

THE IMPACTS OF COMMERCIAL PURSE SEINE FISHING ON THE BIOLOGY AND  
ECOLOGY OF THE SILKY SHARK, (*CARCHARHINUS FALCIFORMIS*): IMPLICATIONS  
FOR SCIENCE BASED MANAGEMENT.

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"You can judge the morality of a nation by the way the society treats its animals"

-Mahatma Gandhi

"This we know: the earth does not belong to man, man belongs to the earth. All things are connected like the blood that unites us all. Man did not weave the web of life, he is merely a strand in it. Whatever he does to the web, he does to himself"

-Chief Seattle

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## ABSTRACT

The silky shark (*Carcharhinus falciformis*) is a circum-tropical species found in all oceans between 20° North and South latitude. This distribution tends to overlap with the preferred habitat of three heavily exploited tropical tuna species (bigeye, *Thunnus obesus*, yellowfin *T. albacares*, and skipjack, *Katsuwonus pelamis*). The western and central Pacific Ocean (WCPO) is the most productive tuna fishery on the globe (2,613,528 mt in 2012) with the largest proportion of tuna landed via purse seine fishing operations (69%). In large part, this productivity is feasible because in the WCPO, the tuna purse seine fishery utilizes drifting fish aggregating devices (FADs) to attract and hold tuna and thereby reduce search times and cost. Juvenile silky sharks also aggregate in large numbers at FADs and, as a result, comprise the largest component of the incidental elasmobranch catch taken in the tropical tuna purse seine FAD fishery. A stock assessment revealed that silky shark spawning biomass, median size and CPUE have all declined and concluded that the WCPO stock(s) are overfished and that overfishing is occurring. Population analysis for this species has shown that high mortality during the juvenile life stages has the largest impact on population growth. Information on the basic biology, behavioral ecology and habitat use of this species is deficient. To fill these data gaps and better manage the impact that fishing has on this species I conducted an analysis of the survival rates and total mortality of sharks captured in purse seine gear. Survival was related to the stage during the fishing operation when sharks were released. This work was augmented by an investigation of the physiological disruptions that lead to mortality when sharks are captured in a purse seine. This was achieved by measuring stress hormones, blood gases and electrolyte concentrations in sharks sampled at various stages of the fishing process and by an analysis of the habitat use and movement behavior of juvenile silky sharks in the Pacific Ocean. Taken together, these data will be crucial to developing meaningful conservation tactics and future bycatch mitigation tools.

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## CHAPTER 1

### GENERAL INTRODUCTION

#### *Background*

The silky shark, *Carcharhinus falciformis* (Müller & Henle 1841) is a cosmopolitan, circumtropical species inhabiting both coastal and pelagic waters in all tropical oceans warmer than 23° C (Compagno, 1984, Last and Stevens, 2009). This distribution typically falls between 20° north and 20° south latitudes (Clarke et al., 2011) and tends to overlap with most of the commercial fishing effort targeting tropical tuna (bigeye *Thunnus obesus*, yellowfin *T. albacares*, and skipjack *Katsuwonus pelamis*). As such, silky sharks make up a large component of the elasmobranch bycatch in both purse seine and longline fisheries worldwide (e.g. Watson et al., 2008; Clarke et al., 2011). In the western and central Pacific Ocean (WCPO) tuna purse seine fishery, silky sharks comprise around 95% of the shark bycatch (Lawson, 2011). These numbers were not this high until relatively recently when a significant expansion of the WCPO purse seine fishery occurred in the 1980's, a result of displaced fishing effort from the eastern Pacific Ocean purse seine fishery when dolphin safe canned tuna requirements were enacted and coastal state jurisdictions were expanded (Doulman, 1987; Hall, 1998; Lennert-Cody & Hall, 2000). At that time purse seine fishing effort in the WCPO was based on targeting unassociated ("free") tuna schools and tuna found in association with natural floating objects (Gillett et al., 2002). During the 1990's the WCPO fishery continued to expand as Asian, Spanish and US fleets grew in number and purse seine effort shifted to the use of drifting Fish Aggregating Devices (FADs) to increase catch rates and reduce search time (Dagorn et al., 2012). Currently, the WCPO is the largest volume tuna fishery in the world, accounting for approximately 59% of total global landings, with the purse seine fishery landing 69% of the catch (Williams & Terawasi, 2013).

The use of FADs in industrial-scale purse seine fishing is a contentious issue because of increased levels of bycatch on vulnerable species such as sharks and sea turtles (Watson et al., 2008). Juvenile silky sharks congregate around these drifting objects and become incidentally caught in purse seine nets targeting skipjack (*Katsuwonus pelamis*) and yellowfin tuna (*Thunnus albacares*) that are harvested primarily for production of canned tuna (Filmlalter et al., 2011). Shark catch rates are typically twice as high in FAD associated sets versus unassociated or 'free school' fishing sets (Clarke et al., 2011). High juvenile silky shark catch rates also have been documented in FAD associated tuna purse seine sets in the

Indian (Amandè et al., 2008), the eastern Pacific (Roman-Verdesoto and Orozco-Zoller, 2005), and the Atlantic Oceans (Amandè et al., 2010).

In the WCPO, concerns have been raised about the bycatch impacts on silky shark populations caused by the FAD associated purse seine fishery because of declining silky shark catch rates, declining size trends in some datasets, and increasing tuna fishing effort (Clarke, 2011). These population trends are most likely a result of large increases in fishing mortality from both purse seine and longline fisheries over the last two decades (Rice and Harley, 2013). Increases in fishing mortality, in combination with the life history characteristics of elasmobranchs (e.g. slow growth, low reproductive output, late ages at maturity), may prevent silky shark populations from recovering from excessive fishing pressure. Additionally, population demographic analyses have shown that high mortality on the juvenile life stages of elasmobranchs has the most profound effect on silky shark population trajectories (Cortes, 2002; Beerkircher et al., 2003; Roman-Verdesoto, 2014). Unfortunately, it is predominantly juvenile silky sharks that are found in association with FADs. Furthermore, a stock assessment of the silky shark population in the WCPO estimated that spawning biomass, total biomass and recruitment had all declined. Conclusions drawn from this are that overfishing is occurring and that fishing mortality has increased to levels far in excess of the maximum sustainable yield (Rice and Harley, 2013). Despite these findings, Regional Fishery Management Organizations (RFMO) have failed to produce any meaningful conservation and management measures to reduce the impact that fishing has on silky shark populations. Current management measures banning finning and requiring no retention of bycatch are easy to implement in theory but are difficult to enforce and monitor and still fail to reduce fishing mortality (Clarke et al. 2012).

Commercial fishing is driving down silky shark (and other shark) populations and virtually nothing is being done to mitigate it, despite the fact that there is a growing body of evidence that disturbances at the top of food webs have repercussions that resonate throughout all trophic levels in marine ecosystems (e.g. Hall et al. 2000, Kitchell et al. 2002, Ferretti et al. 2010, Dulvy et al. 2014). These effects include, but are not limited to, changes to mean body size, lower overall fecundity, which may induce evolutionary responses in life histories (Kuparinen and Merila 2007) and the concomitant predatory release of lower trophic levels (Essington et al. 2002, Hinke et al. 2004). The resultant changing abundances and distributions of mesopredators force further trophic cascades, loss of biodiversity and shifts in entire ecosystems (e.g. Jackson et al. 2001, Bascompte et al. 2005, Shepherd and Myers 2005, Ward and Myers 2005). These shifts may have "dire biological consequences

throughout the ecosystem, far beyond the social, economic and moral costs of depleted fisheries" (Hinman 1998). There is a paucity of information available on the biology and ecology of this species however, so it is impossible to determine exactly what ecosystem effects the loss of this species may have. Furthermore, bycatch species are typically discarded. Discards change the foraging behavior and natural diet of other marine species (e.g. seabirds, marine mammals, sharks and benthic species). Differences in the life history characteristics of discarded species produce unbalanced ecosystem effects, exacerbated by differences in demographic effects of removal of non-target species at different life stages. In the case of the FAD associated purse seine fishery, the systematic removal of juvenile silky sharks has contributed to growth overfishing within two decades.

Since effective management strategies to reduce the impact of fishing on silky sharks have not yet been implemented, it is prudent to look for alternative methods to reduce the incidental take of this species in commercial fisheries. This study consists of two main components: 1) Quantification of the dynamics and causes of survival and mortality of silky sharks incidentally captured in commercial purse seine fisheries and 2) Identifying the behavioral factors that cause silky sharks to be vulnerable to capture. I used physiological and post-release survival studies to identify potential shark bycatch mitigation strategies in the purse seine fishery. During a chartered cruise on board a commercial purse seine vessel conducting typical fishing operations, I investigated the interaction rates, total mortality and post release survival dynamics of incidentally captured juvenile silky sharks. To identify both post-release survival rates and the point in the fishing operation when silky sharks sustain the injuries that lead to mortality, I used a combination of data obtained from satellite linked pop-up tags and from blood chemistry analysis. Further analysis of the stress physiology of this species was undertaken to get a better understanding of the physiological disturbances that correlated with mortality and how these disturbances are reflected in blood chemistry. Electronic tag data were also analyzed to identify the underlying movement behavior and habitat use patterns that make this species vulnerable to capture in purse seine and other tuna fisheries. These data will assist in formulating effective bycatch mitigation strategies.

### *Post-release Survival and Stress Physiology*

The tropical tuna purse seine fishery and other commercial fisheries targeting tuna and swordfish have high rates of incidental shark capture (Gillman, 2012). These sharks are typically unwanted and of low market value and are discarded at sea (Worm et al., 2013). Discarded sharks are often released alive but several studies have shown that they may have

sustained injuries (both physical trauma from capture and handling and physiological disturbances) that can have immediate or delayed effects resulting in mortality (eg. Skomal, 2007; Skomal and Mandelman, 2012). Blood borne biochemical indicators of stress are increasingly being used to elucidate the post release condition of discarded elasmobranchs captured in fisheries (eg. Moyes et al., 2006; Hight et al., 2007; Renshaw et al., 2012). Since blood and white myotomal muscle comprise from 3-6% and 50-60% of the total body mass, respectively, changes in muscle biochemistry caused by a stressful event such as capture in fishing gear are strongly reflected in the blood (Wells et al. 1986). To elucidate the physiological perturbations that occur in silky sharks that have been captured in a purse seine, I measured several blood based indices of stress from animals sampled during each stage of the fishing operation, including sharks that were sampled with a minimal amount of handling and prior to any interaction with purse seine fishing gear.

The elasmobranch stress response closely resembles that of most vertebrates with a few important differences (Skomal and Bernal, 2010; Skomal and Mandelman, 2012). Immediately following the perception of a stressor, the initial neuroendocrine signal follows the hypothalamic-pituitary-interrenal axis to release large concentrations of catecholamines (adrenaline and noradrenaline) into the blood stream from chromaffin tissue in the head kidneys and axillary bodies on the dorsal surface of the cardinal sinus (Butler and Metclaf, 1988). In higher vertebrates adrenaline is secreted from adrenal glands. The presence of adrenaline in circulation increases heart rate and ventilation and acts to mobilize glucose reserves from the liver to the active muscle tissues (Bone 1988). In sharks, normal locomotion is performed by the highly vascularized and perfused red muscle and fueled via aerobic respiration (Heisler 1988). During burst swimming or periods of struggle (as in a purse seine net) the white muscle is activated and glycolysis occurs via anaerobic pathways giving rise to lactic acid. Lactic acid is dissociated relatively quickly and pH drops within the tissues and extracellular compartments (Heisler 1988). Changes in pH are readily reflected in the blood plasma (Mandelman & Skomal 2009, Gallagher et al. 2010, Frick et al. 2012). In conditions of hypoxia or when the animal's ability to ventilate is compromised, further respiratory acidosis occurs as partial pressures of CO<sub>2</sub> rise, and pO<sub>2</sub> falls (Mazeud and Mazeud 1981). Bicarbonate concentrations increase in the plasma and acid-base buffering capacity is diminished. Acidosis of the system increases the permeability of cell membranes thereby increasing the osmolality of the blood. Increases in hemoconcentration following fluid shifts across tissue compartments may also occur (Cliff & Thurman 1984).

