School of Molecular and Life Sciences

Assessing survival rates of discarded sandbar sharks (*Carcharhinus plumbeus*), tiger sharks (*Galeocerdo cuvier*), Port Jackson sharks (*Heterodontus portusjacksoni*) and dusky sharks (*Carcharhinus obscurus*)

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made. This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

There is no declaration, from myself or any co-authors associated with this thesis, of a conflict of interest.

The research presented and reported in this thesis conform to the Australian Code for the Care and Use of Animals for Scientific Purposes 8th Edition (2014). The proposed research study received animal ethics exemption from the Curtin University Animal Ethics Committee. Exemptions were granted to the Department of Primary Industries and Regional Development (DPIRD) for research.

Signature: 

Date: 26/05/2023

Abstract

The decline in shark populations globally has triggered conservation and fisheries management efforts to rebuild stocks. Some shark fisheries have been closed with a range of management measures implemented in others. Due to the slow growth rates and low fecundity of elasmobranchs, stocks of many sharks are still declining. Bycatch has been identified as a possible risk for the continual decline of sharks. Discarded sharks are released alive, although the fate of these shark's post-release is widely unknown. Species-specific post release survival studies are lacking and inhibit a full understanding of the effects on the population.

Post-capture and post-release survival for species of shark commonly caught as bycatch can be assessed in many ways. Post-capture survival rates are often quantified by the survival upon retrieving the individual, although other methods such as time on the hook and blood physiology have also been explored. Stress hormones (e.g., 1ᾳ-hydroxycorticosterone, adrenocorticotropic hormone, and glucocorticosteroids) and some secondary stress responses (e.g., lactate, glucose, pH, urea) have been rarely used, although lactate levels have been used to quantify stress experienced after capture. Species-specific research is needed because each species will have different stress thresholds for mortality.

Post-release survival is often assessed using methods such as satellite tagging, although this method is expensive and is not as readily available. Satellite pop-up archival transmitting tags are a popular due to their ability to quantify mortality as well as monitor depth, light and temperature for set periods. This aids in monitoring the vertical and biological behaviours exhibited by sharks

after release. Post-release predation can also be quantified through the use of tagging methods and is common for species that do not handle stress well.

I investigated a species-specific integrated approach for assessing the survival rates of discarded sharks caught by used commercial longline, gillnet and drumlines fisheries. These fishing methods are also used to assess shark stocks in scientific surveys in Australia.

To assess the survival of sandbar sharks (Carcharhinus plumbeus) in northern Western Australia, sharks were caught on longlines (chapter two). All sharks sampled had experienced stress with hormones indicative of prolonged stress events, suggesting peak hormone levels could have been assessed with hook times under 30 minutes. Secondary stress responses such as lactate levels increased as time on the hook increased. Despite the stress event, the post-release survival was 100%, with sharks diving up to 307 m deep and showing cyclical depth movement patterns with some individuals moving up and down the water column both day and night. In contrast, others moved almost exclusively at night, corresponding with the movement patterns of prey.

The same post-capture and post-release methods were used for sampling tiger sharks, dusky sharks, and Port Jackson sharks (chapter three). Sampling for dusky sharks occurred in longlines in northern Western Australia, Port Jackson sharks in gillnets in southern Western Australia and tiger sharks in longlines in northern Western Australia and drumlines in Queensland. Tiger sharks showed high lactate levels for both methods, despite the short time on hook, although 84% of sharks had strong release conditions and 100% post-release survival. Dusky sharks showed similar stress responses, although more sensitive with 72% being in strong release condition. One tagged dusky shark experienced potential post-release predation with the tag transmitting 0 light and altered temperature readings less than 24 hours after release. Port Jackson sharks are a hardy

species and had relatively low lactate levels and low stress hormones with 100% of individuals having strong release conditions. The sensitivity of the PSAT tags had to be increased for Port Jackson sharks due to depth behaviours indicating mortality. Despite one shark having a tag malfunction, 100% of tagged Port Jackson sharks survived post-release.

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Chapter one

1.1. Background and rationale

The shark family has existed successfully for millions of years, but in recent decades the increase in fishing mortality has caused a 70% decline in global populations with 25% of species being categorised as threatened, or critically endangered (Dulvy *et al*., 2021; Pacoureau *et al*., 2021). Targeted and untargeted catch of sharks occurs worldwide with a mix of commercial and recreational fishing (Dulvy *et al*., 2021). Commercial fisheries tend to value the larger species/individuals decreasing brood stock and potentially impacting the natural replenishment of a population (Davidson *et al*., 2016b; Dulvy *et al*., 2021). Sharks may be deliberately targeted by commercial fishers, caught as bycatch or opportunistically (Oliver *et al*., 2015). Underreporting of bycatch means the fate of many species remains largely unknown. Recreational fishing has historically had a lesser-known effect on the population of sharks, although, with a lack of fishing effort and catch reporting, the total effect on the population is not quantifiable (Oliver *et al*., 2015).

Conservation efforts and the local closure of fisheries have been effective in restoring populations of some species of sharks, although species with a cosmopolitan distribution or large migratory movements still have a higher risk of fishing mortality (Braccini *et al*., 2018, 2019). There are social challenges with shark conservation with a lack of education (Kyne & Feutry, 2017). However, shark and ray ecotourism has played an important role in challenging stereotypes associated with elasmobranchs (Vianna *et al*. 2012). Reef manta rays (*Manta alfredi*), tiger sharks (*Galeocerdo cuvier*) and great white sharks (*Carcharodon carcharias*) have become widely popular and profitable species within the marine ecotourism industry, especially within Western Australia, South Australia, Queensland and Hawaii (Vianna *et al*., 2011, 2012). Fisheries

management is another form of conservation with many governments implementing rules on the discarding of bycatch (Braccini *et al*., 2012, 2018, 2019).

1.1.1 Shark fishing practices and bycatch

Elasmobranchs being targeted by unsustainable fishing is a global threat (Pacoureau *et al*., 2021). In Australia, rules and regulations state that every species of bycatch should be recorded at a species level as well as rated on a survival index (Braccini & Waltrick, 2019). Those species caught as bycatch are to be released as soon as possible to increase survival post-release (Braccini *et al*., 2012; Heberer *et al*., 2010). Studies have shown that shark species are the most common species recorded as bycatch in bait-used fisheries, especially near Fish Aggregating Devices (FADs) which usually have a higher density of targeted fish. A study conducted on silky sharks (*Carcharhinus falciformis*) found there were more sharks present and caught as bycatch around FADs than there were in free schooling areas (Hutchinson *et al*., 2015a). These differences in shark numbers present an increase in the likelihood of capture. Capturing sharks and rays as bycatch is undesirable for the animal, with the physiology of the animal being disrupted and stress increasing causing an increased possibility of mortality (Marshall *et al*., 2012)

1.1.2 Stress physiology

Stress physiology has been used in previous studies to understand the effect fishing practices has on discarded bycatch in commercial fisheries (Braccini *et al*., 2012). Some species of sharks, such as the greater hammerhead shark (*Sphyrna mokarran*), are more sensitive to stress and when caught can show more significant physiological changes which may result in mortality (Butcher *et al*., 2015; Hammerschlag *et al*., 2017). Other species of sharks, such as sandbar sharks (*Carcharhinus plumbeus*) and Australian swellshark (*Cephaloscyllium laticeps*), are robust and

show little signs of stress during capture (Braccini *et al*., 2012; Gulak & Carlson, 2021). Species-specific studies have indicated that blood physiology and survival could be linked to behavioural changes and the stage of capture (i.e., the position of the shark in the fishing gear, i.e., start or end of a longline or gillnet) (Fuller *et al*., 2020; Gulak & Carlson, 2021; Schaefer *et al*., 2021). Most studies concluded that the longer the shark was subject to the fishing practice (for example time on the hook or time in the net) the more likely that shark is to die. A study on silky sharks also found that the survival of the sharks significantly decreased once the lactate reading exceeded 11.3 mmol/L (Hutchinson *et al*., 2015a). Larger species are thought to have a higher tolerance to the amount of lactate with lower mortality until levels of 20 mmol/L or higher (Gulak & Carlson, 2021; Marshall *et al*., 2015). There are other indicators within blood samples commonly used to test stress in animals such as humans, monkeys and other fish species (Anderson, 2012; Atkinson *et al*., 2015; Barton, 2002). These indicators include hormones such as ACTH and corticosterone (Anderson, 2012; Armour *et al*., 1993; Fuller *et al*., 2020). A study on silky sharks had small amounts of data on the ACTH levels within the sharks and showed a high correlation between post-capture mortality and high levels of ACTH, which also correlated to the higher levels of cortisol in the blood (Hutchinson *et al*., 2015a; Schaefer *et al*., 2021). There was also a higher mortality later in the capture stage. Those sharks released in the early stages of retrieval of fishing gear (i.e., seine nets) were 30% more likely to survive than those released in the middle stage and 80% more likely to survive than those found in the bottom of the fishing gear (Hutchinson *et al*., 2015a). The time it took to process the sharks on deck was also taken into consideration, although there was no evidence that the processing times were linked with survival (Hutchinson *et al*., 2015a). Studies of dusky sharks (*Carcharhinus obscurus*) in the United States of America showed similar findings, time on the hook (TOH) was the main factor in mortality rates, with 83% mortality

after 3 hours TOH and 36% mortality for sandbar sharks after the same TOH (Marshall *et al*., 2015). Most capture and release studies measured each shark's time out of water (Fuller *et al*., 2020; Marshall *et al*., 2015). Other studies conducted focused on the difference in survival for sharks that were not affected by gill irrigation or time out of the water (Shaefer *et al*., 2021). These studies found that gill irrigation did not increase survival and as a result, many recent studies do not irrigate the gills or measure time out of the water (Gulak & Carlson, 2021). Studies on stress physiology has been done on many aquatic species, although species specific studies on elasmobranchs is still vastly limited (Skomal and Bernal, 2010)

1.1.3 Post-capture survival

Post-capture survival (PCS; survival observed at the vessel) has been used in nearly every survival analysis study (Fuller *et* al., 2020; Gulak and Carlson, 2021). This form of survival analysis can include quantifying at-vessel survival, release indices, stress physiology, and the use of hook timers. The most effective use of PCS is in an integrated approach with post-release survival to assess the longer term effects of the capture and release process on shark species (Knotek *et al*., 2022).

1.1.4 Post-release survival and tagging

Tagging methods have been used to study the post-release survival (PRS; survival after discard) of many marine species, including elasmobranchs (Skomal, 2007)). A drawback of this method is that it is usually more expensive than post-capture methods due to individual tags costing upwards of $2500 AUD and therefore can be less affordable. Different tags can be used to provide different information about the sharks' behaviour post-release. The most popular method is the use of satellite pop-up archival transmitting (PSAT) tags or smart position-only tags (Hammerschlag *et*

al., 2011). PSAT tagging methods can give light, depth and temperature for a specified amount of time, from days to years, providing vertical and biological behaviour information key to understanding stress recovery. These tags have been used on a range of species around the world with varying success. Smaller species of shark or ray are not suited to this type of tag because it can cause changes in their swimming behaviour (Lynch *et al*., 2017). Tag programming can pose challenges for mortality indicators in demersal sharks and rays that sink to the bottom and remain at a constant depth (±1m) for longer than 24 hours. Despite being alive, these sharks are referred to as 'sinkers' in survival studies and subsequently determined as a mortality (Rogers *et al*., 2013).

1.1.5 Species-Specific Information

Tiger (*Galeocerdo cuvier),* dusky (*Carcharhinus obscurus*), Port Jackson (*Heterodontus portusjacksoni*) and sandbar (*Carcharhinus plumbeus*) are four species of shark that are usually caught as bycatch both recreationally and commercially in Australia (Heithaus, 2001; Last & Stevens, 1994; McAuley *et al*., 2005). In Western Australia, there has been a decrease in population size of some sharks (e.g., dusky and sandbar sharks) due to unsustainable historical fishing (Braccini *et al*., 2018). Dusky shark distribution throughout Western Australia ranges through the temperate waters of the Midwest and southwest, up to the warmer waters of the Pilbara and the Kimberly (McAuley *et al*., 2005). Stock assessments of dusky sharks have shown a great decline in warmer waters and a steady decline in temperate waters (Braccini *et al*., 2019). Due to this decline, the dusky shark has been given the classification of vulnerable and studies are required to determine the causes of the decline (Braccini *et al*., 2019). Research has concluded that the catching of these sharks, both targeted and bycatch, was resulting in a mass decline and fisheries regulators worldwide implemented rules and regulations on the release and capture of this shark

species (Braccini, 2016; Braccini *et al*., 2018). There was also management determination to close the northern shark fishery and joint authority fishery (commonwealth and state managed) in Western Australia to aid in the recovery of mature populations of whaler sharks (Braccini, Molony, and Blay., 2021). Research has provided no clear indication that recreational fishing activities were correlated with the declines and further studies examining what happens to the shark after capture are needed to understand the effect of recreational fishing (Braccini *et al*., 2012; Braccini *et al*., 2021) The sandbar shark is one of the most common bycatch species in northwest Western Australia with a steadily increasing population size, although not at the level expected since the joint authority fishery closure in 2005 (McAuley *et al*., 2005; Oliver *et al*., 2015). Tiger sharks are commonly caught in commercial fisheries as bycatch, but also in recreational game fishing (Heithaus, 2001). It is also the species most commonly caught on trial SMART drumlines deployed in WA and other states around Australia. While many shark species are released after capture and classified as surviving upon release, shark populations are still declining (Dulvy *et al*., 2021). Further research into the survival post-capture is needed to investigate the stress physiology and behaviour post-release (Braccini *et al*., 2012; Sulikowski *et al*., 2020).

1.1.6 The gap in knowledge

More species-specific research is needed to understand the relationship between the decline in stocks and the fate of sharks after discard (Davidson *et al*., 2016b; Dulvy *et al*., 2021; Pacoureau *et al*., 2021). Each species of shark and ray has different tolerances to human interference, with some studies showing the possibility of smaller species having higher mortality rates (Braccini & Waltrick, 2019). Without species-specific information, there is no clear indication that certain sharks survive post-release (Braccini & Waltrick, 2019). Despite numerous previous studies, the

information available gives a narrow picture of the full extent of the effects caused by commercial or recreational fishing. More species-specific data need to be collected to allow for a sustainable fishing practice of sharks and rays as targeted species, as well as management and monitoring plans for those caught as bycatch and released alive (Braccini & Waltrick, 2019). There is a comprehensive body of global research on the impacts of commercial shark fishing (Butcher *et al*, 2015; Marshall, 2021; Shiffman *et al*, 2017). However, species-specific information will allow the development of localised shark and ray management plans to ensure sustainable fisheries. The species-specific approach to the studies of post-capture survival is an important focal point and research will need to be conducted in areas where catch levels are high (Davidson *et al*., 2016b; Dulvy *et al*., 2021).

1.2. Study area

Sample sites were proposed from a list of historical scientific surveys done utilising three different fishing methods by DPIRD and James Cook University (Fig. 1.1)

- Northern WA between Carnarvon and Broome (2020/2021)
- South coast of WA between Lancelin and Esperance (2020/2021)

- QLD, Fraser island and Batt Reef, Port Douglas (2015)

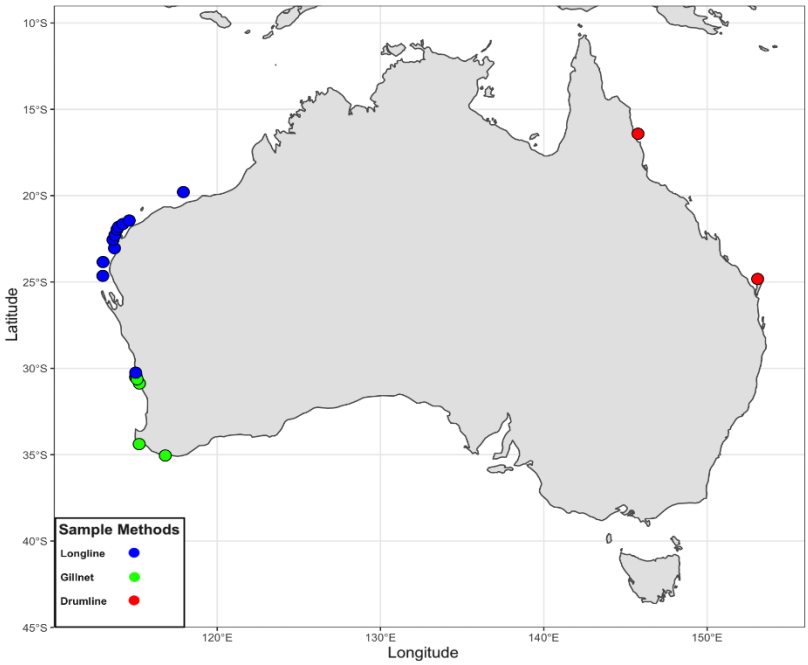

Fig. 1.1: Map of sampling sites for longlines (blue), gillnets (green) and drumlines (red). Sites used previously in historical scientific surveys 2015 and 2020/2021

1.3. Aims and objectives

The overarching objective of the research was to better understand the post-release survival rates of key discarded species of shark.

This research took an integrated approach using hook-time, release condition indexing, PSAT tagging, and blood metabolite analysis to assess PCS and PRS rates for sandbar sharks, tiger sharks, Port Jackson sharks, and dusky sharks. I also aimed to utilise primary stress responses such as ACTH and corticosterone (CORT), and secondary stress responses such as lactate to assess the stress physiology of these species and to assess their PCS and PRS rates and temporal vertical behaviours using spatial pop-up archival transmitting tags.

1.4. An integrated approach to assessing survival of discarded sandbar sharks (*Carcharhinus plumbeus*) caught on longlines (chapter 2)

Chapter two investigates the physiological stress of capture and release on sandbar sharks caught on longlines in northern WA using an integrated approach of hook timers and blood parameters (lactate, ACTH, and CORT), release conditions, and tagging. Data were collected from 10 sites with 5 replicate longlines along the northern continental shelf ranging from 50m - 250m in depth. All gear specifications were kept the same apart from 70 hook timers attached to shorter snoods (resulting in the same total length as non-hook timer snoods) randomly sampled along the longline. Comparisons were made between hook time and blood parameters as well as observing temporal vertical behaviours after release for a short period to quantify survival.

1.5. Assessing survival rates of discarded tiger (*Galeocerdo cuvier*), Port Jackson (*Heterodontus portusjacksoni*) and dusky (*Carcharhinus obscurus*) sharks (chapter 3)

Applying the outcomes of chapter two, I investigated the post-captured post-release survival rates of tiger sharks, dusky sharks, and Port Jackson sharks. All were caught in varying methods of fishing around Australia, but these methods were not compared. Tiger sharks were caught in a mixture of longlines (Northern WA) and drumlines (QLD), dusky sharks in longlines (Northern WA) and Port Jackson Sharks in gillnets (southern WA). We used the same methods of assessing survival as in chapter 2, plus some additional blood parameters for tiger sharks in QLD.

1.6. Thesis structure

This thesis has been structured into four chapters, a general introduction, two data chapters and a general discussion (Fig. 1.2). Data chapter two has been submitted for review to the Journal of *Marine and Freshwater Research*, and data chapter three formatted for the Journal of *Marine and Freshwater Research*. As stand-alone chapters, there is some repetition in the introductions and method sections. Additionally, in chapters 2 and 3, I use “we” or “our”, to reflect that this work has been submitted for review with co-authors.

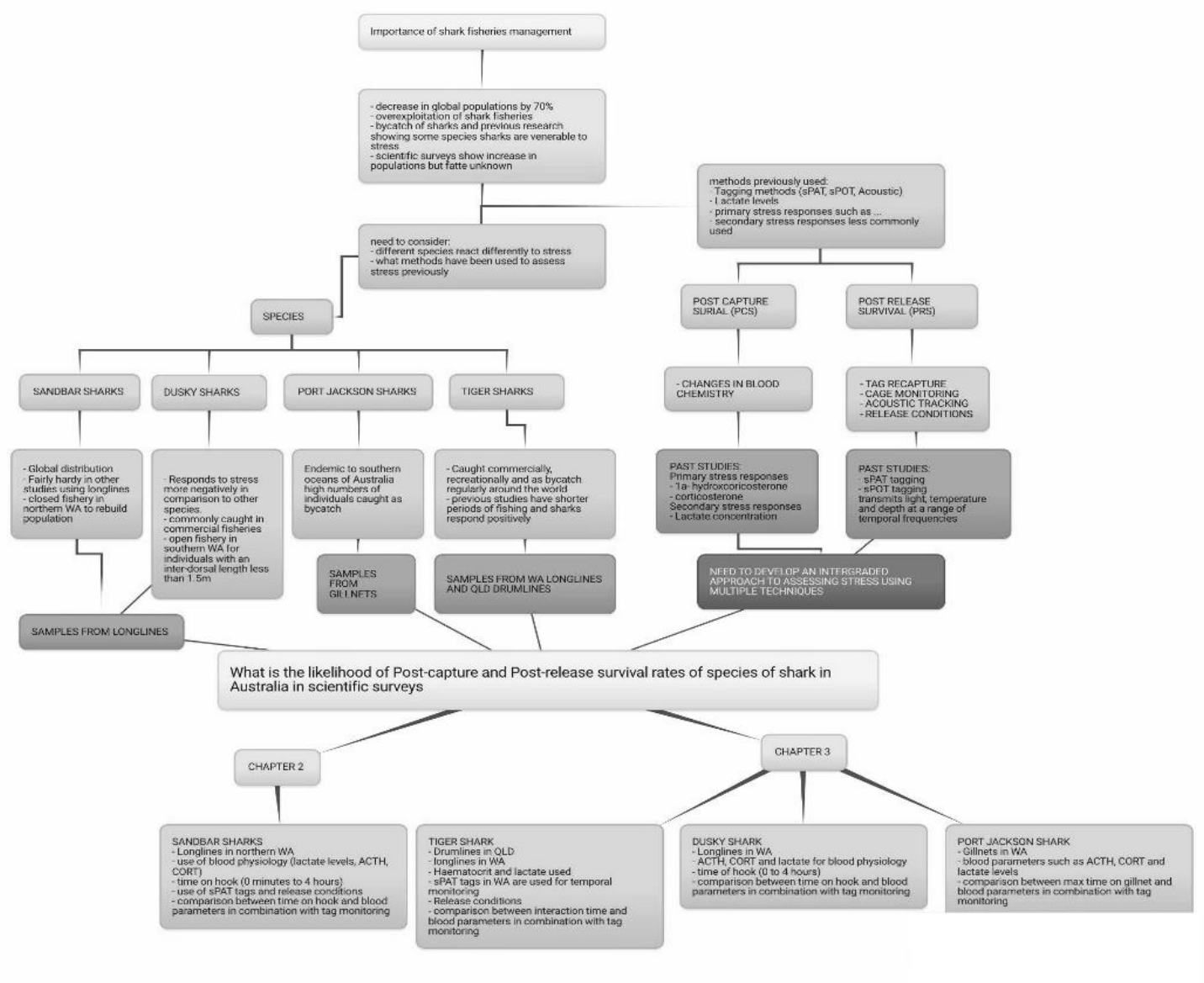

Fig. 1.2: Thesis flow diagram outlining the background and rationale, main research question, and specific aims for each data chapter.

Chapter two

An integrated approach for assessing the survival of discarded sandbar sharks, *Carcharhinus plumbeus*, captured in scientific longlines

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Abstract

Context: The sandbar shark (*Carcharhinus plumbeus*) has a global distribution and is caught by commercial fishers and recreational anglers.

Aims: Assess the stress physiology, release condition, and post-release survival of sandbar sharks caught in longline surveys conducted in Western Australia.

