ± 11.92	203.19 ± 96.84	55.15 ± 19.09
G. cuvier	22	4.5 – 141.6	61.3 ± 43.3	0.99 ± 0.82	126.61 ± 71.40	144.53 ± 95.37	25.19 ± 9.51
C. plumbeus	24	7.1 – 41.5	27.1 ± 14.4	0.92 ± 0.66	35.05 ± 26.35	31.80 ± 24.09	16.75 ± 6.37
C. limbatus	5	9.9 – 49.1	22.0 ± 15.5	0.44 ± 0.09	23.85 ± 13.08	84.64 ± 20.43	19.02 ± 9.78
H. griseus	9	54.7 – 341.6	142.5 ± 83.8	0.56 ± 0.32	26.55 ± 15.39	22.14 ± 10.49	10.49 ± 6.98
E. cookei	5	–	–	0.52 ± 0.34	68.05 ± 50.79	141.55 ± 44.86	14.49 ± 5.88

Body weights (BW) were estimated using published length-weight regressions for each species.

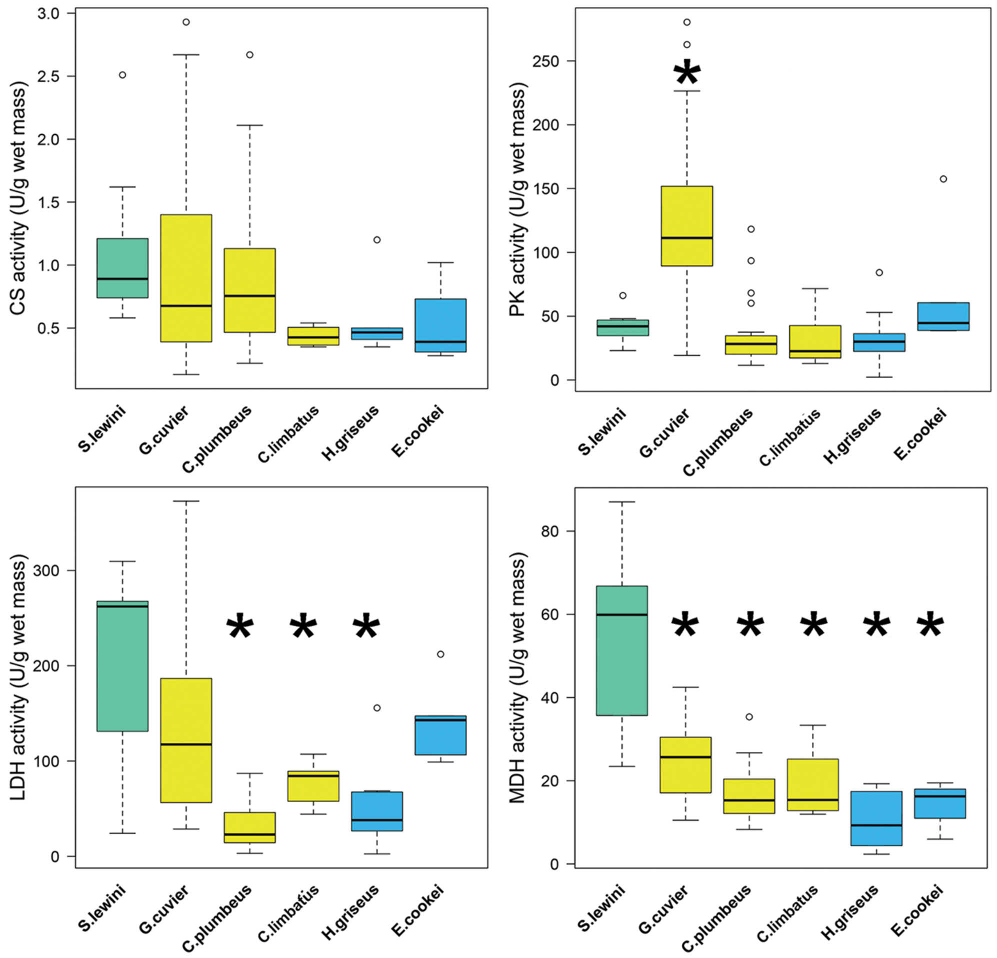

FIGURE 2
Boxplots of white muscle CS, PK, LDH, and MDH activities (U g^{-1} wet mass at 10°C) from (left to right) *Sphyrna lewini* (teal), *three* coastal species (yellow) *(Galeocerdo cuvier*, *Carcharhinus plumbeus*, *Carcharhinus limbatus*), and two deep-water species (blue) (*Hexanchus griseus*, *Echinorhinus cookei*). Thick line in each represents the mean, box ends are the 25th and 75th quantiles, dashed lines are min and max values, circles are outliers. Asterisks (*) indicate significant differences in species means in comparison to *S. lewini* (*P*-values < 0.05, Welsh's ANOVA *post-hoc* Games-Howell test).

conditions. MDH plays a key role in the malate-aspartate cycle, to shuttle electrons across the inner mitochondrial membrane after the conversion of lactate to pyruvate (Ombres et al., 2011). The high MDH activity measured in *S. lewini*, in comparison to all other species examined, suggests a higher potential for this process in the white muscle under aerobic conditions (Suarez et al., 1985; Ombres et al., 2011). This is further supported by the high MDH: CS ratio observed in *S. lewini* (Table 2, Figure 3B). CS activity often scales with whole-body metabolic rate (Childress and Somero, 1979; Dahlhoff, 2004; Drazen and Seibel, 2007). Tiger (*G. cuvier*) and sandbar (*C. plumbeus*) sharks also have high CS activities, but not nearly the correspondingly high MDH activity measured in *S. lewini*, as reflected in the MDH: CS ratios (Figure 3B). MDH and CS are part of the citric acid cycle, and their activities in a given tissue should co-vary with mitochondrial content if their only role is as catalysts in the primary aerobic metabolic pathway (Dalziel et al., 2017). Thus, finding that MDH and the MDH: CS ratio are higher in *S. lewini* than in the other species studied suggests a more important role for MDH in *S. lewini*. The predominant role is likely to facilitate lactate clearance for post-exercise recovery after dives, but it may also be involved in redox balance during high-intensity activity. Although challenging, future studies measuring the production of lactate during dives and its subsequent fate during recovery from intensive activity, would be needed to distinguish

TABLE 2 Ratios (mean ± standard deviation) of MDH to CS enzyme activity in white muscle of each species.

Species	N	MDH/CS
S. lewini	10	57.1 ± 22.9
G. cuvier	20	39.5 ± 26.2
C. plumbeus	22	24.8 ± 23.2
C. limbatus	5	48.4 ± 24.9
H. griseus	7	24.7 ± 20.2
E. cookei	3	24.8 ± 11.2

Statistical comparisons of MDH: CS ratios between *S. lewini* and all other species except *E. cookei* were not significant (all *P*>0.05).

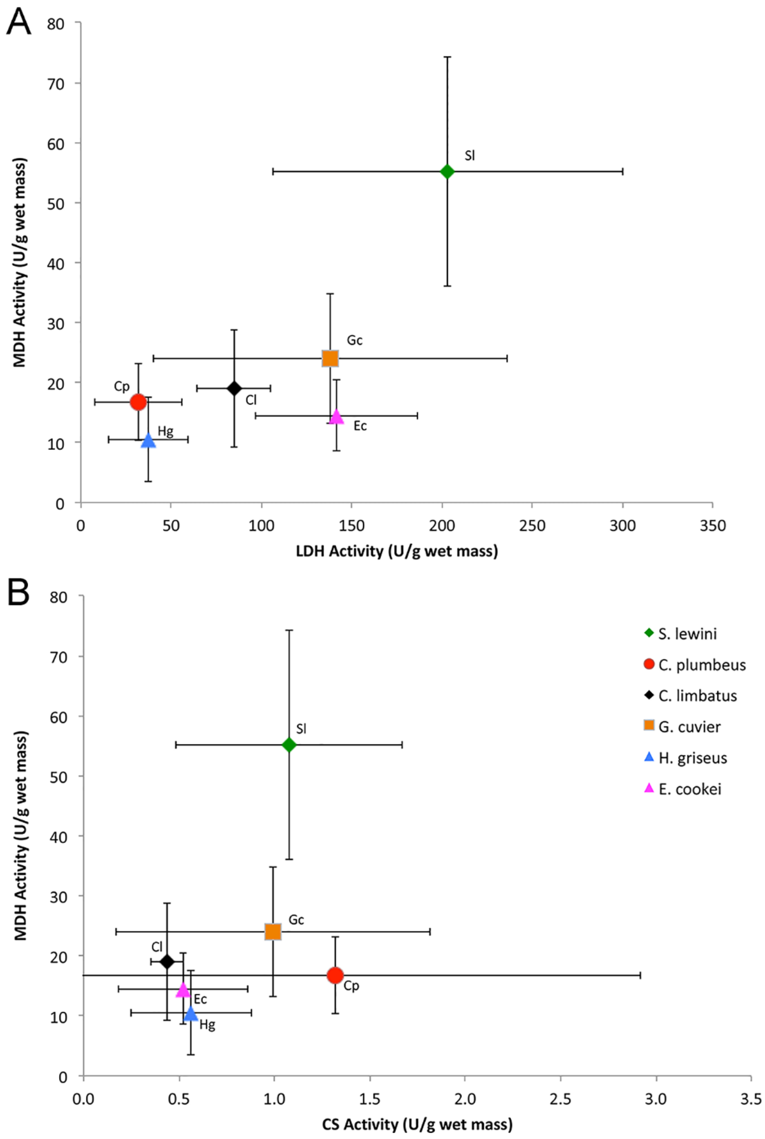

FIGURE 3
Comparison of mean white muscle activities (U g^{-1} wet mass at 10°C) of **(A)** LDH versus MDH and **(B)** CS versus MDH in the six shark species studied. Error bars indicate standard deviations. Though the statistical comparisons of MHD: CS ratios between *S. lewini* and all other species except *E. cookei* were not significant (all *P*>0.05), it is noticeable that *G. cuvier* and *C. plumbeus* also have high CS activity but not the correspondingly higher MDH activity observed in *S. lewini*.

among these alternatives and fully understand which biochemical processes underly the diving and foraging behavior of *S. lewini*.

Lactate processing may be initiated when *S. lewini* reaches the "inflection point" of the dive ascent at ~250 m, which marks reduced swimming activity and a slower rate of ascent, associated with rapidly cooling muscle temperatures (Meekan and Gleiss, 2023; Royer et al., 2023). Available evidence suggests that this is the moment when *S. lewini* resumes ram-ventilation in adequately oxygenated waters, which would allow rapid repayment of any oxygen debt incurred and to remove lactate accumulated during the dive (Meekan and Gleiss, 2023; Royer et al., 2023). Converting lactate into glycogen via gluconeogenesis *in situ* under aerobic conditions during the inter-dive period would allow *S. lewini* to reduce lactate concentration and replenish white muscle glycogen stores for subsequent deep dives (Iosilevskii et al., 2022). Gluconeogenesis could also occur in other tissues such as the liver and kidney (Suarez et al., 1985). Future studies should investigate the activities of key enzymes in the gluconeogenesis pathway in multiple tissues of *S. lewini* and other sharks. Although evidence for gluconeogenesis in white muscle has been observed in several teleosts (Milligan and Wood, 1986; Girard and Milligan, 1992), a study of activities of several key gluconeogenic enzymes in the white and red locomotor muscle, liver, and heart in mako, blue, and leopard sharks found no evidence for gluconeogenic capacity (Backey, 2007). Nevertheless, rapid removal of lactate by conversion to pyruvate and oxidation of pyruvate in the citric acid cycle would be beneficial for repeated dives by *S. lewini*.

In comparison to sympatric coastal and deep-water shark species, the higher capacity of *S. lewini* for aerobic and anaerobic metabolism and hence high activity during deep dives may confer a competitive advantage in actively foraging for deep-dwelling prey which may be relatively sluggish due to low body temperatures and metabolic rates (Childress, 1995; Seibel et al., 2000; Seibel and Drazen, 2007). Previous studies revealed a significant decline in tissue anaerobic capacities of the white muscle of deep-dwelling teleosts and cephalopods with increasing minimum depths of occurrence (Childress and Somero, 1979; Sullivan and Somero, 1980; Drazen and Seibel, 2007). For the sympatric coastal species that are adapted to warm-temperate waters, the deleterious physiological effects of moving into colder water make these deep prey resources generally inaccessible. Deep-water sharks (e.g. *H. griseus* and *E. cookei*) on the other hand are able to permanently inhabit these cold depths with access to deep prey patches, with the trade-off of living with reduced metabolic capacity. In contrast, by maintaining a high capacity for burst swimming and briefly maintaining a warm body temperature (Royer et al., 2023), *S. lewini* are able to conduct their intensive deep-dives to exploit an ecological niche that is inaccessible to sympatric coastal species (e.g., coastal carcharhinids), and have a more competitive, albeit transient, hunting capacity than the shark species that permanently inhabit these deep depths. The tradeoff for *S. lewini* is that they must have the energy reserves needed to power these brief and intensive deep dives (Meekan and Gleiss, 2023). Another potential trade-off is the increased vulnerability to predation during the inter-dive recovery period, similarly to beaked whales after their deep dives (Aguilar de Soto et al., 2020; Siegal et al., 2022).

Important caveats must be considered when interpreting the results of this study. Although it is known that enzyme activity scales with body size in many fishes, we did not detect any scaling effects in our study, most likely due to low sample sizes and limited size ranges for each species. Enzymatic activity can change based on a number of factors such as sampling period, time of day, recent feeding or mating events, and fish activity level (Yang and Somero, 1993; Dahlhoff, 2004; Li et al., 2012) - none of which are known for any of the sharks that were sampled for this study, but could explain some of the variability in the data. However, the same factors affect other published studies of fish samples from wild populations. Tissue samples were collected from live sharks under challenging field conditions and with rapid handling times to ensure proper animal welfare upon release, especially for species that are more sensitive to capture stress, particularly *S. lewini* (Hutchinson et al., 2023).

Conclusions

The scalloped hammerhead shark possesses enzyme characteristics that probably facilitate anaerobic metabolism during deep dives and rapid recovery during inter-dive intervals through the rapid aerobic breakdown of anaerobic end products. The high aerobic and anaerobic metabolic capacities measured in the white muscle of *S. lewini* are likely crucial for conducting repeated high-activity, deep dives while the shark “holds its breath” by suppressing gill function. High levels of MDH likely facilitate clearance of lactate and possibly restoration of glycogen stores in the white muscle when the sharks are in well-oxygenated surface waters. The results also raise further questions about the oxygen storage capacity of *S. lewini*, and whether hypoxia occurs during deep foraging dives (Meekan and Gleiss, 2023). A greater understanding of the metabolic pathways and oxygen demands during dives and post-dive recovery may explain the paradox of how this species is capable of routinely exerting itself to conduct intense deep dives while suppressing gill function, yet is also highly vulnerable to rapid stress-induced mortality from fishing capture. This ability to forage actively in deep waters and to rapidly recover from periods powered by anaerobic metabolism may be key to the ecological success of *S. lewini*, which has historically been circumglobally abundant in coastal and offshore waters (Rigby et al., 2019).

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 approved by Shark capture and sampling activities were carried out in accordance with the animal use protocols of the University of Hawai'i Institutional Animal Care and Use Committee (IACUC) and were approved under IACUC protocols #05-053 and #11-1242. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

MR: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. DG: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Writing – review & editing. KD: Conceptualization, Data curation, Formal Analysis, Methodology, Resources, Software, Supervision, Validation, Writing – review & editing. KW: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. CM: Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing. KH: Funding acquisition, Resources, Supervision, Writing – review & editing, Validation. JD: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing.

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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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Love bites? Pre-copulatory behaviours of whale sharks (*Rhincodon typus*) at Ningaloo Reef, Western Australia

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Very little is known about the reproductive behaviours of whale sharks (*Rhincodon typus*). Here, we describe field observations of courtship behaviour by a whale shark at Ningaloo Reef, Western Australia. We witnessed and recorded following and biting behaviours by a sexually mature male of a smaller female. Following and biting are common events during courtship and copulation of other species of elasmobranchs. Our observations are consistent with earlier reports of courting behaviours in whale sharks provided by fishers, the pilot of a light plane and observations of courtship by a sexually mature male towards females held in aquaria.

KEYWORDS

courtship, reproduction, elasmobranch, sexual segregation, mating activity, intra-specific aggression, sex ratio, marine megafauna

Introduction

Whale sharks (*Rhincodon typus*) aggregate in coastal waters at tropical and warm temperate locations around the world (Araujo et al., 2022). Although these near-shore aggregations provide the opportunity to access these animals for research, they are typically dominated by juvenile males (with some exceptions (see summaries: Norman et al., 2017; Araujo et al., 2022). For this reason, research on the ecology of the species tends to be based on data that are biased towards this subset of the population. Our knowledge of adult whale sharks, particularly their reproductive and behavioural ecology, is largely derived from chance observations of sharks caught by fisheries or from animals held in aquaria (Pierce et al., 2021). For example, the only pregnant shark that has ever been documented was harvested by a (now closed) Taiwanese fishery in 1994 (Joung et al., 1996; Hsu et al., 2012). This individual showed that the species is ovoviviparous and contained 300 young in various stages of development (Joung et al., 1996). Large female whale sharks with distended abdomens thought to be indicative of pregnancy have been recorded in the waters of Holbox Island, Mexico (Ramírez-Macías et al., 2012) Baja California Peninsula, Mexico (Ramírez-Macías et al., 2007), the Archipelago of São Pedro and São Paulo, Brazil (Macena and Hazin, 2016), the Galapagos Archipelago, Ecuador (Acuña-Marrero et al., 2014; Hearn et al., 2016), and at St. Helena, Central South Atlantic (Perry et al., 2020). However, hormone testing and ultrasound examination of these females in the Galapagos found them to be sexually mature, but not pregnant (Matsumoto et al., 2023). Thus, to date, the female captured in Taiwan remains the sole doumented example of pregnancy in the species.