In this study I measured the following blood parameters from animals that were sampled during each stage of the fishing operation: adrenaline, pH, blood gases (O<sub>2</sub> and CO<sub>2</sub>), lactate, glucose, osmolality, hematocrit (as percent packed cell volume), sodium, potassium, and calcium. Disruptions in these parameters provide an indication of the physiological perturbations caused over the course of the fishing procedure. Silky sharks also are a large component of the shark bycatch in longline fisheries and in some regions, including the WCPO where longline fishing has higher silky shark catches due to the level of effort than purse seining fishing. The longline fishery also discards silky shark bycatch at sea, so a similar study on post release mortality rates using the stress parameters identified in this study will be useful in understanding the impacts of the longline fishery.

#### *Habitat Use & Movement Behavior*

The most obvious and effective means of reducing the impacts that the tropical tuna purse seine fishery has on silky shark populations would be total avoidance. Thus, identifying geographical areas and bio-physical factors that are indicative of essential habitat or regions of core habitat use are essential to silky shark conservation. Presently, silky shark movement behavior is relatively unknown. Although they are found in all tropical waters and despite having few barriers to their movements, fishery landing data provide an indication of spatial and temporal structure by size and sex (Watson et al. 2008) and life history studies demonstrating regional differences in vital rates (Branstetter 1987, Bonfil et al. 1993, Oishitani et al. 2003, Joung et al. 2008, Sanchez-de Ita et al. 2011, Hall et al. 2012). To elucidate the movement behavior and habitat use of juvenile silky sharks in the WCPO region, data from satellite tagged sharks were analyzed for horizontal and vertical movement patterns in time and space.

Developments in satellite linked pop-off archival transmitting tag technologies have increased our ability to garner information on animal movement patterns - including those from remote regions of the oceans such as those where commercial tuna fisheries operate. Archival tags collect time, depth, temperature and light data from free-ranging animals and deliver accurate depictions of the corresponding environmental conditions (Breed et al. 2012). The availability of these data provide insight to residency and migration patterns (e.g., Jonsen et al. 2012, Pedersen et al. 2011), the environmental factors that affect movement (Jonsen et al. 2012, Papastamatiou et al. 2013) and the extent of individual and population ranges (Jorgensen et al. 2009, Howey-Jordan et al. 2013). They can also inform population

level models on habitat use and how distributions might be affected under climate change (Jonsen et al. 2012).

State space modeling approaches (SSM) are now the tools of choice for analyzing animal telemetry data (Breed et al. 2012). Using both light based and Argos based location estimates, the positional data provided by these tags can be used to generate models of horizontal movement and to estimate the underlying behavioral states that lead to the observed displacement of tagged animals. For marine animals that do not spend a significant portion of time at the surface (e.g. sharks and teleosts) accurate location fixes can be difficult to acquire. Light-based geo-location algorithms and movement “filters” can be used to estimate position and resolve several potential sources of error due to local environmental conditions (e.g. cloud cover, water clarity) and the dive behavior of the animal. To estimate position data for each individual silky shark and to identify discrete behavioral states, I utilized a spatial Hidden Markov Model (Pedersen et al. 2011). The model can be applied to improve geo-location estimates by incorporating sea surface temperature data from the tags to reveal the behavioral states underlying the horizontal displacement of each animal (e.g. migrating or resident: Pedersen et al. 2011, Jonsen et al. 2013). Model predictions can then be mapped to identify hot spots of biological importance such as nursery, foraging or breeding grounds (Jonsen et al. 2012).

Marine animals move in three dimensions and so understanding the vertical component of movement behavior is equally important for devising effective conservation strategies. Identification of not only the preferred depth ranges but also the biotic and abiotic factors that affect juvenile silky shark vertical positions would provide a better understanding of their role in the ecosystem. Evidence of diel shifts in depth and temperature preferences allow us to make inferences regarding the species' foraging tactics and movements of prey items (Carey et al. 1990, Merten et al. 2014), and to identify potential behavioral responses to physiological states (e.g. behavioral thermoregulation - Holland et al. 1992 , Howey-Jordan et al. 2013). Furthermore, distinguishing patterns in vertical movement are useful in designing future bycatch mitigation techniques. During this study, vertical movement and displacement data were collected using satellite linked archival tags attached to silky sharks released at the study sites.

Despite growing conservation concerns about silky shark population declines worldwide, management entities have thus far failed to produce effective conservation and management measures that will reduce mortality from fishing (e.g. catch limits, reductions in capacity, restrictions on FADs). To reduce the impact that purse seine fishing has on the

population I set out to quantitatively assess the post release survival and total mortality rates of silky sharks captured in purse seine fishing gear. The aim was to identify the point in the fishing operation where the physiological perturbations lead to mortality occur and to elucidate the underlying behaviors that lend silky sharks vulnerable to purse seine capture. These data provide invaluable insight into the development of meaningful silky shark conservation tactics and future bycatch mitigation tools.

### *Dissertation Organization*

Each chapter of this dissertation with the exception of the introduction chapter, was written for publication in peer-reviewed journals. The various journal formats have been changed into one format for consistency. There is some overlap of the material that is presented within each chapter. Chapter 2 is the initial investigation of the purse seine fishery interaction with silky sharks using some of the data from subsequent chapters to quantitatively assess mortality rates. In chapter 3 I further explore the physiology of the silky shark as the fishing procedure progresses. In chapter four I investigate the movement behavior of juvenile silky sharks through the use of satellite tag technologies to identify the underlying behaviors that lend silky sharks vulnerable to capture.

CHAPTER 2:

POST-RELEASE SURVIVAL OF JUVENILE SILKY SHARKS CAPTURED IN A  
TROPICAL TUNA PURSE SEINE FISHERY

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## **Abstract**

Juvenile silky sharks, *Carcharhinus falciformis*, comprise the largest component of the incidental elasmobranch catch taken in tropical tuna purse seine fisheries. During a chartered cruise on board a tuna purse seine vessel conducting typical fishing operations we investigated the post release survival and interaction rates of incidentally captured silky sharks using a combination of satellite linked pop-up tags and blood chemistry analysis. To identify trends in survival probability and the point in the fishing interaction when sharks sustain the injuries that lead to mortality, animals were sampled during every stage of fishing procedure. The total mortality rates of silky sharks captured in purse seine gear was found to exceed 84%. We found survival to precipitously decline once the silky sharks had been confined in the sack portion of the net just prior to loading. Additionally, shark interactions recorded by the scientists were markedly higher than those recorded by vessel officers and the fishery observer. Future efforts to reduce the impact of purse seine fishing on silky shark populations should be focused on avoidance or releasing animals while they are still free swimming.

**Keywords:** Bycatch, Fish aggregating device, stress physiology

## Introduction

Nearly 82% of the total global tuna landings are consumed as a canned product and the European Union and the United States are by far the largest consumers (83% combined, Gillman and Lundin, 2008). The western and central Pacific Ocean (WCPO) tuna fishery is the largest volume tuna fishery in the world and 69% of the total tuna caught in the region are landed via purse seine fishing (Williams & Terawasi 2013) and destined for a cannery. A significant amount of this purse seine fishing effort is focused on the use of drifting Fish Aggregating Devices (FADs) to increase catch rates and reduce search time (Dagorn et al. 2012). These FADs are often outfitted with sophisticated GPS and sonar buoys, which inform the vessel of the FAD's proximity and a rough estimate of the biomass underneath it. The use of FADs in industrial-scale purse seine fishing is a contentious issue because they not only attract the target tuna species but also several other 'non-target' pelagic species. At FADs, bycatch rates of vulnerable species such as sharks and sea turtles (Watson et al. 2008) and juvenile bigeye tuna, *Thunnus obesus* (Fonteneau et al. 2000) are much higher than fishing sets conducted on free-schools of tuna. Juvenile silky sharks, *Carcharhinus falciformis* congregate around these drifting objects and become incidentally caught in purse seine nets targeting skipjack, *Katsuwonus pelamis* and yellowfin, *Thunnus albacares* tunas (Filmlalter et al. 2011). In the WCPO FAD-associated (when purse seine nets are set around a FAD) purse seine fishery, silky sharks compose 95% of the total elasmobranch bycatch (Lawson 2011). Shark catch rates are typically twice as high in FAD-associated sets versus unassociated or 'free school' fishing sets (Clarke et al. 2011). High juvenile silky shark catch rates also have been documented in FAD-associated tuna purse seine sets in the eastern Pacific (Roman-Verdesoto & Orozco-Zoller 2006), the Atlantic (Amandè et al. 2010) and the Indian Oceans (Amandè et al. 2008). In the Indian Ocean, Poisson et al. (2014) demonstrated that post release survival among purse seine captured silky sharks was less than 20%.

The scientific committee to the Western Central Pacific Fishery Council (WCPFC) has raised concerns about silky shark bycatch rates in the FAD-associated purse seine fishery because of declining catch rate trends and declines in median sizes despite increased fishing effort (Clarke 2011). Additionally, the WCPO stock assessment concluded the silky shark stock is overfished and that overfishing is currently occurring (Rice & Harley 2013). These factors, in combination with the life history characteristics of elasmobranchs (e.g. slow growth, low reproductive output, late ages at maturity) may prevent silky shark populations from recovering from excessive fishing pressure. Furthermore, population demographic analyses have shown that high mortality on the juvenile life stages of elasmobranchs has the most profound effect on silky shark population growth or decline (Cortes 2002; Beerkircher et al. 2003; Roman-Verdesoto 2014). It is predominantly juvenile silky sharks that are found in association with FADs, which lends this population more vulnerable to over-exploitation via this fishing modality.

Similar to most commercial tuna fisheries, sharks that are captured incidentally are typically discarded at sea (Worm et al. 2013). Discarded sharks are often released alive but several studies have shown that sharks may sustain injuries, both physical trauma from capture and handling and physiological disturbances that can have immediate or prolonged effects resulting in mortality (eg. Skomal 2007; Skomal & Mandelman 2012). Blood borne biochemical indicators of stress are increasingly being used to elucidate the post release condition of discarded elasmobranchs captured in fisheries (eg. Moyes et al. 2006; Hight et al. 2007; Renshaw et al. 2012). Physiological effects typically involve respiratory and metabolic acidosis, changes in the osmotic structure of the blood plasma, hemodilution and elevated glucose levels (Renshaw et al. 2012). Accordingly, lactate, pH, and other divalent electrolytes in the plasma have been shown to correlate with capture induced stress and mortality in several shark species (e.g. Moyes et al. 2006; Marshall et al. 2012).

In this study we sought to quantify total mortality and post release survival rates of incidentally captured silky sharks and to identify the point in the purse seine fishing operation when sharks sustain injuries that lead to mortality. We were able to do this using data collected onboard a commercial tuna purse seine vessel conducting normal fishing practices in the WCPO. To quantitatively assess fishery induced shark mortality, we used lactate concentrates found in the blood in combination with satellite linked pop-up archival transmitting (PAT) tags to validate the fate of released animals. Other results from the cruise allowed us to investigate the vertical habitat use of silky sharks to identify potential bycatch mitigation strategies and to assess the accuracy of shark catch rate estimates made by the observer and the vessel officers.

## **Methods**

### *Field methods*

Sharks were captured incidentally in purse seine sets for tropical tuna in association with drifting FADs from May through June 2012, during a chartered trip in the western and central Pacific Ocean aboard the U.S. flagged purse seine vessel, M/V Cape Finisterre. This vessel measures 239 ft LOA (222 ft waterline) and is rated at 1434 GRT with a total fish hold capacity of 1149 metric tons held in 19 brine-refrigerated holds. The purse seine net measures 1719 m long at the corkline and is approximately 330 m deep (stretched) (Itano et al. 2012 a). Thirty-one sets were conducted (30 around drifting FADs-FAD associated and one on a free school feeding at the surface-unassociated or free school) over the course of 41 days following normal fishing practices (Fig. 1). To assess post release survival and to identify the point in the fishing operations when animals sustain the injuries that result in mortality, we sampled and tagged animals prior to commencement of fishing (Pre-set) and animals that were landed during each stage of the fishing operations. For a general description of the purse seine fishing procedure see Box 1. The landing stages and control groups identified for this

experiment were; (1) Pre-set - sharks were captured at FADs using dip nets or baited hooks from a small work boat, prior to the onset of fishing to establish a reference curve of blood biochemistry indicators and to release some sharks that were exposed to a minimal amount of handling. (2) Encircled - these animals had been encircled by the purse seine net and the net hauled back to at least half net. These sharks were fished out of the net using hook and line, handlines and dipnets during the net 'haul back' while it was still large enough for the animals to swim freely. They were tagged and sampled onboard the small workboat and then released outside the purse seine net. (3) Entangled - sharks that had become entangled (gilled or meshed) in the purse seine net during haul back and are removed by the fishermen as the net emerges from the water. These animals get landed early, before sacking up and brailing. (4) First Brail - sharks that were brought onto the vessel during the first brail. These sharks would have been on the top of the sack. (5) Later Brails - these sharks were landed during any subsequent brails and were confined in the sack for longer periods of time and subjected to the weight of the catch on top of them. Sharks that were recovered during the onboard species composition spill sampling by the observer (Itano et al., 2012a) or recovered from the lower well deck were also considered to have been landed during a later brail.