Methods: Assessed the post-release survival of sandbar sharks caught in longlining surveys using an integrated approach that combined the use of hook-timers, qualitative release conditions, satellite-tagging, and blood physiology.

Key results: Of 57 individuals examined, there was 100% post-capture survival after a maximum of four hours on the hook. Most of these animals (88%) displayed a strong release condition, exhibiting minimal behavioural impairment. All 13 satellite-tagged individuals survived 30-days post-capture. Sharks dived up to 307m deep and showed cyclical depth movement patterns with some individuals moving through the water column both day and night, while others moved almost exclusively at night. The concentration of blood metabolites did not significantly change with time-on-hook.

Conclusion: 100% post-capture and post-release survival after up to four hours on hooks suggests the use of longlines for surveying sandbar shark abundance has no deleterious effects on captured sharks.

Implication: This will support future stock assessments of sharks by quantifying the survival rates in the methods used for long-term monitoring of sandbar shark populations.

Keywords: stress physiology, PSAT tags, release condition, Chondrichthyes

1. Introduction

Elasmobranchs are one of the most vulnerable vertebrate groups living in our oceans. An increasing number of elasmobranchs are being classified as either Endangered or Threatened by the International Union for Conservation of Nature Red List due to the life history characteristics (slow growth rates, late age at maturity, low fecundity, and long-life span) that make the group vulnerable to overfishing (Clementi *et al*., 2020; Dulvy *et al*., 2021, Pacoureau *et al*., 2021). The economic value of fins and other elasmobranch by-products has resulted in increased fishing pressure worldwide (Cardeñosa *et al*., 2020; Clarke *et al*., 2007; Clarke *et al*., 2006). Sharks are directly targeted for the fin trade and 'flake' consumption but are caught and discarded by fishers targeting other species (Clarke, Milner-Gulland and Bjørndal, 2007; Dulvy *et al.*, 2021; Oliver et al., 2015).

Empirical estimates of discard mortality are often unavailable when estimating commercial or recreational fishing mortality, and the fate of discarded sharks is largely unknown (Braccini *et al*., 2021; Clarke *et al*., 2006; Davidson, Krawchuk and Dulvy, 2016). This information is key for the accurate assessment of stocks and the development of effective fisheries management and conservation policies (Braccini *et al*., 2021). Different approaches have been used to assess the fate of discarded sharks upon release, either by quantifying post-capture survival (PCS; survival observed at the vessel) or post-release survival (PRS; survival after discarding). For example, changes in blood chemistry (e.g. lactate, glucose, pH, urea) can be used to estimate the likelihood of discard survival (Dapp *et al*., 2016), while methodologies such as cages to monitor survival (Rulifson, 2007), tag-recapture (Hueter *et al*., 2006), and electronic tracking (Kneebone *et al*., 2013) are also used (Braccini, Van Rijn and Frick, 2012; Ellis, McCully Phillips and Poisson,

2017). However, estimates of both PCS and PRS are needed to quantify the overall mortality of the captured sharks (Braccini, Van Rijn and Frick, 2012; French *et al*., 2015; Hutchinson *et al*., 2015; Hutchinson and Bigelow, 2019).

Several blood physiology indicators have been used to quantify how sharks respond to the catch-and-release. In recent years adrenocorticotropic hormone (ACTH) levels have been used to evaluate the role of ACTH, a pituitary hormone responsible for stimulating the release of glucocorticoids (GCs) associated with physical stress events. Although, these studies are very limited in sharks and their roles are not entirely understood concerning capture stress (Fuller et al., 2020). While 1α-hydroxycorticosterone (1α-OH-B) is the primary internal corticosteroid produced by elasmobranchs (Anderson, 2012) to help the body to respond to physical stress (Anderson, 2012; Armour, O'Toole and Hazon, 1993; Ruiz-Jarabo et al., 2019; Schoen et al., 2021), corticosterone (CORT) has also been linked to initial stress coping mechanisms in elasmobranchs (Iki et al., 2020). Corticosterone constitutes 10% of overall GCs levels in sharks and has been used to assess the stress response in conjunction with other metabolites. However, CORT can be separately expressed with maturity and reproductive state, which should be considered when evaluating concentrations. These initial endocrine coping mechanisms to stress trigger a range of secondary stress responses such as lactate, glucose, and hydromineral balance (Hammerschlag et al., 2020; Heberer et al., 2010; Karsten, 2000).

Satellite tagging, such as with pop-up satellite archival transmitting (PSAT) tags, is an effective method for assessing PRS (Mohan *et al*., 2020, Schaefer *et al*., 2021). However, the cost of satellite tags limits the number of individuals tagged (French *et al*., 2015; Kohler and Turner, 2001). The PSAT tag transmits light, temperature, and depth data in a range of temporal frequencies which

can be altered to suit the species of shark studied (Lynch *et al*., 2017; Sulikowski *et al*., 2020). Many charismatic species have been monitored using this style of tag, with varying results in PRS and spatiotemporal movements (French *et al*., 2015; Hutchinson and Bigelow, 2019; Hutchinson *et al*., 2015; Sulikowski *et al*., 2020).

Sandbar sharks (*Carcharhinus plumbeus*) have a global distribution across temperate and tropical waters and are captured in commercial and recreational fisheries worldwide (McAuley *et al*., 2005). Historically, commercial shark fisheries in northern Western Australia (WA) targeted sandbar sharks, with a peak of >700 tonnes landed in 2004 – 2005 (Braccini, Molony and Blay, 2019; McAuley *et al*., 2005). Annual scientific shark surveys in northern WA have been conducted for >20 years (commencing in 2001) to monitor sandbar shark abundance (Braccini, Molony and Blay, 2019). Since large spatial closures were implemented in 2005 and the cessation of operations in the remaining northern shark fisheries, these scientific surveys indicate that there has been an increase in the abundance of sandbar sharks along the northwest coast (stock statuses: over-exploited in 2005 and recovering in 2021) (Braccini, Molony and Blay, 2019; Newman *et* al., 2021). Sandbar sharks are routinely tagged-and-released as part of these surveys, but there are limited reports of recaptured individuals from either recreational or commercial fisheries, and little is known about the fate of tagged sandbar sharks upon release in the survey area.

In the USA, sandbar sharks caught from demersal longlines exhibited variable PRS (29% to 96%) due to differences in gear specifications, shark size, soak times, and fishing depth (Gulak and Carlson, 2021; Marshall *et al*., 2015; Whitney *et al*., 2021). Therefore, results from previous studies may not be representative of the PRS of individuals discarded from all line fisheries (Gulak and Carlson, 2021; Marshall *et al*., 2015). Hence, further research on the fate of sandbar sharks

tagged-and-released from longlines, including experiment survey gear, is required for a more thorough understanding of its potential deleterious effects, particularly here in WA with a recovering population. The scope of this study was to assess whether experimental surveys influence the recovering population, considering other forms of commercial fishing in the region may have less influence (i.e., due to shark fishing regulations and cessation of the northern shark fisheries). We took an integrated approach using qualitative release condition, PSAT tagging, and analysis of blood physiological parameters (i.e., ACTH, GC, and lactate) to assess the likelihood of survival of sandbar sharks caught on demersal longline surveys.

2. Methods

2.1 Onboard sampling

Scientific demersal longline surveys were conducted in 2020 and 2021 at 10 fixed stations along the continental shelf of northern WA (**Error! Reference source not found.**).

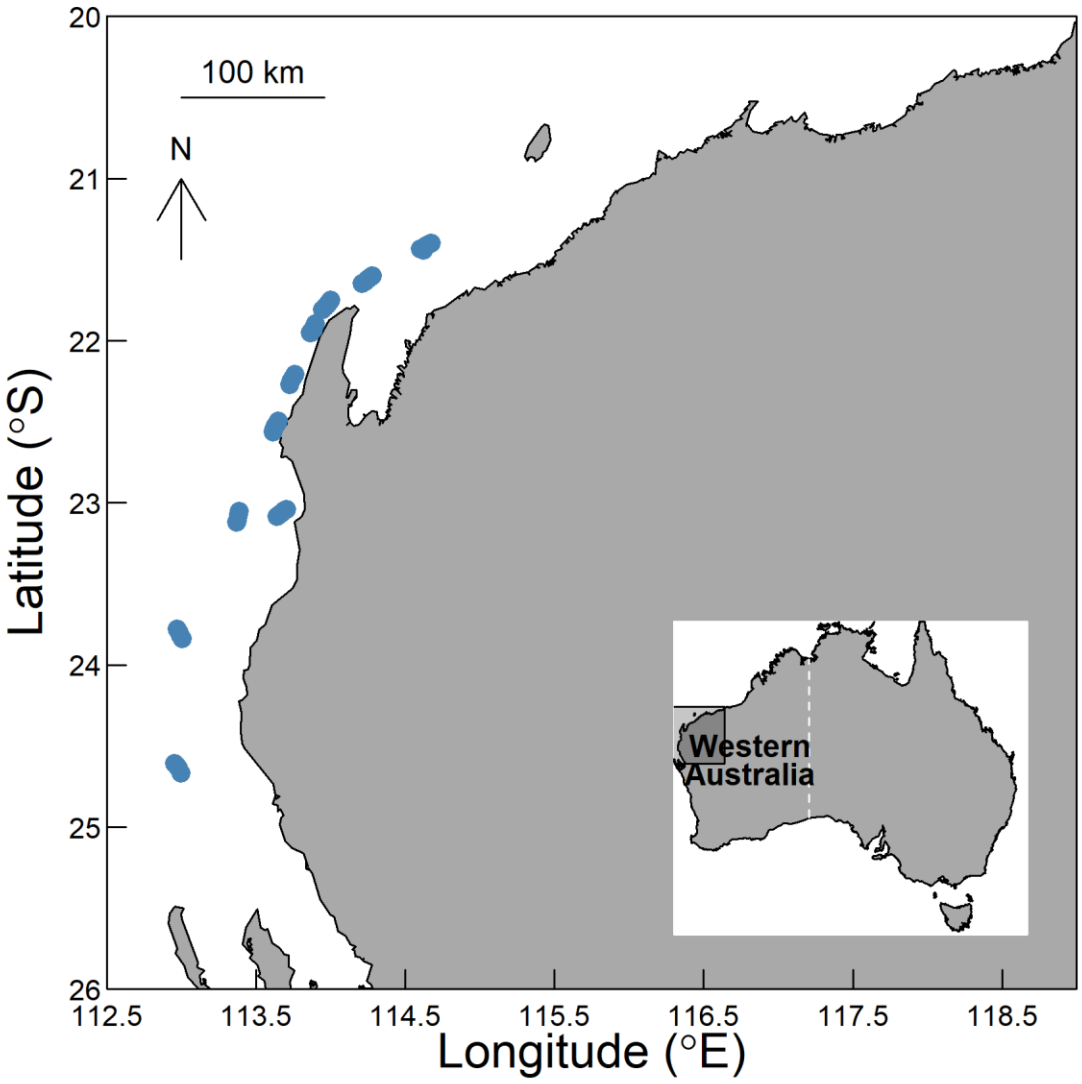

Fig 2.2: Map of demersal longline (blue) survey area in northern Western Australia. Each site has five replica longline sets.

Approximately 250 hooks were deployed each day. Each deployment comprised three to five longlines of ~500 m long each. 12/0 J-shaped hooks baited with sea mullet (*Mugil cephalus*) (cut into two to three pieces, ~20 cm) were attached to ~2 m metal snoods to the mainline. This

configuration was on a smaller scale than tradition commercial methods and for further details on gear configuration and deployment, refer to Braccini, Molony and Blay (2019). To determine time on hook (TOH, i.e., time spent on the line after hooking), hook timers (Lindgren-Pitman Inc.) were randomly attached to 16 snoods on each deployment. The start and end latitude and longitude, depth, water temperature, and time of day were recorded for each longline deployment. Thirty-nine longline sets were conducted over ten sites with soak times ranging from three to four hours.

Upon capture, sandbar sharks were brought on deck and the hook removed. The TOH was recorded, and blood samples (5 ml) were taken immediately from the caudal vein in 57 individuals randomly selected for PCS analysis by studying blood/plasma physiology, of which this process took approximately one minute. Sharks were then measured (fork length cm, FL) and sexed. Whole-blood lactate levels were measured *in situ* in all individuals, while the whole-blood samples from 29 of these sharks were centrifuged for five minutes at 8000 rpm, and plasma separated and stored at -20ºC for further analysis of ACTH and GC levels. Thirteen of the sharks sampled for further blood/plasma physiology were opportunistically fitted (with considerations of distributions across TOH) with survivorship pop-up satellite archival transmitting (PSAT) tags (Wildlife Computers Inc., WA, model: sPAT) to quantify PRS. Gills were not irrigated during handling to mimic commercial fishing operations. The PSAT tags were tethered to a nylon intramuscular anchor inserted at the base of the dorsal fin. All sharks captured were tagged with a plastic fin tag and released. At the time of release, the condition of each individual was classified as: (1) alive and strong, (2) alive, but weak/disoriented, (3) moribund (eye and jaw response when stimulated, otherwise exhausted and unresponsive), or (0) dead (Braccini and Waltrick, 2019).

2.2 Post capture survival (PCS) - Blood/plasma physiology

2.2.1 Adrenocorticotropic hormone (ACTH)

Plasma levels of ACTH were analysed using an ELISA kit (CUSABIO TECHNOLOGY LLC, Houston, TX, Product Code: CSB-E15926Fh). Absorbance was measured with a microplate reader (Stat Fax 303 Plus Awareness Technology, Inc. FL) at a wavelength of 450 nm and converted to pg.ml-1 using a four-parameter logistic curve. Plasma samples and assay standards, 50 µl, were assayed in duplicate in a single kit. The assay kit was validated by assessment of the slope of serial dilutions of plasma samples against the assay standards. The standard curve consisted of five ACTH concentrations ranging from 75 to 1200 pg.ml-1 and the serial dilutions consisted of two new standard curves spiked with two different 10 µl pools of plasma samples, each plasma pool constructed by adding 5 µl of ten individual samples (Fuller *et al*., 2020). Spiked standard curves showed good parallelism with the assay standard curve (Supplementary Material: Fig 1). For further information on calculating ACTH, refer the Supplementary Material, Adrenocorticotropic hormone.

2.2.2 Glucocorticosteroids

Total GCs were analysed using a CORT ELISA Kit (Cayman Chemical, Ann Arbor, M, Product code: 501320). This kit was previously validated to quantify 1α-OH-B, by using the cross-reactivity of the CORT antibody with 1α-OH-B and excluding other GCs by mass spectrometry (Evans *et al*., 2010; Iki *et al*. 2020; Lyons and Wynne-Edwards, 2019;). The cross-reactivity between CORT antibody and 1α-OH-B was reported to be 1.49% (Iki *et al*., 2020) and 5.2% (Evans *et al*., 2010). As reported by Cayman Chemical, the CORT antibody has high cross-reactivity with CORT (100%), 11-Deoxycorticosterone (15.8%), 11-dehydrocorticosterone (2.9%), and cortisol (2.5%). As we did not exclude other GCs, we assumed the CORT assay would

reflect total GC levels of all components that immunoreacted with the COT antibody, expressed in CORT units. For further information on calculating GCs, refer to the Supplementary Material; Glucocorticosteroids.

2.2.3 Lactate

A hand-held Lactate Pro 2 analyser (Arkray LT-1730) was used to measure whole-blood and plasma lactate concentrations *in situ* following the manufacturer's instructions. The Lactate Pro 2 reads in the range of 0.8–25 mmol/L-1 displaying 'high' for values higher than the upper range limit.

2.3 Post-release survival (PRS) – Satellite tagging

All PSAT tags were programmed to detach from the sharks after a 30-day monitoring period. The tags are designed to record depth, temperature, and light information which was archived at 24-hour intervals for the full 30-day deployment, and ten-minute intervals for the last five days of deployment. The PSAT tags were programmed to activate once the individual swam at a depth >10 m and to prematurely release (i.e., before the 30-day specified period) if the individual remained at a constant depth (± 2 m) for 24 consecutive hours. The tag transmits data to the ARGOS satellite system. Raw data were analysed by Wildlife Computers, which provided a report indicating the pop-off date, location, and daily values for temperature, light, and depth, as well as movement data at 10-minute resolution for the final five days (Drymon and Wells, 2017).

2.4 Data analyses

A two-way ANOVA was used to assess for differences in the blood/plasma metabolite concentrations between release condition and sex. Quantitative and semi-quantitative analytical methods such as Pearson's correlations and means plots were used to assess how well the concentrations of blood metabolites can be used as a survival predictor. Based on previous work on changes in plasma/whole blood metabolites with time (Fuller *et al*., 2020; Iki *et al*., 2020), ACTH, GCs and lactate levels were binned in 15-minute increments for the first hour on the hook, and then bins were expanded to 30-minute increments through four hours (i.e., the maximum time on the hook).

For the 13 PSAT tagged sharks, PRS was estimated using the transmitted depth data for the full 30-day deployment unless the tag detached prematurely. Premature PSAT tag release occurs in one of three situations: sharks that sink to the seafloor and remain below a certain depth (referred to as a 'sinker'), sharks sit at a constant depth ('sitter'), or if a tag was floating at the surface (maximum depth was ≤ one metre) ('floater') (Hutchinson *et al*., 2015; Lynch *et al*., 2017). Two mortality scenarios accounting for the five "floater" tags were considered: "floaters" were (S1) considered premature tag detachments from surviving sharks, or (S2) premature detachment was the result of mortality (and scavenging) or a predation event. . Kaplan-Meier (KM) survival models fit the data under these two scenarios and were used as a non-parametric approach to estimating survival. For each PSAT tagged shark, depth profiles were plotted for the last five days (one record per ten minutes) of the deployment periods. Changes in light, temperature, and depth values in the final five days of deployment were reviewed to classify the fate of 'floater' tags as predation events or premature detachments.

All data analysis was performed using R software (R version 4.2.3 (2023-03-15), R Foundation for Statistical 213 Computing, Vienna, Austria, see https://www.R-project.org/, accessed 12 April 2023) with packages: car (Fox and Weisberg, 2019), ggplot2 (Wickham, 2016), tidyverse (Wickham *et al*., 2019), survival (Therneau, 2023), ggpubr (Kassambara, 2022a), rstatix (Kassambara, 2022b), lubridate (Grolemund and Wickham, 2011), ggpmisc (Aphalo, 2022), maptools (Bivand and Lewin-Koh, 2023), RODBC (Ripley and Lapsley, 2022), chron (James and Hornik, 2023), stringr (Wickham, 2022), data.table (Dowle and Srinivasan, 2022), mgcv (Wood, 2017), mgcViz (Fasiolo *et al*., 2018), PBSmapping (Schnute, Boers and Haigh, 2022), geosphere (Hijmans, 2022).

3. Results

A total of 240 sandbar sharks (mean FL ± SE: 136.7 ± 0.3 cm) were captured with TOH ranging from 46 min to 250 mins (n=28, mean TOH ± SE: 105 ± 23 mins) (Supplementary Material: Table 1). Most sharks (98.8%) were hooked cleanly (i.e., through the side or bottom jaw), with only three sharks entangled in the line (all of which had blood samples taken). Two of these entangled sharks exhibited a physical injury to their gill slits. Fifty-seven sharks had blood samples (29 whole blood and 28 lactate only) collected (mean FL: 138.2 ± 0.5cm ; range: 82 – 162 cm), of which 13 were tagged with PSAT tags (mean FL: 138.2 ± 1.0cm ; range: 119 – 162 cm) (Fig 2.2).

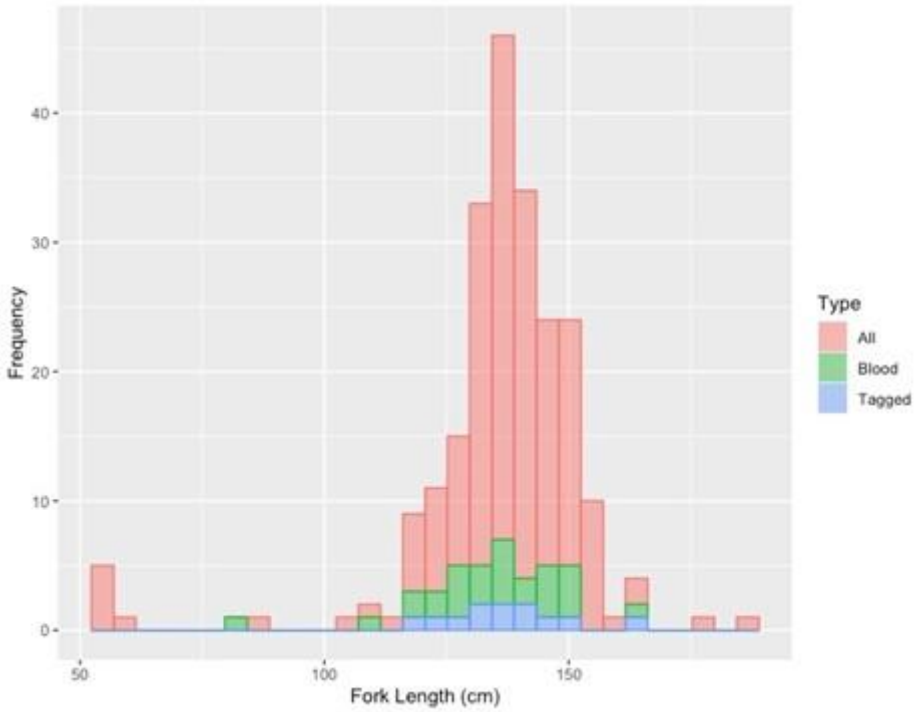

Fig 2.2: Length-frequency distribution (fork length FL cm) of all sandbar sharks (mean FL 136.7 ± 0.3 cm, n=240), with sharks that were blood sampled (mean FL 138.2 ± 0.5 cm, n=57) and tagged sharks (mean FL 138.2 ± 1.0 cm, n=13)

3.1 Post capture survival (PCS) - Blood/plasma physiology

Out of the 240 sandbar sharks caught in the surveys, 74% were assigned to release category 1. Most of the blood-sampled sandbar sharks (n=49) were assigned to release category 1, three in category 2, and five were in category 3. 100% PCS were observed in this study, and all animals were released after sampling. An ANOVA was not performed on release conditions due to a limited number of samples in conditions 2 and 3 for all blood metabolites, resulting in little statistical relevance. Conditions 2 and 3 were combined to compare sharks released in good

condition to those not in good conditions, although the sample size was still limited for the latter category.

No significant difference was detected between sexes for any blood physiology metric (ACTH: t=0.6, df=10, *P*=0.6; GCs: t=0.6, df=10, *P*=0.6; and lactate: t= 2.2, df=4, -*P*= 0.10).

Adrenocorticotropic Hormone values ranged from 62.6 to 109.0 $pg.ml^{-1}$ with variability observed at each time interval. No significant differences were observed in ACTH mean values through time (t=0.54, df=23, *P*=0.6). Glucocorticosteroid values ranged from 316.8 to 2013.7 $pg.ml^{-1}$, and although no significant differences (t=1.8, df=23, *P*=0.1) were seen through time, two higher values were recorded in the 181 min to 240 interval and these higher values corresponded to females (**Error! Reference source not found.**). Whole-blood lactate continued to rise with increased TOH (t=2.6, df=24, *P*=0.02) (**Error! Reference source not found.**).