Mating and courting behaviours of whale sharks have been observed in the wild at two locations. In the Atlantic Ocean, aggregations of whale sharks occur in the waters surrounding St Helena Islands and can include sexually mature males and females (Perry et al., 2020). Fishers have provided anecdotal evidence of male whale sharks in this aggregation swimming alongside females and rotating ventral side up in a ‘belly to belly’ position below the female, probably to insert their claspers (Perry et al., 2020). Researchers also reported what was presumed to be courtship behaviour, where a male followed a female and nudged the caudal fin of the female (Perry et al., 2020). At Ningaloo Reef, Western Australia, the pilot of a light plane photographed a larger (estimated size 9 m) whale shark attempting to grasp the pectoral fins of a smaller (estimated size 6.5 m) individual, and orient ventral-side-up below the smaller shark. As this was observed from the air, it could not be confirmed that the interaction involved a male and female shark (Gudgeon, 2019). Within aquaria, mating attempts by mature males towards unreceptive, immature females have shown the same processes of body inversions, biting of females, and the additional insight of clasper flexion (Pierce et al., 2021).

Given the rarity of observations of reproductive behaviour in whale sharks, any reports provide valuable insights into the ecology of this Endangered species (International Union for the Conservation of Nature Red List; Pierce and Norman, 2016). Here, we describe observations of what is assumed to be courtship behaviour between a mature male shark and a female of indeterminate sexual maturity in the waters off Ningaloo Reef in Western Australia.

Methods

Annual two-week field expeditions in early May (the peak of the seasonal aggregation of whale sharks) occurred at Ningaloo Reef, Western Australia, from 2009-2024 (Supplementary Table S1). A light plane was used to spot whale sharks and to direct our research vessel towards a sighting. Once in the vicinity of the shark, the research team entered the water to record body dimensions of sharks with stereo cameras (Sequeira et al., 2016; Meekan et al., 2020), take images for photo identification (Speed et al., 2007; Lester et al., 2020), use ultrasound scanners for internal images (Meekan et al., 2024), collect eDNA (Dugal et al., 2022), deploy satellite tags to understand movement behaviour (Sleeman et al., 2010; Thums et al., 2013; Meekan et al., 2015; D’Antonio et al., 2024) and collect tissue biopsies and parasitic copepods for trophic studies (Marcus et al., 2019; Meekan et al., 2022; Osorio et al., 2023). When we could obtain a clear view of the pelvic fins, we recorded the sex of each shark based on the presence or absence of claspers (Awruch et al., 2008). We recorded males as mature when their claspers were calcified and extended beyond the trailing edge of the pelvic fins (Norman and Stevens, 2007). We determined female sexual maturity based on total length (> 10 m; Nozu et al., 2015; Meekan et al., 2020; Matsumoto et al., 2023). If we were unable to observe the pelvic fins before the shark departed from surface waters the sex was recorded as unknown.

A sex ratio of males to females was calculated from the annual sightings data. Repeated sightings of the same sharks within each season of field work (identified by the presence of a recent scar from a tissue biopsy and by photo-identification) and sharks of unknown sex (approximately 20% of individuals) were excluded from this analysis.

Results

On the 14th of May 2024, our research vessel (Isurus; 7 m length) was directed by the spotter plane to a whale shark sighted at the surface offshore of the reef slope of Ningaloo Reef in waters ~ 40 m deep (22° 04’ 80” S 113° 52’ 51” E). Researchers entered the water at 13:15 AWST to sample a 7 m (total length) female whale shark. After length and photo-ID measurements were collected, a second whale shark, identified as a male, appeared and swam 2-3 m behind the caudal fin of the female. Photo-ID and length measurements of this male shark were also collected. The calcified claspers (Figure 1A) and size of the male (8.5 m) suggested that it was an adult (Norman and Stevens, 2007; Meekan et al., 2020). The behaviour of the male following the female was recorded by video (GoPro V. 11; Supplementary Video S1A; Figure 1B). One researcher also sampled the skin microbiome of the male (see Doane et al., 2023 for methods), during which time it maintained a close trajectory behind the female shark and appeared to be indifferent to the activity of the researcher (Supplementary Video S1B). Once the sampling was complete, researchers followed the male whale shark, which had remained swimming close to the caudal fin of the female. The male was observed to open its mouth and lunge forward towards the caudal fin of the female. The male shark increased its swimming speed and lunged again at the caudal fin of the female, this time making contact and briefly biting the tail (Supplementary Video S1C). The female responded by rapidly pivoting with pectoral fins pointing downwards to face the male (Supplementary Video S1C;

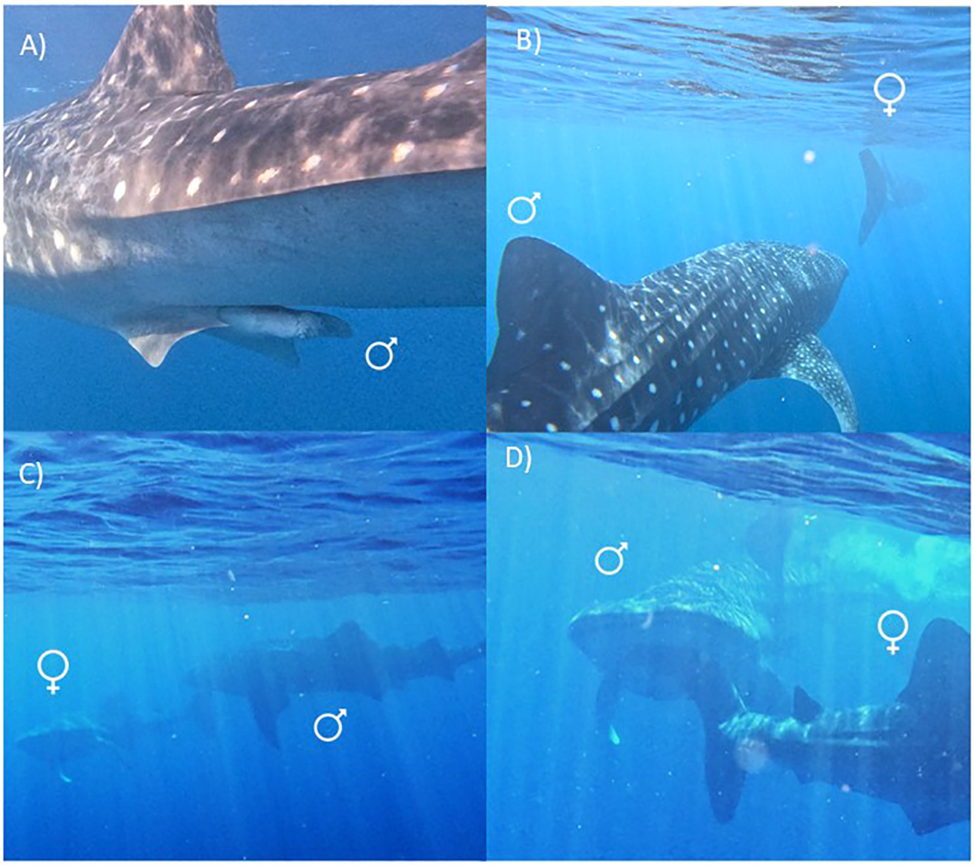

FIGURE 1
Whale sharks (*Rhincodon typus*) engaging in possible courtship behaviors at Ningaloo Reef. **(A)** Calcified clasper of the male shark. **(B)** An ~8.5 m male whale shark following closely behind a ~7 m female whale shark. **(C)** The female shark (left) rapidly pivoting after being bitten on the caudal fin by the male (right). **(D)** The caudal fin of the female whale shark (right) bending as it makes contact with the snout of the male shark (left). Key: ♀ = female, ♂ = male.

Figure 1C). After a brief pause in forward movement, the female again turned rapidly, with contact occurring between the snout of the male and the caudal fin of the female (Figure 1D). The female then rapidly descended to depth followed by the male (Supplementary Video S1C). At this point, the researchers lost sight of the sharks from the surface.

We recorded the sex of 1047 whale sharks at Ningaloo Reef across 2014-2024 (Supplementary Table S1). The mean sex ratio during this time was one female to three males (Figure 2).

Supplementary Video S1 available at: https://youtu.be/PhLx_7NHGCQ

Discussion

The pre-copulatory behaviours of male sharks commonly include the following and biting of females (Parsons et al., 2008). Our observations of whale sharks at Ningaloo are very similar to pre-copulatory behaviours witnessed in this species in aquaria and reported by fishers in the waters around St Helena Island (Macena and Hazin, 2016; Gudgeon, 2019; Perry et al., 2020; Pierce et al., 2021). Similar behaviours have also been reported for many other species, including scalloped hammerhead, *Sphyrna lewini* (Salinas-de-León et al., 2017), oceanic white tip, *Carcharhinus longimanus* (Talwar et al., 2023), tiger, *Galeocerdo cuvier* (Rangel et al., 2023) and basking *Cetorhinus maximus* (Curtis et al., 2024) sharks. Notably, male zebra sharks (*Stegostoma fasciatum*) – the closest living relative to whale sharks – have also been observed biting the tails of female zebra sharks in an effort to slow the movement of the female down, likely so they can position themselves for clasper insertion (Birt et al., 2019). The consistency of our observations with earlier reports of courtship in whale sharks and a wide variety of other species is evidence that we witnessed pre-copulatory behaviour.

At Ningaloo Reef, the behaviours we observed and those reported by Gudgeon (2019) did not appear to result in successful mating. Although it was possible that mating could have occurred after the sharks that we followed descended to deep water, the female appeared to actively resist the attention of the male. This is not surprising as the females in our study and Gudgeon (2019) report were unlikely to be sexually mature, based on their small sizes (6 - 7 m). These are much smaller than sizes at which maturity for

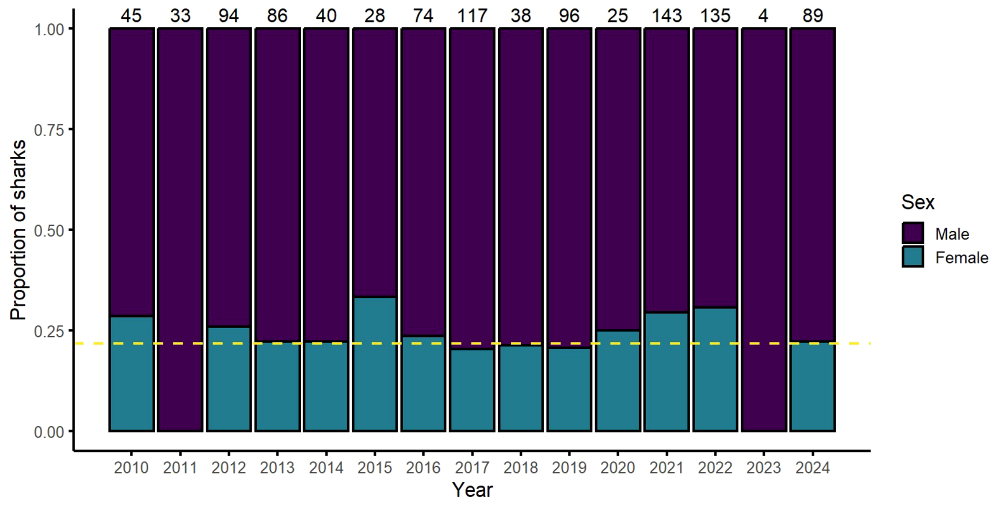

FIGURE 2
The proportionate sex of whale sharks (*Rhincodon typus*) encountered on annual research expeditions (see Supplementary Table S1) from 2010-2024. The yellow dashed line (x = 0.22) represents the mean sex ratio across the sampling period. Numbers along the top represent the number of individual whale sharks where sex was identified per year.

female whale sharks is thought to occur (~ 10 - 12 m; Nozu et al., 2015; Meekan et al., 2020; Matsumoto et al., 2023). Ningaloo (and many other locations) is male dominated, a pattern consistent across several decades (Meekan et al., 2006; Norman and Stevens, 2007). Studies between 1992 – 2004 estimated that only 15% (Norman and Stevens, 2007) and 17% (Meekan et al., 2006) of the sharks sighted at Ningaloo Reef were females, with a sex ratio of approximately 1:5 females to males. More recent data collected on our sampling trips between 2010-2024 found that on average, 22% of the sharks we encountered were females, a ratio of approximately 1:3 females to males (Figure 2). As the sex ratio of males and females at birth is roughly 1:1, as it is for most other species of sharks (Schmidt et al., 2010), this implies that some female whale sharks may be actively avoiding aggregation sites.

Segregation of habitats by size and sex is typical of many populations of elasmobranchs (Speed et al., 2010; Klimley et al., 2023; Wearmouth and Sims, 2010; Sims, 2005) and for juvenile female whale sharks, the energetic costs of unwanted attention from males may be one reason that there is a strong male bias at aggregation sites. This idea is consistent with social factor hypotheses (Wearmouth and Sims, 2008) as drivers of spatial patterns of sexual segregation in elasmobranchs. However, a variety of other hypotheses have been proposed to account for this spatial phenomenon (Wearmouth and Sims, 2008), which is very common in elasmobranchs (Wearmouth and Sims, 2010). Of these, the predation-risk hypothesis involves sharks segregating to avoid intra-species depredation from the larger sex and cannibalism of young (Wearmouth and Sims, 2008) and is obviously not applicable for a species that is a filter-feeder. Similarly, the thermal niche-fecundity hypothesis, which suggests that females choose different thermal habitats to increase reproductive success (Wearmouth and Sims, 2008) and the activity budget hypothesis, which argues that sexual dimorphisms and/or resource allocation into reproduction leads to different habitat requirements (Wearmouth and Sims, 2008) are also unlikely, given that the females at Ningaloo are mostly immature (with no mature length females recorded in our data) and have been observed feeding with males. Although these hypotheses can be discounted, it is still possible that there is some other advantage for females that choose to forage offshore (the foraging selection hypothesis; Wearmouth and Sims, 2008), beyond the avoidance of unwanted attention by males in inshore environments. Determining if this is the case will require observations of foraging of both male and female sharks in offshore habitats, a task that remains a major logistical challenge.

Intra-species competition and aggression could provide an alternate explanation for the biting behaviour we witnessed. This seems unlikely however, as no aggression seems to occur among whale sharks that are occasionally seen feeding in groups of two-to-three individuals at the water surface at Ningaloo Reef. These sharks typically circle around each other in a ‘yin-yang’ pattern (Gudgeon, 2019). Furthermore, cameras deployed on five whale sharks at Ningaloo Reef (see Barry et al., 2023) recorded no antagonistic interactions in 23 hours of footage, and no aggression has been reported in the literature.

Although the observed behavioural interaction reported here did not likely culminate in mating, our observations suggest that pre-copulatory behaviors of whale sharks resemble those of many other species of shark. Such records do not just expand our understanding of reproductive behaviors but may also provide insights into the potential drivers of sexual segregation reported in populations of whale sharks at many coastal aggregations.

Data availability statement

The data used in this study is available at: https://apps.aims.gov.au/metadata/view/57d5b0e7-c0f7-4e3d-bb3e-4ecb6ca8638f.

Ethics statement

The animal study was approved by Animal ethics approval was provided by permit 2019/RA/3/100/1715 of the University of Western Australia. All aspects of the field work program were approved by the management agency, the Western Australia Department of Biodiversity, Conservation and Attractions. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

CB: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Visualization, Writing – original draft, Writing – review & editing. EL: Conceptualization, Investigation, Methodology, Project administration, Writing – review & editing. MD: Conceptualization, Data curation, Investigation, Writing – review & editing. LF: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. MT: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. AG: Investigation, Methodology, Supervision, Writing – review & editing. MM: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

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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 Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2024.1507072/full#supplementary-material

Supplementary Video S1 available at: https://youtu.be/PhLx_7NHGCQ.

SUPPLEMENTARY TABLE 1

Annual sampling effort of whale shark (*Rhincodon typus*) sex at Ningaloo Reef across 2010-2024.

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New findings into the genetic population structure of two commercially valuable and threatened sharks, *Mustelus mustelus* (Linnaeus, 1758) and *M. punctulatus* (Risso, 1827), allow refining management strategy in the Central Mediterranean Sea

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Elasmobranch species are the direct or accidental catch of fisheries and can have a commercial importance. In the Mediterranean Sea, a long-term period of overfishing brought several demersal elasmobranchs to be depleted and threatened by extinction, due to vulnerability related to their life history traits. In such exploited species, information on genetic diversity and connectivity is lacking and should be collected to identify management units. In this study, we focused on two threatened smooth-hound species, *Mustelus Mustelus* (Linnaeus, 1758) and *M. punctulatus* (Risso, 1827), whose abundance and distribution showed a decline at the Mediterranean regional level in the last century. Thanks to an opportunistic yet extensive sampling, we obtained the largest subregional collection of specimens for genetic analysis so far. In total, 86 and 214 specimens of *M. mustelus* and *M. punctulatus* were collected between 2016 and 2020 in the Adriatic Sea and the Strait of Sicily. We assessed the population genetic structure typing 17 microsatellites and sequencing part of the mitochondrial control region in both species. We observed a substantial nuclear and mitochondrial genetic structure when accounting for the geographical sampling area for both species. Our results indicate the presence of at least two genetic stocks for each of the two species: one in the Strait of Sicily and the other in the Adriatic Sea. This study provides valuable data that should be integrated into a broader approach to define management units, improving the development of an effective management strategy for these threatened species in the Central Mediterranean Sea.