All sharks were placed upside down in a cradle and ventilated with running seawater while morphometrics were recorded, tags attached and blood withdrawn. Sharks were then released over the side of the vessel and the condition at the time of release was recorded (Table 1). Release conditions were qualitative and based on a scale from 4-0, where (4) or Excellent Condition was recorded for animals that swam away rapidly without any obvious signs of distress or physical trauma. Sharks released in Good Condition (3) swam away but appeared slower or disoriented. Fair Condition (2) was assigned to sharks whose swimming appeared laborious and/or they exhibited other visible signs of trauma. Sharks in Poor

Condition (1) were able to right themselves and made efforts to swim, while sharks released with a (0) or Dead sank upside down.

Three different types of satellite pop-up archive transmitting (PAT) were used to verify post release survival and to elucidate habitat use and movement behavior; X PAT (Microwave Telemetry Inc. Columbia, MD.), mini PAT and survival PAT (sPAT; Wildlife Computers Inc., Redmond, WA.). The first two tag types continually record and archive detailed behavioral data for user-programmable deployment periods whereas the latter type of tag (sPAT) is designed specifically to provide only summary behavioral data from which survival or mortality can be inferred over a 30 day deployment. Programming and data for these tags are proprietary and are provided by Wildlife Computers to the tag owner post processing. The X and mini PATs were programmed to record temperature, depth, and light level data at specific time intervals and to release after periods of 100-360 days. On the scheduled pop-off dates, the tags detach from the tether, float to the surface and transmit the archived data to the Argos satellite system. In the event of a mortality where the animal sinks through the water column, the PATs have an external guillotine device that severs the tag attachment at ~1680 m, before the tag reaches crushing depths. Depth records that went to 1680 m indicated the animal was sinking and presumed dead. The sPATs transmit the daily maximum and minimum depth and temperature experienced by the animal during the deployment. They are programmed to identify the fate of the tag and thus the animal by one of three designations; (1) Floater: the tag begins to transmit because it was shed by the animal (e.g., due to attachment failure). (2) Sinker: the animal dies and sinks to 1680 meters or dies and remains on the ocean floor at a constant depth for two days. (3) Survivor: the sPAT completes the deployment, the tag initiates a release at 30 days. All sharks that were released alive were also tagged with a wire through metal dart, ID tag, (Hallprint, South Australia).

Blood sampling consisted of a small volume of blood (3 mL) taken from the caudal vein into heparinized syringes and placed on ice until analysis. Blood chemistry analysis was conducted onboard the vessel immediately after the conclusion of the haul (10-120 minutes post blood withdrawal). To quantify concentrations of lactate, the CG 4+ cartridge was used with the I-STAT portable automated blood chemistry analyzer (Abbott Laboratories, IL, USA) (Cooke et al. 2008, Gallagher et al. 2010).

## Data Analysis

### *Post Release Survival*

Survival and mortality events for PAT tagged animals were interpreted using the transmitted depth records from the tags. In this study, animals that survived  $\geq 10$  days were considered to have survived the fishing interaction. Animals (tags) that sank to the critical threshold depth (1680 m) within that time period were considered mortalities. Tags that were shed (Floaters) from sharks that had also been blood sampled within the 10 day period were not used in the post release survival analysis because the reasons the tag detached could not be determined (n=1). Wilcoxon rank sum tests were used to determine which blood parameters differed significantly between survivors and moribund animals (determined from animals that were both blood sampled and satellite tagged; Table 2). Logistic regression models and maximum likelihood estimation was used to predict the probability of survival for animals that had blood drawn but were not satellite tagged (Fig. 3). The fitted values ( $\hat{\pi}$ ) were then used to predict survival rates by fishing operation stage and release condition for all of the sharks captured during the cruise (Tables 3 & 4).

### *Silky Shark Catch Rate Comparisons*

Comparisons of the silky shark catch rates recorded by the scientists, the fisheries observer and the purse seine vessel's officers were conducted to elucidate any differences in reported shark interactions (catch) using repeated measures analysis of variance (ANOVA) followed by Tukey's post-hoc multiple comparisons (Fig. 4). In addition, a linear regression analysis was used to investigate whether or not the weight (tonnage) of the total tuna catch had an effect on the mortality rates of sharks *per set*. Predicted mortality rates using lactate levels and landing stage (as determined above) were compared to the tuna catch sizes derived from the observer data. All statistical data analysis was performed using the R software package (R Development Core Team).

#### *Habitat Use and Vertical Behavior*

The transmitted vertical movement data from the miniPAT satellite tags was analyzed to identify diel vertical shifts in depth or temperature preferences. Analysis and illustration of the tag data was completed using IgorPro 6.3 (WaveMetrics Inc. Portland, Or.).

### **Results**

A total of 295 juvenile silky sharks with length based age estimates of 0 - 4 years old ( $115.2 \pm 17.5$  cm mean total length) were captured during 31 fishing sets conducted over 45 days at sea (Fig. 1). Ages were determined from Joung et al. (2008). Landing stage and release condition information was recorded for each shark (Table 1). Twenty-eight silky sharks landed during different stages of the fishing operation were tagged with satellite tags (15 sPATs, 11 miniPATs and 2 MT X-PATs; Table 2). The two MT X PATs did not report whereas all the sPATs and miniPATs transmitted deployment records. Blood samples were collected from 87 sharks, 17 of which were also satellite tagged and released to establish

biochemical stress indices that correlate with mortality for this species. To date, none of the animals tagged with conventional ID tags (n=132) have been recaptured and/or reported.

### *Post Release Survival*

Shark morphometrics, stage of capture, release condition, lactate concentrations, PAT fate and tag deployment periods for satellite tagged sharks are given in Table 2. Significant differences in lactate concentrations were found (Wilcoxon rank sum, n= 14, lactate:  $P < 0.01$ ) between satellite tagged sharks that survived the fishing encounter (tag deployment  $\geq 10$  days, n = 9) and those that died post release (0-9 days, n = 5). There were four delayed mortalities of satellite tagged sharks at 15, 25, 30 and 129 days (Table 2). These mortalities cannot be directly attributed to the fishing encounter and so were considered survivors. Two of the tags on animals that had also been blood sampled did not report and one was shed after only 5 days so these were not used in this analysis. Logistic regression analysis showed that lactate concentrations are a good predictor of survival for animals that had been blood sampled but were not satellite tagged (Lactate: logit survival  $\sim -0.891 * [\text{Lactate mmol/L}] + 10.5757$ ,  $P < 0.001$ ,  $r^2 = 0.76$ ).

To obtain the probability of survival ( $\hat{\pi}$ ) (for animals that were not satellite tagged) the maximum likelihood estimates ( $b_0 = 10.5757$ ,  $b_1 = -0.891$ ) for the lactate survival curve were substituted into the response function:

$$\hat{\pi} = \frac{\exp(b_0 + b_1 * X)}{1 + \exp(b_0 + b_1 * X)}$$

Survival was set at  $\hat{\pi} > 0.5$ , and the lactate concentration at  $\hat{\pi} = 0.5$  was 11.3 mmol/L (Fig. 2). Thus, all animals with a lactate value of 11.3 mmol/L and higher were considered moribund. Using these criteria, mean survival probabilities were then estimated for each landing stage and release condition for the sharks whose blood was sampled. These values were then applied to the rest of the data set (animals that were not blood sampled) to extrapolate

survival rates by landing stage and release condition for all of the sharks encountered during this cruise (n = 295; Tables 3 & 4).

Animals caught at the FADs during pre-set fishing excursions and those sampled after having been encircled by the purse seine net showed 100% post-release survival, indicating there was no measureable effect from the tagging and blood sampling procedures or from the encirclement process. We found higher survival rates (68.7%) for animals that had become entangled in the net than for sharks that came up in the first brail (16.7%). Total survival rates were lowest (6.7%) for animals loaded during any of the later brails. This culminated in an overall post release survival rate of 15.8% for sharks landed during typical fishery operations (i.e., pre-set and encircled animals excluded), which corresponds to a total mortality rate of 84.2% (Table 3) for purse seine captured silky sharks. I also found that observing and documenting the landing stage and/or release condition of each animal can be a reliable predictor of post-release fate (Table 4).

#### *Silky shark catch comparisons*

The shark catch data recorded by the observer, the vessel officers and the scientists were compared for sets 14- 31 (Fig. 3). Sets 1-13 were omitted from this analysis because the observer and scientists were initially comparing shark catch data and so they were not independent estimates. A one-way ANOVA revealed significant differences in recorded shark catch between the scientists, the observer and the vessel's logbook ( $F = 9.592$ ,  $P = 0.0005$ ). Tukey - Kramer multiple comparisons show both the observer and the vessel reported significantly fewer sharks than the scientists (observer *versus*. scientists:  $-4.722$ ,  $P < 0.05$  and vessel *versus*. scientists:  $-7.611$ ,  $P < 0.001$ ). While the observer consistently recorded more sharks per set than the vessel, the difference was not significant ( $2.889$ ,  $P > 0.05$ ).

We also investigated the possibility that the mortality rates of silky sharks captured in the purse seine were dependent on the size of the total tuna catch (tonnage). We used linear regression analysis to describe the relationship between catch sizes (taken from the observer data) and predicted mortality rates of silky sharks caught during each set. The model showed that catch size (tonnes) does not explain a significant component of the shark mortality rates (mortality rate =  $0.622 + 0.00233$  total catch,  $F_{1,26} = 2.39$ ,  $P = 0.134$ ,  $r^2 = 0.084$  ).

#### *Habitat Use and Vertical Behavior*

The satellite data acquired from the 26 PATs that transmitted resulted in 575 days of data. Our analysis of the tag data revealed diel vertical patterns within the upper 100m of the water column with irregular and rare deeper dives beyond the thermocline (Figs. 4 & 5). Preferred daytime depths are shallower (0-40 m) than night time depths (60-80 m) with increased dive frequencies beyond 100 m at night (Fig. 4). All animals spent the greatest proportion of their time within the upper mixed layer, which extends to about 110 m in this region. Tag data also showed silky sharks occupying a very narrow temperature range of 29.0°C (  $\pm 0.58^\circ$  C sd).

#### **Discussion**

Silky sharks are among the oceans most heavily exploited elasmobranch species. They are captured as bycatch in several commercial fisheries worldwide but also are targeted for their fins and meat in several regions. The most recent stock assessment of silky sharks from the WCPFC concluded that overfishing is occurring and that the population is in a state of decline primarily due to large increases in fishing mortality (Rice & Harley 2013). Longline fishing was attributed with the greatest impact on the silky shark population due to the relative level of fishing effort compared to that of the purse seine fishery, but bycatch

from sets on drifting FADs was also found to significantly contribute to silky shark fishing mortality. Many of the studies dealing with shark bycatch and discard mortality have been focused solely on the effects of capture in longline fisheries (Moyes et al. 2006, Hight et al. 2007, Marshall et al. 2012) while purse seine fisheries have received little attention (Poisson et al. 2014). In this study we reveal the post release survival rates of sharks tagged and released at different purse seine fishing stages. By relating this to blood biochemistry, we were able to extrapolate the survival rates observed in the tagged sharks by landing stage and release condition and thereby obtain overall estimates of total mortality and identify the point in the fishing interaction when animals sustain the injuries that lead to mortality. This, in combination with the vertical habitat use data from the satellite tags gave us some insight into where efforts to mitigate shark bycatch in this fishery should be focused.