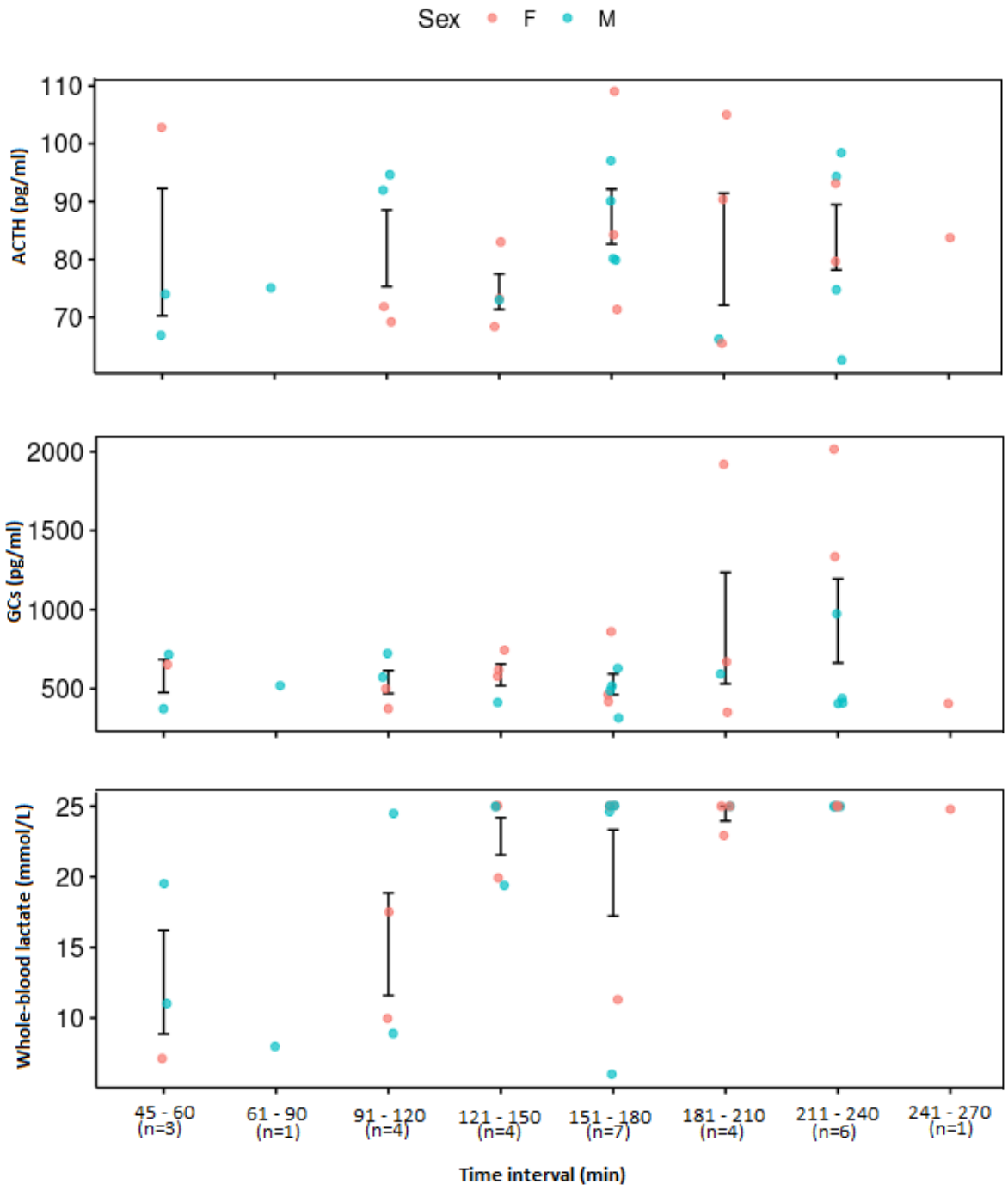

Fig 2.3: Mean whole-blood/plasma metabolite concentration of 29 sub-sampled sharks with all three blood/plasma metabolite values for time intervals Time on Hook (TOH) in minutes (Adrenocorticotropic hormone [ACTH], Glucocorticoids [GCs]). Means were calculated for

sample sizes ≥ 3. Error bars are standard error. Sex: Females (red), Males (blue). Lactate field values ranged from 0 - Hi (Hi ≥ 25 mmol/L); Hi values were given a value of 25.

Whole-blood lactate was measured for all PSAT tagged sharks (n=13, mean: 16.1 ± 7.2 mmol/L), although only 11 tagged sharks had hook times available (**Error! Reference source not found.**). Whole-blood lactate had higher mean values after two hours (before two hours: 13.9 ± 6.6 mmol/L ; after two hours: 21.9 ± 6.1 mmol/L).

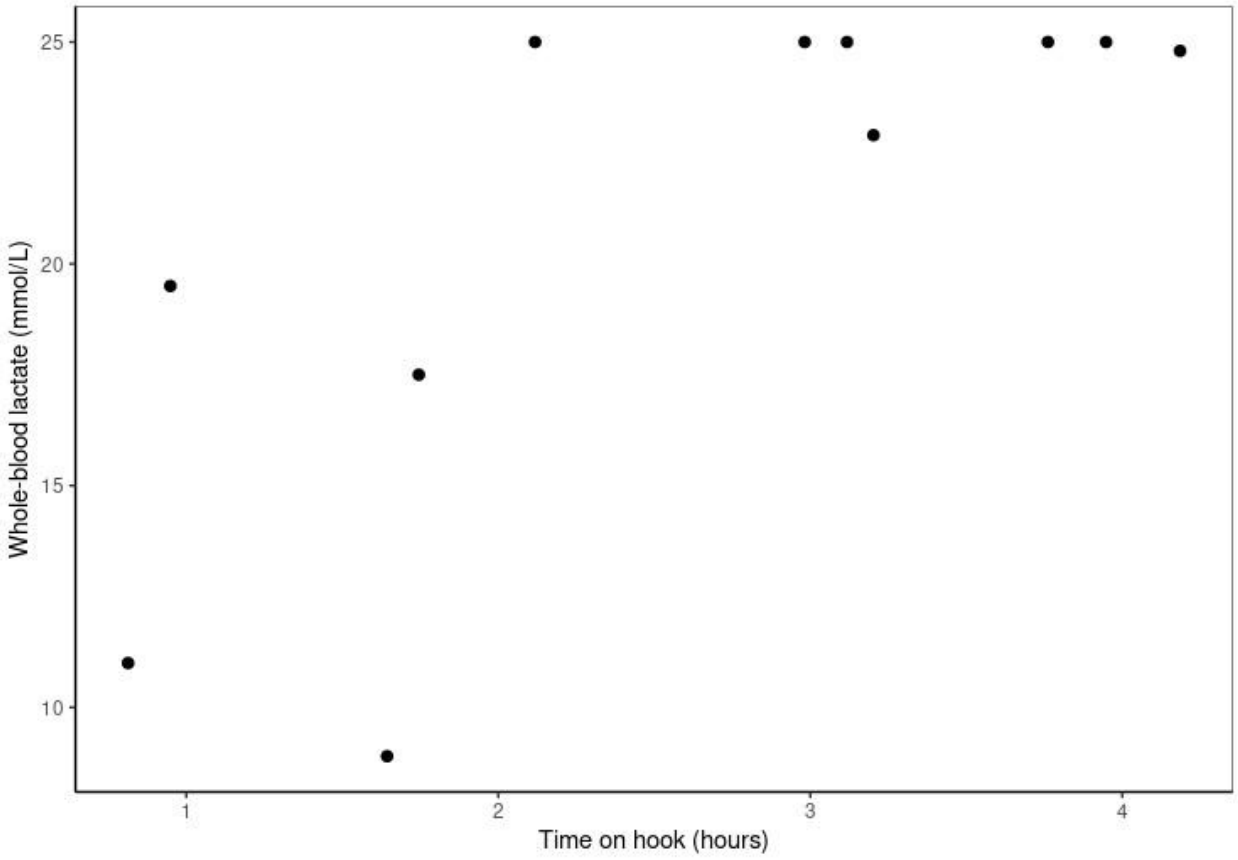

Fig 2.4: Whole-blood lactate concentration of 11 tagged sharks in comparison to time on hook (TOH) (hours). Lactate field values ranged from 0 - Hi (Hi ≥ 25 mmol/L); Hi values were given a value of 25.

3.2 Satellite Tags

3.2.1 Post-release survival (PRS) - Satellite tagging

Post-release survival varied from 100 to 61.5% (Scenarios #1 and 2, respectively) depending on the interpretation of premature PSAT tag releases associated with the five “floater” tags (Supplementary Material: Fig 3). Under scenario 1 (i.e., mortality equals 'sinker'), there was no mortality after 30 days so the KM model could not be fitted due to lack of contrast. Under scenario 2, five of the tagged sharks had premature tag releases (i.e., mortality equals ‘floater’) and are assumed to be mortalities in KM models. The five tagged sharks that had premature tag releases were primarily released in condition 1 (n=4), with TOH ranging from 103 to 222 mins (Supplementary Material: Table 1).

3.2.2 Depth profiles and vertical behaviour

For the last five days of deployment tagged sandbar sharks moved in the water column between the surface (0 m) and 276 m depth (Supplementary Material: Fig 4). There was a diel movement pattern with some individuals moving deeper during the day (06:00 - 18:00) and towards shallower waters at night (18:00 - 06:00) (Supplementary Material: Fig 4). Some sharks remained at a more constant depth during the day (e.g., SS #1) and others had larger vertical behaviours (e.g., SS #11). Shark SS #11 had the most visible diel patterns with strong peaks in depth in the last five days of tag deployment (Fig 2.5).

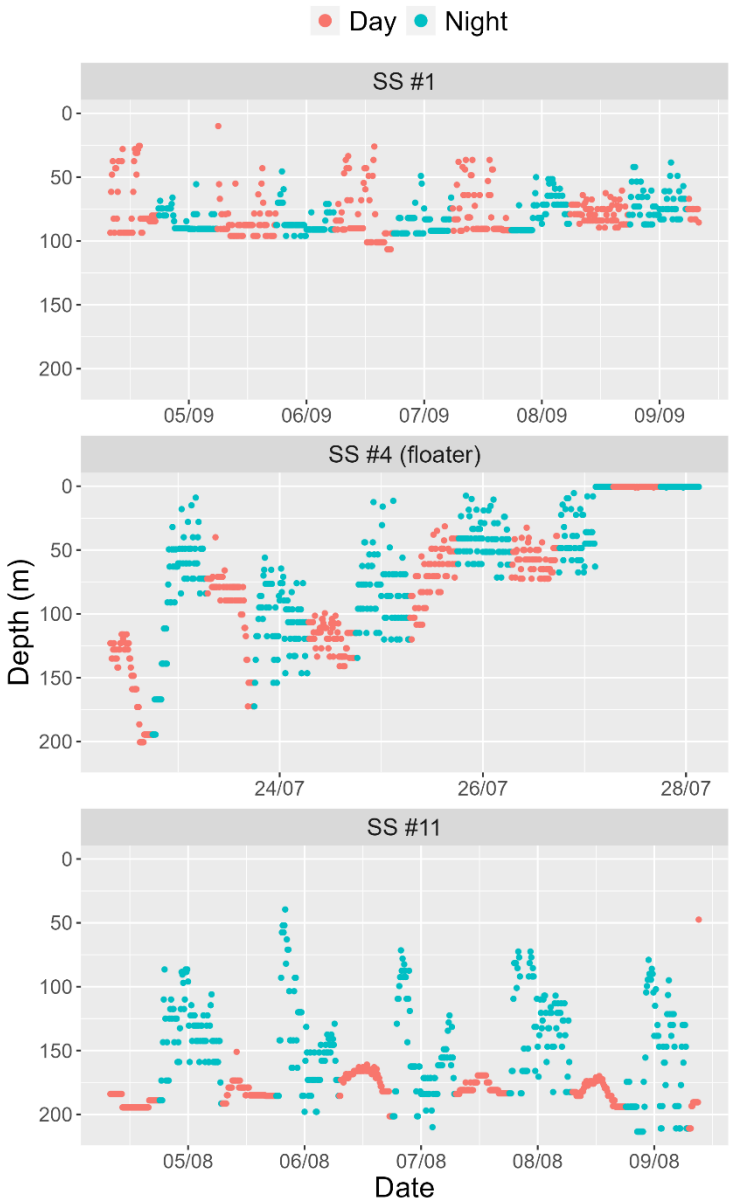

Fig 2.5: Three types of temporal patterns in depth (constant, floater, and diel) for the last five days of deployment for a sub-set of satellite-tagged sharks (day: 06:00 - 18:00; night: 18:00 - 06:00; date: day/month) .

Five sharks (tag numbers SS #4 (Fig 2.5), and SS #2, 6, 8, and 13 (Supplementary Material: Fig 4)) showed characteristics categorised as ‘floaters’ with depth being constant at 0.5m for 24 hours. All ‘floater’ tags’ temperature and light patterns remained consistent with tags at similar depth

ranges that remained attached for the 30-day period. These patterns in light and temperature in the 'floater' tags did not conform to the values usually seen in predation events, such as an expected in changes in vertical movement profiles, and attenuation in temperature and light level variation from ingestion. There was also no evidence of a mortality event, followed by immediate scavenging and tag detachment (i.e., the animal sinking to the seafloor for a brief period (brief constant depth) before the tag detached.) For results on the minimum and maximum depth profiles of the 13 tagged individuals over the entire 30-day period, refer to Supplementary Material: Fig 5.

4. Discussion

The scope of this study was to assess whether experimental surveys influence the recovering population of sandbar sharks and subsequently address if the limited recapture reports from annual scientific surveys were due to capture-related mortality. We combined the use of satellite telemetry and stress physiology to assess if the low recapture rate of sandbar sharks was due to a low PRS in the scientific surveys (8.5% recaptured) as hypothesised by (Bartes *et al*., 2021). We found that all captured animals survived a maximum four hours hooking time.

4.1 Post capture survival (PCS) - Blood/plasma physiology

Hormones are the first indication of stress responses in vertebrates (Armour, O'Toole and Hazon, 1993; Fuller *et al*., 2020). In a controlled environment after an acute stress event, we would have expected to observe an early peak in ACTH followed by an increase in GCs levels upon ACTH stimulation (Sapolsky, Romero and Munck, 2000). However, considering sharks were brought on board and sampled after at least 45 min on the hook, we were not able to register the initial hormonal behaviour at the beginning of the stress response as shown in other shark studies

performed in controlled conditions (Fuller *et al*., 2020; Iki *et al*., 2020). Previous work on ACTH as a stress parameter in the Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*) showed similar levels (ACTH: 50 to 120 pg.ml^{-1}) to our study (ACTH: 62.64 to 109.04 pg.ml^{-1}) (Fuller *et al*., 2020). In our study, ACTH showed variable values over time: the higher values could be a persistent capture stress, and/or a new acute stress event experienced by sharks whilst on the hook (e.g., presence of a predator). The lower values after 50 min could be an indication of the beginning of ACTH clearance after the initial stress response as excess GC levels negatively feedback the production of ACTH (Barton, 2002; Cain and Cidlowski, 2015). The time response to GCs following the production of ACTH is variable among species, but in teleost fishes, it typically take minutes (Pankhurst, 2011). In this study no clear inhibition of ACTH levels were observed, likely because sharks remained stressed over the entire TOH or differing levels of energy expression in individuals (i.e., routinely swimming vs short bursts of escape behaviours) (Bouyoucos et al., 2018; Gallagher, Staaterman and Cooke, 2017). Levels of GCs in elasmobranch species were reported to increase less than an hour after an acute stress event in Japanese banded houndshark (*Triakis Scyllium;* Iki *et al*., 2023) and blacktip reef sharks (*Carcharhinus melanopterus;* Schoen *et al*., 2021) and up to 24hrs after the initial stress event (cururu stingrays, *Potamotrygon wallacei*) (Brinn *et al*., 2012). Variable PCS rates in sandbar sharks have yet to be explored concerning hormone production. The limitation of sample size in this study and other studies reduces the ability to observe these hormonal differences between individuals and different species. The number of samples in this study used for hormonal blood analysis was driven by equipment shortages and difficulty with taking blood (clotting of blood) and future studies should focus on larger sample sizes (n = 64) to avoid this limitation ($\alpha = 0.05$, f = 0.25, power = 0.8).

During capture, increased energy expenditure and/or impaired respiration (e.g., restricted motility due to length of gangion or net entanglement) leads to anaerobic metabolism, and its by-products, such as lactate (Brill *et al*., 2008; Skomal and Mandelman, 2012). Although lactate measurements *in situ* cannot be used to forecast survival following a stress event by capture, the whole-blood lactate values in this study (16.1 ± 7.2 mmol/L) are comparable to sandbar shark (11.5 ± 7 mmol/L) and to that of other Carcharhinidae (10.2 ± 11 mmol/L) in Marshall *et al*'s study(2012). Marshall *et* al (2012) compared Hematological indicators in 11 species in both pelagic and demersal longlines with varying soak times of 2-12 hours, although did not use hook timers. Varying PCS was observed with 9% PCS in Atlantic sharpnose sharks (Rhizoprionodon terraenovae) and 93.2% in blue sharks (*Prionace glauca*). With this study and the addition of hook timers, we build on the understanding of the physiological effects of TOH and PCS of sandbar sharks (Gulak and Carlson, 2021; Mandelman and Skomal, 2009).

Most of the sandbar sharks swam strongly after release, with only a few being in worse-release conditions (i.e., categories 2 or 3). Consequently, the elevated concentrations of GCs and ACTH over time reflect an adequate survival response for sandbar sharks caught in demersal longline surveys (Romero and Beattie, 2022). Due to the small number of blood samples (n=5, n=3) and tagged sharks (n=1, n=0) in release conditions 2 or 3, release condition could not be assessed as an indicator of PCS or PRS. Release conditions vary between species and gear types with dusky sharks have 52% in pelagic longline studies (Sulikowski et al., 2020) and Atlantic sharpnose sharks had 4% in rod and reel studies (Fuller *et al*., 2020) of catch recorded in release condition 1 (or equivalent rating), whereas sandbar sharks in Marshall *et al* (2015) study had a total of 87.4%, and this study had 88% in release condition 1. Release conditions have been a useful predictor in PRS

for other Carcharhinids, such as juvenile silky sharks(*Carcharhinus falciformis*), where larger sample sizes were available in each release condition category (Hutchinson *et al*., 2015b). Whilst qualitative metrics are difficult to compare, further research should aim to increase sample size (n=30, d=0.37, $\alpha = 0.05$, power = 0.8)to assess if release condition can explain the variation in PRS.

4.2 Satellite tags

4.2.1 Post-release survival (PRS) - Satellite tagging

Tagged individuals had 100% PRS. Sandbar sharks are known to be a robust species, with high rates of PRS (Gulak and Carlson, 2021; Marshall *et al*., 2015; Whitney *et al*., 2021). Whitney *et al*. (2021) reported 96.4% PRS for sandbar sharks caught by longlines in a US fishery after three hours TOH. Further, Marshall et al. (2015) reported variable and lower PRS with longer TOH (i.e., 100% survival with < 3 hours TOH; 80% survival with > 4 hours TOH), but this variability may also be attributed to differences in gear, handling practices, sex, and shark size, relative to other sandbar shark PRS studies (Lynch *et al*., 2017; Marshall *et al*., 2015). Our study also resulted in a higher number of adult sharks (136.7 ± 0.3 cm FL) than Marshall *et al* (2015) (99.5 ± 21 cm FL). For some species, such as silky sharks, mature sharks are more robust with 84.8 % PRS in longline fisheries (Shaefer *et al*., 2021) compared to juveniles with 16% in purse seine fisheries (Hutchinson *et al*., 2015). However, as stated previously, further research would have to be done to fully understand the species specific relationship between different factors and PRS (Knotek *et al*, 2022). Post-release survival rates of sandbar sharks are high compared to the 3% and 12% survival of dusky sharks (*Carcharhinus obscurus*) and 25% survival of Atlantic sharpnose sharks

after three hours TOH in other longline studies (Marshall *et al*., 2012; Marshall *et al*., 2015; Morgan and Burgess, 2007; Sulikowski et al., 2020; Whitney *et al*., 2021).

Previous PSAT PRS studies show that constant depth signatures reflecting a dead animal on the seafloor can be used to infer the fate of PSAT-tagged sharks (Drymon and Wells, 2017; French *et al*., 2015; Hutchinson and Bigelow, 2019; Hutchinson *et al*., 2015). In the current study, however, released individuals remained alive until the PSAT released from the anchor. Of these, five tags released prematurely and floated on the surface as a result of improper anchorage into the tissue or tag malfunction (Hammerschlag, Gallagher and Lazarre, 2011; Kohler and Turner, 2001; Musyl *et al*., 2011). Timing of post-release mortality across studies is consistent (Ellis, McCully Phillips, and Poisson, 2017) with most mortalities occurring within hours of release. Whilst these five individuals had a premature tag detachment days after release, which can indicate mortality/ predation, it is more likely that these 'floater' tags are a result of tag attachment failures. Although there was a chance for predation post-release, given predator abundance in the area, the vertical movement profiles of the five "floater" tags were consistent with surviving sharks and did not display any clear evidence of a predation event/attempt (i.e., attenuated temperature and light levels or brief periods of constant seafloor depth) (Braccini *et al.*, 2021; Mitchell et al., 2018; Ryan et al., 2019). . The larger depth variances seen prior to the 24 hours before tag detachment have previously been linked with surviving sandbar sharks (Marshall *et al*., 2015). This provides support to our assumption that 100% of the sharks tagged in this experiment survived the catch and release process under the longline fishing settings used as part of the survey.

4.2.2 Depth profiles and vertical behaviour

The behaviour exhibited by the satellite-tagged sandbar sharks mirrors that of their natural hunting behaviour (Conrath & Musick, 2008). This further supports that scientific surveys are not having negative impacts on sandbar sharks. The observed day/night cycle is supported by sandbar sharks' predominant prey item of squid, which has strong diel vertical movement patterns (Last and Stevens, 1994; Siwabessy, Penrose and Fox, 2000; Stevens and McLoughlin, 1991). The presence of squid in the mouths of tagged sandbar sharks was also noted during data collection (pers obs).

The maximum depth recorded for the entire 30-day deployment reflects the depth profile of the continental shelf in which the sharks were caught and released (McAuley *et al*., 2007; Siwabessy, Penrose and Fox, 2000). Sandbar sharks are known to inhabit depths from intertidal to 280 m (Stevens and McLoughlin, 1991), with depths of 172 m being recorded in studies by Conrath and Musik (2008). In our study, the data transmitted from the PSAT tags recorded depths of up to 307 m, deeper than previously recorded (Andrzejaczek et al., 2018; Conrath and Musick, 2007; Last and Stevens, 1994).

4.3 Conclusions

By combining the information on the immediate expression of ACTH, GCs and of whole-blood lactate levels continuing to rise with increased TOH (Fuller *et al*., 2020; Hoffmayer and Parsons, 2001), we theorise that the sandbar sharks remained stressed throughout the capture process. However, the post-capture and post-release survival are high under current survey sampling practices indicating this species is resilient to capture and handling during longline surveys. The extended TOH of four hours limited our ability to investigate the expression of ACTH and GCs to examine the relationship between the studied hormones and TOH. A shorter TOH would be required to fully explore this relationship through capturing the rapid expressions of these

circulating hormones. To observe a more clearly defined relationship between lactate levels and TOH, lactate levels should be measured for a longer period of time after the initial stress event (Gulak and Carlson, 2021; McAuley *et al*., 2005). Although a shorter TOH is recommended for testing primary stress responses for sandbar sharks, using whole-blood lactate as a secondary stress response could be an effective method of predicting mortality with longer exposure times, coupled with PRS monitoring to qualify modelling (Braccini, Molony and Blay, 2019; Gulak and Carlson, 2021). The depth profiles of sandbar sharks in the last 5 days of tag deployment support the possibility of sharks following their prey through the water column which could indicate the hardiness of the species after days returning to the ocean after the capture process, with some individuals exhibiting strong diel patterns of diving during the day and coming into shallower waters at night. Our results showed a clear indication that the current longline methods used in shark abundance surveys do not affect the post-capture and post-release survival of sandbar sharks. The combination of blood metabolites as stress parameters, release conditions and satellite tagging methods could be applied to other species or fisheries to develop predictive PRS modelling (Braccini, Van Rijn and Frick, 2012). This could decrease the costs of survival studies as well as create an understanding of how each species reacts to fishing pressures.