KEYWORDS

Triakidae, connectivity, elasmobranch, conservation, management

1 Introduction

The decline of elasmobranchs worsened during the late 20th and early 21st centuries, due to extensive global overexploitation of their populations (Dulvy et al., 2021). Their life history traits, including the slow growth rate and low fecundity, compared to other marine species, make elasmobranchs vulnerable to overfishing (Dulvy et al., 2017). Non-random space use (i.e., site fidelity and philopatry) and reproductive aggregations further contribute to their vulnerability (Hueter et al., 2005; Chapman et al., 2015; Hirschfeld et al., 2021). The conservation status of many elasmobranch species highlights the need for a scientific-tailored management plan (Dulvy et al., 2021). The concept of stock is a fundamental pillar in fishery management defined as an intraspecific group of randomly mating individuals with temporal or spatial integrity (Ihssen et al., 1981). The identification of genetic stock is a key step that could help delineate the geographic boundaries of populations characterized by consistent genetic distinctiveness (i.e., genetic structure) where divergence in allele frequencies is found at both nuclear and mitochondrial loci (Moritz, 1994). Ultimately, the combination of all ecological and biological traits (e.g., phenotype, movement, and genetics) leads to the definition of a management unit at different temporal organization (Abaunza et al., 2008; Cadrin et al., 2014).

Among evolutionary process, gene flow and genetic drift are two opposite forces that determine the genetic structure of a population and the genetic connectivity between adjacent populations. When gene flow occurs, the migratory movement and the successful mating of one or more individuals may contribute to the gene pool of a recipient population (Cadrin et al., 2014). Unlike species with larval stage, elasmobranchs are characterized by an active dispersal that occurs at the late juvenile/adult stage. Migrants may join an adjacent population without contribution to gene flow if successful mating relies on behavioral knowledge to engage with foreign reproductive aggregation (Ovenden, 2013). In elasmobranch species, genetic connectivity is influenced by maximum depth of occurrence, maximum body size, habitat, and species-specific dispersal potential (Hirschfeld et al., 2021).

In the Mediterranean Sea, elasmobranch species are overfished, and their decline was reported (Ferretti et al., 2013; Barausse et al., 2014; Walls and Dulvy, 2021). The common smooth-hound (*Mustelus mustelus*), hereafter *Mm*, and the black-spotted smooth-hound (*M. punctulatus*), hereafter *Mp*, are among the most important landed shark species in the Mediterranean Sea, as bycatch and being seasonally targeted by small-scale fishery, operating in the Northern Adriatic Sea and in the Strait of Sicily, two of the few Mediterranean areas where these species still show viable populations (Barausse et al., 2014; Colloca et al., 2017, Colloca et al., 2020; Carpentieri et al., 2021; Di Lorenzo et al., 2022; Maioli et al., 2023). Indeed, both species suffered a steep decline in the last century, with a strong contraction of their occurrence and abundance (Colloca et al., 2017), and they are classified in the International Union for Conservation of Nature (IUCN) red list as endangered (*Mm*) and vulnerable (*Mp*) at the global level (Jabado et al., 2021a; 2021b), as vulnerable at the Mediterranean level (Farrell and Dulvy, 2016; Dulvy et al., 2016), and endangered by the Italian IUCN (Rondinini et al., 2022). These two benthic species are found in coastal habitats and shelf area, and they both feed on crustaceans, with only adults preying large crustaceans, cephalopods, and small teleosts (Di Lorenzo et al., 2020; Finotto et al., 2023). Their morphology is similar, except for the presence of black dots in *Mp* and for a species-specific mouth shape (Marino et al., 2018). They also differ in the maximum size and the size at sexual maturity, with *Mm* being larger and maturing at a greater size than *Mp* (Riginella et al., 2020; Boscolo Palo et al., 2022). The seasonal movement of *Mm* was highlighted in the Northern-Central Adriatic Sea (Manfredi et al., 2010; Bonanomi et al., 2018; Barbato et al., 2021) and a strong site fidelity was described for both species in the Strait of Sicily (Boscolo Palo et al., 2022). Hybridization between these two species was detected only in a single clutch (Marino et al., 2015b).

Genetic structure differences were identified in other benthic Mediterranean elasmobranch species, linked to habitat fragmentation, heterogeneity, and the presence of oceanographic currents (Gubili et al., 2014; Catalano et al., 2022; Di Crescenzo et al., 2022; Melis et al., 2023). Consequently, a similar hypothesis can be formulated for both *Mustelus* species. To date, a differentiated genetic structure was described between Mediterranean subregions for nuclear DNA (nDNA) only in *M. mustelus* but not for mitochondrial DNA (mtDNA) (Hull et al., 2019).

An opportunistic yet extensive sampling of tissues for genetics, representing the first subregional effort, enabled this research on *M. mustelus* and *M. punctulatus*. This study aimed to 1) evaluate nuclear and mitochondrial diversity of the two species and 2) assess their genetic structure in two Mediterranean subareas, the Adriatic Sea, and the Strait of Sicily. To this end, we used 17 microsatellite loci—the largest panel of nuclear markers applied to these species—and partial sequencing of the control region (CR), a widely used mitochondrial marker. Microsatellites are highly polymorphic nDNA loci with repeated motifs [1–6 base pairs (bp)], typically located in non-coding regions unaffected by selection. Their co-dominant inheritance provides detailed insights into heterozygosity and genetic structure (Dudgeon et al., 2012). The CR, an mtDNA marker, is uniparentally inherited and has variable non-coding regions, less constrained by selection than protein-coding mtDNA genes, enabling analyses of haplotype and nucleotide diversity while complementing population differentiation studies (Phillips et al., 2021; Portnoy and Heist, 2012)

2 Materials and methods

2.1 Sample collection and DNA extraction

During scientific surveys on fishing vessels operating various fishing gears from 2016 to 2020, muscle tissue samples were opportunistically collected from 300 individuals (86 *Mm* and 214 *Mp*), representing populations from the Strait of Sicily (SIC; GSA 16) and the Adriatic Sea (ADRI; GSA 17 and 18). Within ADRI, samples were taken from the Italian Northern-Central coasts (N-ADRI; GSA 17) and the Montenegrin coasts (S-ADRI; GSA 18), with S-ADRI samples collected exclusively in 2020 (Figure 1; Supplementary Figures S1, S2). Sampled individuals were identified using the latest diagnostic morphological features from Marino et al. (2018) such as black spot presence, distance of the nostrils and mouth shape, and, when possible, dermal denticle observation under stereoscopes. Total length (TL; in centimeters), sex, and haul coordinates were recorded for individuals: *Mm* ranged from 40- cm to 150-cm TL (24 females and 14 males) and *Mp* from 30-cm to 120-cm TL (58 females and 68 males) in N-ADRI. In SIC, *Mm* ranged from 30- cm to 150-cm TL (21 females and 17 males) and *Mp* from 30- cm to 90-cm TL (30 females and 21 males). In S-ADRI, only female *Mp* were sampled, ranging from 92-cm to 138-cm TL (Figure 1); only three *Mm* were sampled in S-ADRI and were not included in the analyses. From each individual, a muscle sample was collected and stored in pure grade ethanol at 4 degree Celsius (°C) for further analyses.

Genomic DNA was extracted from 30-mg to 40-mg tissue samples by salting-out protocol (Patwary et al., 1994), and the extract quality was checked by 1% agarose gel in TBE buffer (1×) electrophoresis, with GelRed stain (0.025 µL/mL; Biotium). Extracted DNA was conserved at −20°C.

2.2 Nuclear DNA amplification

A total number of 19 loci were used (Supplementary Table S1.1), starting from previously tested microsatellites (Boomer and Stow, 2010; Chabot and Nigenda, 2011; Giresi et al., 2012; Marino et al., 2015a; Maduna et al., 2017). Among these, four loci were amplified in only one of the two species, according to previous successful amplification and testing (Marino et al., 2015a; Maduna et al., 2017), namely, Mmu9 and Gg22 in *Mm* and MaND5 and Mh29 in *Mp*. Thus, for each species, 17 microsatellites for each species were included throughout the analysis. The microsatellites were divided into three groups and amplified by a Multiplex PCR kit (QIAGEN) according to the published amplification profiles (Marino et al., 2015a; Hull et al., 2019). For each microsatellite, fluorophores were chosen according to length to avoid overlapping (Supplementary Table S1.1). A separate single locus PCR was carried out for the Mmu11 locus, and PCR products were assembled before sending to the genotyping service (see Supplementary Materials for Mmu11 amplification profile). This was necessary only in Mmu11 because the allelic peaks did not match when comparing single and multiplex amplifications. After checking the successful amplification by electrophoresis in 1.8% agarose gel, PCR products were sent to the BMR Genomics (Padua, Italy) for genotyping service where an ABI Prism 3100 Genetic Analyzer and LIZ500 as size standard were used. Scoring to examine the allelic profiles was performed for each sample by the software PEAK SCANNER v1.0 (Applied Biosystems). The binning was done by FLEXIBIN (Amos et al., 2007).

2.3 Mitochondrial DNA amplification

For the CR amplification, the primer pair MaCYB/MaDLP was used (Hull et al., 2019), following the protocols reported therein. After confirming the amplification by electrophoresis, amplicons were purified by EuroSAP - PCR Enzymatic Clean-up (Euroclone, Italy) and then sent for Sanger sequencing at the Eurofins Genomics (Colone, Germany). All sequences were manually checked for quality by FinchTV (Geospiza Inc.) and then trimmed, edited, and forward primer–removed. All sequences were visualized in Mega 6 (Tamura et al., 2013) and aligned by Muscle algorithm (Edgar, 2004) with default parameters.

The sequenced fragment encompassed the last part of cytochrome b, two Transfer Ribonucleic Acid (tRNA), and the first part of the CR (orientation 5′-3′) when compared to the full mitochondrial genome of *Mm*, available on GenBank (accession number MH559351.1) by Blastn (Altschul et al., 1990) and Clustal Muscle alignment (Edgar, 2004). The initial sequenced fragment was composed of 713 bp and the first 230 bp encompassed the cytochrome b and tRNAs. The remaining 483 bp included the beginning of the CR region, which started at base position 15638 of the *Mm* complete genome (Supplementary Figure S3). All the downstream analyses were performed considering only the CR fragment (483 bp).

2.4 Genetic diversity

Microsatellite genotypes were first checked for null alleles using MicroChecker v2.2.3 (Van Oosterhout et al., 2004), and their presence was evaluated on the estimation of genetic structure by FreeNA (Chapuis and Estoup, 2007). GENEPOP ON THE WEB v4.2 (Raymond and Rousset, 1995; Rousset, 2008) was used for testing linkage disequilibrium (LD) and deviations from Hardy–Weinberg equilibrium (HWE). For statistical significance of multiple tests, Benjamini–Hochberg correction (B-H; Benjamini and Hochberg, 1995) was applied with the function *p.adjust* on RStudio 1.3.1093-1 (RStudio Team, 2020). Bayesian structure analysis by STRUCTURE (Pritchard et al., 2000) was run to obtain the most reliable species identification for each specimen, assuming an admixture ancestry model with independent allelic frequencies and without a prior on sample origin (see Supplementary Materials for more details). Using the species identification based on STRUCTURE, for each species, the nDNA genetic diversity was calculated using the R package DiveRsity (Keenan et al., 2013), as the number of alleles observed per locus per population sample (A), the allelic richness (A_R), the observed and expected heterozygosity (H_O and H_E), and the inbreeding coefficient (F_{IS}). For the mtDNA, the CR diversity was calculated by DnaSP (Rozas et al., 2017) on the basis of the same species identification. Total number of haplotypes (H), haplotype diversity (h), and nucleotide diversity (π) were obtained. The haplotype network was created using TCS (Clement et al., 2000) and edited with PopART (Leigh and Bryant, 2015). To avoid that population structure was influenced by family structure, Colony v2.0.6.7 (Jones and Wang, 2010) was used for each species in separate runs selecting for female and male polygamy with inbreeding and cloning, by full-likelihood method at 95% and no prior. Only one sample for each full-sibling pair was kept for further analysis (see Supplementary Materials).

2.5 Population differentiation

To assess the level of genetic structure among the sampling sites in the two species, a Bayesian clustering analysis by STRUCTURE was performed with correlated allelic frequencies together with the abovementioned settings and software to account for uneven sample size.

For both nDNA and mtDNA, genetic differentiation, pairwise F_{ST}, and Φ_{ST} respectively, was determined by ARLEQUIN (Excoffier and Lischer, 2010) with 10^4 permutations across sampling sites. Only for the *Mp*, the comparisons were performed between the ADRI and SIC (pooling together N-ADRI and S-ADRI), and among N-ADRI, S-ADRI, and SIC. When appropriate, B-H correction for multiple comparisons was performed with experiment-wide significance at $p\text{-values} < 0.01$. To assess whether unbalanced sample size in *Mp* may have affected the genetic differentiation, a random subsampling was performed, taking 50 random individuals from N-ADRI (N = 37) and S-ADRI (N = 13) while also balancing the sex ratio; the metrics of genetic differentiation were computed as described above.

The discriminant analysis of principal components (DAPC) (Jombart et al., 2010) was used to investigate population genetic structure. By summarizing between-group variation and ignoring within-group variation, DAPC effectively identified genetic structure within the samples. This multivariate approach assigned individuals to distinct groups and assessed inter-population differentiation without relying on specific population genetics models (e.g., HWE and LD).

3 Results

3.1 Genetic diversity

3.1.1 Nuclear DNA

DNA of satisfactory quality was obtained from the 300 available tissue samples, and it was used to confirm the morphological identification using STRUCTURE. The optimal K was equal to 2 and cluster analysis allowed identifying 86 *Mm* and 214 *Mp*. Overall, four samples were discarded because of too many missing loci (Supplementary Figure S4). Using F_{ST}, the genetic divergence between the two species was 0.63 ($p\text{-value} < 0.001$). The presence of null alleles did not affect the estimates of genetic structure (Supplementary Table S2), and no significant deviation from HWE (Supplementary Table S3) and LD (data not shown) was detected across sampling sites. Thus, all loci were kept for further analyses. In *Mm*, a low genetic diversity emerged at both locations, with A_R around 3.5 and Ho between 0.33 and 0.36, whereas *Mp* samples showed even smaller values, with A_R between 2.14 and 2.33 and with Ho between 0.22 and 0.26. F_{IS} turned out to be positive across locations (Supplementary Table S4).

3.1.2 Mitochondrial DNA

The mtDNA CR of 83 samples of *Mm* and 207 samples of *Mp* was successfully sequenced, whereas 10 samples were not kept for further analyses (Supplementary Table S5). Among the excluded samples, seven *Mp* (N = 3 from the N-ADRI, 1 from the S-ADRI, and 3 from the SIC) and three *Mm* from SIC had poor sequencing quality or failed amplification. One specimen was identified to be an introgressed hybrid and discarded. In detail, the individual (sample #S301) had a CR haplotype belonging to *Mp*, but it was identified as a sexually mature male of 135 cm of TL *Mm* based on morphology, and this species identification was confirmed by STRUCTURE. In this sample, only one of the two *Mm* species-specific microsatellites (Mmu9) was successfully amplified. Even though the sample was excluded from further analysis, being out of the scope of this study, its haplotype was accounted for in the haplotype occurrence.

In *Mm*, a moderate variation was found, with four haplotypes and three or two segregating sites, respectively, in N-ADRI and SIC. In *Mp*, the haplotype number ranged from 3 to 5 and the segregating sites between 2 and 5 (Supplementary Table S5). In *Mm*, the haplotype N3 was the most frequent, present in 63% of individuals, followed by N4. Haplotype N2 and N10 were only found in the SIC and the N-ADRI, respectively (Figure 2, Supplementary Table S6). *Mp* has 83% of individuals with haplotype N1, and N7 was the second most frequent haplotype. *Mp* presented three unique haplotypes in the N-ADRI (N11, N12, and N13), one in the S-ADRI (N9), and two in the SIC (N6 and N8) (Figure 2, Supplementary Table S7).

3.2 Population differentiation

3.2.1 Microsatellites

Without using prior information of sample origin, the cluster analysis in STRUCTURE did not evidence the presence of structure between the ADRI and the SIC (Supplementary Figure S5) for both species, with STRUCTURESELECTOR metrics indicating that the optimal K was equal to 1. From ARLEQUIN analysis, however, microsatellite-derived F_{ST} showed a statistically significant differentiation between the population samples from N-ADRI and SIC in *Mm* ($F_{ST} = 0.02$, p-value = 0.005) and between ADRI and SIC in *Mp* ($F_{ST} = 0.03$, p-value = 0.003); for this latter species, genetic differences did not emerge between N-ADRI and S-ADRI samples ($F_{ST} = 0.002$, p-value = 0.23), whereas divergence was found between S-ADRI and the SIC ($F_{ST} = 0.02$, p-value < 0.001). The result of significant genetic differentiation between ADRI and SIC in *Mp* was further investigated, possibly biased by the unbalanced sample size of the two population samples (N = 163 and 51, respectively). To this end, the analysis was repeated, randomly subsampling 50 ADRI individuals from the genetically homogeneous N-ADRI and S-ADRI population samples (see Materials and Methods) and comparing them with the 51 SIC individuals, confirming our finding ($F_{ST} = 0.03$, p-value < 0.001) (Table 1).

DAPC analyses revealed a clear distinction for *Mm* among the two sampling sites (N-ADRI) and SIC, with a reasonable power to reassign individuals from the two locations using our microsatellites data, albeit with a slight overlap between the two regions (Figures 3A, B). On the other hand, DAPC analyses on *Mp* revealed no distinction between N-ADRI and S-ADRI, but a significant level of correct re-assignment for SIC versus N-ADRI, with still an overlap between the two clusters (Figures 3C, D).