#### *Post release survival*

Several studies have demonstrated that sharks incidentally captured in fisheries and subsequently discarded at sea suffer high rates of post release mortality from the physical and physiological stress experienced during capture (eg Hight et al. 2007, Campana et al. 2009, Mandelman & Skomal 2009, Frick et al. 2010, Poisson et al. 2014). Many of these studies also have revealed several blood chemistry parameters that correlate to the post release fate of pelagic sharks. The nature and extent of these blood chemistry disturbances are species specific and found to correspond to the activity level (swimming speeds) and ecology (e.g., pelagic, coastal, or demersal habitats) of each species (Skomal 2007, Marshall et al. 2012). Yet in many commercial fishery and experimental scenarios lactate concentrations were consistently affected across species (e.g. Mandelman & Skomal 2009, Hyatt et al. 2012). In this study lactate concentrations were significantly different between survivors and moribund sharks and found to be a good predictor of survival. Increased lactate loads lead to metabolic

acidosis when lactate and hydrogen ions are formed in the muscle tissue during anaerobic respiration (Cliff & Thurman 1984). The stress of confinement in a purse seine likely results in a cascade of physiological perturbations from the point in the haul back where the animal begins to struggle against the net to the point where they have been confined in the sack and gas exchange is limited. Accordingly, moribund animals showed high lactate concentrations whereas sharks that survived the capture event had lower lactate concentrations ( $< 11$  mmol/L).

Animals that were captured, while still free-swimming, during the pre-set sampling and from inside the net (encircled sharks) had 100% post release survival rates. This suggests that the effects of tagging and the simple act of surrounding the sharks by the purse seine net did not affect survival. Animals that had become entangled in the net and were released during the net haul had relatively high survival rates (68%) and probably reflected the amount of time between when the animal had become entangled and when that portion of the net was retrieved. Regardless, a higher survival probability for entangled sharks highlights the importance of good handling practices for the safe removal and release of sharks caught in the nets (Poisson et al. 2013, Poisson et al. 2014). A study on the post release survival of silky sharks captured in a purse seine conducted in the Indian Ocean also found higher survival rates in sharks landed via entanglement (81%-Poisson et al., 2014). Here, fewer animals were landed than in this study so higher survival rates for entanglement in the Indian Ocean may reflect differences in fishing techniques (e.g., set times or net specifications) between studies.

We anticipated that animals that were landed during the first brail would also have good survival rates because these sharks would have been at the surface, not exposed to the weight of the catch and released after a much shorter period of time than animals that were deeper in the sack and landed in later brails. Survival rates of first brail sharks, however, was

low (17%) and not much better than those landed in subsequent brails (7%). Direct visual observations carried out using SCUBA gear and documented with high definition video and digital still photography (see Muir et al. 2012) confirmed that silky sharks appeared exhausted from struggling against the net during the final stages of net hauling. At that point they would slide down the edge of the net and become buried beneath the tuna at the bottom before the fishers even began 'sacking up' the net (Muir et al., 2012). Higher lactate concentrations in sharks landed during the later brails corroborate our visual observations of the sharks struggle against the net during the end of the haul. This may explain why only a small proportion of sharks were found in first brails and why the total weight of the haul did not have a significant effect on the mortality rates - crushing under the weight of the tuna catch is not the cause of mortality. The inability to ram ventilate and the stress of confinement probably leads to mortality before they are injured by being crushed. Thus, once the animal has been confined in the sack portion of the net, its chances of survival have been severely compromised.

Overall, total mortality for sharks captured and loaded via typical fishing operations (Pre-set and Encircled stages excluded) was 84%. Poisson et al. (2014) also found high total mortality rates (81%) of silky sharks captured in purse seines. This indicates that only a small proportion of sharks captured and subsequently discarded in purse seine fisheries are going to survive the interaction. These results emphasize the relative ineffectiveness of no retention policies as a management tool for purse seine fisheries. Releasing sharks from the deck after they have been loaded to the vessel through brailing will not reduce fishing mortality in this fishery. Our results do highlight the importance of total avoidance or early release through mechanisms such as a release panel (Itano et al. 2012b) or other method while the animals are still free swimming. Obviously, avoiding the encirclement of sharks altogether is the best means of reducing the impact this fishery has on juvenile silky sharks and points to the

importance of technical solutions to this problem in tandem with other management measures.

### *Catch Comparisons*

Comparison of the number of captured sharks observed by the scientific party and those reported by the vessel and fishery observer revealed that there are significant recording discrepancies regarding the number of sharks impacted by this fishery. We found catch rates were significantly under estimated by both the observer and the vessel logbook. There did not appear to be any deliberate underreporting and differences are most likely due to the nature of the fishing operation; brails are loaded quickly ( $0.5 \text{ ton min}^{-1}$ , pers. obs.), vessel operators are not paying attention to undesirable species, and observers are occupied conducting the various catch sampling and estimation duties in addition to documenting all bycatch. The use of a sorting hopper and having the operator close the trap door to slow down the descent of the catch in to the wells greatly enhanced our ability to see and then pull sharks for sampling before they went down the chute into the holds. Regardless of the causes, the differences in recorded shark catch rates that we observed should be given appropriate attention when conducting stock assessments based on observer and logbook catch data. It is also worth noting that due to the variety of vessels in the tropical tuna purse seine fishery, loading and hold styles vary dramatically and this should be taken into account when considering these estimates.

### *Habitat Use and Vertical Behavior*

Understanding silky shark movement behavior, habitat use and FAD residence times is important when conceptualizing bycatch mitigation techniques. There is a general lack of information on the associative behavior of juvenile silky sharks at FADs but in the WCPO purse seine fishery twice as many silky sharks are captured in FAD associated sets than unassociated sets (Clarke et al. 2011) and aggregations of juvenile silky sharks at FADs have

been documented in several studies (Lennert-Cody & Hall 2000, Watson et al. 2008, Filmlalter et al. 2011). A study of ten juvenile silky sharks acoustically tagged in the Indian Ocean at drifting FADs (Filmlalter et al. 2011) found continuous residence times (periods of absence not exceeding 24 hours) of 0.42 to 10.7 days. The movement data acquired using electronic tags during this cruise gave us some insight into the vertical and horizontal behavior of juvenile silky sharks. The most pertinent result for this fishery was that juvenile silky sharks remain in the upper 100 m of the water column whether they are in association with a FAD or not. Diel shifts were observed, with sharks deeper at night (~70 m) and shallower during the day (0-40 m) and their vertical structure is contained within the upper mixed layer. Purse seine net depths measured using temperature depth recorders placed at three different places on the net during the cruise gave net depths ranging from 142 m at the shallower edges to over 200 m in the center (Itano et al. 2012a). Thus, the depth preferences of silky sharks keep them within the vertical range of the purse seine nets at all times of the day. Specifically, juvenile silky sharks in this study did not exhibit behaviors that would allow them to avoid capture under normal fishing conditions and practices.

## **Conclusions**

Our analysis revealed high total mortality rates of 84.2 % to juvenile silky sharks under current purse seine fishing practices. We found that post release survival was dependent on which stage in the fishing process, the shark was loaded onto the vessel. Our results indicate that early release (entangled sharks or through a release panel built into the net) could significantly reduce the impact FAD-based purse seine fishing has on silky shark populations. This is of particular management interest because all of the sharks encountered during this cruise were juveniles and demographic studies have shown that silky shark population growth is highly dependent on juvenile survival (ages 0-5; Roman -Verdesoto

2014). If the current level of purse seine fishing effort on FADs is not mitigated silky shark populations could be driven beyond the point of recovery.

We documented a significant difference in the shark catch estimates made by the vessels crew, the observer and the scientific party. We suggest that these discrepancies be factored into future stock assessments of silky shark populations. These assessments would also be strengthened by additional data fields recorded by observers. If shark landing stage and release condition information were also documented, better estimates of fishing mortality could be used in future population models.

This study found that silky shark behavior in the net at the end of the net haul in combination with the physiological state of the individuals is what contributes to the high overall mortality rates that we observed. A large proportion of the sharks captured in a purse seine are not going to survive the interaction. In the WCPO, the longline fishery has the largest effect on the overall silky shark fishing mortality due to the level of fishing effort alone. Thus, post release survival studies are necessary for commercial longline fisheries. On the whole, more complete, species-specific information of fishing mortality in these and other fisheries is necessary before effective stock assessment and population level management can result.

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## **Appendix**

### **Purse seine fishing operation overview**

A typical purse seine fishing set begins when the skiff attached to the net at the stern of the purse seiner releases from the vessel and they both drive in opposite directions to surround the school with the net. Surrounding the school takes nine minutes on average. The vessel then begins pursing the net by drawing the chain at the base of the net up to enclose the encircled school within the net (20-25 minutes). The vessel then begins to haul back the net, restacking it at the stern of the vessel with the use of a large hydraulic block (wheel). Once most of the net has been recovered and stacked the crew begins sacking up the rest of the net to get the catch into a very tight long sack at the side of the vessel. Up to this point, the process takes nearly 2 hours (~110 minutes). The catch is then brought on board via a brailer. The brailer is a large dipnet that is lowered into the sack to scoop up the fish on top of the sack. It is then loaded onto the vessel and released into a system of chutes that funnel the catch into the holds below decks with a super-cooled brine solution. The number of brails required to get all of the fish on board depend on the size of the school. The brailing procedure from first brail until the last brail takes 18 minutes on average.

**Table 2-1.** Number and condition of silky sharks released during each fishing stage.

Release Condition	Pre-Set	Encircled	Tangled	1st Brail	Brail	Total
Excellent (4)	9	6	24	0	0	39
Good (3)	1	0	5	1	9	16
Fair (2)	0	1	3	5	12	21
Poor (1)	0	0	1	7	26	34
Dead (0)	0	0	3	14	148	165
Unknown	0	0	1	3	16	20
Total	10	7	37	30	211	295

**Table 2-2.** Satellite tagged animal morphometric, blood chemistry and tag deployment data.

Tag Type	ID	Sex	TL (cm)	Fishing Stage	Lactate (mmol/L)	Release Condition	Pat Fate†	Deployment (Days)
miniPAT	54245	M	105	Pre-set	NA	4	Floater	26
miniPAT	54246	M	104	Encircled	NA	2	Floater	34
miniPAT	54247	M	104	Pre-set	NA	4	Floater	3
miniPAT	54305	M	127	Encircled	NA	4	Floater	6
miniPAT	54249	M	93	Pre-set	NA	4	Floater	15
miniPAT	54267	F	116	Entangled	1.19	4	Floater	5
miniPAT	54270	M	145	Entangled	2.37	4	Sinker	129
miniPAT	54274	M	144	Entangled	NA	4	Floater	32
miniPAT	62937	M	122.5	Entangled	5.3	4	Floater	10
miniPAT	62936	M	133	Entangled	2.19	4	Survivor	100
miniPAT	62941	F	136	Entangled	12.07	3	Sinker	0
sPAT	117916	M	123	Entangled	14.47	4	Sinker	25
sPAT	117917	F	128	1st Brail	17.51	0	Sinker	0
sPAT	117918	M	107	Entangled	NA	1	Sinker	0
sPAT	117919	U	110	Entangled	2.13	4	Survivor	30
sPAT	117920	F	128	1st Brail	NA	2	Sinker	0
sPAT	117921	M	116	Entangled	2.88	4	Survivor	30
sPAT	117922	M	137	Brail	13	2	Survivor	30
sPAT	117923	M	125	Entangled	1.99	3	Sinker	15
sPAT	117924	M	105	1st Brail	NA	1	Sinker	0
sPAT	117925	F	104	Encircled	NA	4	Survivor	30
sPAT	117926	F	119	Pre-set	1.87	4	Sinker	30
sPAT	117927	M	111	Brail	14.91	0	Sinker	0
sPAT	117928	F	111	1st Brail	15	0	Sinker	0
sPAT	117929	M	93	Entangled	NA	4	Floater	23
sPAT	117930	M	107	Brail	13.79	1	Sinker	0
X-PAT	19899	F	128	Entangled	14.61	4	No data	-
X-PAT	52210	M	128	Entangled	14.08	4	No data	-

†Floater denotes a tag that was shed, Sinker is a tag that sank to depth, Survivor is a tag that was released after the pre-programmed deployment period.