Ethics

Shark capture, tagging, and the taking of blood samples were done under exemptions of the *Fish Resources Management Act 1994*. Exemptions were granted to the Department of Primary Industries and Regional Development (DPIRD) for research.

All aspects of this study conform to the Australian Code for the Care and Use of Animals for Scientific Purposes 8th Edition (2014).

Declaration of Competing Interest

None.

Declaration of Funding

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Data Availability

Data is available upon request to the authors. Please contact matias.braccini@dpird.wa.gov.au

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Chapter three

Assessing survival rates of discarded tiger (*Galeocerdo cuvier*), Port Jackson (*Heterodontus portusjacksoni*) and dusky (*Carcharhinus obscurus*) sharks

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Abstract

Quantifying the post-release survival of shark species is important for understanding the fate of sharks caught as bycatch by commercial or recreational fishers, and/or targeted by scientific surveys. We assessed the post-release survival of tiger (Galeocerdo cuvier), Port Jackson (Heterodontus portusjacksoni) and dusky (Carcharhinus obscurus) sharks caught in Western Australia (WA) and Queensland (QLD), Australia. An integrated approach was used that combined the use of hook-timers, release condition staging, satellite tagging, and physiology parameters (adrenocorticotropic hormone, corticosterone, and lactate). Of the 39 tiger sharks examined, 95% were “alive and strong” with 100% of the three satellite-tagged sharks surviving when released after a maximum of 4 hours on the hook. Of the 25 Port Jackson shark individuals examined, 100% were “alive and strong” with 100% of the 5 satellite-tagged sharks' initial survival when released after a maximum of 18 hours in a net. Of the 25 dusky shark individuals examined, 72% were “alive and strong” with 100% initial survival when released after a maximum of 5 hours on the hook. Of the 4 satellite-tagged dusky sharks, 75% survived, with one individual possibly being predated following release. This research suggests that scientific surveys of shark abundance using longlines, drumlines for tiger and dusky sharks and gillnets for Port Jackson sharks have minimal effects on their survival post-capture and post-release. It also suggests that if these species are caught by commercial or recreational fishers and are discarded quickly and carefully, mortality rates should be negligible.

Keywords: stress physiology, PSAT tags, release condition, Elasmobranchs, Tiger shark, Dusky shark, Port Jackson shark.

3.1 Introduction

One-third of shark and ray species are at risk of extinction (Dulvy *et al* 2021) with oceanic shark populations having declined by 71% since the 1970s due to an 18-fold increase in relative fishing pressure (Pacoureau *et al*., 2021). Due to the slow-growth rates and low fecundity of sharks, it is important to determine the fate of discarded sharks to understand the effects that different fishing practices have on the survival of specific species.

Assessing post-capture survival (PCS) and post-release survival (PRS) helps to inform the effects that fishing can have on released sharks, either directly through mortality (Braccini *et al*., 2012; Hutchinson & Bigelow, 2019; Sulikowski *et al*., 2020) or indirectly, through post-release predation of discarded individuals (Hammerschlag *et al*., 2017; Raby *et al*., 2014). A range of approaches has been implemented to assess PCS and PRS. Whilst hook timers and at-vessel survival can be used to assess PCS, changes in blood chemistry (e.g., hormones, lactate, glucose, pH, urea) can be used to study both PCS and PRS (Atkinson *et al*., 2015; Dapp *et al*., 2016). Holding sharks in cages has been used to monitor PRS (Rulifson, 2007), as has tag-recapture (Hueter *et al*., 2006) and acoustic tracking techniques (Kneebone *et al*., 2013; Braccini *et al*., 2012). A combination of PCS and PRS approaches is the most reliable method for assessing the survival of discarded sharks (Braccini *et al*., 2012; French *et al*., 2015; Hutchinson *et al*., 2015a; Hutchinson & Bigelow, 2019).

Post-capture survival is assessed from the moment the shark is caught on the hook to the moment it is released and can be assessed by collecting data on the time spent on the hook (TOH) using hook-timers, at-vessel mortality, release indices, and blood/stress parameters (Butcher *et al*., 2015; Heberer *et al*., 2010; Kneebone *et al*., 2013). Both primary (glucocorticosteroids) and secondary (lactate, glucose, hydromineral balance) stress responses can be used to measure stress events in

shark species (Barton, 2002; Hammerschlag *et al*., 2017; Heberer *et al*., 2010; Karsten, 2000; Schoen *et al*., 2021). Corticosterone (CORT) and adrenocorticotropic hormone (ACTH) (Fuller *et al*., 2020) are considered possible indicators that can be used to determine early stress response (Barton, 2002; Brinn *et al*., 2012; Schoen *et al*., 2021). Although blood physiology may not readily be accessible due to the cost and need for specialised laboratory equipment, the combination of assessing primary and secondary stress responses creates more accurate species-specific studies. This has been demonstrated for sandbar sharks (*Carcharhinus plumbeus*) with stress hormone concentrations fluctuating indicating possible prolonged stress events and lactate levels indicating higher values with a potentially longer TOH (Grosse *et al*., in review).

Post-release survival can be assessed using blood/stress parameters and tagging methods, which can also help determine post-release predation (Drymon & Wells, 2017; Skomal, 2007). The use of satellite tagging methods can be limited in the type of data generated such as Satellite Popup Archival Transmitting (PSAT) tags which monitor a shark's short-term survival after release with data transmitting in a range of frequencies for light, temperature, and depth (Grosse *et al*., in review). These methods are limited by the cost of PSATs (~$2,500 AUD) which reduces the number of individuals tagged (French *et al*., 2015; Kohler & Turner, 2001). As a consequence, it is recommended that both post-capture and post-release methods are used in conjunction with each other to assess the fate of discarded sharks at a species-specific level.

Dusky (*Carcharhinus obscurus*) and tiger (*Galeocerdo cuvier*) sharks are cosmopolitan species with dusky sharks being one of the main target species in commercial shark fisheries (Braccini *et al*., 2021; Heithaus, 2001; Sulikowski *et al*., 2020). Port Jackson sharks (*Heterodontus portusjacksoni*) are endemic to Australian waters and are a common bycatch species in commercial

gillnet and longline fisheries (Braccini *et al*., 2012; Frick *et al*., 2010). Whilst tiger and Port Jackson sharks are not currently assessed for stock status, dusky sharks have management arrangements in Western Australia to aid in the recovery of the population after overexploitation, including maximum size limits that protect larger breeding individuals (McAuley *et al*., 2005). Understanding PRS is important to inform the effectiveness of management actions and implications for the recovery of the stock.

Tiger sharks are sought after by recreational game fishers and are a common bycatch species (Shiffman *et al*., 2017). They are considered to be a hardy species with strong release conditions, although the fate of released sharks is still widely unknown (Afonso & Hazin, 2014; Whitney *et al*., 2021). Port Jackson sharks are also a common bycatch species in commercial gillnet fisheries in Western Australia (Braccini *et al*., 2012). They have spiracles (external respiratory openings) that allow them to gulp and breathe with little in the way of -reported stress characteristics (e.g., lethargy) during the capture and release. With limited studies on the fate of discarded Port Jackson sharks and minimal recreational bycatch reporting, PRS for this species is still unknown (Braccini *et al*., 2012; Frick *et al*., 2010).

The objective of this research is to quantify the fate of tiger, Port Jackson, and dusky sharks that had been caught in scientific surveys or commercial vessels which had observers onboard. We used an integrated approach using release conditions, PSAT tagging, and analysis of blood physiological parameters (i.e., ACTH, CORT, and lactate) to assess the likelihood of survival of tiger, Port Jackson and dusky sharks sampled during the shark abundance surveys and observer surveys conducted on the coast of WA and QLD.

3.2 Methods

3.2.1 Onboard sampling

3.2.1.1 Longlines

Scientific longline surveys were conducted in 2020 and 2021 at 10 fixed stations along the continental shelf of northern WA. Approximately 250 hooks were deployed each day. Each deployment comprised three to five longlines of ~500 m long each. 12/0 J-shaped hooks baited with sea mullet were attached to 70 ~2 m metal snoods to the mainline. For further details on gear configuration and deployment, refer to (Braccini *et al*., 2019). The configuration used is a smaller scale of previously used gear specifications in the tropical shark fishery prior to closing in 2005 (less soak time and a smaller number of hooks). To determine TOH, 16 hook timers (Lindgren-Pitman Inc.) were randomly attached to the snoods on each deployment to assess PCS. The start and end latitude and longitude, depth, water temperature and time of day were recorded for each longline deployment. Thirty-nine longline sets were conducted over ten sites with soak times ranging from three to four hours. Upon capture, dusky and tiger sharks were brought on deck to remove the hook. The TOH was recorded, and sharks were measured (fork length cm, FL) and sexed. Blood samples (5 ml) were taken from the caudal vein in 24 individuals randomly selected for blood/plasma analysis. Whole-blood lactate levels were measured in situ in all 24 individuals using LacPro 2 Analyser, while the blood samples of 15 dusky sharks were centrifuged for five minutes at 8000 rpm, and plasma was separated and stored at -20ºC for further analysis. Four of the dusky sharks and three tiger sharks sampled for blood/plasma parameters were randomly fitted with PSAT tags (Model MK10, Wildlife Computers Inc., WA) to address the PRS. Gills were not irrigated during handling to mimic commercial fishing settings. The PSAT tags were tethered to a

nylon intramuscular anchor inserted at the base of the dorsal fin. All sharks were tagged with a plastic fin tag and released. At the time of release, their condition was classed as: (1) alive and strong, (2) alive, but weak/disoriented, (3) moribund (eye and jaw response when stimulated, otherwise exhausted and unresponsive), or (0) dead.

3.2.1.2 Gillnets

Port Jackson sharks were sampled in the west coast and south coast bioregions of Western Australia by scientifically trained observers in 2020 onboard commercial fishing vessels using gillnets (6- and 7-inch mesh size). Gear was deployed day and night in depths of between 1 and 230 m for between 1 and 26 h. The date, time, GPS location, and bottom depth (in metres) were recorded for each gear deployment. Upon gear retrieval, all individuals were sexed, and their fork length (FL) was measured (in centimetres). Time in net (TIN) was calculated by the maximum time the shark could be on the net using set and haul times to assess PCS. For further details on gear configuration and deployment, refer to (Braccini & Taylor, 2016) and (Braccini, 2016). Whole-blood lactate levels were measured in situ in all individuals using LacPro 2 Analyser, and the blood samples of 17 sharks were centrifuged for five minutes at 8000 rpm, and plasma was separated and stored at -20ºC for further analysis. As described above, five of the sharks sampled for blood/plasma physiology were randomly fitted with survivorship pop-up satellite archival transmitting (PSAT) tags. The gills of sharks were not irrigated during handling to mimic commercial fishing settings. All sharks were tagged with a plastic fin tag and released. At the time of release, a release condition was assigned.

3.2.1.3 Drumlines

Scientific drumline and line fishing surveys targeting tiger sharks were conducted by OCEARCH (www.ocearch.org) in 2014 near Fraser Island and Batt Reef near Port Douglas, Queensland. Tiger sharks caught near Fraser Island (3 sharks) were all caught using a hook, line, and buoy, mimicking configuration of drumlines used by the Queensland state government in the shark control program. They were then brought up on the OCEARCH vessel platform. All sharks had their gills irrigated and were kept on the platform for a maximum of 15 minutes.

All tiger sharks caught on Batt Reef were via drumlines, which were checked regularly in 30–60-minute intervals. Eight to ten single-hook drumlines were deployed between sunrise and sunset (~5:30 h to 18:30 h), at 2–20 m depth (refer to Barnett *et al*., 2022 for details on gear configuration). Sharks were measured (Total length cm, TL and later converted to FL (Fig. 3.1)) using conversion factors (De Wysiecki & Braccini, 2017) and sexed. Blood samples (5 ml) were taken from the caudal vein analysis of blood/plasma parameters. Whole-blood lactate levels were measured in situ in all individuals using LacPro 2 Analyser, while the blood samples of 10 sharks were centrifuged for five minutes at 8000 rpm, and plasma was separated and stored at -20ºC for further analysis. All sharks were tagged with a plastic fin tag and released. At the time of release, release conditions were assigned.

3.2.2 Stress Physiology

3.2.2.1 Adrenocorticotropic hormone (ACTH)

Plasma levels of ACTH were analysed using an ELISA kit (CUSABIO TECHNOLOGY LLC, Houston, TX, Product Code: CSB-E15926Fh) (Fuller *et al*., 2020; Grosse *et al*., in review).

Briefly, plasma samples and assay standards, 50 µl, were assayed in duplicate in a single kit. The standard curve consisted of five ACTH concentrations ranging from 75 to 1200 pg.ml-1 and the serial dilutions consisted of two new standard curves spiked with two different 10 µl pools of plasma samples, each plasma pool constructed by adding 5 µl of ten individual samples. Samples showed good parallelism with the assay standard curve. Absorbance was measured with a microplate reader (Stat Fax 303 Plus Awareness Technology, Inc. FL) at a wavelength of 450 nm and converted to pg.ml-1 using a four-parameters logistic curve. Samples were assayed in duplicate in a single kit. The intra-assay coefficient of variation for the assay was 7.15%, the accuracy of the assay was 1.20% (± 1.62 SE), and the detection limit (80% B/B0) was 72 pg.ml-1. No cross-reactivity between fish ACTH and other plasma components has been reported by the manufacturer, however, some cross-reactivity may still exist.

3.2.2.2 Corticosterone (CORT)

Corticosteroids were analysed using an ELISA Kit (Cayman Chemical, Ann Arbor, M, Product code: 501320). This CORT kit was previously validated to quantify 1α-hydroxycorticosterone (1α-OH-B), by using the cross-reactivity of the CORT antibody with 1α-OH-B and excluding other corticosteroids by mass spectrometry (Evans *et al*., 2010; Iki *et al*., 2020; Lyons & Wynne-Edwards, 2019). The cross-reactivity between the CORT antibody and 1α-OH-B was reported to be 1.49% (Iki *et al*., 2020)and 5.2% (Evans *et al*., 2010). As reported by Cayman Chemical, the CORT antibody has high cross-reactivity with CORT (100%), 11-Deoxycorticosterone (15.8%), 11-dehydrocorticosterone (2.9%), and cortisol (2.5%). As we did not exclude other corticosteroids, we assumed that the CORT assay would reflect relative corticosteroid levels measured in CORT units.

Plasma samples, 200 µl, were extracted twice with diethyl ether (1:5), vortexed for 30 sec, and frozen at -20 °C for an hour to separate the phases (Grosse *et al*., in review). Briefly, the diethyl ether phase was evaporated by nitrogen and reconstituted with 100 µl of ELISA buffer (fractions were twice concentrated due to the general low values of CORT reported in elasmobranchs). For the assay, 50 µl of each reconstituted sample was assayed in duplicate in a single kit. The absorbance of each well was measured with a microplate reader at a wavelength of 410 nm and converted to pg.ml-1 by a four-parameters logistic curve and linearised using a logit transformation. The recovery of spiked CORT from dusky and Port Jackson sharks' plasma was 87%. Validation of the assay kit was done by comparing the serial dilution of plasma samples with the assay standards. The intra-assay CV was 9%, the accuracy of the assay was 1.09 % (± 1.54 SE) and the detection limit was 30 pg.ml-1. Primary stress responses (ACTH and CORT) were not possible to measure in tiger sharks (Results: Table 3.1) and therefore some information is not available for comment.

3.2.2.3 Lactate

A hand-held Lactate Pro 2 analyser (Arkray LT-1730) was used to measure whole-blood and plasma lactate concentrations in situ following the manufacturer's instructions. The Lactate Pro 2 reads in the range of 0.8–25 mmol/L-1 displaying "Hi" for the values above. These "Hi" values were given a value of 25 mmol/L for analysis.

3.2.3 Post-capture survival (PCS)

At-vessel survival was assessed by observing living and deceased sharks upon retrieval of the fishing gear (longlines, gillnets and drumlines). Any deceased sharks were retained on board for

full necropsy which included reproductive status, gut contents, genetic testing and blood parameter analysis if applicable. As stated in section 3.2.1, TOH and TIN was taken to assess PCS against blood parameters in the three species of shark.

3.2.4 Post-release survival (PRS) - Satellite tagging

All PSAT tags were deployed with a detaching period of 30 days. The tags are designed to record depth, temperature, and light information which was archived at 24-hour intervals for the full 30-day deployment, and ten-minute intervals for the last five days of deployment. The PSAT tags were programmed to activate once the individual swam below 10 m in depth and to release prematurely (i.e., before the 30-day specified period) if the individual remained at a constant depth (± 1 m) for 24 hours, indicating mortality. The first Port Jackson shark tagged had its tag prematurely detach after 24 hours, and due to the nature of the shark (limited vertical depth behaviour and low movement), it was assumed the original programming was not suited to this species. The tag parameters were changed for the subsequent Port Jackson sharks to activate on the shark reached below 5 m from the surface and to prematurely release if the individual remained at a constant depth (± 2 m) for 48 hours. The tag transmits data to the ARGOS satellite system. Raw data were analysed in-house by Wildlife Computers, which provided a report indicating the pop-off date, location, and daily values for temperature, light, and depth (Drymon & Wells, 2017). For the last 5 days of tag deployment, values were measured in ten-minute time intervals.

3.2.5 Data Analysis

Means plots were used to assess how well the concentrations of blood metabolites can be used as a survival predictor for these three species. For tiger sharks caught on drumlines, blood metabolites

were compared to the time between capture and release. For dusky, tiger, and Port Jackson sharks caught in WA, blood metabolites were compared to hook timers for dusky and tiger sharks if available or the minimum and maximum possible amount of time spent on longline or in a gillnet for all species.

For the PSAT-tagged sharks, PRS was estimated using the transmitted depth data for the full 30-day deployment unless the tag detached prematurely. Premature PSAT tag release occurs in one of three situations: sharks that sink to the seafloor and remain below a certain depth (referred to as a 'sinker'), sharks sit at a constant depth ('sitter'), or if a tag was floating at the surface or if the maximum depth was ≤ one metre ('floater') (Hutchinson *et al*., 2015b; Lynch *et al*., 2017). There were two tagged sharks categorised as 'sitters' or 'sinkers', one Port Jackson and one dusky shark. Therefore, we considered two scenarios with scenario one (S1) setting mortality equal to 'sinkers' only and scenario two (S2) setting mortality equal to 'sinkers' or 'floaters'. Kaplan-Meier (KM) survival models fit the data under these two scenarios. For each PSAT-tagged shark, depth profiles were plotted for the full 30-day period (one record of min and max depth per day) and the last five days (one record per ten minutes) deployment periods. An ANOVA was not performed on release conditions due to a limited number of samples for all blood metabolites in conditions 2 and 3, resulting in little statistical relevance. All data analysis was performed using R software (version 1.4.1103: 2018) with packages: car, ggplot2, dplyr, tidyverse, survival, ggpubr, rstatix, lubridate, ggpmisc, maptools, RODBC, chron, stringr, data.table, mgcv, mgcViz, PBSmapping, geosphere.

3.3. Results

A total of 27 tiger sharks were caught on longlines (mean FL [±SE]: 199 cm [±1.7]) and 10 on drumlines (mean FL: 298 cm [±2.4]) with 34 sharks subsampled for stress physiology (32) and

PRS (11) (Table 3.1). Due to small samples sizes to assess PRS, these results are considered as preliminary.

Table 3.1: Catch records (species, number, fishing method), and the mean parameters for stress physiology, post-capture survival, and environmental conditions

Species	Method	Number Caught	Number of Lactate samples	Number of full blood samples	Number of PSAT tagged	FL (cm)	TOH/TIN (mins)	Release Condition	ACTH (pg/ml)	GCs (pg/ml)	Whole blood Lactate (mmol/L)	Depth (m)	Water Temperature (°C)
Tiger shark	Longline	27	24	0	3	199 ± 1.7	220 ± 1.5	1	N/A	N/A	17.4 ± 0.5	N/A	25.5 ± 0.2
	Drumline	10	10	0	0	298 ± 2.4	31 ± 1.2	1	N/A	N/A	9.9 ± 0.6	N/A	N/A
Port Jackson shark	Gillnet	23	22	17	5	71.1 ± 0.8	677 ± 3.0	1	97.5 ± 1.0	2115.5 ± 8.2	2.0 ± 0.2	N/A	N/A
Dusky shark	Longline	25	24	15	4	244.6 ± 0.9	220 ± 0.2	1	140.7 ± 1.0	676.8 ± 3.7	21.7 ± 0.5	N/A	25.6 ± 0.2

Tiger shark's TOH ranged from 80 min to 225 min for longlines and nine minutes to 62 min for drumlines. Two tiger sharks were foul hooked (i.e., not hooked through the mouth). Thirty-four tiger sharks had blood samples (24 lactate only) collected (mean FL: 240.1 cm [±1.7]; range: 78 – 362.7 cm), of which three were tagged with PSAT tags (mean FL: 222.3 cm [± 4.6]; range: 155 – 286 cm). Most of the blood-sampled tiger sharks (n=21 longlines, n=10 drumlines) were assigned to release condition category 1, four in category 2, and two in category 3. An ANOVA was not performed on release conditions due to a limited number of samples for all blood metabolites in conditions 2 and 3, resulting in little statistical relevance. There were 23 Port Jackson sharks caught in gillnets (mean FL: 71.18 cm [±0.82]). Port Jackson shark's maximum time in the net (TIN) ranged from 05:46 min to 17:02 min. All Port Jackson sharks had release conditions of category 1. Twenty-two Port Jackson sharks had blood samples (5 lactate only) collected (mean FL gillnet: 71.1 cm [±0.8]; range: 55 – 89 cm (excluding longlines)), of which five were tagged with PSAT tags (mean FL: 87.9 cm [± 1.3]; range: 79.5 – 95 cm). All of the blood-sampled Port Jackson sharks (n=22) were assigned to release condition category 1. An ANOVA was not performed on release conditions due to no samples for all blood metabolites in conditions 2 and 3, resulting in little statistical relevance. There were 25 dusky sharks caught on longlines (mean FL: 244.6 cm [±0.9]). Dusky shark's TOH ranged from 02:42 mins to 05:18 mins. In total, 72% of dusky sharks had release conditions in category 1. Twenty-four dusky sharks had blood samples (11 lactate only) collected (mean FL: 245.2 cm [±1]; range: 205 – 294 cm), of which 4 were tagged with PSAT tags (mean FL: 257.3 cm [± 2.5]; range: 220 – 275 cm). Most of the blood-sampled dusky sharks (n=18) were assigned to release condition category 1, five in category 2, and two in category 3.

3.3.1 Stress physiology

3.3.1.1 Adrenocorticotropic hormone

Adrenocorticotropic Hormone (ACTH) values of Port Jackson sharks ranged from 69.98 to 144.83 pg.ml-1 with low and high values observed over maximum TIN (Fig. 3.1). There was no correlation between ACTH and TIN (t= 1.42, df= 12, p-value= 0.18).

The ACTH values of dusky sharks ranged from 103.98 to 195.50 pg.ml-1 with low and high values observed over TOH. No correlation was observed in ACTH mean values through time (t=-0.56, df=12, p-value=0.59).