3.2.2 Control region

CR-derived Φ_{ST} values showed significant differentiation in both species sampled in the ADRI and the SIC ($\Phi_{ST} = 0.12$, p-value = 0.001, in *Mm*; and $\Phi_{ST} = 0.086$, p-value < 0.001 in *Mp*). When accounting for uneven sample size in *Mp*, randomly subsampled sequences from N-ADRI and S-ADRI confirmed the

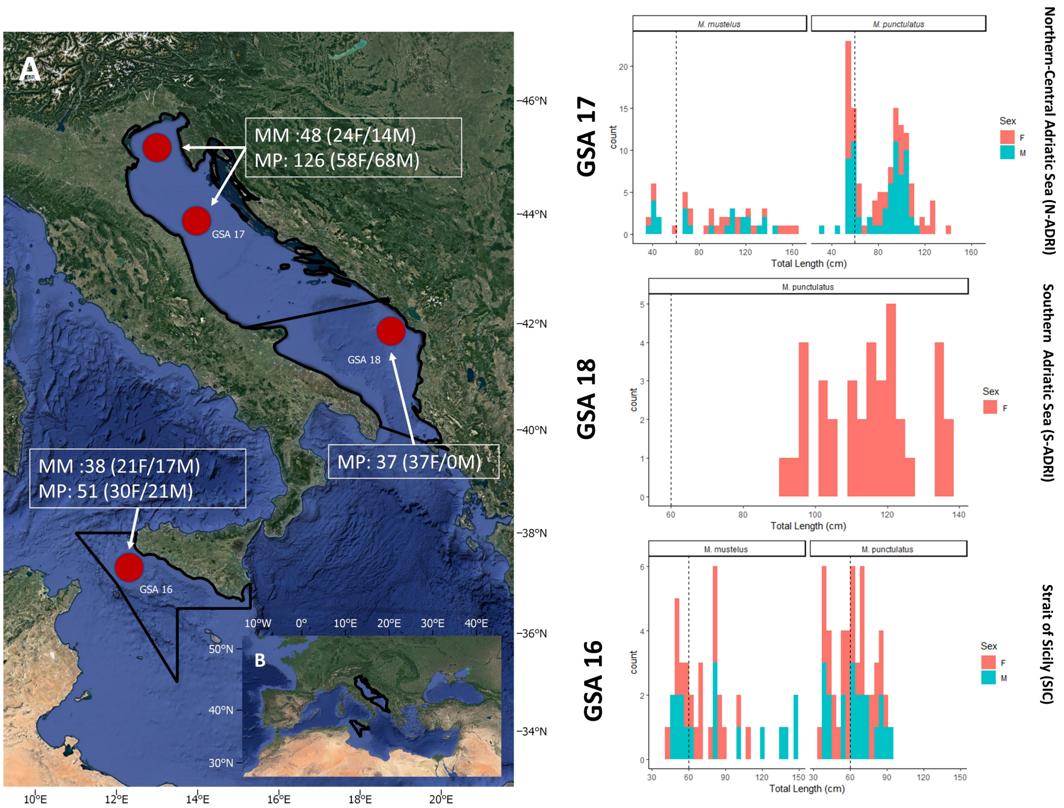

FIGURE 1
(A) Red points indicate cumulatively all sampling coordinate points in the North-Central Adriatic Sea (GSA17), defined as N-ADRI, the Southern Adriatic Sea (GSA18), defined as S-ADRI, and the Strait of Sicily (GSA16), defined as SIC, in the Mediterranean Sea (Google Maps, 2023); **(B)** length frequency distribution of the sampled *M. mustelus* (Mm) and *M. punctulatus* (Mp) females (red) and males (green) from each sampling locations (red circles in the map A). Plots were created by 'ggplot2' R package (Wickham, 2016).

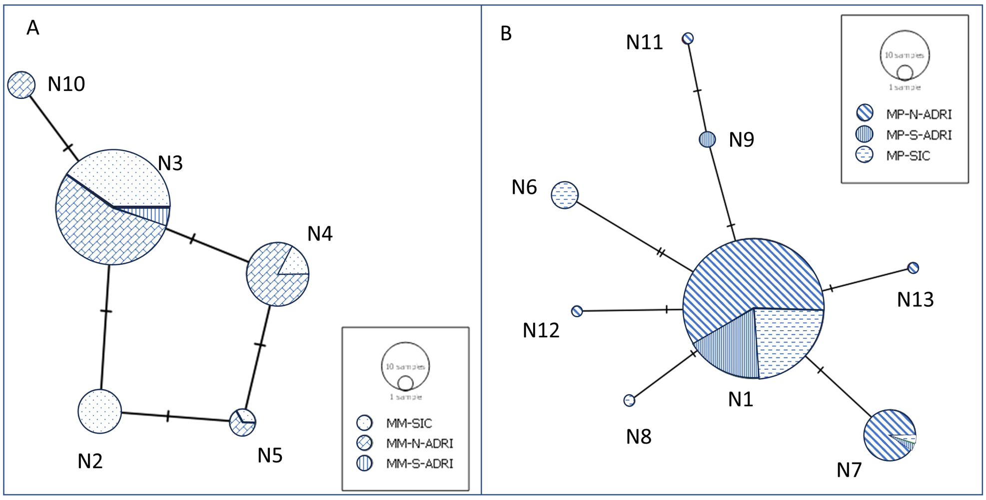

FIGURE 2
Haplotype network of the sequenced control region fragment of *M. mustelus* **(A)** and *M. punctulatus* **(B)**. The size of the circles represents the number of samples belonging to a unique haplotype.

differences with SIC (Φ_{ST} = 0.07, p-value < 0.001). After B-H correction, significant genetic divergence emerged only in *Mp* between the N-ADRI and the SIC (Φ_{ST} = 0.09, p-value < 0.001), whereas no statistical significance was observed between the N-ADRI and S-ADRI (Φ_{ST} = 0.03, p-value = 0.026) and between the S-ADRI and the SIC (Φ_{ST} = 0.06, p-value = 0.026) (Table 1).

4 Discussion

4.1 Genetic diversity

A low genetic diversity emerged in this study, particularly evident in the nDNA of *Mp* with an expected heterozygosity of about 0.25 and only about two alleles expected per locus. For *Mm*, the genetic diversity was slightly higher, with about 35% expected heterozygotes and 3.5 alleles per locus, and, to a small extent, greater than what previously found in *Mm* with only nine microsatellites (Hull et al., 2019). The low nuclear diversity found in our study is in line with the one found in other benthic elasmobranchs in the Mediterranean populations such as the *Galeus melastomus* in the southern Adriatic Sea and in Sicily (A_R = 2.7–2.8 and H_E = 0.36–0.4; Di Crescenzo et al., 2022) and the *Raja asterias* in the northern Adriatic Sea (A_R = 3.35 and H_E = 0.56; Catalano et al., 2022). On the other hand, a higher nuclear diversity (A_R = 5.2–6.5, H_E = 0.56–0.76) was reported in the northern Adriatic Sea for other benthic elasmobranch species, such as the *Schyliorhinus canicula* (Gubili et al., 2014), the *R. clavata* (Melis et al., 2023), and the epibenthic *Squalus acanthias* (Gračan et al., 2020). The mtDNA diversity found in our study mirrors the pattern observed with nDNA, showing a very low variability in *Mp* (h = 0.16–0.31 and π = 0.0003–0.001) and higher values in *Mm* (h = 0.52–0.54 and π = 0.001); in *Mm*, despite the shorter sequenced fragment in our study, CR showed similar diversity values to those previously found (Hull et al., 2019). CR diversity, however, is known to vary between closely related species and within the same species (Subramanian

TABLE 1 Pairwise F_{ST} and Φ_{ST} values were calculated, respectively according to microsatellite on nuclear DNA and to controlo region (CR) on mitochondrial DNA comparing the sampling origins (N-ADRI, Northern-Central Adriatic Sea; S-ADRI, Southern Adriatic Sea; SIC, Strait of Sicily); B-H correction was applied only for *M. punctulatus* when comparing multiple sampling sites, marked by the asterisk, and statistical significance was set at p-values < 0.01, reported in bold when significant.

		M. mustelus	*M. punctulatus*			
		N-ADRI-SIC	ADRI/SIC	N-ADRI/S-ADRI	N-ADRI/SIC	S-ADRI/SIC
nDNA	**F_{ST}**	**0.02**	**0.03**	0.002	**0.03**	**0.02**
	p-value	**0.005**	**0.003**	0.23*	**<0.001***	**<0.001***
	Sample size	48:38	163:51	126:37	126:51	37:51
CR	**Φ_{ST}**	**0.12**	**0.086**	0.03	**0.09**	0.06
	p-value	**0.001**	**<0.001**	0.026*	**<0.001***	0.026*
	Sample size	48:35	160:48	123:36	123:48	36:48

B-H correction was applied only for M. punctulatus when comparing multiple sampling sites, marked by the asterisk. Statistical significance was set at p-values < 0.01, reported in bold when significant. N refers to the sample size.

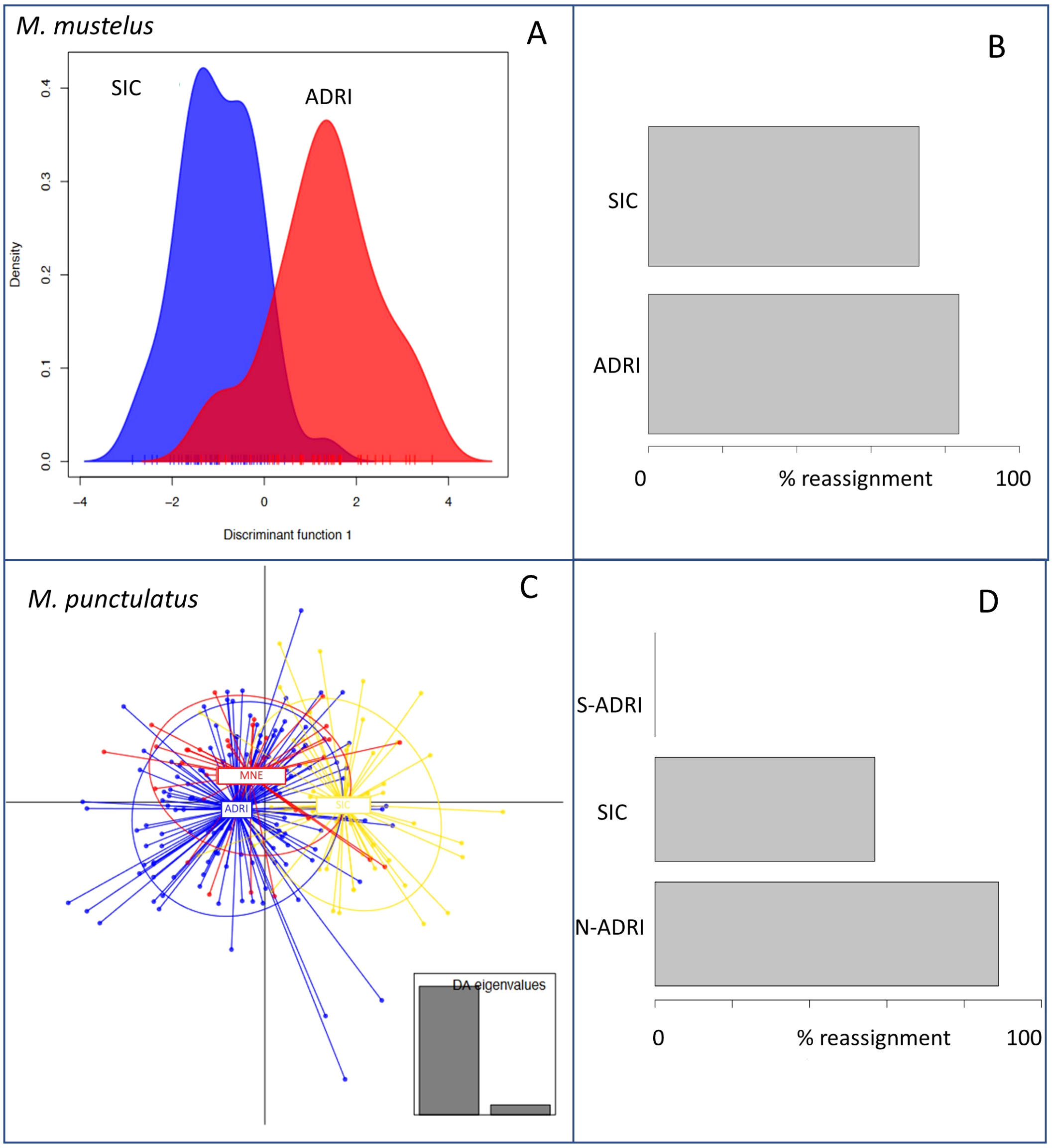

FIGURE 3
DAPC analysis accounting for the sampling origin for *M. mustelus* **(A, B)** and *M. punctulatus* **(C, D)**. For the two studied species, the principal component diagram **(A, C)** and the bar plot of the percentage of reassignment **(B, D)** are shown. The average percentage of correct reassignment for *M. mustelus* is 0.9, which indicates a high degree of discrimination between SIC and ADRI clusters. On the other hand, we have an average reassignment around 0.68 for *M. punctulatus*. This value is due to the low level of discrimination between N-ADRI and S-ADRI (e.g., samples from Montenegro) clusters. Population codes: ADRI (Adriatic Sea); N-ADRI (North Adriatic Sea); S-ADRI (South Adriatic Sea, Montenegro coast); SIC (Strait of Sicily).

and Lambert, 2011). Accordingly, several studies in other elasmobranch species reported very different patterns with a moderate to high CR genetic diversity in some species [reviewed by Hull et al. (2019); h = 0.78–0.99 and π = 0.004–0.35], including *S. canicula* from the Adriatic Sea (h = 0.71 and π = 0.003; Gubili et al., 2014).

For the aforementioned benthic elasmobranch species, whose genetic diversity was investigated in many Mediterranean subareas (Gubili et al., 2014; Catalano et al., 2022; Di Crescenzo et al., 2022; Melis et al., 2023), including the two smooth-hound species of the present study, their steep decline found support in literature (Colloca et al., 2017; Ferretti et al., 2013; Dulvy et al., 2021). This calls for further research to deepen the understanding of fishery-induced effect of the genetic diversity of over-exploited elasmobranch species (Domingues et al., 2018)

4.2 Population differentiation

The evidence of genetic structure found in our study for *Mm* confirms and extends what was previously known at the global level for this species (Hull et al., 2019). In fact, although, as expected, our extended microsatellite panel confirms the existence of genetic differentiation between the Adriatic Sea and the Strait of Sicily, our study adds new support for the occurrence of a genetic structure between these two Mediterranean areas that, thanks to our higher sample size, were detected, for the first time, using also mtDNA. In *Mm*, the occurrence of one high-frequency shared haplotype in SIC and ADRI could be due to a recent common ancestor, whereas the other shared haplotypes could be the result of recent immigration. However, the existence of the unique haplotype (N10) points out to the occurrence of some level of isolation reflecting either localized adaptation or bottlenecks in N-ADRI. In *Mp*, although one prevailing ancestral haplotype was found, multiple unique haplotypes were detected in both N-ADRI, S-ADRI, and SIC, highlighting a higher degree of isolation and genetic drift. Through sequencing a longer CR fragment, focused research could discern whether mtDNA divergence could also result from a sex-biased dispersal (Hirschfeld et al., 2021; Phillips et al., 2021). However, the haplotype network and the value of Φ_{ST} and F_{ST} could potentially be explained by alternative hypothesis such as female-mediate gene flow, incomplete lineage sorting, or strong population genetic declines, as also discussed at global level in Hull et al. (2019). This result of genetic differentiation is in line with what reported for this species at the global scale, *Mm* displayed a significant level of isolation shaped by the effect of oceanic currents or other biogeographical barriers, despite the capability to undertake rare and long migrations (Mann and Bullen, 2009; da Silva et al., 2013; Maduna et al., 2016). In South African water, the combination of tagging and genetic methods in *Mm* contributed to find a contrasted dynamic between a strong site fidelity in a relatively short period and a significant gene flow at evolutionary scale (Klein et al., 2022).

With regard to *Mp*, our study provided the first evidence for a significant structure between SIC and ADRI, with a pattern that mirrors the *Mm* structure. It is worth noting that seasonal and permanent currents occur in this area; in particular, the ADRI circulation consisted in wind-driven currents, one from south to north along the eastern coast and another one from north to the south in the western coast, and three seasonal gyres (Russo and Artegiani, 1996). The ADRI hydrodynamics did not appear to influence the genetic differentiation between N-ADRI and S-ADRI in *Mp* while being responsible for the genetic structure of many marine species with larval dispersal (Papetti et al., 2013; Matić-Skoko et al., 2018). Similarly, other populations of *Mustelus* species appeared to be genetically structured, including the gummy shark (*M. antarcticus*) in Australian waters despite a wide movement range (238–900 km) (Braccini et al., 2017) and the brown smooth-hound (*M. henlei*) in the Gulf of California (Sandoval-Castillo and Beheregaray, 2015). Our analyses of re-assignment using DAPC further supported the concept of two distinct and yet slightly connected populations, highlighting the presence of a semi-permeable barrier between the Adriatic Sea and the Mediterranean Sea.

Despite being opportunistic, our sampling represents the first coordinated subregional effort, yielding the largest genetic dataset for these exploited species. Combining microsatellite genotyping with CR sequencing, we detected genetic diversity and a statistically significant population structure between the Adriatic Sea and the Strait of Sicily, confirming two distinct genetic stocks for *Mm* (SIC) and *Mp* (ADRI) for the first time. Our results were in line with previous analyses on Mediterranean and benthic elasmobranch species that showed significant genetic structuring between Mediterranean areas (Gubili et al., 2014; Catalano et al., 2022; Di Crescenzo et al., 2022; Melis et al., 2023). The diversity of habitats present between ADRI and SIC, encompassed by deep water and the presence of the Strait of Otranto, seemed to act as an only semi-permeable dispersal barrier for many populations of elasmobranch species, highlighting the importance of identifying genetic stock. Behaviors like philopatry and sex-biased dispersal may play a role in shaping the genetic structure and contribute to the connectivity in elasmobranch (Chapman et al., 2015; Phillips et al., 2021), but, due to the opportunistic sampling, this study was not able to address this goal and needs to be further investigated. Considering the stock definition (Ihssen et al., 1981), fishery management of these two stocks could be improved tailoring stock specific strategies that consider different growth rate, reproduction and nursery areas, and harvesting dynamic (Cadrin et al., 2014). The commercial relevance and the conservation issues of *Mm* and *Mp* in the two Mediterranean populations require the delineation of management units. Albeit a holistic approach is needed, our study provides solid evidence in relation to the genetic stocks of these exploited and threatened shark species.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material. The datasets generated during and/or analysed during the current study are available in the Figshare repository: https://doi.org/10.6084/m9.figshare.22652257.