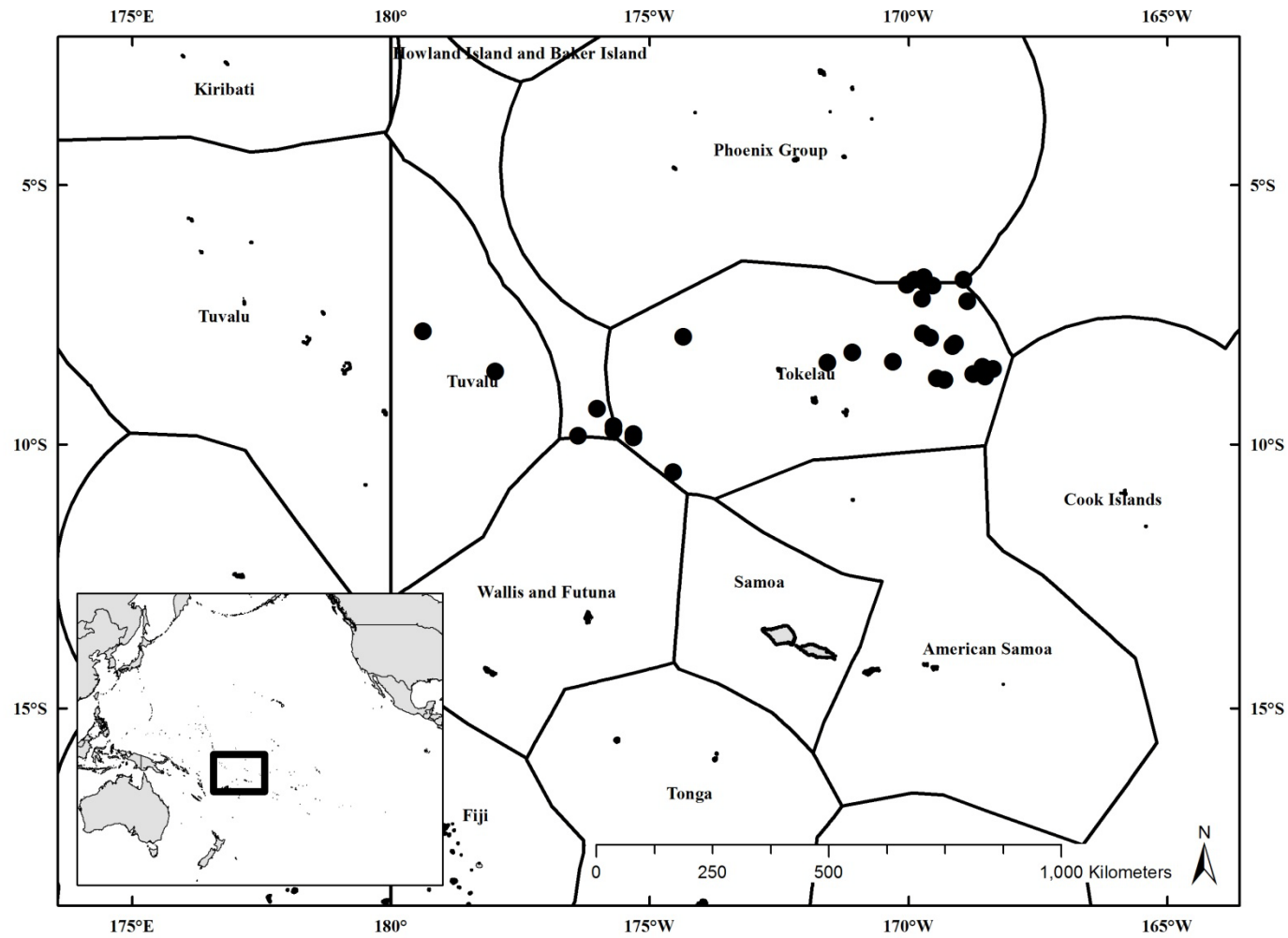
**Table 2-3.** Predicted survival by stage of fishing operation. Number of animals landed and predicted survival for each stage. Numbers in parentheses represent total survival for regular fishing operations (pre-set fishing and encirclement stages removed).

Landing Stage	Pre-Set	Encircled	Tangled	1st Brail	Brail	Total
n	10	7	37	30	211	295 (278)
Predicted Survival	100%	100%	68.4%	16.7%	6.67%	20.68% (15.83%)
No. Survivors	10	7	25	5	14	61 (44)
Total mortality rate for juvenile silky sharks landed during typical fishing stages = 84.17%						

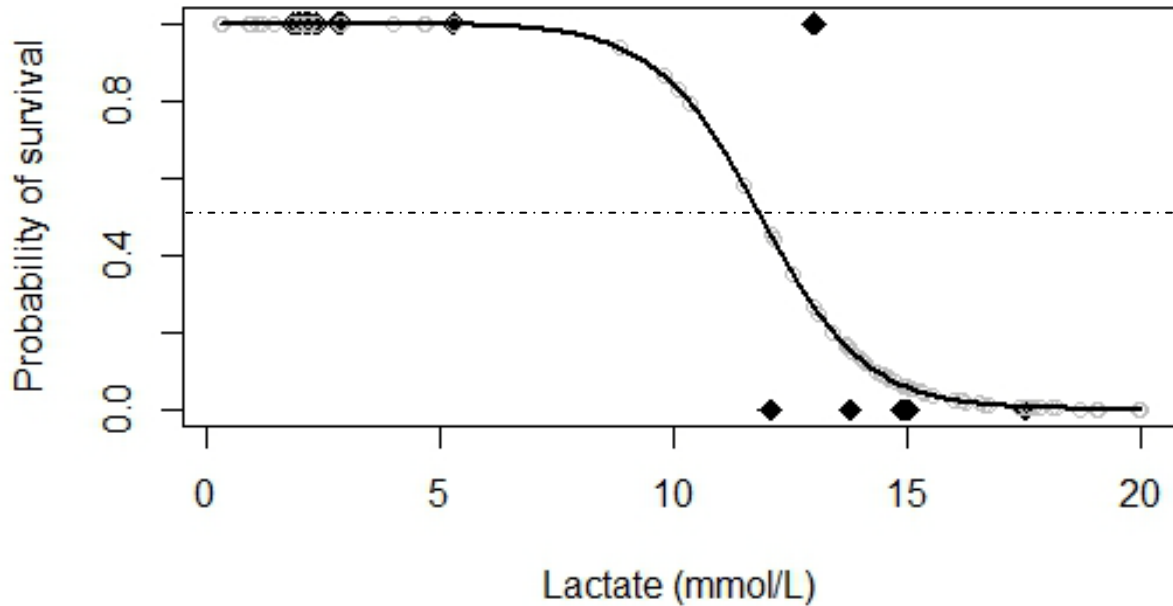
**Table 2-4.** Predicted survival rates by release condition. Some animals were released without a release condition recorded.

Release Condition	Excellent	Good	Fair	Poor	Dead
n	39	16	21	34	165
Predicted Survival	91.67%	11.49%	6.897%	0%	2.94%
No. Predicted Survivors	35.75	1.84	1.45	0	4.85

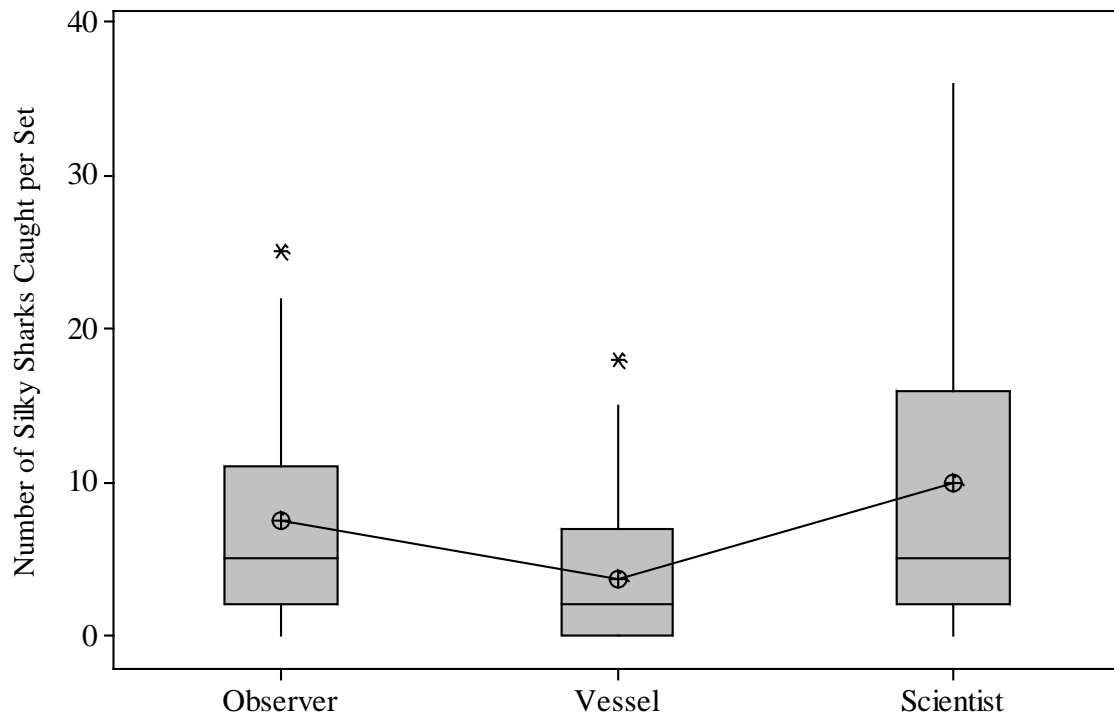
**Figure 2-1.** Fishing set locations (black circles). Lines denote the exclusive economic zones of small island nations in the region.



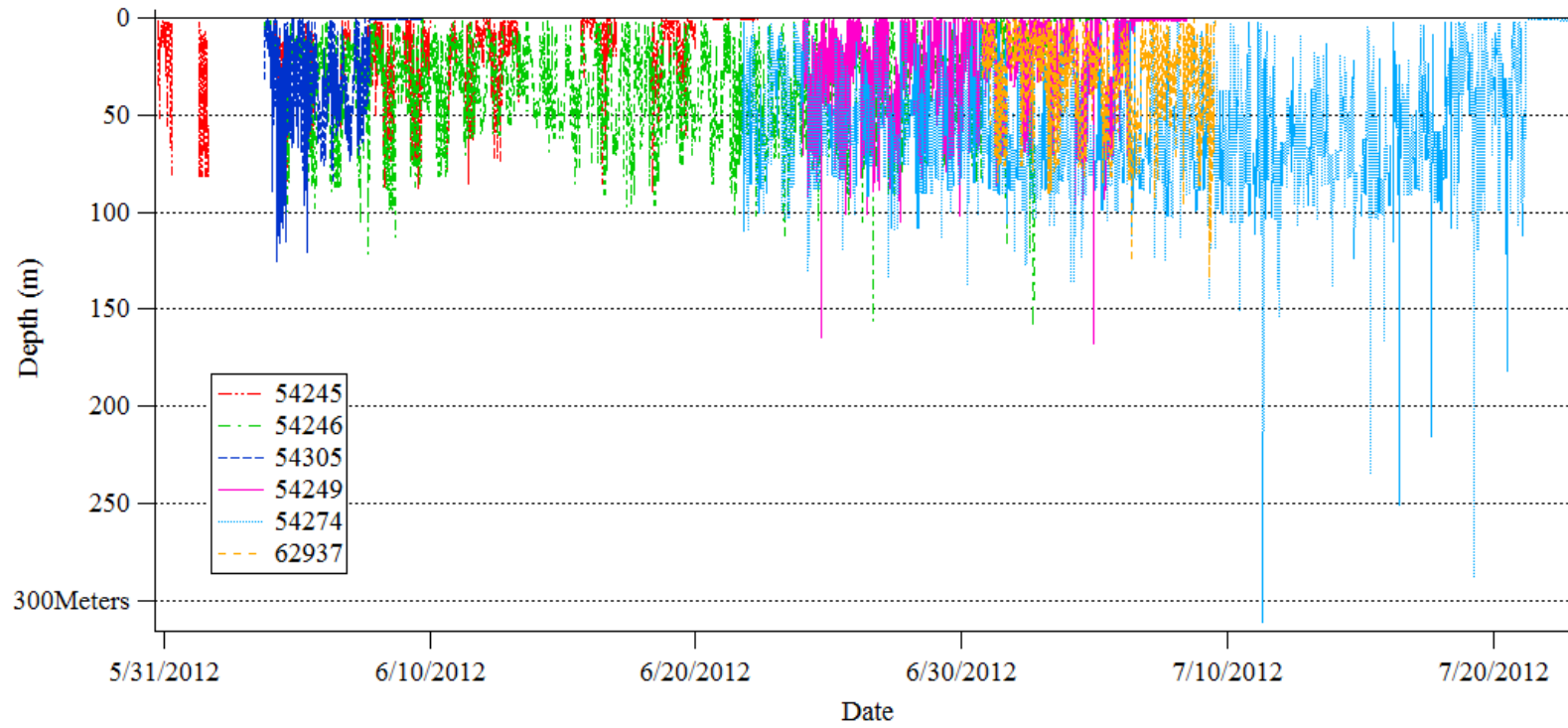
**Figure 2-2.** Survival probability curve using lactate concentrations (mmol/L). Lactate values for satellite tagged animals (black diamond) that either survived the fishing interaction (1) or died post release (0). The fitted probabilities (grey open circles) represent animals that had blood drawn but were not satellite tagged. Probability values  $> 0.5$ , or above the dashed horizontal line reflect sharks predicted to have survived the fishing event, those below the line are mortalities.