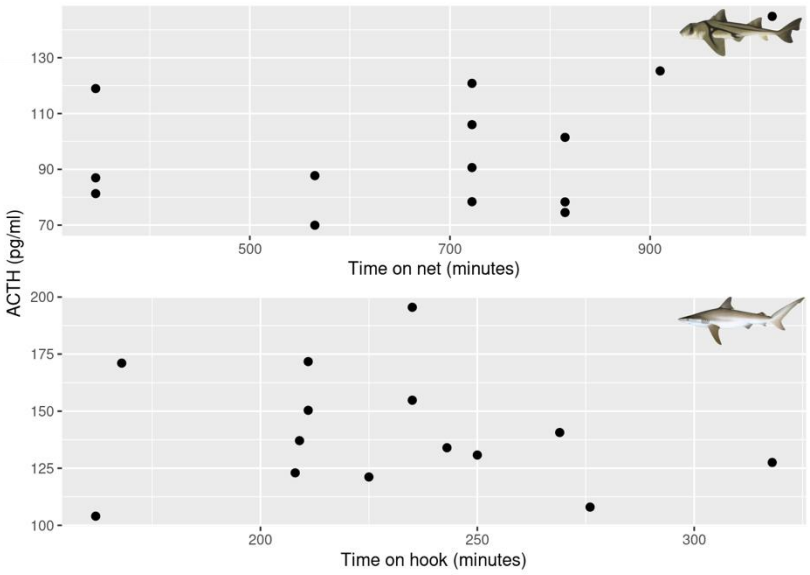

Fig. 3.1: ACTH levels of Port Jackson sharks and dusky sharks over time on hook/time in net (minutes).

3.3.1.2 Glucocorticosteroids

The GCs values of Port Jackson sharks ranged from 548.45 to 5012.84 pg.ml-1, and although a correlation (t=0.29, df=12, p-value=0.78) was seen through TIN, the higher GCs values were recorded in between 500 - 700 mins and these higher values corresponded to females (Fig. 3.2).

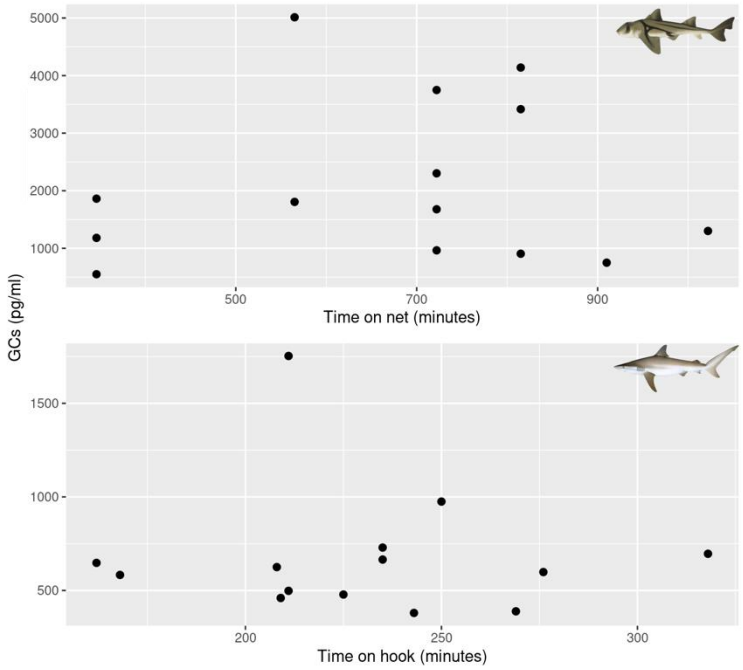

Fig. 3.2: GCs levels of Port Jackson sharks and dusky sharks over time on hook/time in net (minutes).

The GCs values of dusky sharks ranged from 379.79 to 1753.04 pg.ml-1, and although no correlation (t=-0.25, df=12, p-value=0.81) was seen through TOH, one higher value was recorded between 200 and 250 minutes (Fig. 3.2).

3.3.1.3 Whole-blood lactate

There was no correlation between whole-blood lactate and TIN/TOH for Port Jackson sharks (t=-1.47, df=18, p-value=0.16), for dusky sharks (t=-1.02, df=21, p-value=0.32) or tiger sharks caught on longlines (t=-0.17, df=16, p-value=0.87), or drumlines (t=-0.12, df=8, p-value=0.9) (Fig. 3.3).

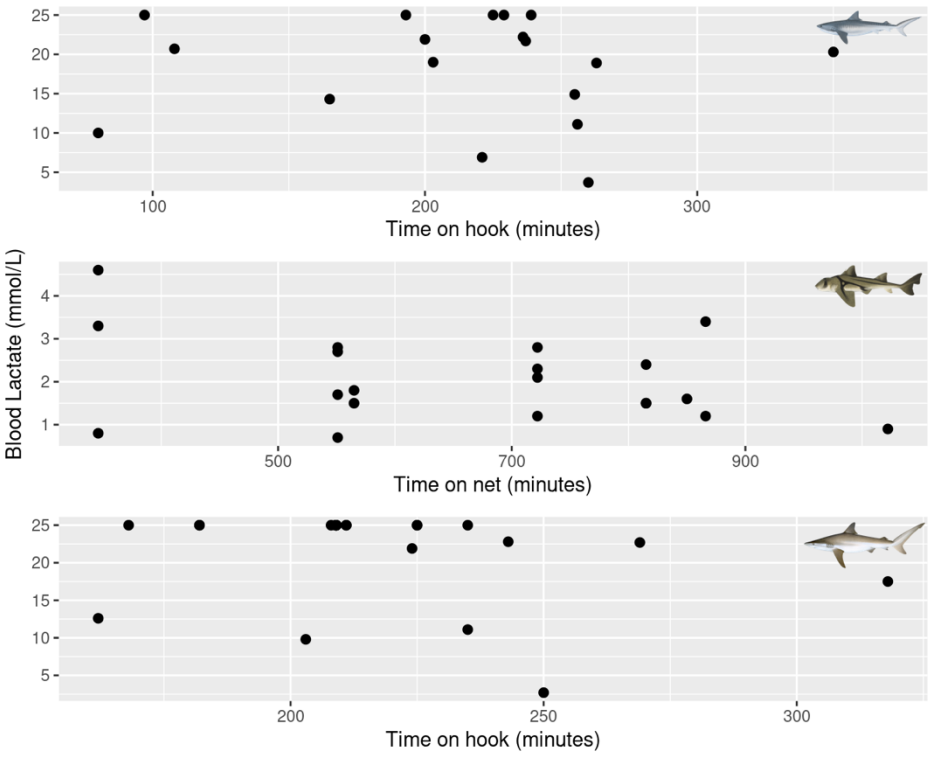

Fig. 3.3: Whole-blood lactate levels of tiger sharks, Port Jackson sharks and dusky sharks over time on hook/time in net (minutes). 'Hi' lactate levels were given the value of 25 mmol/L but could have exceeded that.

3.3.2 Post-capture survival (PCS)

There was 100% at-vessel survival for all of the tiger, Port Jackson, or dusky sharks observed in this study.

3.3.3 Satellite Tags

3.3.3.1 Post-release survival (PRS) - Satellite tagging

Post-release survival (PRS) varied depending on the interpretation of premature PSAT tag releases (Fig. 3.4). For more information on each tagged shark, please refer to supplementary material: Table 2.

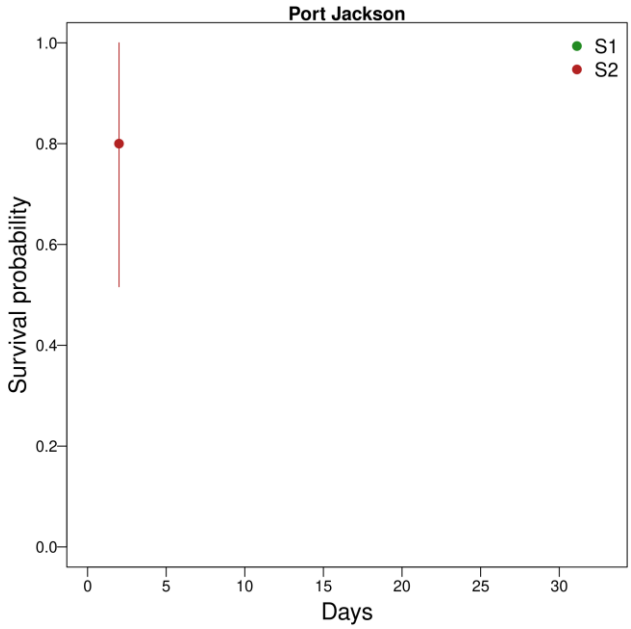

Fig. 3.4: Kaplan-Meier survival plot illustrating median survival probability over time (days) for tagged Port Jackson sharks released with PSAT tags. Where S1 is categorised as death = sinker and S2 is categorised as death = premature tag release. The vertical lines are the 95 % CI

Under scenario 1 (i.e., mortality equals 'sinker'), there was no mortality after 30 days so the Kaplan-Meier (KM) model could not be fitted due to lack of contrast. Under scenario 2, one tagged Port Jackson shark had premature tag releases (‘sitter’) assumed to be mortalities. Tiger sharks had

100% survival; therefore, KM model was not fit for purpose, and dusky sharks also did not have a fit for purpose KM model despite mortality.

3.3.3.2 Post-release predation

One tagged dusky shark (19P1640) showed tag anomalies after release within 5 hours of tag deployment. The tag sunk to the sea floor initially and did not transmit depth, light or temperature for 24 hours and then transmitted depth and temperature readings, although light showed no change, remaining at ~ 0 ΔE for the remainder of deployment (Fig. 3.5). The 19P1640 depth in the first five hours dropped to 100 m rapidly before some movement before the tag not transmitting. Light temperature and movement all rose just before the tag not transmitting, suggesting a scavenging event.

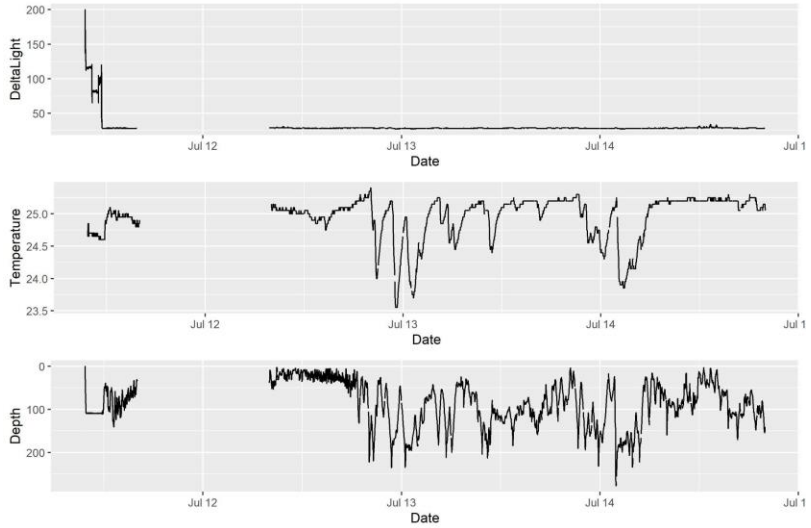

Fig. 3.5: tag transmissions (light (ΔE), temperature (°C), depth (m)) for full 30-day deployment from suspected consumed dusky shark (19P1640)

3.3.3.3 Depth profiles and vertical behaviour

For the last five days of deployment, tagged tiger sharks moved in the water column between the surface (0 m) and 78m depth, Port Jackson sharks between the surface and 46 m, and dusky sharks between the surface and 213 m (Fig. 3.6).

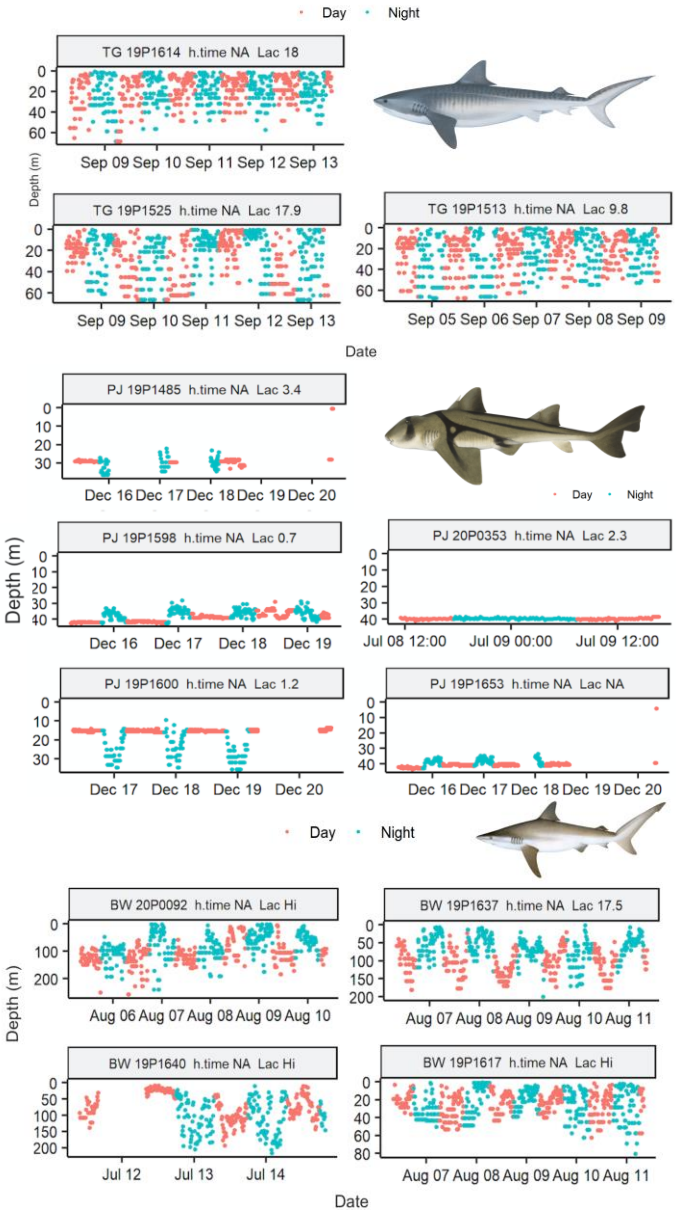

Fig. 3.6: Temporal patterns in depth for the last five days of deployment for satellite-tagged tiger (top), Port Jackson (middle), and dusky sharks (bottom) (day: 06:00 - 18:00; night: 18:00 - 06:00;

h.time: hook time (hours:minutes:seconds); Lac: lactate level, ranging between 0 mmol/L and Hi (≥25 mmol/L)).

Whilst tiger sharks showed no depth patterns between night and day, all tagged individuals occupied depth ranges between surface and approximately 80m. One Port Jackson shark (tag number 20P0353) showed characteristics categorised as 'sinker' with depth being constant at 0.5m for 24 hours. Port Jackson sharks remained at a more constant depth during the day, and some had larger vertical behaviours at night (18:00 - 06:00) (e.g., 19P1600). Shark 19P1600 had the most visible diel patterns with strong peaks in depth in the last five days of tag deployment (Fig. 3.6).

3.4. Discussion

3.4.1 Stress physiology

Stress events trigger hormonal changes as a first response in vertebrates (Anderson, 2012; Armour *et al*., 1993; Fuller *et al*., 2020). In a controlled environment after an acute stress event, it is expected to observe an early peak in ACTH followed by an increase in GCs levels upon ACTH stimulation (Sapolsky *et al*., 2000). However, considering sharks were brought on board and sampled after at least 150 minutes (mins) on the hook for dusky sharks or 330 mins in the net for Port Jackson sharks, it is probable that we were not able to register the initial hormonal behaviour at the beginning of the stress response as shown in other shark studies performed in controlled conditions (Fuller *et al*., 2020; Iki *et al*., 2020). Previous work on ACTH as a stress parameter in the sharpnose sharks (*Rhizoprionodon terraenovae*) showed similar levels to this study, ACTH levels peak 15 min after capture to remaining high for 60 min where a mix of low and high levels was observed (Fuller *et al*., 2020). In this study, ACTH showed low and high values over time for

all species, the high values could be persistent capture stress, and/or a new acute stress event experienced by sharks still on the hook (e.g., the presence of a predator). This remains unknown due to no video observations available of sharks whilst on the hook or net. The GCs values for Port Jackson sharks were relatively high in comparison to the dusky sharks in this study and the sandbar sharks (Grosse *et al*. in review), with some individuals presenting upwards of 5000 pg/ml. The cause of this is unknown but could be due to limited studies on the species, or it could be a species-specific stress characteristic. When coupled with low lactate levels, the high GC values are uncharacteristic of the usual secondary stress trends witnessed in elasmobranch species (Iki *et al*., 2020). The lower values for dusky sharks after 150 min could be an indication of the beginning of ACTH clearance after the initial stress response as excess GC levels negatively feedback the production of ACTH (Barton, 2002; Cain & Cidlowski, 2015). The time it takes for ACTH levels to spike through the production of GCs is variable among shark species. Teleost fishes typically take minutes, although also this varies within species (Pankhurst, 2011). In this study, no clear decrease in ACTH levels was observed, possibly because sharks remained stressed over the entire TOH/TIN. Low and high GC values were observed over time, although the higher levels were observed for Port Jackson sharks 450 mins after capture and dusky sharks 196 mins after capture. Levels of GCs were reported to increase less than an hour after an acute stress event (Iki *et al*., 2020; Schoen *et al*., 2021) and up to 24 hrs after the initial stress event (Brinn *et al*., 2012). Stress has been widely shown to increase lactate concentrations in elasmobranch species (Skomal & Mandelman, 2012). However, lactate measurements alone cannot be used to forecast survival following a stress event by capture (Chapman & Renshaw, 2009; Mandelman & Farrington, 2007; Mandelman & Skomal, 2009). With ‘Hi’ whole-blood lactate values given a value of 25 mmol/L, the whole-blood lactate values are comparable to other shark studies (McAuley *et al*., 2005)

providing insights into the effects of an extended TOH/TIN on these three species. For tiger and dusky sharks, a slight increase over time in lactate levels may have been present without the limitation of 'Hi' values. Although further investigation into this would need to be done. Port Jackson sharks are a hardy species with very low lactate levels (Braccini *et al*., 2012; Frick *et al*., 2010). There is no trend in lactate levels with increased TIN for Port Jackson sharks, and with high TIN studied, it is unlikely there will be correlations observed in this species.

Most of the sharks swam strongly after release, with only a few being in release categories 2 or 3. Consequently, the elevated concentrations of GCs and ACTH over time most likely reflect an adequate survival response to stress conditions (Romero & Beattie, 2022). Due to the low number of blood-sampled sharks in release conditions 2 or 3 (tiger sharks: n=4, n=3, Port Jackson sharks: n=0, n=0, dusky sharks: n=5, n=2)), release conditions could not be assessed as an indicator of PRS. For other related species, such as juvenile silky sharks (*Carcharhinus falciformis*), for which larger sample sizes were available for the different release condition categories, release condition was a useful predictor of post-release survival (Hutchinson *et al*., 2015b).

3.4.2 Post-capture survival (PCS)

Whilst there was 100% at-vessel survival of all three species in this study, species specific studies would have to be conducted further to understand the relationship of TOH and TIN with PCS. Previous research has shown many different species of shark, such as hammerheads and silky sharks, can have varying at-vessel survival depending on fishing methods and stress physiology (Gallagher *et al*., 2014; Shaefer *et al*., 2021).

3.4.3 Satellite tags

3.4.3.1 Post-release survival (PRS) - Satellite tagging

Tagged tiger and Port Jackson sharks had 100% post-release survival, assuming the tag did not indicate a true mortality. These species are known to be robust, with high rates of PRS ranging between 93.1-100% for tiger sharks and 100% for Port Jackson sharks (Braccini *et al*., 2012; Frick *et al*., 2010; Whitney *et al*., 2021).

Gulak and Carlson's (2021) found little difference in the at-vessel survival rates of tiger sharks caught on longlines with sharks caught on J-hooks having and circle hooks (93.1-100% PRS respectively). Similarly, Whitney *et al*., (2021) also recorded high rates of survival for tiger sharks (98%) and suggest that no-take regulations for shark fisheries may be beneficial to this species due to their high survival rate.

Dusky sharks are known to be more sensitive to stress ranging between 3-71.4% PRS (Butcher *et al*., 2015; Marshall *et al*., 2015; Sulikowski *et al*., 2020). Seventy-five percent of tagged dusky shark individuals survived post-release after 4 hours TOH in this study. Differing gear has been shown to at-vessel affect the survival of dusky sharks with ranges of 14.3% (J hooks)- 71.4% (circle hooks)(Gulak & Carlson, 2021). Despite similar TOH to this study, Marshall *et al*'s 2015 research had very low PRS comparatively (3% after three hours) which may have been a result of differences in gear, depth, handling practices, and the size of the sharks caught. They also set longlines in depths of 5–19m compared to this study where depths ranged from 50–200m. The spatial location may also be a factor, with areas in this study resulting in more mature sharks (244.64 cm [±0.94] FL) in comparison to Marshall *et al*'s study (109 cm [± 21] FL) which could

alter the survival rate (Marshall *et al*., 2019), although more research would need to be done to support this assumption. The survival rates of dusky sharks in this study are in the high range compared to the collective 3-71.4% survival of dusky sharks after similar TOH (Gulak & Carlson, 2021; Marshall *et al*., 2015). This study also has more dusky sharks in release condition 1 (72%) compared to the 52% reported by Sulikowski *et al*. (2020) and 60% by Marshall *et al*.(2015) and highlights the effects that gear specifications have on PRS.

Previous studies on PRS and information from the tag supplier, Wildlife Computers Inc., indicated that mortalities are depicted by sharks sinking to the seafloor and remaining there for a specified time (24 hours), causing the tag to release and transmit (Drymon & Wells, 2017; French *et al*., 2015; Hutchinson *et al*., 2015a; Hutchinson & Bigelow, 2019). As the one premature released tag of a Port Jackson shark was a 'sinker', they conform to the usual definition of mortality. One possibility that conforms with the observed depth profile is the tag was not programmed properly for this species of shark. Port Jackson sharks are bottom-dwelling sharks that use spiracles to breathe, meaning they do not need to swim constantly to stay alive (Braccini *et al*., 2012). The individual was also released in the early morning, and this species is not known to be as active during the day as at night and the species is known to remain at a constant depth for greater than 24 hours (Braccini *et al*., 2012). The tag used for this individual was originally programmed with the standard parameters to prematurely release if the shark does not move ±1m within a 24-hour period, indicating mortality. Subsequent to this, later tagged Port Jackson sharks had tags programmed to accommodate the species' behaviour by extending the time period to 48 hours and no further issues were observed. Other tagged Port Jackson sharks also showed very limited depth variation throughout the deployment, often not in excess of 1 m. This provides support to our

assumption that 100% of the sharks tagged in this experiment survived the catch-and-release process under the gillnet fishing settings used as part of the observer survey.

3.4.3.2 Post-release predation

Post-release predation (PRP) is a commonly overlooked form of mortality. Although shark species have varying responses to stress, the recovery from a stress event may increase the risk of PRP (Dulvy *et al*., 2017; Raby *et al*., 2014; Simpfendorfer *et al*., 2001). The tagged dusky shark (19P1640) showed characteristics of a predation/scavenging event and with analysts' confirmation from Wildlife Computers, the depth profile for the total deployment suggests a scavenging event occurring within the first 24 hours of release. 19P1640 sank to the seafloor and remained there, indicative of poor stress management and lethargy (Marshall *et al*., 2015). No changes in light transmission after 12 hours is indicative of the tag being consumed by another animal. Due to no visual observations available, the identification of the predatory species and the level of predation is unknown, although another shark species or predatory fish is likely (Raby *et al*., 2014). Vertical behaviour is also inconclusive as to what species may have been involved.