Ethics statement

Samples from the Adriatic Sea were collected from dead specimens caught during professional fishing activity and, therefore, no approval from the local ethics committee was necessary. In the strait of Sicily, all procedures carried out were approved by the international authorities (EU/DG Mare, FAO/GFCM). All specimens and methods were caught and performed in accordance with the relevant guidelines and regulations. In the cases the animal was alive when it arrived on the vessel during the scientific survey (MEDITS-DCF, EU Reg. 199/2008), it was suppressed in compliance with the recommendation of Decree Law n. 26 of 4 March 2014. All efforts were made to minimize suffering. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

MB: Writing – original draft, Writing – review & editing, Conceptualization, Data curation, Formal analysis, Investigation, Methodology. SB: Funding acquisition, Writing – review & editing. DB: Investigation, Writing – review & editing. IC: Investigation, Writing – review & editing. FC: Investigation, Writing – review & editing. MD: Investigation, Writing – review & editing. IM: Conceptualization, Data curation, Investigation, Methodology, Writing – review & editing. CM: Conceptualization, Funding acquisition, Writing – review & editing. AP: Investigation, Writing – review & editing. AS: Funding acquisition, Writing – review & editing. LZ: Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing. MM: Conceptualization, Investigation, Methodology, Supervision, Validation, Writing – review & editing.

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Conflict of interest

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Evidence of cascading ecosystem effects following the loss of white sharks from False Bay, South Africa

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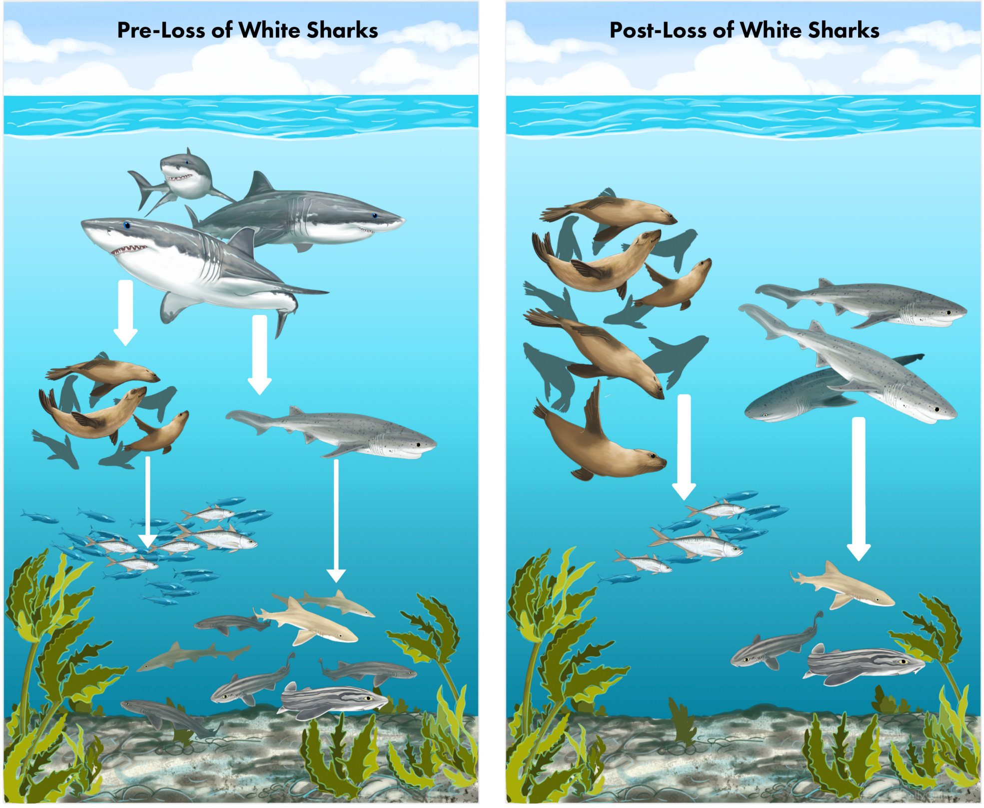
Introduction

Top predators can affect the density (Creel and Winnie, 2005), physiology (Leach and Taylor, 1977; Woodley and Peterson, 2003), and behavior (Werner et al., 1983) of prey. Such direct impacts on prey may indirectly impact multiple trophic levels (Ripple et al., 2001; Croll et al., 2005; Hammerschlag et al., 2019). Apex predators are among the most threatened species globally, experiencing widespread global population declines (Ripple et al., 2014). Accordingly, this has led to a growing concern for and need to understand the associated ecological consequences of apex predator loss on food webs (Estes et al., 2011).

Sharks are upper-level predators in virtually all marine environments (Cortes, 1999). Research has shown that spatial and temporal variations in shark presence has the potential to alter the abundance and behavior of their prey, indirectly impacting habitat structure (reviewed by Dedman et al., 2024). For example, on a Fijian coral reef, Rasher et al. (2017) found that differences in topography and tidal state created patches of reef that varied spatially and temporally in shark presence. At locations and times where shark encounter rates were high, herbivorous fish reduced grazing activity. This predator-driven alteration to fish behavior created spatial refuges for seaweed from grazing, resulting in higher seaweed biomass (Rasher et al., 2017). In light of global shark population declines from overfishing, understanding and predicting the consequences of shark loss on ecosystems been identified as a research priority (Jorgensen et al., 2022; Dedman et al., 2024). However, empirical evidence of the ecological impacts of large shark declines on prey populations in the wild are limited (Ruppert et al., 2013; Barley et al, 2017a; Barley et al., 2017b, Hammerschlag et al., 2018, Hammerschlag et al., 2019, Hammerschlag et al., 2022) and may be context-dependent (Sandin et al., 2022). For example, on the great barrier reef, Desbiens et al. (2021) did not find an impact of shark density on either density or biomass of teleost mesopredators and prey; instead, many functional groups, including sharks, were more associated with environmental drivers. Moreover, evidence for indirect cascading ecosystem effects of shark removals across multiple trophic levels is scarce (Ferretti et al., 2010; Myers et al., 2007). These knowledge gaps likely stem from the methodological,



GRAPHICAL ABSTRACT
This study provides empirical evidence of a trophic cascade following the loss of white sharks (*Carcharodon carcharias*) from False Bay, South Africa. Arrow thickness represents the relative strength of top-down predation effects (thicker arrows indicate stronger effects, thinner arrows indicate weaker effects). **Left Panel:** Historically, white sharks occupied the apex of the food web, preying on Cape fur seals (*Arctocephalus pusillus pusillus*) and both competing with and feeding on sevengill sharks (*Notorynchus cepedianus*). Seals primarily preyed on schooling fishes, while sevengill sharks primarily preyed on benthic sharks. **Right Panel:** Following the decline and eventual disappearance of white sharks from False Bay, both seals and sevengill sharks have increased in relative abundance, coinciding with declines in small fish that seals feed on and smaller sharks that sevengills prey upon. Illustration by Kelly Quinn / Canvas of the Wild.

logistical, and financial challenges of conducting experimental research on large, highly mobile predators in vast marine environments (Hammerschlag, 2019).

Here we evaluated a unique dataset collected as part of a 20+ year monitoring program from False Bay, South Africa, which documented the decline and ultimate disappearance of white sharks (*Carcharodon carcharias*) from the system (Hammerschlag et al., 2019, Hammerschlag et al., 2022). While the exact reason behind the loss of white sharks from False Bay is unclear (Gennari et al., 2024), known removals of white sharks by the KwaZulu-Natal Sharks Board's lethal shark control program are high enough to drive regional population declines (Bowlby et al., 2022). Although not yet recorded from False Bay, instances of white shark mortality due to specialized shark-eating orcas (*Orcinus orca*) have been documented elsewhere along the coastline (Towner et al., 2022), likely contributing to the decline (Gennari et al., 2024).

In False Bay, standardized boat-based surveys of white shark sightings at Seal Island occurred between 2000-2020 providing a metric of relative abundance over time based on the number of individual white sharks sighted per hour of observational effort (Skubel et al., 2018; Hammerschlag et al., 2019). The overall trend in the relative abundance of white sharks was relatively stable in the first fifteen years of monitoring; however, their relative abundance began to precipitously decline after 2015, leading to a complete disappearance of white sharks from the standardized surveys since mid-2018 (Hammerschlag et al., 2022). This rapid decline and subsequent loss of white sharks from False Bay coincided with rapid behavioral and physiological response in their prey, Cape fur seals (*Arctocephalus pusillus pusillus*), including reductions in seal stress levels and increasing seal rafting distances from Seal Island, a behavior that would have rendered seals vulnerable to shark attack (Hammerschlag et al., 2022). The loss of white sharks from False Bay also coincided with the novel emergence of sevengill sharks (*Notorynchus cepedianus*) in boat-based surveys at Seal Island (Hammerschlag et al., 2019). Historically, this large-bodied shark would have been an important prey of and competitor with white sharks in False Bay.

Based on ecological theory, here we investigated for potential trophic cascades resulting from the rapid decline and loss of white sharks in the system. Given established predator-prey dynamics in False Bay, we hypothesized that the loss of white sharks in the system would coincide with increases in the relative abundances of Cape fur seals and sevengill sharks, which in turn would be associated with decreases in the relative abundances of their prey, such as small pelagic fishes in the case of seals and smaller benthic sharks in the case of sevengill sharks.

Methods

Study site

Located off South Africa, False Bay is nearly 1000 km^2 (Pfaff et al., 2019; Figure 1). Historically, white sharks could be found year-round in False Bay, spending more time within inshore areas during warmer months, while during colder months spending relatively more time patrolling the waters surrounding Seal Island, a rocky outcrop situated 10 km off the Bay's northern shoreline (Kock et al., 2013). Seal Island may be inhabited by as many as 60,000 Cape fur seals that were targeted prey of white sharks during colder months, whereas white sharks more time within inshore areas during warmer months (Kock et al., 2013), presumably to increase foraging on large teleosts and elasmobranchs, such as sevengill sharks (Martin et al., 2005; Kock et al., 2013).

Boat-based surveys at Seal Island

Between 2000 and 2020, shark relative abundance at Seal Island was monitored from standardized boat-based observation surveys (details in Hammerschlag et al., 2019). While surveys occurred year-round, most observations occurred during colder months (May through September) (Hammerschlag et al., 2006; Fallows et al., 2012). During boat-based surveys, sharks were attracted to the boat using a line baited with fish and/or a seal decoy. Individual sharks were identified based on a combination of visual makers, including unique scarring, presence/absence of claspers, and individual variation in pigmentation patterns on the gill flaps, pelvic fins, and caudal fins. The duration of each baited survey was recorded, along with the number of different individual sharks observed during this period. Using these data, the number of different individual sharks observed per hour of baited survey was calculated as a metric of shark relative abundance (Hammerschlag et al., 2019). We used these data to calculate annual means of shark relative abundance at Seal Island, as well as overall means in the period prior to and following the decline of white sharks from False Bay (2000-2015 *vs* 2016-2020, i.e. pre-loss *vs* post-loss period).

Seal sightings

Sightings data of Cape fur seals in False Bay were derived from the Global Biodiversity Information Facility (GBIF) database (Telenius, 2011). Only geo-referenced occurrences deriving from 'Human Observation' and those occurring within or along the coastline of False Bay were selected, producing reports on Cape fur seal sightings by location and date. The majority of these GBIF data were derived from the iNaturalist citizen science application. Because few reports included the number of individual seals observed at a given time, we focused our analysis on the number of reports of seal sightings, rather than the number of seals observed across reports. The number of visitors to False Bay are likely to influence the number of seal sightings reported to iNaturalist and GBIF (i.e., more visitors equates to more reports). To address this, we obtained data on the number of visitors to the Cape Point Nature Reserve, which spans the western shoreline of False Bay. These data were derived from Wesgro Research (http://www.wesgro.co.za/) sourced from South African Tourism and Table Mountain National Park. While visitor data from the Cape Point Nature Reserve may underestimate the total number of visitors to False Bay, it serves as a reliable relative proxy as

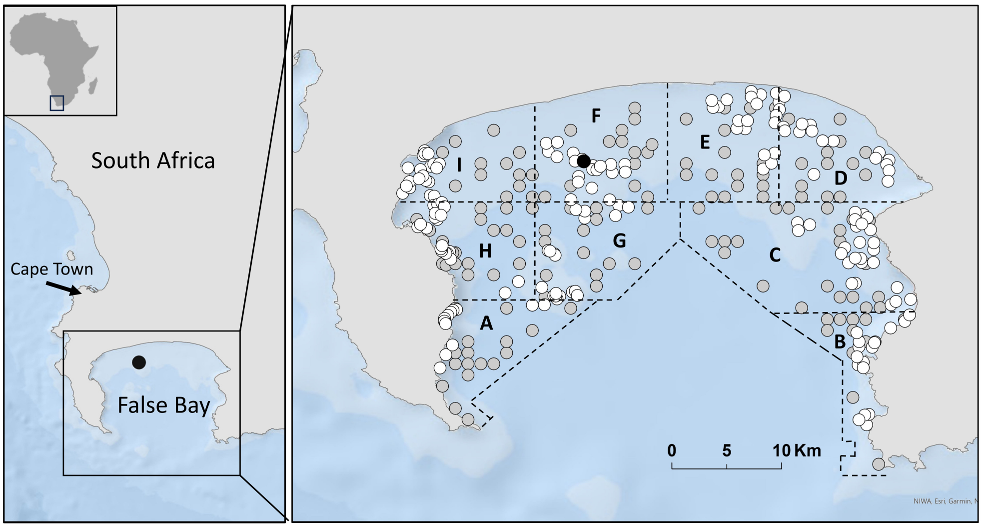


FIGURE 1
False Bay, South Africa. Seal Island is located with a black circle; white and grey circles represent Baited Remote Video Station (BRUVS) sampling sites in the pre-loss (white circles; 2012) and post-loss (grey circles; 2020/21) periods. Sampling zones are labelled **(A–I)**.

the number of visitors to the Cape Point Nature Reserve will scale with the number of visitors to False Bay. It also stands to reason that individuals visiting a nature reserve would be more likely to report natural observations of wildlife to citizen science databases, such as those used here. Thus, to evaluate if sightings of seals have increased over time, we calculated the annual number of seal reports to GBIF divided by the number of annual visitors to the Cape Point Nature Reserve as a standardized proxy of relative seal occurrence in False Bay over time and space. Visitor data were only available beginning 2009, therefore we focused on data from 2009 to 2022. To also evaluate if the spatial distribution of seal sightings has changed across the width of False Bay over time, we calculated the annual longitudinal range in reported seal sightings by subtracting the minimum and maximum longitudinal value of reported seal sightings by year. We then plotted the longitudinal degree range of seal sightings annually, from 2009 to 2022.

Baited remote underwater video stations

BRUVS were deployed in False Bay in both the pre-loss and post-loss period using the same methods and equipment of De Vos et al. (2015a). Each BRUVS consisted of a GoPro HD camera fixed to a metal base, facing a perforated PVC bait canister (130 mm × 110 mm with 10 mm perforations). The bait-facing camera was positioned 1 m away from the bait canister, which was filled with 1 kg of chopped sardine (*Sardinops sagax*). For deployments, BRUVS were lowered to the seafloor via a retrievable float line and left for at least 65 minutes. The footage retained for analysis was standardized to 60 minutes, starting from the moment the BRUVS settled on the seafloor (De Vos et al., 2015a).

As outlined in De Vos et al. (2015a) False Bay was divided into nine sampling zones, each encompassing a range of depths and habitats to facilitate deployment of BRUVS (Figure 1). BRUVS were deployed in depths of 5 to 50 m, as visibility was compromised in shallower depths due to turbulence and insufficient light at deeper depths. Deployment site selection followed a random stratified design to ensure that each zone, depth range, and habitat type was sampled during winter and summer of both periods. The number of deployments chosen for each zone was proportional to the size of the zone, with fifty percent of deployments within each zone allocated to reef and sand, respectively (see De Vos et al., 2015a for more details). The minimum distance between daily sampling sites was restricted to a minimum of 250 m, but averaged 500 m, to permit statistical independence of deployments (Cappo et al., 2001, Cappo et al., 2003) and to reduce the chance of overlapping bait plumes (Bernard and Götz, 2012).

Between June and December of 2012, 173 BRUVS were deployed in False Bay (summer = 85, winter=88), comprising pre-loss period sampling (Figure 1). Between July 2020 and February 2021, 150 BRUVS were deployed (summer=82, winter=68), comprising post-loss period sampling (Figure 1). Videos from each deployment were analyzed for MaxN, which is the maximum number of individuals of the given species in any single frame for the duration of the 60-minute video at a site (Albano et al., 2021). This provided a value of MaxN per hour for each deployment that was then averaged across samples, providing a metric of relative abundance (Cappo et al., 2001, Cappo et al., 2003).

While BRUVS tend to under-sample larger-bodied shark species (Santana-Garcon et al., 2014; Albano et al., 2021), possibly due to the type and amount of bait used (1 kg of sardines), any white sharks or sevengill sharks recorded on the BRUVS were analyzed for MaxN as a means of independently corroborating patterns found in the boat-based surveys. We primarily used BRUVS data to examine for changes in relative abundance of important prey for seals and sevengill sharks. Based on David (1987) and Huisamen et al. (2012), important prey for seals that were also detected on our BRUVS was Cape horse mackerel (*Trachurus capensis*). Based on Ebert (1991) important prey for sevengill sharks in the region that were also detected in our BRUVS were pyjama catsharks (*Poraderma africanum*) and smoothound sharks (*Mustelus mustelus*).