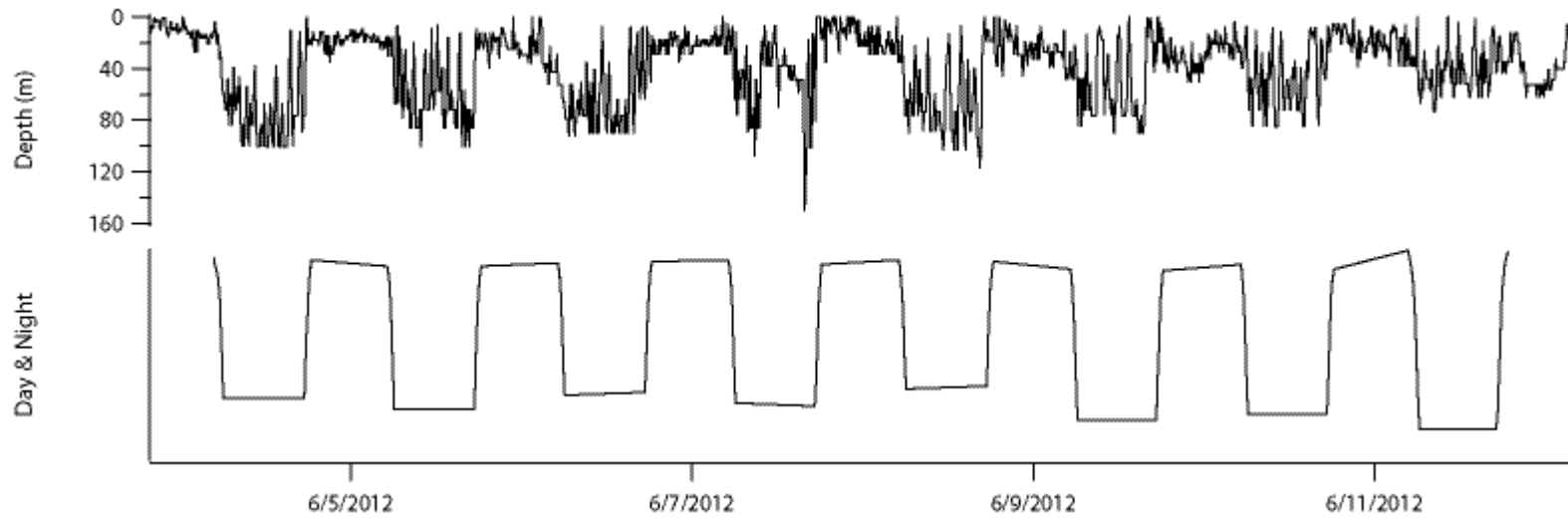
**Figure 3-3.** Shark catch rate comparisons between the fishery observer, vessel log and the scientists. The boxplots show the interquartile range, median line and means (connected by the line) of the total shark catches recorded per set. Asterisks denote outliers.



**Figure 3-4.** Vertical habitat use of six silky sharks tagged with miniPATs.



**Figure 2-5.** Diel vertical movement behavior of silky shark 54246. The top line shows the depth time series over a period of seven-24 hour day/night cycles. The bottom line shows light levels to infer dawn, day time, dusk and night times.



## CHAPTER 2

### PHYSIOLOGICAL RESPONSES OF JUVENILE SILKY SHARKS, *CARCHARHINUS FALCIFORMIS*, TO CAPTURE IN TUNA PURSE SEINE FISHERIES.

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## **Abstract**

The tropical tuna purse seine fishery and other commercial fisheries have high rates of incidental shark capture. In the western central Pacific Ocean (WCPO) purse seine fishery, juvenile silky sharks comprise greater than 95% of the shark bycatch. These sharks are of low market value and are discarded at sea. While discarded sharks are often released alive, several studies have shown that they may have sustained injuries (both physical trauma from capture and handling and physiological disturbances) that can have immediate or delayed effects that result in mortality. Blood borne biochemical indicators of stress are increasingly being used to elucidate the post release condition of elasmobranchs released after being captured in commercial fisheries. To identify the physiological perturbations that occur in silky shark bycatch in a purse seine, we quantified several blood borne indices of stress including: pH, lactate, glucose, adrenaline, blood gases, electrolytes and osmolality, from animals sampled during every stage of the fishing operation, including sharks that were sampled with a minimal amount of handling prior to interaction with purse seine fishing gear. The results show increasing lactate concentrations and decreases in pH as the fishing procedure progressed, this suggests that metabolic acidosis takes place following prolonged exposure to netting procedures. The levels of the potassium and calcium were higher in moribund sharks landed later in the fishing operations, suggesting intracellular leakage. Overall, we found that irreparable physiological damage (and ultimately, mortality) occurred once the sharks have been confined in the sack portion of the net. We conclude that sharks discarded after purse seine capture have low probability of post-release survival.

**Key words:** Bycatch, stress, blood chemistry, post release mortality.

## Introduction

The tropical tuna purse seine fishery and other commercial fisheries have high rates of incidental shark capture (Gillman, 2012). These sharks are typically unwanted and of low market value and are discarded at sea (Worm et al., 2013). In the western central Pacific Ocean (WCPO) tuna purse seine fishery, juvenile silky sharks are captured in high rates and comprise > 95% of the total elasmobranch bycatch (Lawson, 2011). In this and other fisheries, discarded sharks may be released alive but several studies have shown that they may have sustained injuries (both internal and external) that can have immediate or delayed effects that result in mortality (eg. Skomal, 2007; Skomal and Mandelman, 2012). Blood borne biochemical indicators of stress are increasingly being used to elucidate the post release condition of discarded elasmobranchs captured in fisheries (eg. Moyes et al., 2006; Hight et al., 2007; Renshaw et al., 2012). Since fish blood and white myotomal muscle comprise from 3-6% and 50-60% of the total body mass respectively, changes in muscle biochemistry from stress or injury occurring during capture in fishing gear are strongly reflected in the blood (Wells et al. 1986). To identify the magnitude and temporal dynamics of the physiological perturbations that occur in silky sharks captured in a purse seine we quantified several blood borne indices of stress from animals sampled during every stage of the fishing operation, including sharks that were sampled with a minimal amount of handling prior to interaction with purse seine fishing gear.

A stress response refers to the physiological changes in an organism exposed to a stimulus or stressor – be it intrinsic or extrinsic – which causes disruption of homeostasis (Pickering, 1981; Asterita, 1985; Wendelaar-Bonga, 1997). It is considered an integrated response because it typically involves all levels of organismal organization (central, peripheral, autonomic and somatic nervous systems; Wendelaar-Bonga, 1997). There are three phases of the integrated stress response: the primary response includes the activation of brain centers and the autonomous nervous system resulting in a release of catecholamines and corticosteroids immediately after perception of a stressor. This leads to a cascade of physiological changes during the secondary response which include increases in heart rate and ventilation, dilation of blood vessels and the rapid mobilization of energy reserves to respond to the perceived stressor. These physiological disturbances can have long term effects on the health of the individual and may lead to an inability to tolerate additional stressors and to reduced immune function (Skomal

and Mandelman, 2012). Individual effects may extend to the level of the whole population if the stressor causes reduced reproductive output.

The stress response is conserved amongst vertebrates of both aquatic and terrestrial taxa (Pickering, 1981; Wendelaar-Bonga, 1997). Although the conceptual framework for the integrated stress response was based on mammalian studies (Cannon, 1929; Selye, 1950), and later expanded to studies on teleosts (e.g. Mazeaud et al., 1977; Pickering, 1981; Wendelaar-Bonga, 1997) only recently have studies begun to focus on elasmobranchs (e.g. Cliff and Thurman, 1984; Wells and Davie, 1985; Heisler, 1988; Skomal and Bernal, 2010). These studies suggest that the elasmobranch stress response closely resembles that of teleosts and mammals but with a few differences. Immediately following the perception of a stressor, the primary response is neuroendocrine and signaling follows the hypothalamic inter-renal axis to stimulate the release of catecholamines and corticosteroids into the blood stream. In higher vertebrates adrenaline is secreted from adrenal glands but in elasmobranchs catecholamines (adrenaline and noradrenaline) are released from chromaffin tissue in the kidneys and axillary bodies on the dorsal surface of the cardinal sinus (Butler and Metclaf, 1988). The presence of circulating adrenaline increases heart rate and ventilation and acts to mobilize glucose reserves from the liver to the active muscle tissues (Bone 1988). In sharks, normal locomotion is performed by the highly vascularized and perfused red muscle and fueled through aerobic respiration (Heisler 1988). During burst swimming or periods of struggle (as in a purse seine net) the white muscle is activated and glycolysis occurs via anaerobic pathways giving rise to lactic acid. Lactic acid dissociates relatively quickly because of a low pK'a value into  $\text{La}^-$  (lactate) and  $\text{H}^+$  and pH levels drop within the tissue followed by the extracellular compartments (Heisler, 1988). Changes in pH are readily reflected in the blood plasma (Mandelman & Skomal 2009, Frick et al. 2012). In conditions of hypoxia or when the animal's ability to ventilate is compromised, further respiratory acidosis occurs as the partial pressure of  $\text{CO}_2$  rises, and  $\text{pO}_2$  falls (Mazeaud and Mazeaud, 1981). Bicarbonate concentrations decrease as carbonic acid increases in the plasma and the acid base buffering capacity is diminished. Lactic acidosis of the intracellular spaces increases the permeability of cell membranes thereby affecting increases in osmolality of the blood (Mandelman and Skomal, 2009) and hemoconcentration caused by fluid shifts across tissue compartments may also occur (Cliff & Thurman 1984).

The magnitude of these changes are species specific and depend on the physiological ecology of the animals including their thermal biology, metabolic scope, swimming speeds, and the type of habitat they occupy (e.g. Marshall et al., 2012, Mandelman and Skomal, 2009). Silky sharks are found in several habitats including shallow inshore reefs and pelagic waters and may occupy different ecological niches throughout ontogeny (e.g. Compagno 1984; Branstetter 1987; Bonfil 1993; Watson et al., 2008). They are also captured in several fisheries targeting various species and using a variety of techniques (e.g. pelagic and demersal longlines, purse seines, gill nets, trawl). Mortality rates for silky sharks captured on longlines in the Atlantic Ocean and Gulf of Mexico was found to be 66% at the point where the animals arrive at the boat as the gear was retrieved (Beerkircher et al., 2002). Total mortality in tuna purse seines was shown to be high in both the Pacific Ocean (84%; Hutchinson et al. 2014) and in the Indian Ocean (81%; Poisson et al., 2014). In this study I characterize how silky sharks respond physiologically to each stage of purse seine capture by measuring the magnitude of change in several blood parameters including; pH, lactate, glucose, adrenaline, blood gases, electrolytes and osmolality at these various stages. Furthermore, I show how these physiological disturbances lead to and/or reflect morbidity and identify parameters that can be used to predict post release survival in future studies.