3.4.3.3 Depth profiles and vertical behaviour

The behaviour exhibited by the satellite-tagged sharks of all three species supports that scientific and observer surveys are not having negative impacts on these sharks. Tiger sharks move through the water column with no patterns, typical of this species as opportunistic predators, feeding on a large variety of prey (Heithaus, 2001; Last & Stevens, 1994; Simpfendorfer *et al*., 2001). Some Port Jackson sharks swam deeper and some shallower during the night, although this may be indicative of their benthic habitat and food source (Bass *et al*., 2021; Powter & Gladstone, 2008).

Some dusky sharks showed strong diel vertical movement patterns (tag numbers 20P0092, 19P1637, 19P1617), a pattern associated with dusky shark hunting and feeding(Last & Stevens, 1994). The observed day/night cycle is supported by dusky sharks' varying diet, in which some prey species have strong diel vertical movement patterns (Braccini, 2016; Last & Stevens, 1994; McAuley *et al*., 2005; Stevens & McLoughlin, 1991). Maximum depth for both tiger sharks and Port Jackson sharks was in line with other studies of 110m and 46m, respectively (Last & Stevens, 1994). All sharks' maximum depth largely correlated with the depth at which they were released. The maximum depth for dusky sharks recorded for the last five days of tag deployment reflects the depth profile of the continental shelf in which the sharks were caught and released (Siwabessy *et al*., 2000). Dusky sharks are known to inhabit depths from intertidal to 400 m (Last & Stevens, 1994; Stevens & McLoughlin, 1991), with depths of 355 to 400m being recorded in other studies (Carlson & Gulak, 2012; Rogers *et al*., 2013). In our study, the data transmitted from the PSAT tags recorded depths of up to 413m.

3.4.3 Conclusions

By combining the information on high ACTH, GCs and whole-blood lactate levels continuing to rise with increased TOH/TIN (Fuller *et al*., 2020; Hoffmayer & Parsons, 2001), it is theorised that these three shark species remained stressed throughout the capture process. However, the post-capture and post-release survival are high under current survey sampling practices indicating these species can support the use of longlines, drumlines and gillnets for sampling abundance and bycatch monitoring. The extended TOH/TIN of four hours to 26 hours decreased the ability to examine the relationship between the studied hormones and TOH/TIN. A shorter TOH/TIN would be required to fully explore this relationship. To observe a greater relationship between lactate

levels and TOH/TIN, lactate levels should be measured for a longer period after the initial stress event (Marshall *et al*., 2015). Although a shorter TOH/TIN is recommended for testing primary stress responses for these species of shark, using whole-blood lactate as a secondary stress response would be an effective form for predicting mortality with longer exposure times (Braccini *et al*., 2019; Gulak & Carlson, 2021). The depth profiles of these sharks following the usual patterns seen in the respective species through the water column show the hardiness of the species when returning to the ocean after the capture process. Tiger sharks showed no patterns in the day and night depth behaviours, characteristic of this species (Heithaus, 2001; Heithaus *et al*., 2007; Simpfendorfer *et al*., 2001). Port Jackson sharks showed clear diel patterns and could have a possible relationship of movement depending on benthic habitat (i.e., rock/coral or sand), but these trends would need to be further explored (Powter & Gladstone, 2008). Some dusky sharks exhibit strong diel patterns of diving during the day and coming into shallower waters during the night. The combination of blood metabolites as stress parameters, release conditions and satellite tagging methods can create species-specific models to predict PCS rates (Braccini *et al*., 2012), decreasing the costs of survival studies as well as creating an understanding of the fate after the discard of each species. The results of this study indicate the three species studied are hardy and have high survival rates in varying survey sampling processes.

Ethics

Shark capture, tagging, and the taking of blood samples were done under exemptions of the *Fish Resources Management Act 1994*. Exemptions were granted to the Department of Primary Industries and Regional Development (DPIRD) for research.

All aspects of this study conform to the Australian Code for the Care and Use of Animals for Scientific Purposes 8th Edition (2014).

Declaration of Competing Interest

None.

Declaration of Funding

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Data Availability

Data is available upon request to the authors. Please contact matias.braccini@dpird.wa.gov.au

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Chapter four

General Discussion

4.1. Summary of findings

My research aimed to determine the post-capture and post-release survival of four species of shark (sandbar, tiger, Port Jackson and dusky sharks) commonly caught as bycatch within Australian waters (Oliver *et al*., 2015; Salini *et al*., 2007). Shark populations have decreased by 71% since the 1970s with many species being listed as threatened on the IUCN red list. As a consequence, it is important for conservation and fisheries managers to understand the fate of sharks caught by commercial and recreational fishers and discarded (Dulvy *et al*., 2021; Pacoureau *et al*., 2021). While different shark species experience stress differently, post-capture survival can be dictated by the types of gear used, handling processes and species biology (Gulak & Carlson, 2021). Multiple sampling techniques have been used to assess post-capture and post-release survival (blood physiology, hook timers, release conditions, cage monitoring, and satellite tagging). Limitations and biases of each technique, such as equipment programming, may influence their ability to sample specific species depending on their reaction to stress (Drymon & Wells, 2017; Whitney *et al*., 2021).

In chapter two, I assessed the survival rates of sandbar sharks post-capture and post-release through a holistic sampling strategy integrating data from satellite tags, hook timers, an assessment of release conditions and blood physiology (Fig 4.1). Sandbar sharks were caught in the northwest coast of Western Australia (WA) using longlines that soaked for up to 4 hours. Sharks were retrieved and brought on deck for processing, tagging and then released. The outcomes of this

study showed sandbar sharks are a hardy species with 100% survival post-release and no lethal thresholds met in blood physiology. The stress hormone levels of individual sharks (adrenocorticotropic hormone (ACTH) and corticosterone (CORT)) indicated there may have been potential peaks in stress during the initial hooking phase and then again after two hours on the hook. This may be indicative of a prolonged stress event or a secondary stress response to an environmental factor (i.e., the presence of a predator). Further research would need to be done with less time on the hook to understand the hormonal response in this species (Butcher *et al*., 2015; Gulak & Carlson, 2021). Lactate levels were not conclusive because of the limitations of capping values at 25 mmol/L on the in-field device. These 'High' values can be addressed in future studies with the addition of plasma lactate levels or blood dilution to create a formula to calculate values over 25 mmol/L (Awruch *et al*., 2011).

Satellite-tagged individuals showed strong diel patterns in the last five days of tag deployment with many swimming at shallower depths at night and diving deeper in the day. These patterns follow that of squid, a common food source for sandbar sharks (Last & Stevens, 2009; McElroy *et al*., 2006). Five tagged individuals showed tag anomalies in which the shark/tag floated for 24 hours before the tag detaching. Upon investigating the depth data and previous vertical behaviour exhibited by each individual, these tags were ruled as other malfunctioning tags or a possible unreported recapture of the shark. It was concluded that the methods used in scientific surveys had no deleterious consequences on sandbar sharks.

In chapter three, I investigated the survival rates of three more species of shark (tiger shark, Port Jackson shark and dusky shark) using an integrated approach of maximum time on hook/in net, blood physiology, satellite tagging, and release conditions (Fig 4.1). Each species was targeted

using different methods via scientific and observer surveys on commercial vessels (longlines and gillnets in WA, and drumlines in Queensland (QLD)).However, because there was little cross-over of methods among species, the methods were not compared.

Tiger sharks were caught in scientific surveys on both longlines in WA and drumlines in QLD and onboard sampling methods for blood were the same. Tiger sharks' lactate levels were consistent over the maximum time on the hook with high and low values present in all time intervals. Like sandbar sharks, there were no lethal thresholds met and the limitation of 'Hi' values hindered any trends in lactate levels from being observed (Awruch *et al*., 2011). Hormone levels were not available for this species. Most tiger sharks (84% in release condition 1) were alive and strong upon release and with 100% survival post-release. I conclude that the current methods used in scientific surveys in both WA and QLD have no deleterious consequences on this species.

Port Jackson sharks were caught on commercial vessels in the south coast of WA in gillnets deployed for up to 24 hours. These vessels were undertaking scientific surveys and had an observer on board at all times. The maximum time in the net was used to compare the blood physiology between individuals. Glucosteroid levels for Port Jackson sharks were higher than all other species of shark studied and was coupled with very low lactate levels. This blood physiological response to stress did not follow the trends usually seen in most other elasmobranch species (Skomal & Mandelman, 2012; Whitney *et al*., 2021). Due to the biology of Port Jackson sharks (i.e., the presence of spiracles), the increased time in the net did not affect the survival rates post-capture or post-release. Sharks with properly fitted tags showed strong movement during the night, probably following the movements of popular prey items such as crustaceans and small fish. Port Jackson sharks had 100% post-release survival.

Dusky sharks were sampled from longlines in the northwest coast of WA. Following the same methods used for sandbar sharks and having similar biology, the expected physiological response to stress was very similar to sandbar sharks with high and low values of ACTH and CORT over increased time on the hook. The limitation of 'High' lactate values was more prevalent with this species, therefore further studies should be undertaken to fully understand the relationship between lactate levels and increase time on the hook (Awruch *et al*., 2011). One tagged individual had an observed post-release predation event where the tag ceased transmitting for 24 hours. Upon retrieval of the tag, the observed light and temperature readings were different to that of the other tagged dusky sharks and depth patterns not conforming with the usual vertical behaviour observed in the species. We hypothesise that this shark was predated upon. It is uncertain what species predated on the dusky shark, but the event resulted in the dusky sharks having 75% post-release survival. This is still a greater percentage than previous studies on dusky sharks. With evident recovery of dusky shark populations within the resource, the methods used in scientific surveys are appropriate (Butcher *et al*., 2015; Marshall *et al*., 2015; Sulikowski *et al*., 2020).

Overall, the species of shark studied are hardy and with current methods used in scientific and observer surveys, the survival of released sharks is high.

4.2 Limitations of the research

The major limitation presented in both chapters was the sampling of whole blood lactate with in situ reading capping at 25 mmol/L. Studies by Awruch *et al*. (2011) addressed this issue using samples of plasma lactate. However, due to limited resources in the field, there were not enough plasma samples to confidently use plasma lactate to determine whole blood lactate levels through linear regression (Awruch *et al*., 2011). Another potential solution to unknown 'High' levels in

lactate was dilution of blood to allow successful readings, although again limitations of equipment such as distilled water meant there was little confidence in these readings. Given this experience, if I were undertaking this sort of research again, I would ensure I had extra blood sampling equipment to allow for plasma samples or dilution with distilled water for those samples with very high lactate values (Awruch *et al*., 2011).

While the PSAT tags highlighted interesting patterns increased sampling effort using these tags would give greater confidence to the conclusions that can be drawn. I would suggest increasing the number of PSAT tags, and, if budget permits, to increase the potential sample size including all possible survival measures (hook-timers, release conditions, blood sampling).

4.3 Future research

Future species-specific research into less hardy species of shark, such as hammerhead sharks, would aid in quantifying post-release mortality for future resource management (Butcher *et al*., 2015; Drymon & Wells, 2017) (Fig 4.1). Utilising an integrated approach to sample stress and survival is more favourable, although with further studies using blood physiology as the main stress measure and reducing the limitations of lactate sampling, survival rates can be determined more cost-effectively (Awruch *et al*., 2011; Whitney *et al*., 2021). This could be achieved through shorter times on hooks for all the shark species to understand the initial stress response through hormones. Based upon the information presented in this thesis I predict that stress peaks in the first 20 minutes of capture. The times of this initial response are likely to be species-specific and will need further investigation (Fuller *et al*., 2020; Sadoul & Geffroy, 2019). Lactate could be more broadly studied through a range of hook times to gain an extensive understanding of the relationship between TOH and survival for each species of elasmobranch commonly encountered

in fishing. Creating a species-specific equation for lactate values over 25 mmol/L could also aid in the cost-effectiveness of the sampling method by reducing lab equipment needed to counter the limitation of the in-field LacPro (Awruch *et al*., 2011).

There is also a need to quantify the fate of sharks and rays caught and discarded by recreational fishers. The methods used in recreational fishing can differ from commercial fishing greatly across different fisheries and the handling and release process could cause increased stress events. This may be harder to quantify with recreational fishers not having access to tagging and sampling equipment or general non-interest in participating.

4.4 Conclusions

The data and outcomes of my research, in conjunction with previous studies globally can be used to communicate the appropriate thresholds for commonly caught shark species and best fishing practices to increase post-release survival of these four species of sharks (Gulak & Carlson, 2021; Hutchinson & Bigelow, 2019; Marshall *et al*., 2015). I conclude that whilst blood physiology is a viable and affordable way to assess the post-capture survival of all four species of shark studied, the use of an integrated approach of satellite tagging and blood physiology allows for a complete understanding of the fate of these sharks' post-capture and release. The results of my research support the current methods used in scientific surveys and commercial fishing practices to reduce shark bycatch mortality (Fig 4.1).

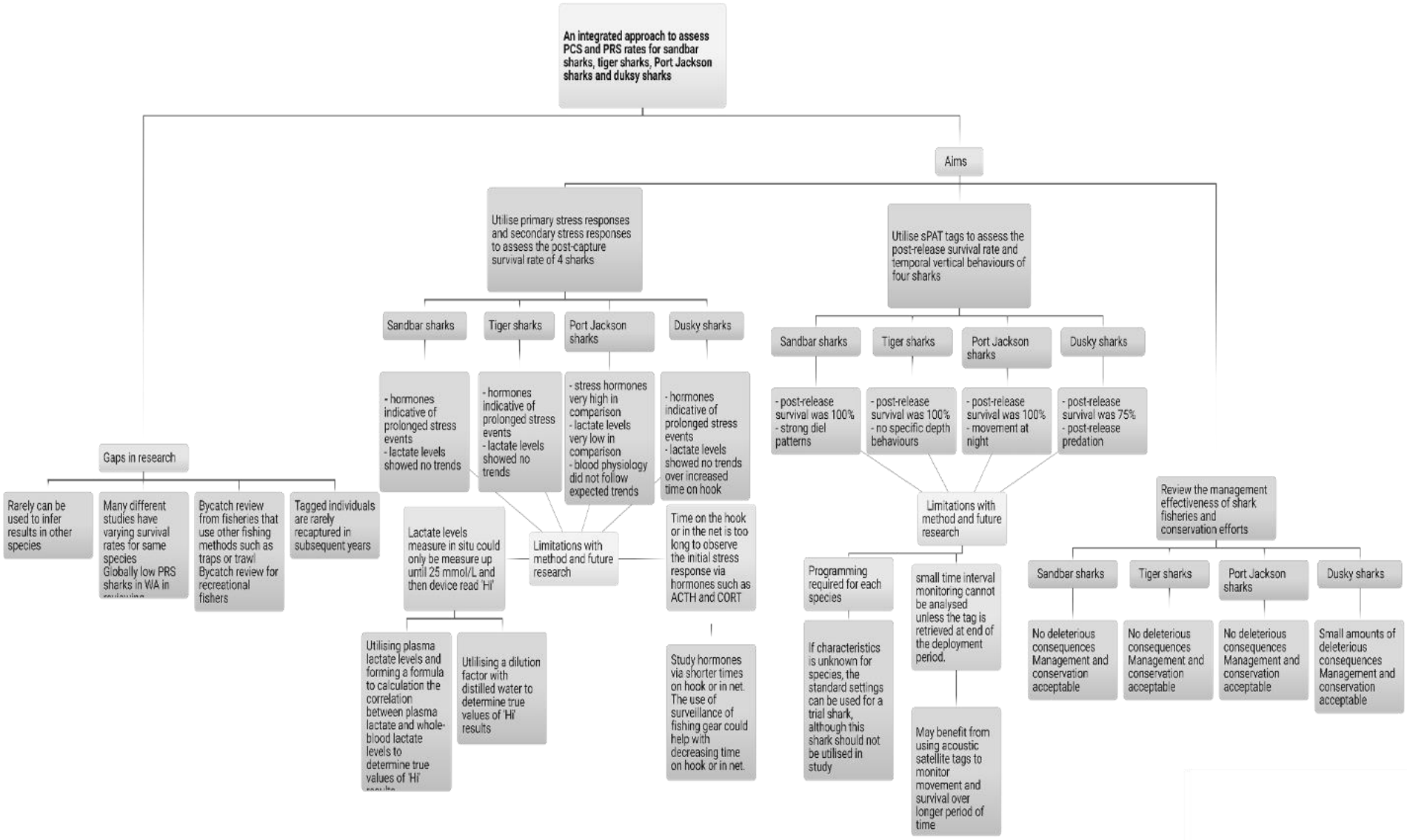

Fig. 4.1 Thesis flow diagram outlining the research outcomes, limitations, and further research for each data chapter.

Literature cited

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Afonso, A. S., & Hazin, F. H. V. (2014). Post-release survival and behavior and exposure to fisheries in juvenile tiger sharks, Galeocerdo cuvier, from the South Atlantic. *Journal of Experimental Marine Biology and Ecology*, *454*, 55–62. https://doi.org/10.1016/j.jembe.2014.02.008

Anderson, W. G. (2012). The endocrinology of 1α-hydroxycorticosterone in elasmobranch fish: a review. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*, *162*(2), 73–80. https://doi.org/10.1016/j.cbpa.2011.08.015

Andrzejaczek, S., Gleiss, A. C., Pattiaratchi, C. B., & Meekan, M. G. (2018). First Insights Into the Fine-Scale Movements of the Sandbar Shark, Carcharhinus plumbeus. *Frontiers in Marine Science*, *5*, 483. https://doi.org/10.3389/fmars.2018.00483

Armour, K. J., O'Toole, L. B., & Hazon, N. (1993). Mechanisms of ACTH- and angiotensin II-stimulated 1 alpha-hydroxycorticosterone secretion in the dogfish, Scyliorhinus canicula. *Journal of Molecular Endocrinology*, *10*(3), 235–244. https://doi.org/10.1677/jme.0.0100235

Atkinson, S., Crocker, D., Houser, D., & Mashburn, K. (2015). Stress physiology in marine mammals: how well do they fit the terrestrial model? *Journal of Comparative Physiology. B, Biochemical, Systemic, and Environmental Physiology*, *185*(5), 463–486. https://doi.org/10.1007/s00360-015-0901-0

Awruch, C. A., Simpfendorfer, C., & Pankhurst, N. W. (2011). Evaluation and use of a portable field kit for measuring whole-blood lactate in sharks. *Marine and Freshwater Research*, *62*(6), 694–699. https://doi.org/10.1071/MF10149

Bartes, S. *et al.* (2021) Conventional tagging of sharks in Western Australia: The main commercial species exhibit contrasting movement patterns. Marine and Freshwater Research. [Online] 72 (11), 1643–1656.

Barnett, A., Fitzpatrick, R., Bradley, M., Miller, I., Sheaves, M., Chin, A., Smith, B., Diedrich, A., Yick, J. L., Lubitz, N., Crook, K., MatTINe, C., Bennett, M. B., Wojtach, L., & Abrantes, K. (2022). Scientific response to a cluster of shark bites. *People and Nature (Hoboken, N.J.)*, *4*(4), 963–982. https://doi.org/10.1002/pan3.10337

Barton, B.A. (2002) 'Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids', Integrative and comparative biology, 42(3), pp. 517–525. Available at: https://doi.org/10.1093/icb/42.3.517.Bass, N. C., Day, J., Guttridge, T. L., Mourier, J., Knott, N. A., Vila Pouca, C., & Brown, C. (2021). Residency and movement patterns of adult Port Jackson sharks (*Heterodontus portusjacksoni*) at a breeding aggregation site. *Journal of Fish Biology*, *99*(4), 1455–1466. https://doi.org/10.1111/jfb.14853

Bouyoucos, I.A. *et al.* (2018) 'Exercise intensity while hooked is associated with physiological status of longline-captured sharks', Conservation physiology, 6(1), p. coy074. Available at: https://doi.org/10.1093/conphys/coy074.

Braccini, M. (2016). Temporal patterns in the size of the main commercial shark species of Western Australia. *Marine and Freshwater Research*, *68*(6), 1112–1117. https://doi.org/10.1071/MF16117

Braccini, M., Blay, N., Hesp, S. A., & Molony, B. (2018). *Resource assessment report: temperate demersal elasmobranch resource of Western Australia*. http://www.fish.wa.gov.au/Documents/research_reports/frr294.pdf

Braccini, M., Denham, A., O'Neill, M. F., & Lai, E. (2021). Spatial and temporal patterns in catch rates from multispecies shark fisheries in Western Australia. *Ocean & Coastal Management*, *213*, 105883. https://doi.org/10.1016/j.ocecoaman.2021.105883

Braccini, M., Kangas, M., Jaiteh, V., & Newman, S. (2021). Quantifying the unreported and unaccounted domestic and foreign commercial catch of sharks and rays in Western Australia. *Ambio*, *50*(7), 1337–1350. https://doi.org/10.1007/s13280-020-01495-6

Braccini, M., Molony, B., & Blay, N. (2019). Patterns in abundance and size of sharks in northwestern Australia: cause for optimism. *ICES Journal of Marine Science: Journal Du Conseil*, *77*(1), 72–82. https://doi.org/10.1093/icesjms/fsz187

Braccini, M., & Taylor, S. (2016). The spatial segregation patterns of sharks from Western Australia. *Royal Society Open Science*, *3*(8), 160306. https://doi.org/10.1098/rsos.160306

Braccini, M., Van Rijn, J., & Frick, L. (2012). High post-capture survival for sharks, rays and chimaeras discarded in the main shark fishery of Australia? *PloS One*, *7*(2), e32547. https://doi.org/10.1371/journal.pone.0032547

Braccini, M., & Waltrick, D. (2019). Species-specific at-vessel mortality of sharks and rays captured by demersal longlines. In *Marine Policy* (Vol. 99, pp. 94–98). https://doi.org/10.1016/j.marpol.2018.10.033

Brill, R., Bushnell, P., Schroff, S., Seifert, R., & Galvin, M. (2008). Effects of anaerobic exercise accompanying catch-and-release fishing on blood-oxygen affinity of the sandbar shark (*Carcharhinus plumbeus*, Nardo). *Journal of Experimental Marine Biology and Ecology*, *354*(1), 132–143. https://doi.org/10.1016/j.jembe.2007.10.011

Brinn, R. P., Marcon, J. L., McComb, D. M., Gomes, L. C., Abreu, J. S., & Baldisseroto, B. (2012). Stress responses of the endemic freshwater cururu stingray (*Potamotrygon cf. histrix*) during transportation in the Amazon region of the Rio Negro. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*, *162*(2), 139–145. https://doi.org/10.1016/j.cbpa.2011.07.004

Butcher, P. A., Peddemors, V. M., Mandelman, J. W., McGrath, S. P., & Cullis, B. R. (2015). At-vessel mortality and blood biochemical status of elasmobranchs caught in an Australian commercial longline fishery. *Global Ecology and Conservation*, *3*, 878–889. https://doi.org/10.1016/j.gecco.2015.04.012

Cain, D. W., & Cidlowski, J. A. (2015). Specificity and sensitivity of glucocorticoid signaling in health and disease. *Best Practice & Research. Clinical Endocrinology & Metabolism*, *29*(4), 545–556. https://doi.org/10.1016/j.beem.2015.04.007

Cardeñosa, D., Fields, A. T., Babcock, E. A., Shea, S. K. H., Feldheim, K. A., & Chapman, D. D. (2020). Species composition of the largest shark fin retail-market in mainland China. *Scientific Reports*, *10*(1), 12914. https://doi.org/10.1038/s41598-020-69555-1

Carlson, J. K., & Gulak, S. J. B. (2012). *Habitat use and movements patterns of oceanic whitetip, bigeye thresher and dusky sharks based on archival satellite tags*. iccat.int. https://iccat.int/Documents/CVSP/CV068_2012/n_5/CV068051922.pdf