For these focal prey species, we tested for potential differences between periods in MaxN by fitting a zero-inflated Generalized Linear Mixed Model (GLMM) using the R package 'glmmTMB' (Brooks et al., 2017). Since previous work in False Bay by De Vos et al (2015a) revealed differences in the seasonal occurrence of these lower-trophic level species (Cape horse mackerel and smoothound shark = summer; pyjama catshark = winter) as well as habitat preferences for two of the species (smoothound sharks = sand, pyjama catsharks = reef), we modelled individual counts in the form of MaxN as a function of the period (pre-loss *vs* post-loss of white sharks), sampling season (summer *vs* winter) and sampling habitat (reef *vs* sand), including an interaction between period and season. We incorporated sampling zone as a random effect in our models to account for any potential spatial effects and variability on occurrences of the focal prey species. See the Supplementary File 1 for additional details about the GLMMs.

Additionally, from each sampling method (boat-based surveys, seal sightings, BRUVS), we calculated percent change and percent difference in average metric values between periods using the following formulas:

$$\%\ \text{change}\ =\ [V_2 - V_1/V_1]\,x\,100$$

$$\%\ \text{difference}\ =\ [V_2 -\ V_1/((V_2 +\ V_1/2))]\,x\,100$$

where V_i and V_2 are the average value of relative abundance for given species in the pre-loss versus post-loss period. Analyses, calculations, and plots were completed using R Version 4.2.2 (R Core Team, 2024).

Results and discussion

Between 2000 and 2015 (hereafter the pre-loss period), white shark relative abundance in boat-based surveys averaged 1.64 ± 0.03 (mean ± se) shark sightings per hour, whereas between 2016 to 2020 (hereafter as the post-loss period), white shark relative abundance declined to 0.3 ± 0.02 per hour (Figures 2, 3), representing a 82% decrease and -138% difference between periods (Figure 4). No white sharks were sighted in False Bay since August 2018. While BRUVS likely under sampled white sharks, results from our deployments throughout False Bay mirrored those from the boat-based surveys at

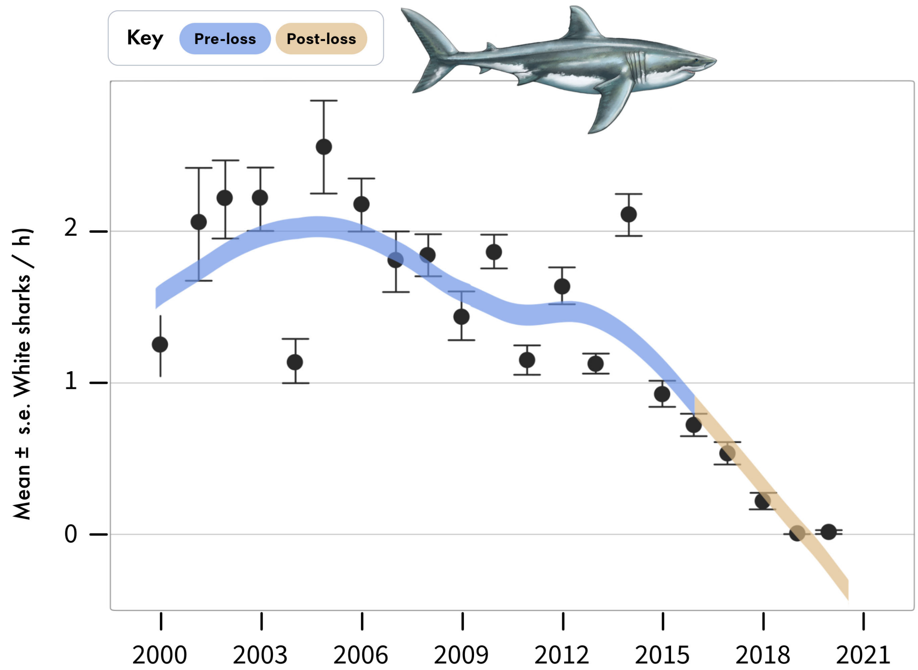


FIGURE 2
Annual sightings of white sharks during standardized boat-based surveys at Seal Island, in False Bay. Data are mean ± standard error of white shark sightings per hour averaged across sampling days for each year.

Seal Island (Figure 3). Specifically, while only three white sharks were detected on 323 BRUVS, these only occurred in the pre-loss period (2012), with no detections of white sharks on BRUVS deployment in the post-loss period (2020/21; Figure 3).

Coinciding with the decline of white sharks, seal observations reported from False Bay have increased over the same period (Figure 5), growing from a mean of 10.52 ± 2.27 reported seal sightings per million visitors to the area during the pre-loss period to 65.21 ± 32.55 in the post-loss period (Figure 5), representing a 520% increase and a 144% difference between periods (Figure 4). Of note, both visitor numbers at the Cape Point Nature Reserve and reported seal sightings dipped in 2020, likely due to Covid-19 lockdowns and associated restrictions on movement, which would likely curtail outdoor activities (Supplementary File 2, Supplementary Figure S1). However, in 2021, despite continued low visitation to the nature reserve, reported seal sightings reached a peak (Supplementary File 2, Supplementary Figure S1). This discrepancy may be explained by a relaxation of lockdown restrictions, which enabled more localized outdoor activities and associated wildlife observations, while few people still avoided public spaces, like the Cape Point Nature Reserve (Supplementary File 2, Supplementary Figure S1). In addition to increases in the frequency of reported seal sightings in the post-loss period, the spatial extent of reported seal sightings has expanded across the width of False Bay (Figure 5). For example, all reported seal sightings spanned only 0.045 degrees of longitude (4.14 km) in 2016, but increased to 0.426 degrees of longitude (39.18 km) in 2019, an increase of 846%. Notably, both the number of reported seal sightings and the spatial extent of these sightings were consistently relatively low for years, until the documented onset of white shark decline, after which values began to rapidly increase (Figure 5, Supplementary File 2, Supplementary Figure S1).

Cape fur seals in the region primarily feed on small pelagic fishes, such as anchovies (*Engraulis encrasicolus*) and to a lesser extent Cape horse mackerel. While anchovies were not detected in our BRUVS, Cape horse mackerel were. Cape horse mackerel have been found to be among the most abundant fish species detected in BRUVS studies within our study region (e.g. Roberson et al., 2015). Coinciding with changes in seal sightings between periods, we detected a reduction in the relative abundance of Cape horse mackerel on our BRUVS (Figure 3), a 22% decrease and -33% difference between the pre- and post-loss periods (Figure 4). This reduction occurred primarily in the summer, when Cape horse mackerel were historically most abundant in False Bay (De Vos, 2021). Their summer relative abundance declined from a mean MaxN of 24.62 ± 0.54 in the pre-loss period to 13.05 ± 0.4 during the post-lost period, representing a 44% decline and -61% difference (Figure 3, 4). This decline emerged as statistically significant in the zero-inflated GLMM (Supplementary Table S1, Supplementary Table S2, Supplementary Table S3), where a strong interaction between period and season was detected (Wald X^2 = 8.71, p = 0.003). As Cape horse mackerel are prey of seals in False Bay (David, 1987), these findings are consistent with a trophic cascade arising from predation release on seals due to the loss of white sharks; a pattern in agreement with previously demonstrated changes in seal behaviors and reductions in seal physiological stress levels associated with the declines of white sharks in False Bay (Hammerschlag et al., 2022).

Sevengill sharks were not observed in boat-based surveys at Seal Island in the pre-loss period, but averaged 0.96 ± 0.03 sightings per

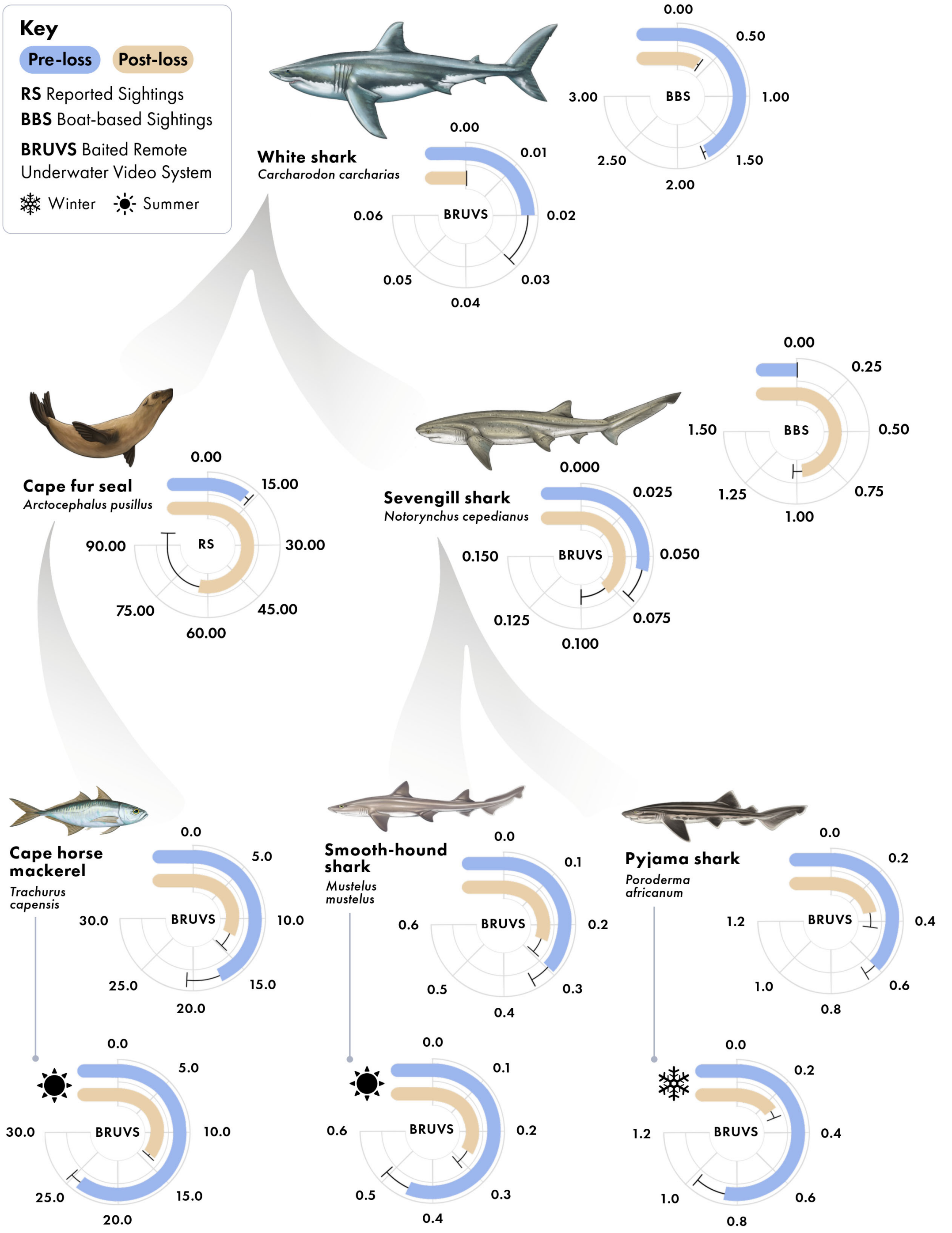


FIGURE 3
Predator-prey interactions in False Bay, South Africa, with average specie-specific relative abundance values between periods (pre-loss and post-loss of white sharks). Sampling methods producing each radial plot are indicated. Values in radial plots are mean ± standard error; for RS, data are the annual number of Cape fur seal sightings reported in False Bay, per million visitors to the Cape Point Nature Reserve; for BBS, data are the number of individual sharks observed per hour of baited survey; for BRUVS, data are the average MaxN (maximum number of individuals of the given species in any one frame for the duration of the 60-minute sampling video). For Cape horse mackerel, pyjama catsharks, and smoothhound sharks, overall data are provided, along with data for the specific season in which each species is prevalent, denoted by icons.

hour following the decline of white sharks from the system (Figure 6). Similarly, the relative abundance of sevengill sharks detected on BRUVS was consistent with the findings from the boat-based data (Figure 3). For two important prey species of sevengill sharks detected in our BRUVS (pyjama catsharks and smoothound sharks), we found reductions in relative abundance that fell by 40% and 21%, respectively, between periods, a -50% and -23% difference (Figures 3, 4). Similar to the findings for Cape horse mackerel, declines for both species primarily occurred in one particular season: winter for pyjama catshark and summer for smoothound.

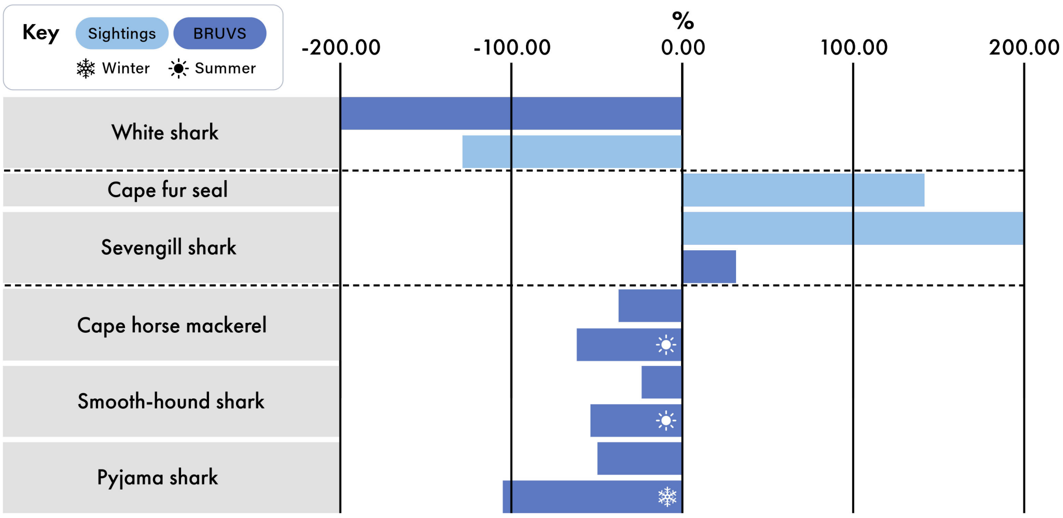


FIGURE 4
Percent difference between pre- and post-loss period for known predator-prey interactions. Darker blue bars are based on baited remote underwater video stations (BRUVS) and lighter blue bars are based on standardized sightings data. Horizontal dashed lines separate trophic levels. For Cape horse mackerel, pyjama catsharks, and smoothhound sharks, overall data are provided, along with data for the specific season in which each species is prevalent, denoted by icons.

For pyjama catsharks, winter relative abundance declined from 0.86 ± 0.10 in the pre-loss period to a mean of 0.24 ± 0.06 during the post-lost period (Figure 3), representing a 72% decline and -113% difference between periods (Figure 4). This decline also emerged as statistically significant in the GLMM (Supplementary Table S1, Supplementary Table S2, Supplementary Table S3), where a strong interaction between period and season was detected (Wald X^2 = 9.70, p = 0.002). For smoothound sharks, their summer relative abundance declined from 0.45 ± 0.07 in the pre-loss period to 0.26 ± 0.06 during the post-lost period (Figure 3), representing a 42% decline and -54% difference between periods (Figure 4). The interaction between period and season did not emerge as statistically significant in the GLMM for smoothound (Wald X^2 = 2.01, p = 0.156), although contrast analysis indicates a potentially relevant change for the species during the summer (Supplementary Table S1, Supplementary Table S2, Supplementary Table S3).

The three prey species – Cape horse mackerel, pyjama catsharks, and smoothounds – are known to exhibit seasonal differences in their occupancy of False Bay (De Vos et al., 2015a; De Vos, 2021). Interestingly, we primarily detected declines between periods in the seasons in which these species were historically more abundant in False Bay. It is thus possible that their predators (seals and sevengill sharks) are primarily targeting these prey species during the seasons in which their prey are most abundant. Accordingly, predation pressure would vary seasonally as would be the expected responses by prey, which would explain our results. Such patterns have been found in other predator-prey systems. For example, experiments with predatory birds and artificial lizards in tropical savanna woodlands have revealed higher predation by birds during the wet season driven by increased prey availability, not by increased predators (Nordberg and Schwarzkopf, 2019). Moreover, observations of big cats in Kenya showed that seasonal changes in prey abundance influenced predation; lion (*Panthera leo*) and cheetah (*Acinonyx jubatus*) switched their hunting focus to wildebeest (*Connochaetes taurinus*) during seasons of increased wildebeest abundance (Vettorazzi et al., 2022).

We note that changes in species relative abundance found here following the loss of white sharks are likely not exclusively or primarily due to alterations in consumer-induced mortality; but rather, are also a result of behavioral adjustments associated with alterations to predation risk. Prey will typically reduce risk of predation by increasing refuge use and/or reducing conspicuous foraging behavior (Werner et al., 1983) as has been documented in the case of white sharks and seals (e.g. De Vos et al., 2015b; De Vos et al., 2015c). According, we suspect that increases in species relative abundance patterns are also driven by increased foraging behavior and/or decreased refuging in the post-loss period associated with predation release. We suspect that given the relatively short time span over which white sharks were lost from False Bay, the observed changes across trophic levels are likely driven more by changes in antipredatory behavior.

The trophic cascade hypothesis posits that the magnitude of top-down effects of predators are strongest at trophic levels immediately below them; but, diminish successively down the food web (Shurin et al., 2002; Ripple and Beschta, 2004; Borer et al., 2005). Our findings were consistent with this ecological pattern (Figure 4), with the largest magnitude in relative differences between pre- and post-loss periods recorded in seals and sevengill sharks, with a relatively lesser magnitude in relative differences seen in lower trophic levels.