## **Methods**

### *Field Methods*

Sharks were caught on the commercial purse seine vessel Cape Finisterre during May through June 2012. To quantify blood chemistry parameters, blood samples (3 mL) were taken via caudal venipuncture into heparinized syringes from sharks that were landed during each stage of the fishing operations. The landing stages and control groups identified for this experiment were; (1) Pre-set- Sharks captured at FADs using dip nets or baited hooks from a small work boat prior to the onset of fishing. These were used to establish a reference curve of blood biochemistry indicators and to release some sharks that were exposed to a minimal amount of handling. (2) Encircled- Animals that had been encircled by the purse seine net and the net hauled back to at least half net. These sharks were fished out of the net using hook and line, handlines and dipnets during the net haul back while it was still large enough for the animals to swim freely. They were tagged and sampled onboard the small workboat and then released

outside the purse seine net. (3) Entangled- Sharks that had become entangled (gilled or meshed) in the purse seine net during 'haul back' and are removed by the fishermen as the net emerges from the water. These animals get landed relatively early, (i.e., before sacking up and brailing). (4) First Brail- Sharks that were brought onto the vessel during the first brail. These sharks would have been on the top of the mass of fish in the sack. (5) Later Brails - Sharks landed during any subsequent brails and were confined in the sack for longer periods of time and subjected to the weight of the catch on top of them.

To collect blood samples, all sharks were placed upside down in a cradle and ventilated with running seawater while sex and length measurements were recorded and while blood was withdrawn. Sharks were then released over the side of the vessel and their condition, as observed at the time of release, was recorded. Release conditions were qualitative and based on a scale from 4-0, where (4) Excellent Condition was recorded for animals that swam away rapidly without any obvious signs of distress or physical trauma. Sharks released in Good Condition (3) swam away but appeared slower or disoriented. Fair Condition (2) was assigned to sharks whose swimming appeared laborious and/or they exhibited other visible signs of trauma. Sharks in Poor Condition (1) were able to right themselves and made efforts to swim, while sharks released with a (0) or Dead sank upside down (Table 1).

### *Blood Variables*

Blood samples were kept on ice and processed immediately after the conclusion of each fishing set (time to analysis was 10 - 140 minutes). Each sample was tested for blood gases and metabolites with an I-STAT portable automated blood chemistry analyzer (Abbott Laboratories, IL, USA). Partial pressure of dissolved carbon dioxide ( $p\text{CO}_2$ ), partial pressure of dissolved oxygen ( $p\text{O}_2$ ), total carbon dioxide ( $\text{TCO}_2$ ), bicarbonate ion ( $\text{HCO}_3^-$ ), base excess (BE), saturated oxygen ( $s\text{O}_2$ ), pH, and lactate concentrations were determined using the CG4+ cartridge. Blood chemistry and electrolyte concentrations of sodium ( $\text{Na}^+$ ), potassium ( $\text{K}^+$ ), chloride ( $\text{Cl}^-$ ), ionized calcium ( $i\text{Ca}$ ), total Carbon Dioxide ( $\text{TCO}_2$ ), glucose, blood urea nitrogen (BUN) and creatinine were measured using the CHEM 8+ cartridge (Table 2). 0.95 $\mu\text{L}$  of whole blood was added to the CG4+ cartridge as per the manufacturer's instructions, while blood used in the CHEM 8+ cartridge was diluted with  $\text{dH}_2\text{O}$  1:1 to return results that were within the reportable ranges of the cartridge for most variables (Cooke et al., 2008). Packed cell volume (%PCV or, hematocrit) was

quantified in triplicate using heparinized, glass micro-hematocrit tubes with the StatSpin centrifuge, micro-hematocrit rotor, and card reader (Iris Sample Processing, Inc. MA. USA). The remaining 2 mL whole blood sample was centrifuged at 10,000 g for 6 minutes to separate plasma from red blood cells and stored at -20°C for later analysis of osmolality and stress hormone (catecholamine) levels. The total solute concentration of the plasma (osmolality) was quantified using a Vapro 5520 vapor pressure depression osmometer (Wescor, Inc., UT. USA). Plasma adrenaline (catecholamine) was quantified using an enzyme linked immunosorbent assay (ELISA) (Eagle Biosciences, Inc., NH. USA) following manufacturer specifications. Optical density of samples were measured at 450 nm with a Spectramax M<sup>2</sup> microplate reader.

### *Conversions and calculations*

The i-STAT system is built for the analysis of mammalian blood in hospitals and veterinary clinics so each sample is heated by the machine to 37°C prior to analysis. In addition, the reportable ranges for several of the measured blood parameters are only applicable to mammalian systems and don't adequately encompass the physiological ranges of fishes. To get pertinent blood chemistry data from the i-STAT CHEM8+ cartridge it was necessary to dilute the shark blood samples with distilled water in a 1:1 ratio. Cooke et al. (2008) found that glucose and the electrolytes (Na<sup>+</sup>, Cl<sup>-</sup>, K<sup>+</sup>) diluted for measurement with the i-STAT were comparable to values determined using standard laboratory techniques and concluded that the i-STAT could be used for field diagnostics of condition of ectothermic fishes. In cases where the sample concentrations fell outside of the reportable ranges, the i-STAT returns either an error message '\*\*\*' or gives truncated values showing that less (<) or more (>) than the reportable range of the cartridge (see Table 2 for ranges). Additionally, there were differences in the time between blood withdrawal to analysis for some sharks. pH will decrease on standing via the anaerobic activity of the erythrocytes at a rate of 0.03 pH units per hour (Abbott Point of Care, IL, USA ). Measured pH values were therefore adjusted according to the amount of time between withdrawal and analysis; these time adjusted values were then used in temperature corrections. Furthermore, the algorithms used to determine several parameters may not account for differences in certain blood characteristics (e.g. nucleated red blood cells, hemoglobin isoforms, Bohr effects). Despite these issues, several studies on the post release condition of elasmobranchs have shown that while the parameter estimates may not be accurate in absolute

values, they do reflect relative differences that are meaningful estimates of physiological status (reviewed by Harter et al., 2014; Stoot et al., 2014). I did not, however, include the sO<sub>2</sub>% or hemoglobin measurements in the analysis due to proven inaccuracies in studies on sharks and teleosts because of the differences in red blood cell characteristics stated above (e.g. Harter et al., 2014).

Blood gas concentrations (and pH) are temperature dependent quantities so temperature corrections were necessary for the pH, pO<sub>2</sub> and pCO<sub>2</sub> results taken from the analyzer. Sea surface temperatures (SST) were used as the proxy for *in vivo* temperatures for each shark sample. The temperature correction algorithms suggested by the manufacturer (Abbott Point of Care, IL, USA) were used for pH, pO<sub>2</sub> and pCO<sub>2</sub>. Bicarbonate (HCO<sub>3</sub><sup>-</sup>) and base excess (BE) were then found using the temperature (and time for pH<sup>3</sup>) corrected values with equations based on the Henderson-Hasselbalch theorem where αCO<sub>2</sub> = 0.03 and pKa' = 6.1

$$1. \quad \text{pH}_{(\text{SST})} = \text{pH}_{(\text{ISTAT})} - 0.0147(\text{T}_{\text{SST}} - 37) + 0.0065(7.4 - \text{pH}_{(\text{ISTAT})})(\text{T}_{\text{SST}} - 37)$$

$$2. \quad \text{pO}_{2(\text{SST})} = \text{pO}_{2(\text{ISTAT})} \times 10^{\frac{5.49 \times 10^{-11} \text{PO}_2^{3.88} + 0.071}{9.72 \times 10^{-9} \text{PO}_2^{3.88} + 2.30} (\text{SST} - 37)}$$

$$3. \quad \text{pCO}_{2(\text{SST})} = \text{pCO}_{2(\text{ISTAT})} \times 10^{0.019(\text{SST} - 37)}$$

$$4. \quad \text{HCO}_3^- = 0.03 \times \text{pCO}_{2(\text{SST})} \times 10^{(\text{pH} - 6.1)}$$

$$5. \quad \text{BE} = \text{HCO}_3^- - 24.8 + 16.2(\text{pH}_{(\text{SST})} - 7.4)$$

The blood gas and electrolyte chemistry values are reported in Tables 3 A & B and 4 A & B.

### *Statistical analysis*

Adrenaline concentrations were found by generating standard curves using the fully automated Excel spreadsheet for 4- and 5- parameter logistic curve fitting of bioassay calibrations (Swart, 2014; Fig. 1). Comparisons amongst parameters across fishing stages and release conditions were performed using a one-way Analysis of Variance (ANOVA) when parametric assumptions were met followed by Tukey multiple comparisons amongst the groups.

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<sup>3</sup> pH<sub>(ISTAT)</sub> are the time corrected values.

For distributions that failed the test of equal variances Welch's test was performed and followed by Games-Howell pairwise comparisons. Analyses of differences in pH was conducted using the upper estimates of pH, where individuals with pH values < 6.5 were excluded. Sharks with pH values < 6.5 were all dead upon release so it was determined that leaving these out of the analysis would not change any inferences we derive regarding survivability. By using the predicted survival estimates from Hutchinson et al. (2014), I was also able to test for significant differences amongst each variable for animals that were predicted to have survived or were determined to be moribund using one way student's t-tests. Mann -Whitney tests of medians were used when distributions were not Gaussian. Statistical analyses were conducted using Minitab 17 (Minitab Inc. PA. USA) and R v.3.1.1 (R Development Core Team, 2014). Statistical significance was determined at  $\alpha = 0.05$  and results are presented as mean  $\pm$  SD unless stated otherwise.

## **Results**

### *Primary Stress Response*

I was only able to determine adrenaline concentrations for nine animals, so comparisons between the various landing stages and release conditions were not possible for this variable. However, adrenaline concentration comparisons between animals that were predicted to have survived ( $n = 5$ ) and those that were moribund ( $n = 4$ ) did reveal significantly higher concentrations in moribund sharks (Table 5; Fig. 2). Glucose concentrations did not differ by landing stage or between release conditions. Measurements of hematocrit as percent packed cell volume (PCV) also did not differ by landing stage, release condition or between survivors and moribund sharks (Table 4A, 4B & 5).

### *Acid-Base Perturbations*

Differences in plasma lactate concentrations by landing stage were significant ( $F_{4,20,26} = 385.46$ ,  $P < 0.001$ ; Fig. 3a). Lactate concentrations were significantly larger between the control group and animals that had interacted with the net (Pre-set vs. Entangled,  $P < 0.001$ ; Pre-set vs. First brail:  $P < 0.001$ ; Pre-set vs. Later brails:  $P < 0.001$ ). Also, lactate concentrations increased significantly as the fishing procedure progressed (Encircled vs. Entangled,  $P < 0.001$ ; Encircled vs. 1st brail,  $P < 0.001$ ; Encircled vs. Later brail,  $P < 0.001$ ; Entangled vs. 1st brail,  $P < 0.001$ ,

Entangled vs. Later brails,  $P < 0.001$ ). Lactate concentration comparisons between condition of sharks at release also showed significant differences ( $F_{4,18.42} = 42.20$ ,  $P < 0.001$ ; Fig 3b), where concentrations were significantly lower in sharks released in Excellent Condition versus those that were released in any other condition (Excellent vs. Good,  $P < 0.01$ ; Excellent vs. Fair,  $P < 0.001$ , Excellent vs. Poor,  $P < 0.001$ ; Excellent vs. Dead,  $P < 0.001$ ). Lactate concentrations were also significantly lower for sharks released in Good Condition compared to Dead ( $P < 0.05$ ). Lactate concentration was used to predict survivorship so tests to identify differences amongst the means were not conducted.

pH values were found to decrease significantly as the fishing procedure progressed ( $F_{4,11} = 40.70$ ,  $P < 0.001$ ; Fig. 4a). Pair-wise comparisons amongst landing stages showed significant differences in pH for the control group of sharks and sharks that interacted with the gear at any point; (Pre-set vs. Entangled,  $P < 0.05$ ; Pre-set vs. First brail,  $P < 0.01$ ; Pre-set vs. Later brail,  $P < 0.001$ ). Similarly, sharks that were encircled by the net and still free swimming had significantly higher pH values than sharks that came up in the brails, (Encircled vs. First brail:  $P < 0.05$ ; Encircled vs. Brail:  $P < 0.05$ ). pH values were significantly more alkaline in sharks that were entangled in the net than those that were landed via later brails ( $P < 0.05$ ). Significant differences were revealed in pH values among animals released in different conditions ( $F_{4,15.26} = 38.79$ ,  $P < 0.001$ ; Fig. 4b). Games-Howell pairwise comparisons found that animals released in Excellent (4) Condition had significantly higher (alkaline) pH values than animals released in any other condition (4 vs. 3,  $P < 0.01$ ; 4 vs. 2,  $P < 0.01$ ; 4 vs. 1,  $P < 0.01$ ; 4 vs. 0,  $P < 0.01$ ). Sharks that were released in Good Condition (3) had significantly higher pH values than animals that were released Dead (0) ( $P < 0.01$ ). pH values by predicted survival were higher for animals that were predicted to survive than those that were moribund ( $T = -10.27$ ,  $P < 0.01$ ; Fig. 4c).