Chapman, C. A., & Renshaw, G. M. C. (2009). Hematological responses of the grey carpet shark (*Chiloscyllium punctatum*) and the epaulette shark (*Hemiscyllium ocellatum*) to anoxia and re-oxygenation. *Journal of Experimental Zoology. Part A, Ecological Genetics and Physiology*, *311*(6), 422–438. https://doi.org/10.1002/jez.539

Clarke, S. C., McAllister, M. K., Milner-Gulland, E. J., Kirkwood, G. P., Michielsens, C. G. J., Agnew, D. J., Pikitch, E. K., Nakano, H., & Shivji, M. S. (2006). Global estimates of shark catches using trade records from commercial markets. *Ecology Letters*, *9*(10), 1115–1126. https://doi.org/10.1111/j.1461-0248.2006.00968.x

Clarke, S., Milner-Gulland, E. J., & Bjørndal, T. (2007). Social, Economic, and Regulatory Drivers of the Shark Fin Trade. *Marine Resource Economics*, *22*(3), 305–327. https://doi.org/10.1086/mre.22.3.42629561

Clementi, G. M., Babcock, E. A., Valentin-Albanese, J., Bond, M. E., Flowers, K. I., Heithaus, M. R., Whitman, E. R., Zinnicq Bergmann, M., Guttridge, T. L., O'Shea, O. R., Shipley, O. N., Brooks, E. J., Kessel, S. T., & Chapman, D. D. (2020). Anthropogenic pressures on reef-associated sharks in jurisdictions with and without directed shark fishing. *Marine Ecology Progress Series*. https://doi.org/10.3354/meps13607

Conrath, C. L., & Musick, J. A. (2008). Investigations into depth and temperature habitat utilization and overwintering grounds of juvenile sandbar sharks, *Carcharhinus plumbeus*: the importance of near shore North Carolina waters. *Environmental Biology of Fishes*, *82*(2), 123–131. https://doi.org/10.1007/s10641-007-9263-0

Dapp, D. R., Huveneers, C., Walker, T. I., Drew, M., & Reina, R. D. (2016). Moving from Measuring to Predicting Bycatch Mortality: Predicting the Capture Condition of a Longline-Caught Pelagic Shark. *Frontiers in Marine Science*, *2*, 126. https://doi.org/10.3389/fmars.2015.00126

Davidson, L. N. K., Krawchuk, M. A., & Dulvy, N. K. (2016). Why have global shark and ray landings declined: improved management or overfishing? *Fish and Fisheries* , *17*(2), 438–458. https://doi.org/10.1111/faf.12119

De Wysiecki, A. M., & Braccini, J. M. (2017). Shark length–length relationships: Studying morphology allows the detection of bias in routine fisheries sampling. *Regional Studies in Marine Science*, *16*, 290–293. https://doi.org/10.1016/j.rsma.2017.10.005

Drymon, J. M., & Wells, R. J. D. (2017). Double tagging clarifies post-release fate of great hammerheads (*Sphyrna mokarran*). *Animal Biotelemetry*, *5*(1), 28. https://doi.org/10.1186/s40317-017-0143-x

Dulvy, N. K., Pacoureau, N., Rigby, C. L., Pollom, R. A., Jabado, R. W., Ebert, D. A., Finucci, B., Pollock, C. M., Cheok, J., Derrick, D. H., Herman, K. B., Sherman, C. S., VanderWright, W. J., Lawson, J. M., Walls, R. H. L., Carlson, J. K., Charvet, P., Bineesh, K. K., Fernando, D., … Simpfendorfer, C. A. (2021). Overfishing drives over one-third of all sharks and rays toward a global extinction crisis. *Current Biology: CB*, *31*(21), 4773–4787.e8. https://doi.org/10.1016/j.cub.2021.08.062

Dulvy, N. K., Simpfendorfer, C. A., Davidson, L. N. K., Fordham, S. V., Bräutigam, A., Sant, G., & Welch, D. J. (2017). Challenges and Priorities in Shark and Ray Conservation. *Current Biology: CB*, *27*(11), R565–R572. https://doi.org/10.1016/j.cub.2017.04.038

Ellis, J.R., McCully Phillips, S.R. and Poisson, F. (2017) 'A review of capture and post-release mortality of elasmobranchs', Journal of fish biology, 90(3), pp. 653–722. Available at: https://onlinelibrary.wiley.com/doi/abs/10.1111/jfb.13197.

Evans, A. N., Rimoldi, J. M., & Gadepalli, R. S. V. (2010). Adaptation of a corticosterone ELISA to demonstrate sequence-specific effects of angiotensin II peptides and C-type natriuretic peptide on 1α …. *The Journal of Steroid Biochemistry and Molecular Biology*. https://www.sciencedirect.com/science/article/pii/S0960076010001160?casa_token=-Ps26SUIaQQAAAAA:yZqm5hbI9UVfiLlqacZKcy5Or0SigLO_K3PLPgKenRWyAX5BS-kcypEHIU3pIq6AgH_gjcD7

Fasiolo, M. et al. (2018) 'Scalable visualisation methods for modern Generalized Additive Models', Arxiv [Preprint]. Available at: https://arxiv.org/abs/1809.10632.

Fox, J. and Weisberg, S. (2019) 'An R Companion to Applied Regression'. Thousand Oaks {CA}: Sage. Available at: https://socialsciences.mcmaster.ca/jfox/Books/Companion/.French, R.P. et al. (2015) 'High survivorship after catch-and-release fishing suggests physiological resilience in the endothermic shortfin mako shark (Isurus oxyrinchus)', Conservation physiology, 3(1), p. cov044. Available at: https://doi.org/10.1093/conphys/cov044.

French, R. P., Lyle, J., Tracey, S., Currie, S., & Semmens, J. M. (2015). High survivorship after catch-and-release fishing suggests physiological resilience in the endothermic shortfin mako shark (*Isurus oxyrinchus*). *Conservation Physiology*, *3*(1), cov044. https://doi.org/10.1093/conphys/cov044

Frick, L. H., Reina, R. D., & Walker, T. I. (2010). Stress related physiological changes and post-release survival of Port Jackson sharks (*Heterodontus portusjacksoni*) and gummy sharks (*Mustelus antarcticus*) following gill-net and longline capture in captivity. *Journal of Experimental Marine Biology and Ecology*, *385*(1), 29–37. https://doi.org/10.1016/j.jembe.2010.01.013

Fuller, L., Stell, E., Leary, C., & Parsons, G. (2020). Circulating adrenocorticotropic hormone levels, lactate levels, hematocrit and osmolality in relation to capture stress in Atlantic sharpnose sharks, *Rhizoprionodon terraenovae*. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*, *243*, 110655. https://doi.org/10.1016/j.cbpa.2020.110655

Gallagher, A.J., Staaterman, E.R. and Cooke, S.J. (2017) 'Behavioural responses to fisheries capture among sharks caught using experimental fishery gear', Canadian Journal of [Preprint]. Available at: https://cdnsciencepub.com/doi/abs/10.1139/cjfas-2016-0165.
Grolemund, G. and Wickham, H. (2011) 'Dates and Times Made Easy with lubridate', Journal of statistical software, 40(3), pp. 1–25. Available at: https://www.jstatsoft.org/v40/i03/.
Gulak, S. J. B., & Carlson, J. K. (2021). Less soak time saves those upon the line: Capture times and hooking mortality of sharks caught on bottom longlines. *North American Journal of Fisheries Management*, *41*(3), 791–808. https://doi.org/10.1002/nafm.10592
Hammerschlag, N., Gallagher, A. J., & Lazarre, D. M. (2011). A review of shark satellite tagging studies. *Journal of Experimental Marine Biology and Ecology*, *398*(1), 1–8. https://doi.org/10.1016/j.jembe.2010.12.012
Hammerschlag, N., Meÿer, M., Seakamela., S. M., Steve, K. S., Fallows, C., & Creel, S. (2020). Erratum. *Ecology*, *101*(1), e02898. https://doi.org/10.1002/ecy.2898
Heberer, C., Aalbers, S. A., Bernal, D., Kohin, S., DiFiore, B., & Sepulveda, C. A. (2010). Insights into catch-and-release survivorship and stress-induced blood biochemistry of common thresher sharks (*Alopias vulpinus*) captured in the southern California recreational fishery. *Fisheries Research*, *106*(3), 495–500. https://doi.org/10.1016/j.fishres.2010.09.024
Heithaus, M. R. (2001). The Biology of Tiger Sharks, Galeocerdo Cuvier, in Shark Bay, Western Australia: Sex Ratio, Size Distribution, Diet, and Seasonal Changes in Catch Rates. *Environmental Biology of Fishes*, *61*(1), 25–36. https://doi.org/10.1023/A:1011021210685
Heithaus, M. R., Wirsing, A. J., Dill, L. M., & Heithaus, L. I. (2007). Long-term movements of tiger sharks satellite-tagged in Shark Bay, Western Australia. *Marine Biology*, *151*(4), 1455–1461. https://doi.org/10.1007/s00227-006-0583-y
Hijmans, R.J. (2022) geosphere: Spherical Trigonometry, R package version 1.5-18.
Hoffmayer, E. R., & Parsons, G. R. (2001). The physiological response to capture and handling stress in the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*. *Fish Physiology and Biochemistry*, *25*(4), 277–285. https://doi.org/10.1023/A:1023210620904
Hueter, R. E., Manire, C. A., Tyminski, J. P., Hoenig, J. M., & Hepworth, D. A. (2006). Assessing Mortality of Released or Discarded Fish Using a Logistic Model of Relative Survival Derived from Tagging Data. *Transactions of the American Fisheries Society*, *135*(2), 500–508. https://doi.org/10.1577/T05-065.1
Husain, F. A., Martin, M. J., Mullenix, P. S., Steele, S. R., & Elliott, D. C. (2003). Serum lactate and base deficit as predictors of mortality and morbidity. *American Journal of Surgery*, *185*(5), 485–491. https://doi.org/10.1016/s0002-9610(03)00044-8
Hutchinson, M., & Bigelow, K. (2019). *Quantifying post release mortality rates of sharks incidentally captured in Pacific tuna longline fisheries and identifying handling practices to improve survivorship*. Pacific Islands Fisheries Science Center (U.S.). https://doi.org/10.25923/2SXY-S659
Hutchinson, M. R., Itano, D. G., Muir, J. A., & Holland, K. N. (2015a). Post-release survival of juvenile silky sharks captured in a tropical tuna purse seine fishery. *Marine Ecology Progress Series*, *521*, 143–154. https://doi.org/10.3354/meps11073
Iki, A., Anderson, W. G., Deck, C. A., Ogihara, M. H., Ikeba, K., Kataoka, H., & Hyodo, S. (2020). Measurement of 1α hydroxycorticosterone in the Japanese banded houndshark, Triakis scyllium, following exposure to a series of stressors. *General and Comparative Endocrinology*, *292*, 113440. https://doi.org/10.1016/j.ygcen.2020.113440
James, D. and Hornik, K. (2023) chron: Chronological Objects which Can Handle Dates and Times, R package version 2.3-61.
Karsten, A. H. (2000). *Stress effects on fecal corticosterone levels in the epaulette shark, Hemiscyllium ocellatum*. https://search.proquest.com/openview/323873e556b1097b8cb93462d9a6ddf9/1?pq-origsite=gscholar&cbl=18750&diss=y
Kneebone, J., Chisholm, J., Bernal, D., & Skomal, G. (2013). The physiological effects of capture stress, recovery, and post-release survivorship of juvenile sand tigers (*Carcharias taurus*) caught on rod and reel. *Fisheries Research*, *147*, 103–114. https://doi.org/10.1016/j.fishres.2013.04.009
Kassambara, A. (2022) ggpubr: 'ggplot2' Based Publication Ready Plots. Available at: https://rpkgs.datanovia.com/ggpubr/.
Kassambara, A. (2022) rstatix: Pipe-Friendly Framework for Basic Statistical Tests. Available at: https://rpkgs.datanovia.com/rstatix/.
Knotek, R.J. et al. (2022) 'Post-release mortality, recovery, and stress physiology of blacknose sharks, Carcharhinus acronotus, in the Southeast U.S. recreational shark fishery', Fisheries research, 254, p. 106406. Available at: https://doi.org/10.1016/j.fishres.2022.106406.Kohler, N.E. and Turner, P.A. (2001) 'Shark tagging: a review of conventional methods and studies', in T.C. Tricas and S.H. Gruber (eds) The behavior and sensory biology of elasmobranch fishes: an anthology in memory of Donald Richard Nelson. Dordrecht: Springer Netherlands, pp. 191–224. Available at: https://doi.org/10.1007/978-94-017-3245-1_12.
Kohler, N. E., & Turner, P. A. (2001). Shark tagging: a review of conventional methods and studies. In T. C. Tricas & S. H. Gruber (Eds.), *The behavior and sensory biology of elasmobranch fishes: an anthology in memory of Donald Richard Nelson* (pp. 191–224). Springer Netherlands. https://doi.org/10.1007/978-94-017-3245-1_12
Kyne, P. M., & Feutry, P. (2017). Recreational fishing impacts on threatened river sharks: A potential conservation issue. *Ecological Management & Restoration*, *18*(3), 209–213. https://doi.org/10.1111/emr.12266
Last, P. R., & Stevens, J. D. (1994). *Sharks and rays of Australia*. East Melbourne, Vic, CSIRO Australia. https://publications.csiro.au/rpr/pub?list=BRO&pid=procite:2fac975f-ca8c-4b0a-a7b0-c297cf2d4ad1
Lynch, S. D., Marcek, B. J., Marshall, H. M., Bushnell, P. G., Bernal, D., & Brill, R. W. (2017). The effects of pop-up satellite archival tags (PSATs) on the metabolic rate and swimming kinematics of juvenile sandbar shark *Carcharhinus plumbeus*. *Fisheries Research*, *186*, 205–215. https://doi.org/10.1016/j.fishres.2016.08.013
Lyons, K., & Wynne-Edwards, K. E. (2019). Legacy environmental polychlorinated biphenyl contamination attenuates the acute stress response in a cartilaginous fish, the Round Stingray. *Stress* , *22*(3), 395–402. https://doi.org/10.1080/10253890.2019.1570125

Mandelman, J. W., & FarringTIN, M. A. (2007). The estimated short-term discard mortality of a trawled elasmobranch, the spiny dogfish (*Squalus acanthias*). *Fisheries Research*, *83*(2), 238–245. https://doi.org/10.1016/j.fishres.2006.10.001
Mandelman, J. W., & Skomal, G. B. (2009). Differential sensitivity to capture stress assessed by blood acid-base status in five carcharhinid sharks. *Journal of Comparative Physiology. B, Biochemical, Systemic, and Environmental Physiology*, *179*(3), 267–277. https://doi.org/10.1007/s00360-008-0306-4
Marshall, H., Skomal, G., Ross, P. G., & Bernal, D. (2015). At-vessel and post-release mortality of the dusky (*Carcharhinus obscurus*) and sandbar (*C. plumbeus*) sharks after longline capture. *Fisheries Research (Amsterdam)*, *172*, 373–384. https://doi.org/10.1016/j.fishres.2015.07.011
Marshall, H. et al. (2012) 'Hematological indicators of stress in longline-captured sharks', Comparative biochemistry and physiology. Part A, Molecular & integrative physiology, 162(2), pp. 121–129. Available at: https://doi.org/10.1016/j.cbpa.2012.02.008.
McAuley, R. B., Lenanton, R., Chidlow, J., Allison, R., & Heist, E. (2005). *Biology and stock assessment of the thickskin (sandbar) shark, Carcharhinus plumbeus, in Western Australia and further refinement of the dusky shark, Carcharhinus obscurus, stock assessment*. Fisheries research report. Fisheries Western Australia.
McAuley, R. B., Simpfendorfer, C. A., Hyndes, G. A., & LenanTIN, R. C. J. (2007). Distribution and reproductive biology of the sandbar shark, *Carcharhinus plumbeus* (*Nardo*), in Western Australian waters. *Marine and Freshwater Research*, *58*(1), 116–126. https://doi.org/10.1071/MF05234
McElroy, W. D., Wetherbee, B. M., Mostello, C. S., Lowe, C. G., Crow, G. L., & Wass, R. C. (2006). Food habits and ontogenetic changes in the diet of the sandbar shark, *Carcharhinus plumbeus*, in Hawaii. *Environmental Biology of Fishes*, *76*(1), 81–92. https://doi.org/10.1007/s10641-006-9010-y
Mitchell, J. D., McLean, D. L., Collin, S. P., & Taylor, S. (2018). Quantifying shark depredation in a recreational fishery in the Ningaloo Marine Park and Exmouth Gulf, Western Australia. *Marine Ecology* . https://www.int-res.com/abstracts/meps/v587/p141-157
Mohan, J.A. et al. (2020) 'Capture stress and post-release mortality of blacktip sharks in recreational charter fisheries of the Gulf of Mexico', Conservation physiology, 8(1), p. coaa041. Available at: https://doi.org/10.1093/conphys/coaa041.
Morgan, A. and Burgess, G. (2007) 'At-vessel fishing mortality for six species of sharks caught in the northwest Atlantic and Gulf of Mexico'. Available at: https://aquadocs.org/bitstream/handle/1834/29440/MorganBurgess_GCFI_Pelagics.pdf?sequence=1 (Accessed: 21 July 2023).Musyl, M.K. et al. (2011) 'Performance of pop-up satellite archival tags', Marine ecology progress series, 433, pp. 1–28. Available at: https://doi.org/10.3354/meps09202.
Molina, J. M. C. (2012). Trends in shark bycatch research: current status and research needs. *Reviews in Fish Biology and Fisheries*, *22*(3), 719–737. https://doi.org/10.1007/s11160-012-9269-3
Musyl, M. K., Domeier, M. L., Nasby-Lucas, N., Brill, R. W., McNaughTIN, L. M., Swimmer, J. Y., Lutcavage, M. S., Wilson, S. G., Galuardi, B., & Liddle, J. B. (2011). Performance of pop-up satellite archival tags. *Marine Ecology Progress Series*, *433*, 1–28. https://doi.org/10.3354/meps09202
Newman, S.J., Wise, B.S., Santoro, K.G. and Gaughan, D.J. (eds). 2021. Status Reports of the Fisheries and Aquatic Resources of Western Australia 2020/21: The State of the Fisheries. Department of Primary Industries and Regional Development, Western Australia
Oliver, S., Braccini, M., Newman, S. J., & Harvey, E. S. (2015). Global patterns in the bycatch of sharks and rays. *Marine Policy*, *54*, 86–97. https://doi.org/10.1016/j.marpol.2014.12.017
Pacoureau, N., Rigby, C. L., Kyne, P. M., Sherley, R. B., Winker, H., Carlson, J. K., Fordham, S. V., Barreto, R., Fernando, D., Francis, M. P., Jabado, R. W., Herman, K. B., Liu, K.-M., Marshall, A. D., Pollom, R. A., Romanov, E. V., Simpfendorfer, C. A., Yin, J. S., Kindsvater, H. K., & Dulvy, N. K. (2021). Half a century of global decline in oceanic sharks and rays. *Nature*, *589*(7843), 567–571. https://doi.org/10.1038/s41586-020-03173-9
Pankhurst, N. W. (2011). The endocrinology of stress in fish: an environmental perspective. *General and Comparative Endocrinology*, *170*(2), 265–275. https://doi.org/10.1016/j.ygcen.2010.07.017
Powter, D. M., & Gladstone, W. (2008). Habitat preferences of Port Jackson sharks, *Heterodontus portusjacksoni*, in the coastal waters of eastern Australia. *Proceedings of the Linnean Society of New South Wales*, *129*, 151–165. https://search.informit.org/doi/abs/10.3316/INFORMIT.754479992744404
Raby, G. D., Packer, J. R., Danylchuk, A. J., & Cooke, S. J. (2014). The understudied and underappreciated role of predation in the mortality of fish released from fishing gears. *Fish and Fisheries* , *15*(3), 489–505. https://doi.org/10.1111/faf.12033
Rasmussen, L.E.L. and Crow, G.L., 1993. Serum corticosterone concentrations in immature captive whitetip reef sharks, Triaenodon obesus. Journal of Experimental Zoology, 267(3), pp.283-287.
Ripley, B. and Lapsley, M. (2022) RODBC: ODBC Database Access R package version 1.3-20.
Rogers, P. J., Huveneers, C., Goldsworthy, S. D., Mitchell, J. G., & Seuront, L. (2013). Broad-scale movements and pelagic habitat of the dusky sharkCarcharhinus obscurusoff Southern Australia determined using pop-up satellite archival tags. *Fisheries Oceanography*, *22*(2), 102–112. https://doi.org/10.1111/fog.12009
Romero, L. M., & Beattie, U. K. (2022). Common myths of glucocorticoid function in ecology and conservation. *Journal of Experimental Zoology. Part A, Ecological and Integrative Physiology*, *337*(1), 7–14. https://doi.org/10.1002/jez.2459
Ruiz-Jarabo, I., Barragán-Méndez, C., Jerez-Cepa, I., Fernández-Castro, M., Sobrino, I., Mancera, J. M., & Aerts, J. (2019). Plasma 1α-Hydroxycorticosterone as Biomarker for Acute Stress in Catsharks (Scyliorhinus canicula). In *Frontiers in Physiology* (Vol. 10). https://doi.org/10.3389/fphys.2019.01217
Rulifson, R. A. (2007). Spiny Dogfish Mortality Induced by Gill-Net and Trawl Capture and Tag and Release. *North American Journal of Fisheries Management*, *27*(1), 279–285. https://doi.org/10.1577/M06-071.1

Ryan, K. L., Taylor, S. M., McAuley, R., Jackson, G., & Molony, B. W. (2019). Quantifying shark depredation events while commercial, charter and recreational fishing in Western Australia. *Marine Policy*, *109*, 103674. https://doi.org/10.1016/j.marpol.2019.103674

Sadoul, B., & Geffroy, B. (2019). Measuring cortisol, the major stress hormone in fishes. *Journal of Fish Biology*, *94*(4), 540–555. https://doi.org/10.1111/jfb.13904

Salini, J., McAuley, R. B., Blaber, S., Buckworth, R., Chidlow, J., Gribble, N., Ovenden, J., Peverell, S., Pillans, R., Stevens, J., & Others. (2007). *Northern Australian sharks and rays: the sustainability of target and bycatch species, phase 2*. CSIRO. http://www.frdc.com.au/Archived-Reports/FRDC%20Projects/2002-064-DLD.pdf

Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews*, *21*(1), 55–89. https://doi.org/10.1210/edrv.21.1.0389

Schaefer, K., Fuller, D., Castillo-Geniz, J. L., Godinez-Padilla, C. J., Dreyfus, M., & Aires-da-Silva, A. (2021). Post-release survival of silky sharks (*Carcharhinus falciformis*) following capture by Mexican flag longline fishing vessels in the northeastern Pacific Ocean. *Fisheries Research*, *234*, 105779. https://doi.org/10.1016/j.fishres.2020.105779

Schnute, J.T., Boers, N. and Haigh, R. (2022) PBSmapping: Mapping Fisheries Data and Spatial Analysis Tools. Available at: https://github.com/pbs-software/pbs-mapping.