Limitations and alternative hypotheses

A limitation of any correlative study in the wild is that our results could be driven by confounding factors including fishing, pollution, invasive species, climate change, and natural stochastic

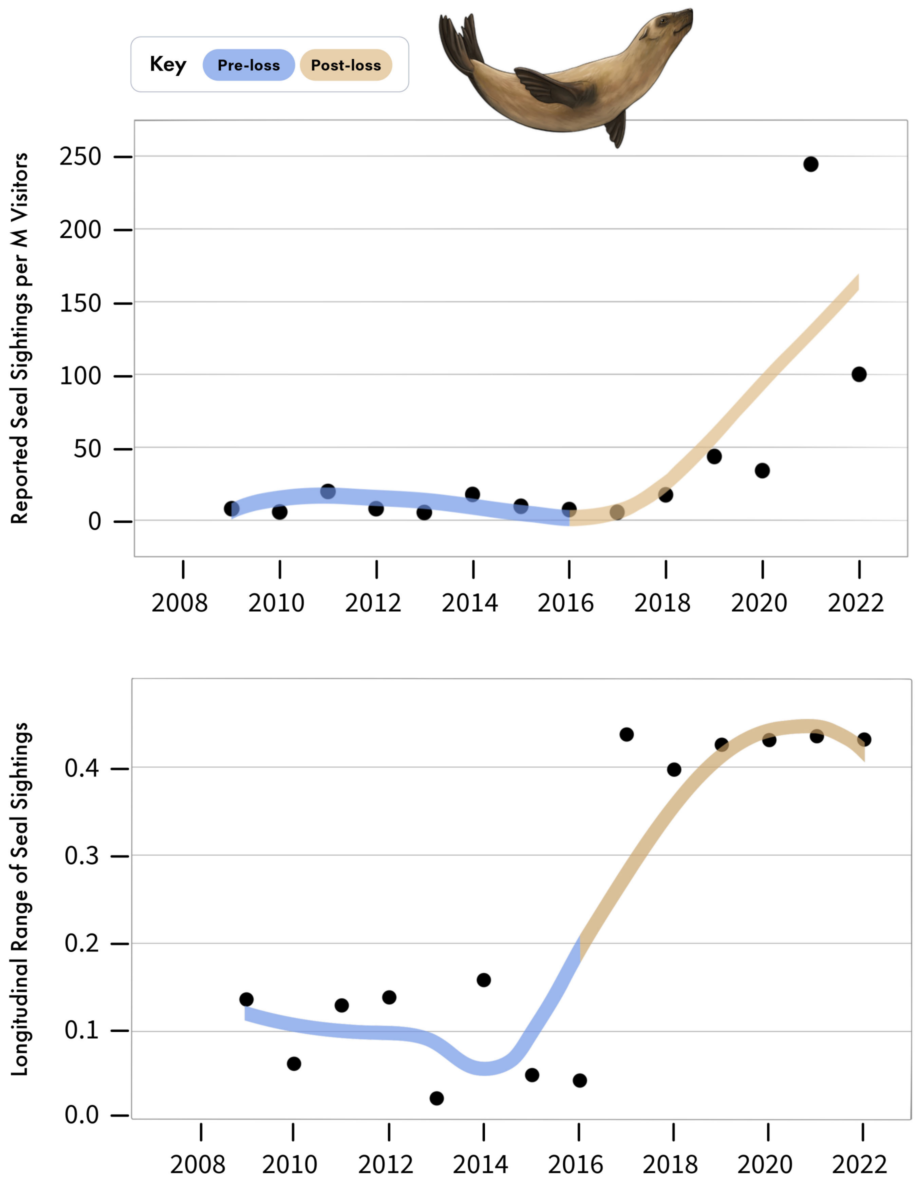


FIGURE 5
(Above) Annual number of Cape fur seal sightings reported in False Bay in GBIF, per million visitors to the Cape Point Nature Reserve. (Below) Longitudinal range, in degrees, of seal sightings reported annually to GBIF. Note that 0.1 degrees longitude is equivalent to approximately 9.2 km.

processes. However, for the species evaluated here, we found patterns that were inconsistent with long-term population trajectories in the region that we believe would otherwise confound our results. For example, we documented a marked increase in the frequency and spatial scale of seal sightings in the post-loss period, despite the seal population in False Bay having been on a declining trajectory since the late 1990s (Kirman et al., 2013). We realize that seal sightings data are dependent on reporting via citizen science applications, like iNaturalist, that have likely become more accessible due to smartphones in more recent decades. However, the number of smartphone users have steadily increased since 2009 (Sandesara et al., 2022), which is inconsistent with the patterns of reported seal sightings documented here, which only began to rapidly increase following the loss of white shark from False Bay after 2017.

While we documented a concurrent decrease in the relative abundance of Cape horse mackerel during the post-loss period, a recent assessment indicated that the stock status of this species for the South African coastline is optimal and that spawning biomass are well above Maximum Sustainable Yield, with commercial catch per unit effort index of abundance in 2018 at its highest on record in South Africa (DEFF, 2020), a pattern inconsistent with the decrease documented in our BRUVS in the post-loss period. A comprehensive study of fisheries data from False Bay, reported no clear temporal trends in relative abundance for sevengills or pyjama catsharks between 1897 and 2011, whereas significant increases

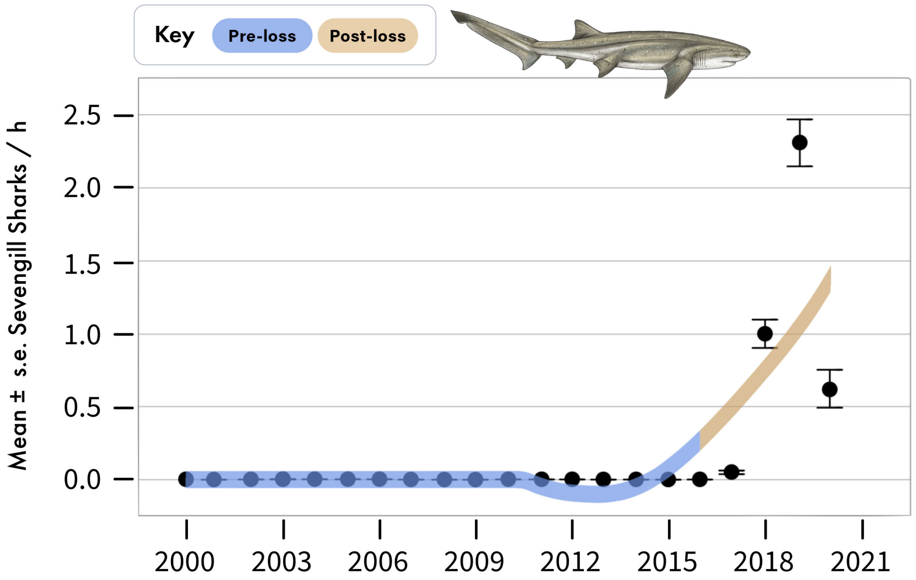


FIGURE 6
Annual sightings of sevengill sharks during standardized boat-based surveys at Seal Island, in False Bay. Data are mean ± standard error of sevengill shark sightings per hour averaged across sampling days for each year.

were found for smoothhounds (Best et al., 2013). Although these time series precede our study, those reported long-term trends differ considerably in magnitude and direction from the changes between periods found for these species in the present study. That said, for smoothhound sharks, fisheries data suggest a regional population decline (da Silva et al., 2019). So, local declines due to increased predation are likely exacerbated by regional population declines from overfishing.

While False Bay has experienced significant coastal development over the past 30 years with associated increases in pollution that has likely had lethal and sublethal impacts on varying species (Pfaff et al., 2019), this is unlikely to have caused the observed inverse patterns in relative abundance and frequency at successively lower trophic levels. Since the 1980s, False Bay has also experienced increased upwelling and cooling, which has led to nutrient enrichment of the bay and subsequent increases in kelp, rock lobsters, seabirds, pelagic fish, and several invasive species (Pfaff et al., 2019). While these changes have likely had bottom-up ecosystem impacts in False Bay, these impacts predate our study by several decades and would likely have already largely stabilized prior to the study period.

Temperature is a major driver of fish behavior and could contribute in some way to changes in the relative abundance of the observed species. However, changes in temperature between the periods would not be expected to drive the observed inverse patterns in relative abundance of predators and prey at successively lower trophic levels. While study results are most parsimoniously explained by the loss of white sharks from False Bay, a major limitation of this study is that for species evaluated using BRUVS, we only have two sampling periods, and cannot account for potential changes in detection probabilities. While confidence in our results was strengthened by incorporating multiple datasets for some species (e.g. sevengill sharks), the availability of other fisheries-dependent or independent data that would corroborate patterns for other species (e.g. horse mackerel) are currently unavailable. Accordingly, future work would benefit from additional sampling over time.

Summary

The decline in white sharks that occurred in False Bay within a long-term monitoring program has provided an unparalleled opportunity to gain preliminary insights into ecological changes in the wild due to the loss of a marine apex predator. The documented changes in relative abundance patterns between periods matched with expectations of a trophic cascade caused by the loss of top-down predation from white sharks. This has included increases in species that were historically important prey of white sharks and in turn, decreases in abundances of their prey. Future work at this site would benefit from understanding if and how community structure and function may have been altered and the extent to which they will continue to change through time. While impacts of apex predator declines are difficult to detect in the wild, especially in marine environments, they are likely more widespread than recognized given the pace and extent of apex predator declines globally.

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

Author contributions

NH: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing. YH: Data curation, Formal analysis, Investigation, Writing – review & editing. CF: Data curation, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. TC: Formal analysis, Supervision, Writing – review & editing.

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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.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2025.1530362/full#supplementary-material

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A classification system for wounds and scars observed on white sharks (*Carcharodon carcharias*)

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1 Introduction

The study of large marine organisms is limited due to the difficulty of making field observations throughout the expansive, deep and relatively opaque ocean environment. While significant advances in electronic tagging and molecular techniques have broadened the scope of questions that can be effectively addressed, major aspects of life history remain unknown. White sharks (*Carcharodon carcharias*) in central California, for instance, are one of the most studied elasmobranch species with over 21,000 instrumented tracking days (Andrzejaczek et al., 2022), documented regional population trends (Kanive et al., 2021), and a sequenced genome (Marra et al., 2019). Despite decades of dedicated research, targeted tourism, and natural history observation (Jorgensen et al., 2022), significant gaps remain in our basic knowledge of their natural history – for example, no one knows the gestation period of a white shark, where they give birth, when and where they mate, and how they interact with prey, predators and each other (Jorgensen et al., 2022). Over the course of these efforts, a proliferation of photographic devices with increasing miniaturization and ever-improving picture resolution has resulted in vast image libraries documenting individual white sharks repeatedly photographed over decades (Anderson et al., 2011). Our observations of white sharks off Central California, documented in an image library, reveal a large number of injuries, wounds, and scars that occur and heal at regular intervals. The unique patterns and the timing of these marks may provide novel insights into the life history of white sharks that have remained undescribed. Additionally, similar libraries from other regional populations (Towner et al., 2012) can provide insights into region specific as well as broad comparisons of life history patterns. However, before we can use these scars to better understand white shark life history, the source of the wounds must first be determined and systematically classified.

To date, a modest number of studies focused on wounds or scars on sharks and marine mammals have made progress in identifying their causes. Injuries resulting from boat strikes and subsequent healing rates have been described on white sharks (Towner et al., 2012) as well as whale sharks (Speed et al., 2008) and manta rays (McGregor et al., 2019). Diagnostic wounds made by propeller blades are reflected by sets of evenly spaced parallel cuts approximately perpendicular to the direction of vessel travel (Rommel et al., 2007; Towner et al., 2012). Scarring on white sharks consistent with interaction with large squid have also been reported (Becerril-García et al., 2020). Squid marks bear a distinctive pattern with rows of circular scars tapering in diameter consistent with suckers ringed with sharp teeth, that flank the arms and tentacles of larger squid species. Small bites extracted by cookiecutter sharks, common on open ocean cetaceans, have also been identified on white sharks (Hoyos-Padilla et al., 2013). Killer whales are a known white shark predator (Jorgensen et al., 2019), and a failed predatory attempt can leave the signature impression of their tightly spaced pointed teeth (De Maddalena, 2023).

In this paper we propose a systematic classification system to describe the different types of wounds and scars commonly observed on white sharks in the northeastern Pacific associated with prey handling, parasites, conspecific aggression and anthropogenic impacts. The systematic approach described in this study includes a dichotomous key for more efficient and consistent scar classification which may be applicable to numerous other marine organisms where long-term image documentation is obtainable.

2 Methods

From 1987 to 2024, while conducting seasonal research on white sharks (*Carcharodon carcharias*) around seal rookeries in central California (Año Nuevo Island, Southeast Farallon Island, Point Reyes, Tomales Point, and Monterey Bay), we archived hundreds of hours of underwater video recordings of individual sharks (Anderson et al., 2011; Chapple et al., 2011; Kanive et al., 2015, 2019). White sharks were attracted toward our research boat using a seal decoy made from outdoor carpet and deployed with a standard medium tackle rod and reel. In most cases a small (~2kg) piece of marine mammal blubber (use permitted by National Marine Fisheries Service) was tethered to the boat to provide a localized olfactory attractant to increase the shark's proximity to the boat once they approached. In Monterey Bay, no decoy or attractant was used and sharks were visually spotted at the surface and approached. With the shark near the boat, we recorded video images using a 'dip camera' attached to a 2–4 m pole (camera specifications varied over this period but should not impact the results of this study). Whenever possible, both sides of the shark were imaged, with special attention given to the dorsal fin profile, for individual identification, and the rear ventral area, to determine sex (Anderson et al., 2011).

While individual ID and sex were initially the primary reason for video documentation, many different types of wounds and scars on the white sharks were recorded. To systematically classify the various scars and wounds, we reviewed a selection of clear scar images and matched the wound impressions with candidate causes based on known interactions classes that could result in injury, these included, 1) Conspecific - e.g. these were aggressive bites or mating hold bites, 2) Prey handling - e.g. scratches or bites from their preferred pinniped prey, 3) Environmental – e.g. scrapes from contact with rocky reef, 4) Parasitic - e.g. cookiecutter shark bites or copepod attachment, and 5) Anthropogenic - e.g. gear entanglement or boat strikes. To facilitate standardizing scar classification, we developed a dichotomous key based on a series of yes/no questions about the size, depth and pattern of an observed wound or scar.

3 Scar categories

Between 1987 and 2022 we recorded over 2500 video clips documenting more than 500 unique individual white sharks in central California. Reviewing every scar was beyond the scope of this study, however from a representative collection of scar observations across a range of age, sex, and locations our sorting by similar scar and wound shapes resulted in 12 observed injury sources, each falling under one of the following five broad interaction classes (Table 1).

3.1 Conspecific

The impression of white shark teeth is highly distinctive. Their teeth are serrated, extremely sharp, and are capable of inflicting deep cuts that generally form a crescent shape wound where individual tooth cuts are evenly spaced. If the bite is deeper, the individual tooth impressions connect into a single large and slightly jagged crescent cut.

3.1.1 Mating hold bites (shallow bites)

Many scars and wounds clearly formed the crescent shape impression of a second white shark's jaw. These scars generally fell into two categories; deep injurious bites, or multiple shallow tooth impressions. Lighter tooth impressions were often repeated in proximity resembling multiple light bites, consistent with a 'hold' that was repeatedly adjusted. We categorized these as a 'Hold Bite' (Figure 1A, 1B). Similar wound patterns have been identified in other species such as nurse sharks (*Gangliocytoma cirratum*), blacktip reef sharks (*Carcharhinus melanopterus*) associated with mating activity (Pratt and Carrier, 2001).

3.1.2 Deep bites

These bites are typically large and deep where the individual tooth impressions are connected in a continuous crescent shape (Figure 1C, 1D). These scars can run all the way down the side of the shark, often affecting the gills. What distinguishes these from 'Hold Bites' is the depth of the bite and the connected laceration between individual tooth impressions. Unlike Hold Bites, which appear light with multiple repetitions, the Deep Bites appear as one or more single impression deep into the muscle. Whereas Hold Bites appear restrained with a shallow depth of the tooth impressionism, these Deep bites appear more aggressive in nature.

TABLE 1 A list of the 5 categories of scar types (colored by category) and a general visual description of each to identify the likely source.

Interaction	Injury (source)	Description
Prey Handling	Scratch (seal)	Raking scratches with 1–5 lines fanning or parallel; shallow and thin but blunt cuts; can be long and in a repeating pattern.
	Canine (seal, sealion bite)	Clusters of pencil-width canine punctures in pairs or fours (upper and lower jaws); some top-bottom or left-right symmetry; often flesh chunks removed.
	Squid (Sucker wound)	Rows of shallow suction marks tapering in size and spacing.
Parasitic	Cookiecutter	Circular wounds with missing skin or "partial moon" look; approximately the size of the shark's eye; perfectly round or oval
	Copepod	Tight clusters of small dots of 'raw' skin; adjacent copepods seen
Conspecific	Deep Bite	Distinctive teeth marks connect in a deep crescent wound that is usually singular but can be repeated; often top and bottom jaw paired marks; deep sharp raking cuts, usually parallel.
	Mating Hold Bite	Multiple shallow teeth marks; may form crescent pattern; often repeating patterns; usually space between individual tooth marks; sometimes with long sharp raking cuts, usually parallel
Environmental	Scrape	Raking scrapes that are wide and parallel; scrapes are short or long; mostly shallow and on protruding parts of the body.
Anthropogenic	Propellor (vessel strike)	Equally spaced lacerations from a rotating propeller, sometimes with a long cut from the motor keel perpendicular to the prop cuts; can also be one singular deep laceration.
	Fishing Gear (entanglement)	Obvious fishing line, tackle or rope; or chafing marks from ropes; marks often describe a straight line from the corner of the mouth or the edges of fins.
	Tag	Tag or leader leaves elongated oval rubbing mark parallel to water flow; often has a line at the front end from the leader.

3.2 Prey handling

In central California white sharks aggregated around pinniped rookeries where they were repeatedly observed capturing and consuming northern elephant seals (*Mirounga angustirostris*), harbor seals (*Phoca vitulina*), and California sea lions (*Zalophus californianus*) as the pinnipeds transit back and forth between haulouts and adjacent open water. We observed typical and distinctive prey handling behavior consisted of an ambush bite followed by the release of the pinniped prey and eventual return to the prey after it has been mortally wounded, and has been previously described as 'bite and spit' or 'exsanguination' (McCosker, 1985; Klimley, 1994). As a result, many white sharks appeared to have injuries inflicted by their pinniped prey, typically around the rostrum, gills and pectoral fins in the form of bites or scratches.

3.2.1 Canine punctures

Pinnipeds (seals and sea lions) have retained canine teeth that are similar in shape and size to those of a dog. Wounds that resembled puncture holes often appeared in pairs, consistent with one pair from a pinniped top jaw and another from the lower jaw that are symmetrically opposed from one another (Figure 1E). Sometimes we observed additional scratches potentially caused by the smaller front teeth next to the canine punctures. If a tooth slides across the skin it may leave a line with some skips beginning or ending at a puncture mark. Canine puncture wounds were common and found on the sharks' head, body and fins, both fresh and healed.

3.2.2 Seal scratches

True seals, both northern elephant seals and harbor seals in the North Pacific, have five sharp claws on the ends of their fore flippers (Figure 1F). Whereas sea lions and fur seals have nails far back from the edge. For defense, sea lions therefore can bite but they can not scratch like a true seal. A common scar pattern encountered resembled a small rake with up to five tines pulled against the shark's skin, leaving parallel or converging scratches. The spacing between individual scratch marks in these wounds closely resembled a seal claw in scale and occured around the forward portions of white shark's bodies.

3.2.3 Squid marks

Larger squid species have teeth embedded in their suckers which leave distinctive marks on toothed whales such as sperm whales and have been reported on some white sharks. They appear as a series of circular impressions that taper in size and spacing and can occur in parallel rows. Squid are a potential source of foraging for white sharks while offshore, and scars fitting their description have been reported off Guadalupe Island (Becerril-García et al., 2020), however, the occurrence of scars resembling squid marks on white sharks in central California is very rare.

3.3 Environmental

3.3.1 Scrapes

We recorded evidence of sharks interacting with their physical environment in the form of scrapes that appear to be from abrasive contact with hard substrate (Figure 1G). They appear as large scrapes usually from the first dorsal fin on the flank back to and on the caudal peduncle and fin. These impressions were slightly elongated, with each abrasion much wider than a single pinniped scratch, with frayed wound edges that appear far less clean than raking teeth cuts. They also tended to extend parallel to the body axis, indicating scraping against a hard, relatively dull and stationary object. These were typically not very deep and were mostly observed as white in color unless completely healed and black.

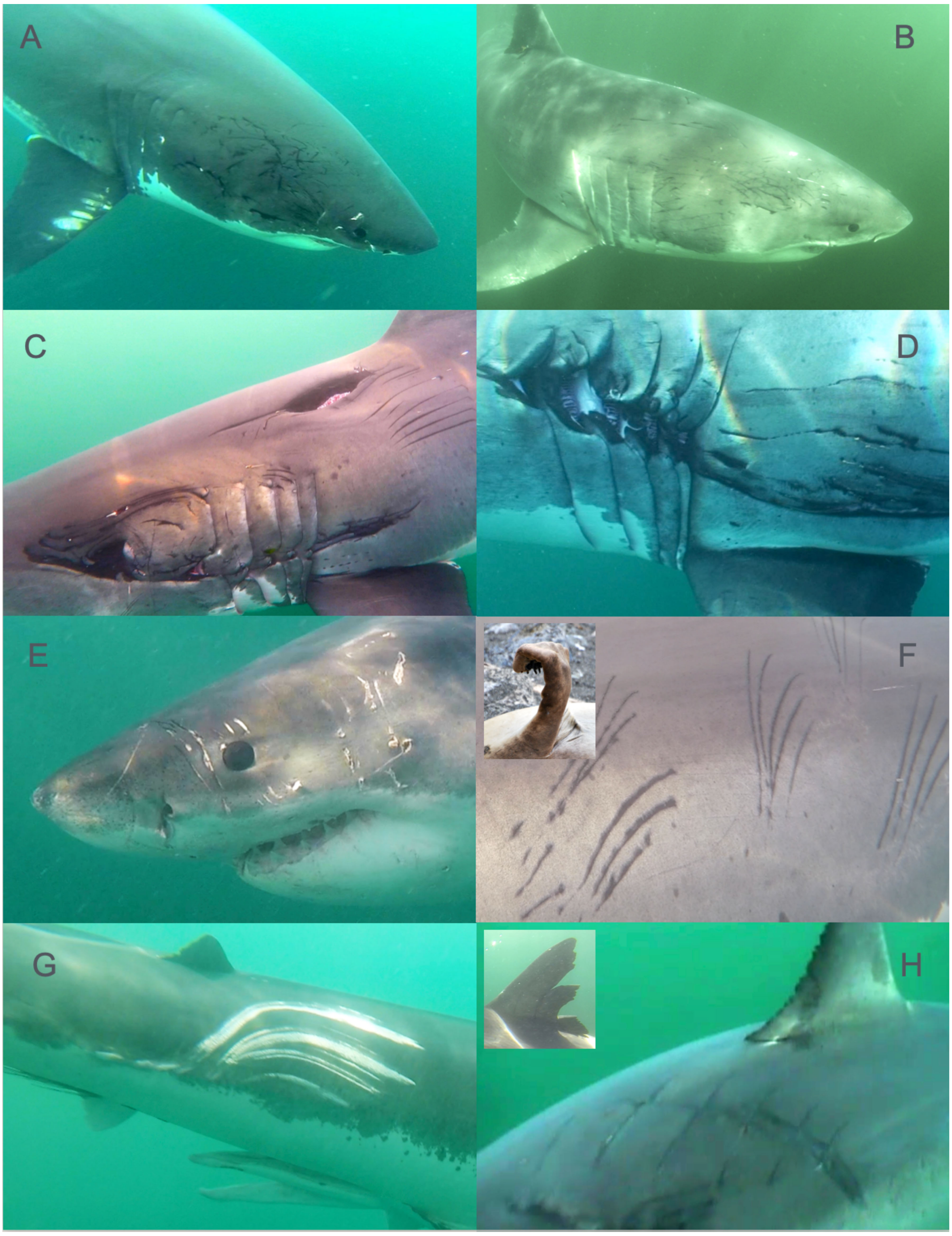

FIGURE 1
Distinctive wounds and scars on white sharks (*C. carcharias*) reflect diverse interactions. Conspecific bite marks on white sharks include repeated shallow bites or 'hold bites' **(A, B)** appear restrained and typically occur on large females and presumably result from mating activity. More aggressive 'deep bites' **(C, D)** occur mostly on males and on some females. Paired puncture wounds resulting from seal or sealion canine teeth **(E)** occur when pinnipeds bite back during prey handling. Another distinctive prey handling injury results from scratches **(F)** from seal claws (F inset) producing up to 5 parallel or fanning raked cuts. Shallow parallel scrapes **(G)** running parallel to the shark's movement result from scraping contact with the reef or other hard substrate. Boat strikes are recognizable from propellor injuries **(H)** that typically result in a series of deep parallel and perpendicular cuts along the trunk or fins (H inset).

3.4 Parasitic

Although large sharks have formidable dermal denticles that protect their skin, they remain vulnerable to some observable external parasites that produce wounds, scars and other markings.

3.4.1 Cookiecutter sharks

The cookiecutter shark (*Isistius brasiliensis*) bites have been well described and were occasionally observed in this study. The small (<50cm) squaliform shark lives in the open ocean and is known as an ectoparasite, with prey ranging from the largest apex predators to small, low trophic level species (Carlisle et al., 2021). With large prey, the cookiecutter shark latches onto their body and spins, removing large plugs of tissue. This ice cream scoop-like action removes a very distinctive circular chunk of flesh from the larger 'host'. The wounds appear as a golf ball to tennis ball size circular bite when successful or like a half circle or a "C" if the parasitic shark fails to remove the bite (Hoyos-Padilla et al., 2013).

3.4.2 Copepods

Copepods are small crustaceans, with parasitic varieties commonly found on many fish and shark species. We observed them on most individuals and on different areas on the shark including the fins, mouth, cloaca and trunk of the body, often appearing clustered in patches. In these patches, they are so close together that they seem to fill the space completely, leaving a pattern of spaced dots where they were attached.

3.5 Anthropogenic

3.5.1 Propeller wounds

Boat propellers cause large traumatic wounds on white sharks which in severe cases are likely to be fatal (Rommel et al., 2007; Towner et al., 2012). While the propeller is rotating, it leaves a series of parallel cuts with even spacing. Propeller cuts are generally clean and straight and evenly spaced (Figure 1H). Most propeller wounds were seen on the middle of the back and dorsal fin, and sometimes the caudal fin as well.

3.5.2 Rope and fishing gear

We have observed a few white sharks with fishing gear with some evidence of entanglement or rolling on a line. A line, if taught, may rub the dermal denticles off the skin leaving a white impression ('rope burn') that sometimes reveals the spiral twist of the line, which may resemble squid marks. The line can hang up on the fins

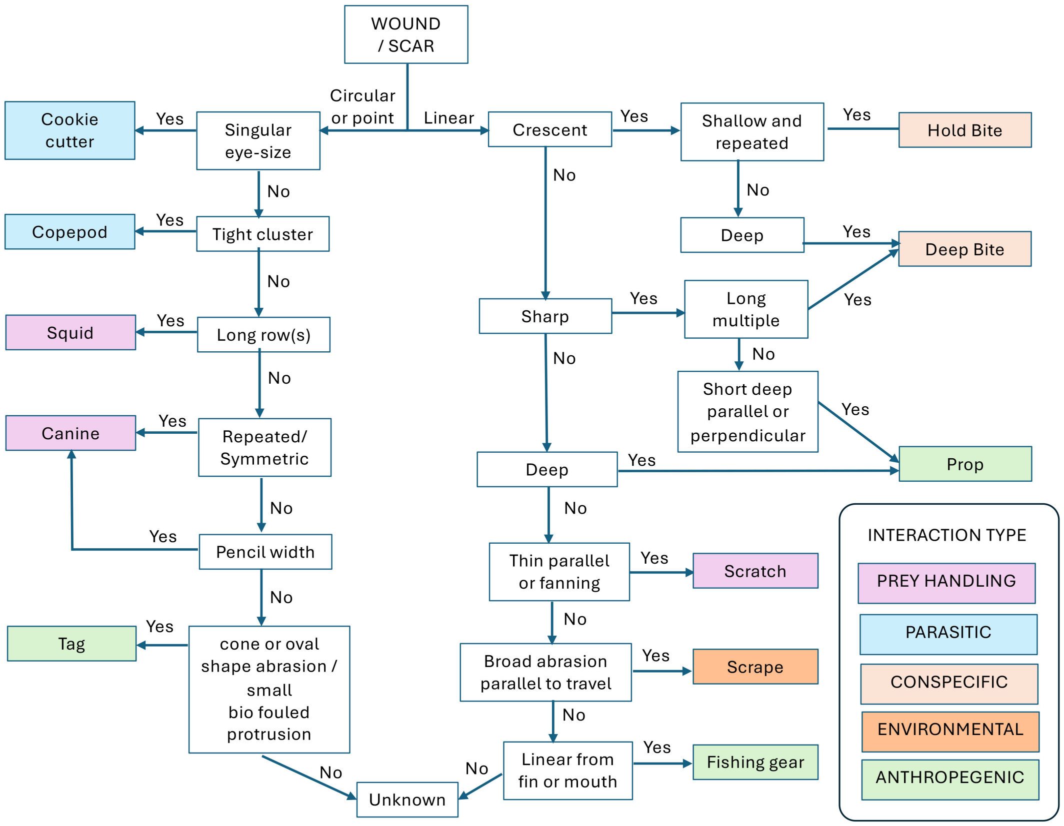

FIGURE 2
Classification schema and dichotomous key for identifying common wounds and scars observed on white sharks (*C. carcharias*). A diagnostic description distinguishes each injury type, and the estimated source of each injury type is sorted by general interaction classes associated with broad life history behavioral categories.

and the shark may roll causing the line to chafe the skin. In many cases the gear can be readily identified, for examples sport fishing gear hooked on their fins or mouths.

3.5.3 Tag scars

We have been tagging white sharks in California waters since 1993 with a variety of tag types attached using a subdermal dart (Boustany et al., 2002). Many of these individuals have been reidentified over the years, providing the opportunity to observe and tag individuals repeatedly over long periods. All tags leave some type of scar that remains visible for some time. Marks are typically from the rubbing of the tag or tag assembly (i.e. leaders). In most cases the darted tags shed over time after one to five years (Chapple et al., 2016) including the tag assembly from pop-off satellite tags. Where the tag rubs on the skin, the dermal denticles are worn off, leaving the white skin exposed. Once healed, the area turns black and finally disappears. The most characteristic tag scars are black, featuring two oval shapes in succession with a line in front where the leader pulled out. Since we aim to put the tag in a small area just below (<.25 m) the first dorsal fin the tag scars are typically easy to identify. We have observed reduced rubbing in tags placed closer to the dorsal fin, presumably where flow is more laminar, resulting in a smaller scar.

4 Dichotomous key

We identified five interaction classes of scars/wounds on white sharks in Central California from underwater video (Conspecific, Prey Handling, Environmental, Parasitic, and Anthropogenic), which each described the interaction attributed to one or more of the distinctive scar types. To more efficiently and consistently classify subsequent wounds and scars, our dichotomous key uses a series of yes/no questions about the size, depth and pattern of an observed wound or scar (Figure 2). Some scars will inevitably be less clear because of poor imaging or due to an unusual shape and cannot be classified. In addition, our scheme does not identify all injury types. Still, this work provides a simple and standardized classification system, identifying the most common and recognizable scars/wounds found in these sharks. We further identify their likely causes, either through direct observation or induction, or building on previous work to create a foundation for future studies utilizing scars/wounds to inform our understanding of white shark life history.

5 Conclusion

The categories of this simplified classification system for scars/wounds on white sharks can also be applied to numerous other species where long-term photographic records can be obtained. For example, many large surface-feeding sharks incur boat-strike injuries (Womersley et al., 2022; Chapple et al., 2024) and interact with static fishing gear and most species are subject to parasite and mating scars. Additional categories may also be added in order to further refine species or population specific scar/wound patterns.

This work sets the foundation for continued quantitative analyses of scars/wounds on white sharks with larger datasets and for other populations globally. White sharks heal at generally predictable rates (Jewell et al., 2011; Towner et al., 2012; Chapple et al., 2015), including animals that have experienced significant injury. All wounds that break the skin undergo a visually distinctive healing progression reflected in the coloration ranging from red or pink when fresh, to white and then black as they heal. The black areas fade over many years to the original skin pigmentation. However, if the wound is not obscured by other newer scars, they may remain visible for decades. The white underside of the shark will eventually heal to white accordingly. As such, future studies can capitalize on both the rate of healing and our categorization scheme to identify when, where, and how various scars/wounds occurred thus informing further understanding of their life history. For example, it is uncertain where northeast Pacific white sharks mate (Jorgensen et al., 2010, 2012). Combining known movement data with data on the occurrence and freshness of mating scars and other scar classifications, researchers can infer the timing and location mating is likely to occur (Gallagher et al., 2024). For instance, if mating marks and cookiecutter bites are healed to a similar degree it could indicate those interactions occur at a similar time and place in the migratory cycle - in the open ocean environment. Conversely, if fresh wounds inflicted by coastal pinniped prey coincide with observed fresh mating bites on females then those interactions might be likely to co-occur in space and time. Similarly, identifying prey handling scars/wounds in smaller sharks can indicate the timing of ontogenetic diet shifts. In summary, this work provides a foundational framework for future studies utilizing available data on scars/wounds to inform our understanding of the cryptic life of white sharks and other elasmobranchs more widely.

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 approved by California State University Monterey Bay IACUC Committee. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

SA: Conceptualization, Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing. PK: Conceptualization, Investigation, Methodology, Resources, Writing – review & editing. TC: Conceptualization, Investigation, Methodology, Resources, Writing – review & editing. SA: Investigation, Resources, Writing – review & editing. BB: Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review &

editing. SJ: Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

Generative AI statement

Publisher's note

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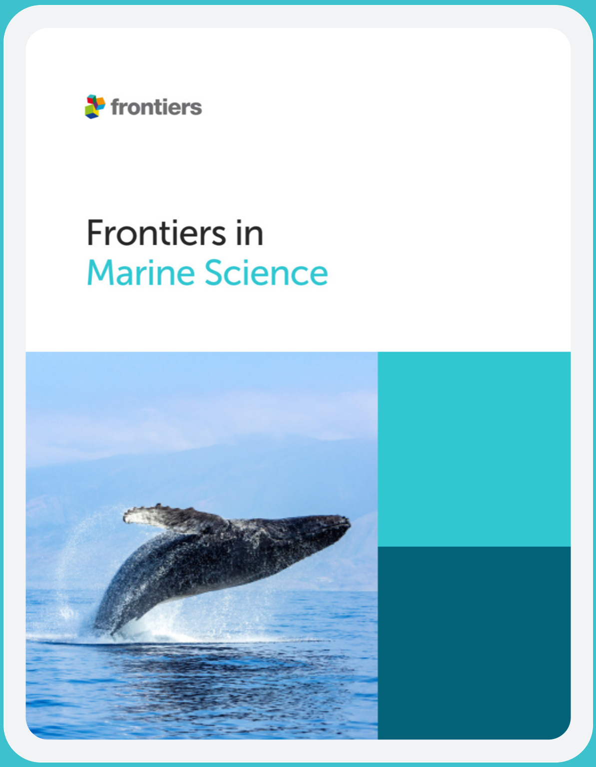

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