Schoen, A. N., Bouyoucos, I. A., Gary Anderson, W., WheaTIN, C. J., Planes, S., Mylniczenko, N. D., & Rummer, J. L. (2021). Simulated heatwave and fishing stressors alter corticosteroid and energy balance in neonate blacktip reef sharks, Carcharhinus melanopterus. In *Conservation Physiology* (Vol. 9, Issue 1). https://doi.org/10.1093/conphys/coab067

Shiffman, D. S., Macdonald, C., Ganz, H. Y., & Hammerschlag, N. (2017). Fishing practices and representations of shark conservation issues among users of a land-based shark angling online forum. *Fisheries Research*, *196*, 13–26. https://doi.org/10.1016/j.fishres.2017.07.031

Simpfendorfer, C. A., Goodreid, A. B., & McAuley, R. B. (2001). Size, Sex And Geographic Variation in the Diet of the Tiger Shark, Galeocerdo Cuvier, From Western Australian Waters. *Environmental Biology of Fishes*, *61*(1), 37–46. https://doi.org/10.1023/A:1011021710183

Siwabessy, P. J. W., Penrose, J. D., & Fox, D. R. (2000). Bottom classification in the continental shelf: a case study for the north-west and south-east shelf of Australia. *, Joondalup, Australia*. https://www.researchgate.net/profile/Paulus_Justy_Siwabessy/publication/236946242_Bottom_Classification_in_the_Continental_Shelf_A_Case_Study_for_the_North-west_and_South-east_Shelf_of_Australia/links/0c96051bb07d6cc4ca000000/Bottom-Classification-in-the-Continental-Shelf-A-Case-Study-for-the-North-west-and-South-east-Shelf-of-Australia.pdf

Skomal, G. B. (2007). Evaluating the physiological and physical consequences of capture on post-release survivorship in large pelagic fishes. *Fisheries Management and Ecology*, *14*(2), 81–89. https://doi.org/10.1111/j.1365-2400.2007.00528.x

Skomal, G. and Bernal, D. (2010) 'Physiological Responses to Stress in Sharks', in Sharks and Their Relatives II. 1st Edition. CRC Press, pp. 475–506. Available at: https://doi.org/10.1201/9781420080483-17.

Skomal, G. B., & Mandelman, J. W. (2012). The physiological response to anthropogenic stressors in marine elasmobranch fishes: a review with a focus on the secondary response. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*, *162*(2), 146–155. https://doi.org/10.1016/j.cbpa.2011.10.002

Stevens, J. D., & McLoughlin, K. J. (1991). Distribution, size and sex composition, reproductive biology and diet of sharks from Northern Australia. *Marine and Freshwater Research*, *42*(2), 151–199. https://doi.org/10.1071/mf9910151

Sulikowski, J. A., Golet, W., Hoffmayer, E. R., Driggers, W. B., Natanson, L. J., Carlson, A., & Sweezey, B. B. (2020). Observing post-release mortality for dusky sharks, *Carcharhinus obscurus*, captured in the U.S. pelagic longline fishery. *Fisheries Research*, *221*, 105341. https://doi.org/10.1016/j.fishres.2019.105341

Therneau, T (2023). _A Package for Survival Analysis in R_. R package version 3.5-3, <https://CRAN.R-project.org/package=survival>.

Vianna, G. M. S., Meekan, M. G., Pannell, D. J., Marsh, S. P., & Meeuwig, J. J. (2012). Socio-economic value and community benefits from shark-diving tourism in Palau: A sustainable use of reef shark populations. *Biological Conservation*, *145*(1), 267–277. https://doi.org/10.1016/j.biocon.2011.11.022

Vianna, G. M. S., Meeuwig, J. J., Pannell, D., Sykes, H., & Meekan, M. G. (2011). The socioeconomic value of the shark-diving industry in Fiji. *Perth: University of Western Australia. 26p*. https://www.academia.edu/download/60490397/Fijisharktourismeconomic-report_November_201120190904-59049-1fq83ig.pdf

Walker, T. I., Hudson, R. J., & Gason, A. S. (2005). Catch evaluation of target, by-product and bycatch species taken by gillnets and longlines in the shark fishery of south-eastern Australia. *Journal of Northwest Atlantic Fishery Science*, *35*, 505–530. https://doi.org/10.2960/j.v35.m515

Whitney, N. M., Lear, K. O., Morris, J. J., Hueter, R. E., Carlson, J. K., & Marshall, H. M. (2021). Connecting post-release mortality to the physiological stress response of large coastal sharks in a commercial longline fishery. *PloS One*, *16*(9), e0255673. https://doi.org/10.1371/journal.pone.0255673

Wickham, H. (2022) 'stringr: Simple, Consistent Wrappers for Common String Operations'. Available at: https://github.com/tidyverse/stringr.

Wickham, H. (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. Available at: https://ggplot2.tidyverse.org.

Wickham, H. et al. (2022) dplyr: A Grammar of Data Manipulation. Available at: https://github.com/tidyverse/dplyr.

Wickham, H. et al. (2019) ‘Welcome to the tidyverse’, Journal of Open Source Software, 4(43), p. 1686. Available at: https://doi.org/10.21105/joss.01686.

Wood, S.N. (2017) Generalized Additive Models: An Introduction with R (2nd edition). Chapman and Hall/CRC.

Supplementary Material

2. Methods

2.2.1 Adrenocorticotropic hormone (ACTH)

The % intra-assay coefficient of variation (CV) was obtained as the average of the individual CVs for each duplicate sample. Individual CVs were calculated as the standard deviation of each duplicate sample, divided by the duplicate mean, and multiplied by 100. The intra-assay coefficient of variation for the assay was 7.15%. The accuracy of the assay expressed as the percent error between the assay-determined value for the assay standards and the assigned value for those standards was 1.20% (± 1.62 SE). The detection limit of the assay (80% B/B0) was 72 pg.ml-1. No cross-reactivity between fish ACTH and other plasma components has been reported by the manufacturer, however, some cross-reactivity may still exist.

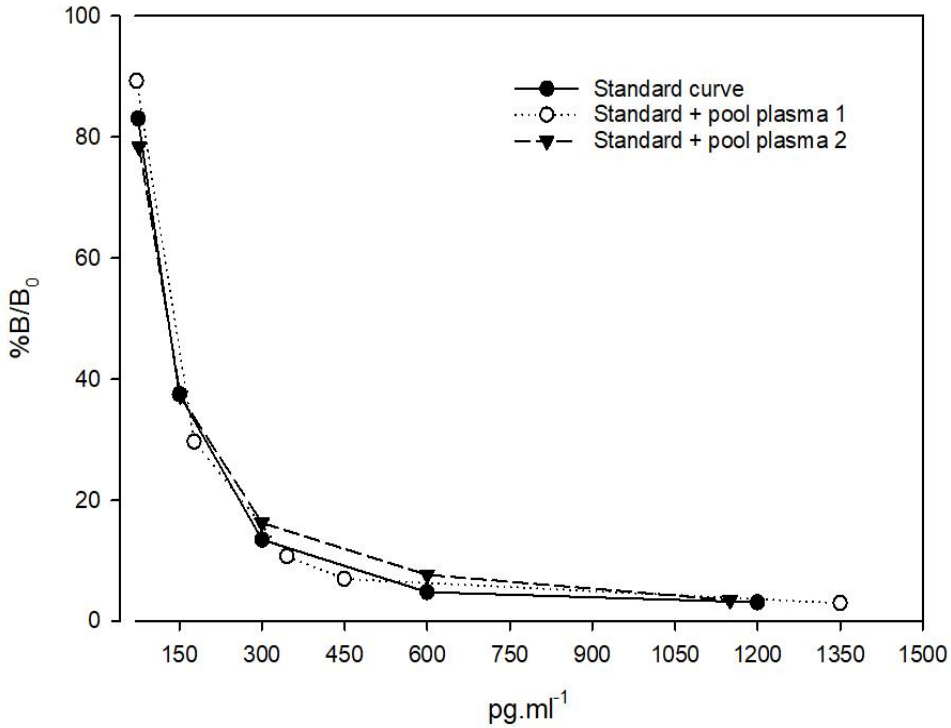


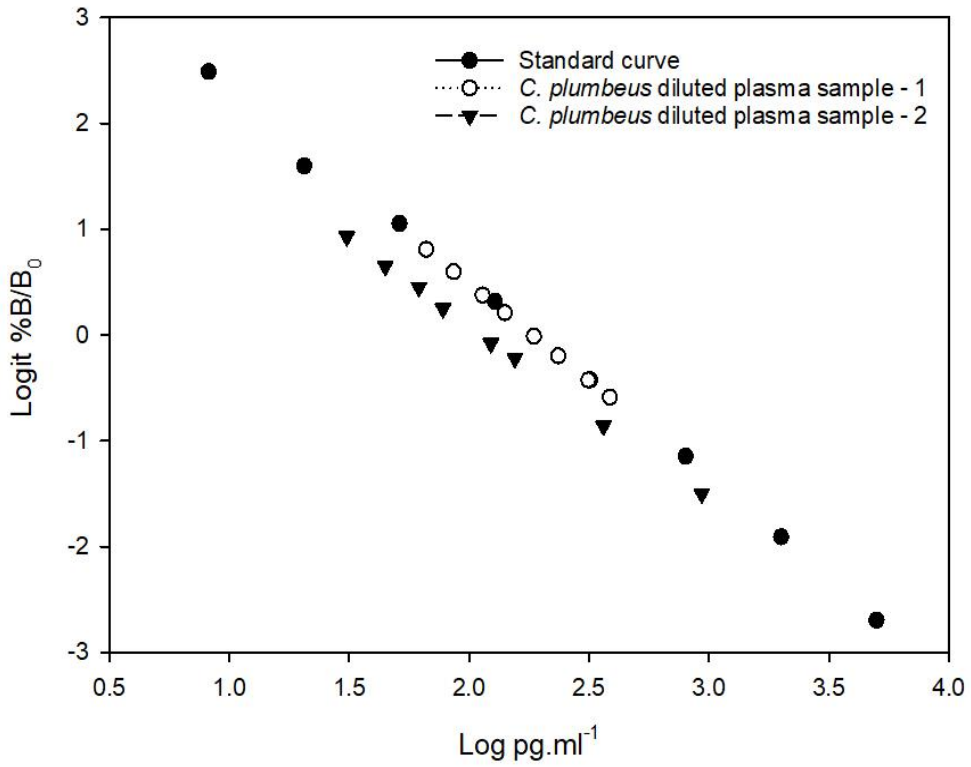
Figure 1: Percent bound (B/B_0) of the ACTH standard curve and two new standard curves spiked with two different pools of plasma samples. Good parallelism is distinguished between the three curves.

2.2.2 Glucocorticosteroids

Plasma samples, 200 µl, were added to a glass tube and diluted 1:5 with diethyl ether, vortexed for 30 sec, and frozen at -20 °C for an hour to separate the phases. After an hour, the top diethyl ether layer was transferred to a clean glass test tube. This step was repeated twice to maximise the recovery of the steroid from the sample. The diethyl ether phase was evaporated by nitrogen and reconstituted with 100 µl of ELISA buffer (fractions were twice concentrated due to the general low values of GCs reported in elasmobranchs). For the assay, 50 µl of each reconstituted sample was assayed in duplicate in a single kit. The absorbance of each well was measured with a microplate reader at a wavelength of 410 nm and converted to pg.ml-1 by a four parameters logistic curve and linearised using a logit transformation. The recovery of

spiked GCs from sandbar sharks' plasma was 87%. Validation of the assay kit was done by comparing serial dilution of plasma samples with the assay standards. The standard curve was made following the manufacturer's instructions consisting of eight points ranging from 5000 to 8.2 pg.ml-1 and the serial dilutions were made by preparing two pools of 200 µl plasma samples, each constructed by combining 25µl from eight individuals, and diluted 1:2.5, 1:5, 1:7.5, 1:10, 1:12.5, 1:15, 1:17.5, 1:20 with Elisa buffer (Supplementary Material: Figure 2.2). The intra-assay CV was determined as above (2.2.1) and was 9%. The accuracy of the assay was 1.09 % (± 1.54 SE) and the detection limit was 30 pg.ml-1.

Figure 2: Percent bound (B/B_0) of the total GCs standard curve and two different diluted 1:2.5



pools of plasma samples. Good parallelism is distinguished between the three curves.

2.3. Results

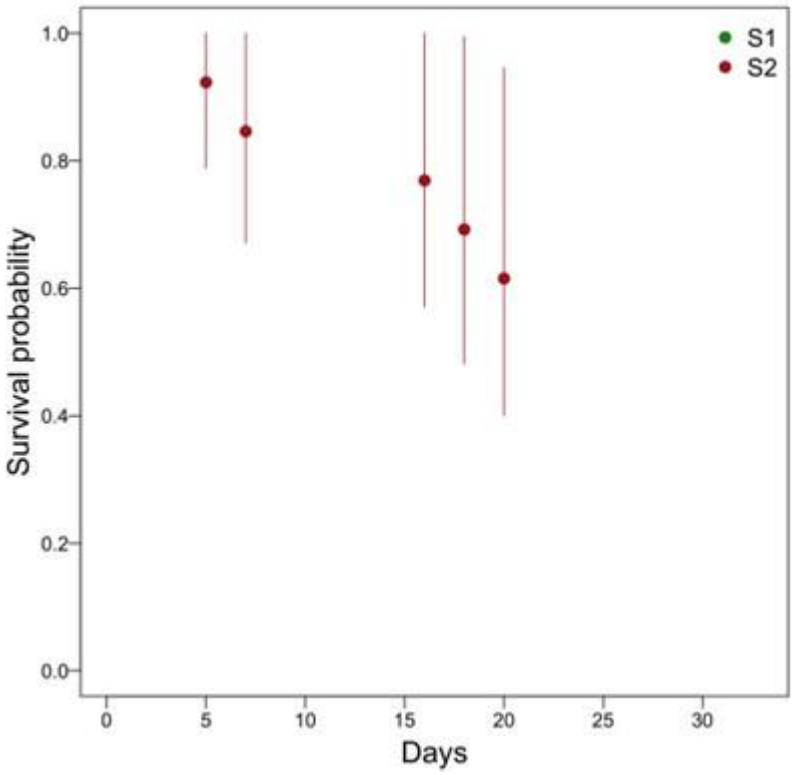


Fig 3: Kaplan-Meier survival plot illustrating median survival probability over time (days) for 13 sandbar sharks tagged and released with PSAT tags. Where S1 is categorised as death = sinker and S2 is categorised as death = premature tag release. The vertical lines are the 95 % CI

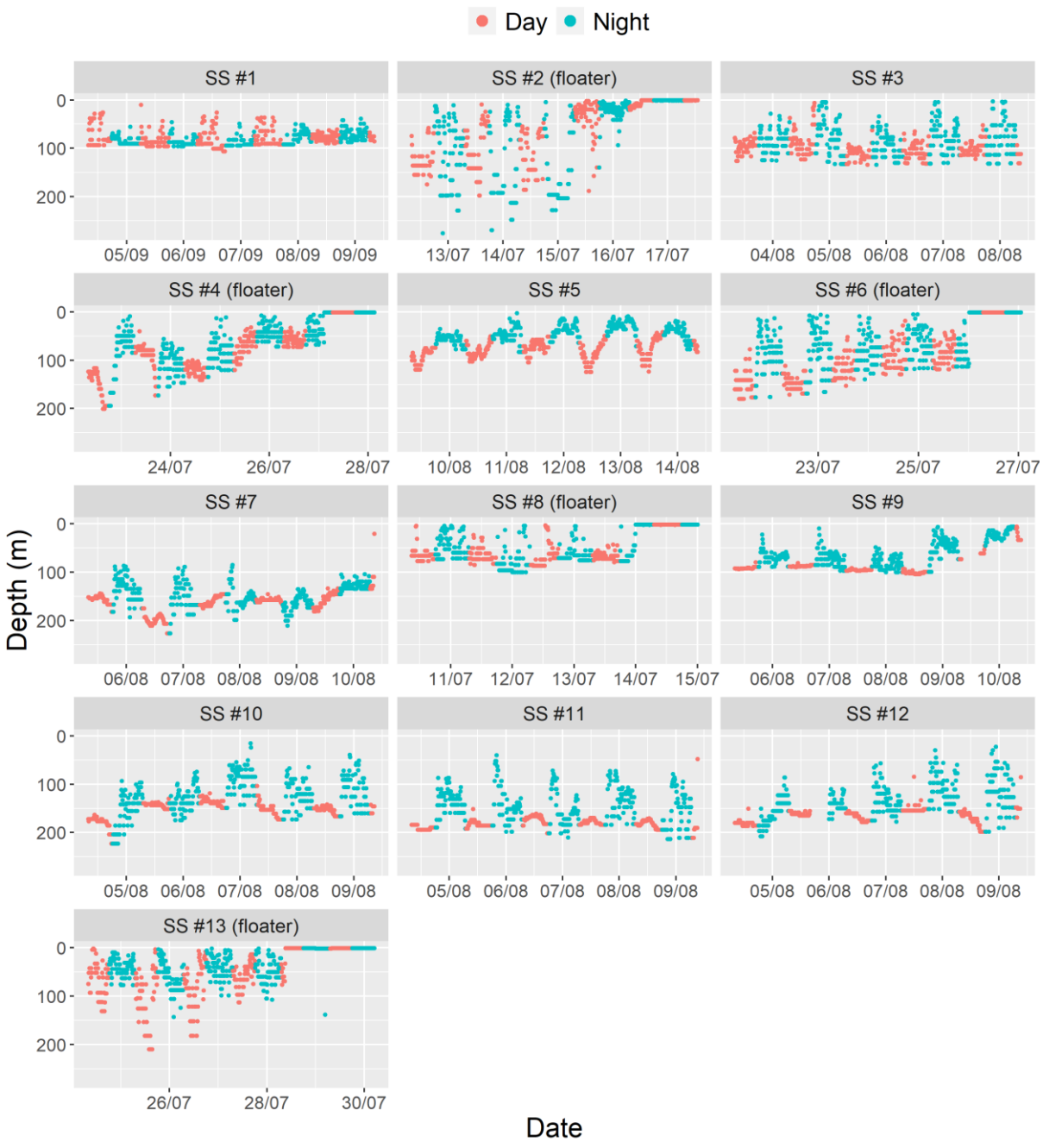


Fig 4: Temporal patterns in depth for the last five days of deployment for satellite-tagged sharks (day: 06:00 - 18:00; night: 18:00 - 06:00; h.time: hook time (hours:minutes:seconds); Lac: lactate level, ranging between 0 mmol/L and Hi (≥25 mmol/L)).

3.2.2 Depth profiles and vertical behaviour

TOH ranged 0:56 to 4:07 hours but did not result in mortality. These individuals showed a variety of depth profiles during the full tag deployment, ranging from the surface (0 m) to 307 m. The depth record of 1200 m (tag number SS #11) is likely to be a tag malfunction. Some samples showed large vertical behaviour, such as 200433 ranging from 10 m (depth of tag activation) to 106.9 m (mean: 80.56 m [±0.14]) and SS #10 ranging from 10 m to 223 m (mean: 137.52 m [±0.21]) (Supplementary Material: Fig 5). There was no significant difference in mean range in depth among sharks (df = 1, p-value = 0.295).

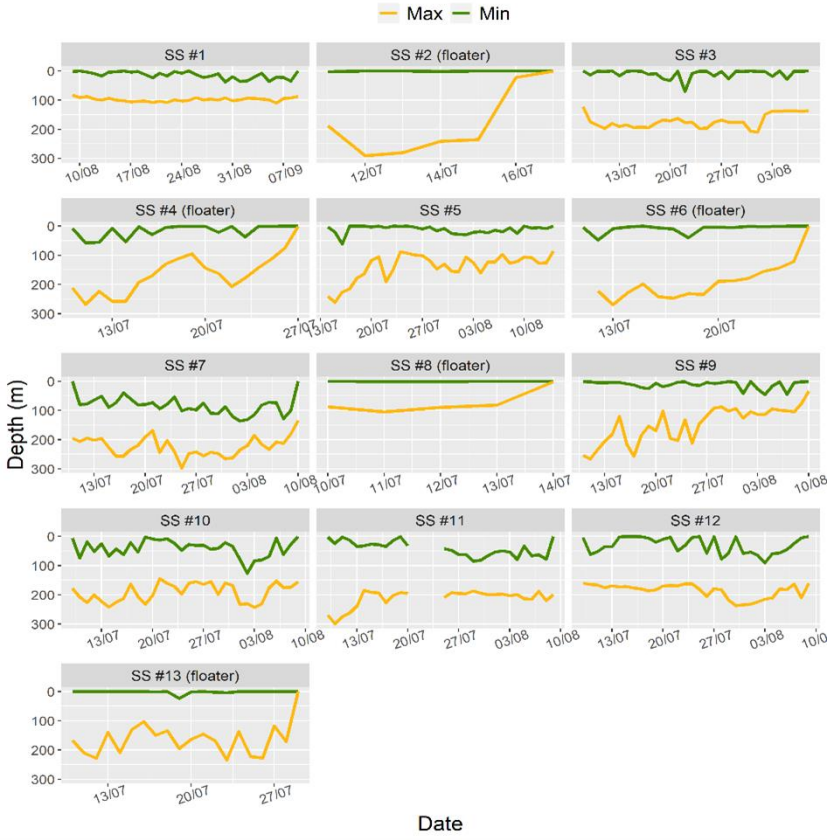


Fig 5: Temporal patterns in depth for the full 30-day deployment (minimum and maximum depth (m).

Table 1: Morphometrics, blood chemistry and time on hook (TOH) from all tags in the survivorship pop-up transmitting tag (PSAT) day and night depth (m) analysis (Figure 6). Hi values were given a value of ≥ 25.

Tag Number	FL (cm)	Sex	Hook Time (mins)	Release Condition	ACTH (pg/ml)	GCs (pg/ml)	Whole-blood Lactate (mmol/L)	Tag deployment (das)
SS#1	135	M	233	1	74.7668	438.65	≥ 25	30
SS#2	127	F	189	1	105.01	350.29	22.9	12
SS#3	134	M	97	1	91.99781	722.35	8.9	30
SS#4	140	M	222	1	62.64	406.31	≥ 25	10
SS#5	119	F	176	1	84.24781	860.31	≥ 25	30
SS#6	152	F	103	1	71.87	374.2	17.5	8
SS#7	162	F	247	1	83.72666	406.31	24.8	30
SS#8	134	F	125	2	83.02	743.01	≥ 25	23
SS#9	136	M	48	1	74.02267	715.65	11	30
SS#10	144	F	227	1	93.0695	2013.68	≥ 25	30
SS#11	140	M	56	1	66.87111	374.2	19.5	30
SS#12	145	M	125	1	NA	NA	≥ 25	30
SS#13	124	M	184	1	66.20	593.08	≥ 25	6

Table 2: Morphometrics, blood chemistry and time on hook (TOH) from all tags in the survivorship pop-up transmitting tag (PSAT) day and night depth (m) analysis (Figure 6). Hi values were given a value of ≥ 25. TG: tiger shark; PJ: Port Jackson shark; BW: dusky shark

Species	Tag Number	FL (cm)	Sex	Hook Time (mins)	Release Condition	ACTH (pg/ml)	GCs (pg/ml)	Whole blood Lactate (mmol/L)	Tag deployment (days)
TG	19P1614	286	M	263	1	NA	NA	18	30
TG	19P1525	275	M	255	1	NA	NA	17.9	30
TG	19P1513	337	F	221	1	NA	NA	9.8	30
PJ	19P1455	79.5	F	866	1	NA	NA	3.4	30
PJ	19P1598	95	F	551	1	NA	NA	0.7	30
PJ	19P1600	64	F	722	1	78.39001	3748.94	1.2	30
PJ	20P0353	58	M	722	1	105.9962	964.45	2.3	1
PJ	19P1653	72	F	910	1	125.2701	750.08	NA	30
BW	20P0092	262	M	225	1	121.1579	478.26	25	30
BW	19P1617	272	F	211	1	171.7279	497.84	25	30
BW	19P1640	275	F	211	2	150.3807	1753.04	25	30

BW	19P1632	220	F	318	2	127.5171	696.06	17.5	30