Partial pressures of oxygen were not different in sharks landed at different stages. Differences between release conditions however, were significantly different ( $F_{4,18.2} = 6.15$ ,  $P < 0.01$ ; Fig. 5a), where pO<sub>2</sub> levels were higher for sharks released in Excellent Condition than those that were released in either Poor or Dead Condition ( $P < 0.05$ ). pO<sub>2</sub> was also significantly higher in animals released in good condition than those that were in poor or dead condition ( $P < 0.01$ ,  $P < 0.05$  respectively). Partial pressures of oxygen were also significantly higher for sharks that were predicted to survive than those that were not ( $P < 0.05$ ; Table 3; Fig.5b).

Comparisons of carbon dioxide partial pressures ( $p\text{CO}_2$ ) between landing stages found that sharks landed later in the fishing operation had higher  $p\text{CO}_2$  levels than animals landed earlier ( $F_{4,11} = 4.871$ ,  $P = 0.0015$ ; Fig. 6a). Tukey HSD post-hoc comparisons showed entangled sharks had significantly lower  $p\text{CO}_2$  vs. those that came up in the later brail ( $P = 0.01$ ). Welch's test of  $p\text{CO}_2$  levels by release condition also found significant differences among levels ( $F_{4,15,26} = 11.04$ ,  $P < 0.01$ ; Fig. 6b). Games-Howell pairwise comparisons found that  $p\text{CO}_2$  levels were significantly lower in animals released in Excellent or Good condition than those released in Poor Condition or Dead (Excellent vs. Poor,  $P < 0.05$ ; Excellent vs. Dead,  $P < 0.01$ ; Good vs. Poor,  $P < 0.05$ ; Good vs. Dead,  $P < 0.01$ ).  $p\text{CO}_2$  was also significantly different between animals predicted to survive and those that were not (Table 3; Fig. 6c).

Bicarbonate ( $\text{HCO}_3^-$ ) values returned significant differences among landing stages (Welch's Test,  $F_{4,11} = 69.78$ ,  $P < 0.001$ ; Fig. 7a). Pairwise comparisons among landing stages with Games-Howell test showed significantly higher  $\text{HCO}_3^-$  concentrations for animals landed while they were still free-swimming (Pre-set vs. Entangled,  $P < 0.001$ ; Encircled vs. Entangled:  $P < 0.01$ ; Pre-set vs. 1st brail,  $P < 0.001$ ; Encircled vs. 1st brail,  $P < 0.001$ ; Pre-set vs. Later brails,  $P < 0.01$ ; Encircled vs. Later brails,  $P < 0.01$ ). Welch's test of the bicarbonate values by release condition also found significant differences ( $F_{4,20} = 69.78$ ,  $P < 0.001$ ; Fig. 7b.). Pairwise comparisons amongst release conditions showed significantly higher  $\text{HCO}_3^-$  concentrations for animals released in excellent condition versus those that were released in any other state ( $P < 0.01$ ) and between sharks released in Fair or Poor Condition versus those released Dead ( $P < 0.05$ ).  $\text{HCO}_3^-$  concentrations were significantly lower in moribund sharks compared to those predicted to survive (Table 3; Fig. 7c).

Base excess (or base deficit) refers to an excess or deficit in the amount of base in the form of bicarbonate present in the blood and can help determine whether pH changes are due to respiratory distress or metabolic inputs from lactic acid build up. Base excess quantities decreased as the fishing procedure progressed and were found to differ significantly among sharks landed at different stages. (Welch's Test,  $F_{4,11,8} = 114.55$ ,  $P < 0.01$ ; Figs. 8A, 10A). Multiple comparisons showed significant differences between animals landed while still free swimming and those that interacted with the gear (Pre-set vs. Entangled,  $P = 0.001$ ; Encircled vs. Entangled,  $P < 0.05$ ; Pre-set vs. 1st brail,  $P < 0.01$ ; Encircled vs. 1st brail,  $P < 0.01$ ; Pre-set vs. Later brail,  $P < 0.01$ ; Encircled vs. Later brail,  $P = 0.01$ ). Sharks that came up entangled also had

higher base excess values than those landed via brailing (Entangled vs. 1st brail,  $P < 0.05$ ; Entangled vs. Later brail,  $P < 0.001$ ). Base Excess values were also significantly different between animals released in different conditions (Welch's Test,  $F_{4,18.2} = 58.95$ ,  $P < 0.01$ ; Fig. 8B 10B). Pairwise tests between release conditions showed that sharks released in Excellent Condition had significantly higher base excess than animals released in any other condition (4 vs. 3,  $P < 0.001$ ; 4 vs. 2,  $P < 0.01$ ; 4 vs. 1,  $P < 0.01$ ; 4 vs. 0:  $P < 0.01$ ). Animals released in Good or Fair Condition also had significantly higher BE values than those that were released Dead ( $P < 0.01$ ,  $P < 0.001$ ). Base excess values were also significantly higher in animals predicted to survive than those deemed moribund (Table 3; Fig. 8c).

The relative contribution of lactate and  $p\text{CO}_2$  to the observed differences in pH levels was explored using stepwise regression (Figures 9 A& 9B). Lactate explained the largest proportion of the variance in pH ( $T = -14.02$ ,  $SS = 4.3566$ ,  $P = 0$ ) but  $p\text{CO}_2$  ( $T = -4.68$ ,  $SS = 0.272$ ,  $P = 0$ ) still had a significant contribution. Consequently, the full model includes both parameters ( $F_{2, 50} = 138.36$ ,  $P < 0.01$ ,  $R^2 = 87.95$ ).

### *Electrolytes*

There were no significant differences found in osmolality or sodium concentrations among sharks landed at different stages, released in different conditions or between survivors and moribund sharks. Potassium concentrations did differ between sharks landed during different stages (ANOVA,  $F_{4,62} = 3.839$ ,  $P = 0.0079$ ; Fig. 11A). Tukey HSD multiple comparisons showed  $\text{K}^+$  values were higher in animals landed via the Brails than those that were sampled Pre-set ( $P = 0.025$ ). Potassium concentrations were also significantly different for animals released in different conditions (ANOVA,  $F_{4,59} = 4.136$ ,  $P = 0.029$ ; Fig. 9B). Tukey HSD multiple comparisons amongst release conditions found  $\text{K}^+$  concentrations to be larger in Dead animals than those released in Excellent Condition ( $P < 0.01$ ), sharks released in Good Condition also had higher  $\text{K}^+$  compared to those released in Excellent Condition ( $P < 0.05$ ). Potassium concentrations were also significantly higher in moribund sharks than those predicted to survive ( $T = 3.44$ ,  $P = 0.002$ ; Fig 9c). Potassium values are truncated at the upper end of the ranges. There were five animals that had potassium values that were reported as greater than 9 mmol/L and these sharks were excluded from statistical analysis. Incidentally, all five of these animals

were sharks landed in later brails and released dead so the means reported for dead sharks may be underestimated (Table 4A & 4B).

Ionized calcium ( $iCa^{2+}$ ) concentrations increased significantly during the course of the fishing procedure ( $F_{4,10.6} = 5.65$ ,  $P = 0.011$ ; Fig. 10A). Calcium concentrations were significantly different in animals released in different conditions ( $F_{4,18.6} = 18.62$ ,  $P << 0.01$ ; Fig. 10B). Games-Howell pairwise comparisons found significantly lower concentrations in animals released in Excellent condition when compared to Fair ( $P < 0.01$ ), Poor ( $P < 0.01$ ) or Dead ( $P << 0.001$ ) conditions. A Mann-Whitney test comparing the median values for survivors and moribund sharks also found higher calcium concentrations in moribund animals ( $W = 1874.5$ ,  $P < 0.001$ ; Fig. 10C).

## Discussion

In this study I describe and quantify the changes in blood chemistry exhibited by silky sharks captured in the tuna purse seine fishery. By measuring the timing and magnitude of these disturbances in parameters that have been identified as key components of the vertebrate stress response, I was able to identify several parameters that correlate with mortality and survivorship in this species and identify when, in the fishing process, these changes occur. I found the use of the i-STAT point of care (POC) device to be suitable for quantification of these blood parameters in a remote field situation. Despite the fact that the i-STAT POC is made for medical applications and the calculation of many parameters are based on constants and algorithms derived for human blood, several studies have now shown that POC devices can be acceptable tools for blood parameter readings in Chondrichthyes (Reviewed by Stoot et al., 2014). The use of the i-STAT POC made the assessment of stress on silky shark bycatch in a commercial purse seine fishery possible.

Because the vertebrate stress response is almost immediate, it is nearly impossible to acquire parameter baseline measurements because simply handling the animals induces changes in blood chemistry, even when the time from capture to sampling is less than two minutes. For comparative purposes, I sampled silky sharks that were collected during pre-set and after encirclement, and these are the best base line data available. The effects of net capture have been shown to lead to a greater degree of acidosis and mortality than longline capture (Hutchinson et al. 2014; Hyatt et al. 2012) and this study benefitted from access to sharks

captured at various fishing stages and levels of stress and is the first to acquire important information about the physiological ranges of several stress-related parameters that were previously unknown.

To interpret the changes that occurred in blood chemistry parameters we must revisit the purse seine fishing procedure and silky shark behavior in the net as fishing progresses. Initially, the net is large enough for encircled sharks to swim freely. As the net gets smaller towards the end of the 'haul back', the sharks begin to struggle violently against the net until it gets condensed to the point (the sack) where they can no longer move. At this point ventilation is impeded until they are brought on board the vessel and subsequently discarded. The condition of sharks that became entangled in the net as it was being hauled back appeared to reflect how long they had been stuck in the net before they were removed. In this context, the following sections examine the dynamics of the various physiological responses.

#### *The primary stress response*

The primary stress response begins with the perception of a stressor and the release of catecholamines (adrenaline and noradrenaline) into circulation to evoke an array of compensatory mechanisms which enable the organism to deal with the stressor (Asterita 1985; Perry and Gilmour, 1996). These mechanisms typically involve catecholamine mediated mobilization of energy reserves and increased oxygen supplies to the active tissues (Wendelaar-Bonga, 1997). The mechanisms associated with adrenergic mediated control of the stress response in elasmobranchs may be different than that of teleosts and other vertebrates and reports vary throughout the literature. Previous studies have concluded that in contrast to bony fish, sharks do not have adrenergic mediated Na<sup>+</sup>/H<sup>+</sup> exchangers at the gill surface, no direct innervation to the heart to increase perfusion, and no splenic ejection of red blood cells to increase oxygen carrying capacity (reviewed in Brill et al. 2008). On the other hand, several studies on the stress response in elasmobranchs have found marked increases in plasma catecholamine levels in response to a stressor and to be indicative of the magnitude of the stressor (e.g. Metcalf and Butler 1988; Hight et al. 2007).

Typical blood adrenaline levels required for regulation of metabolism and cardiovascular function in sharks are on the order of 1 -10 nM. Hight et al. (2007) found increases in catecholamines from 100 to 1600 times baseline levels in pelagic sharks captured on a longline.

In the current study, plasma adrenaline concentrations increased as the fishing procedure progressed and were significantly higher in moribund sharks compared with those that survived. The mean adrenaline concentrations for moribund silky sharks fell within the ranges observed by Hight *et al.* (2007) for moribund blue (*Prionace glauca*) and mako (*Isurus oxyrinchus*) sharks. However, the adrenaline sample size is limited in scope and too small to draw any meaningful conclusions regarding the potential ranges of adrenaline concentrations to be found in silky sharks under various levels of stress.

We expected to see elevated glucose concentrations in silky sharks that had interacted with the net but found no differences among landing stages or in sharks released in any condition other than Excellent. Glucose actually decreased as the fishing operations progressed but differences were not significant. Glucose concentrations were also observed to decrease over time for sharks captured in a gill net (Manire *et al.* 2001). This could be due to depletion of energy stores and morbidity may be due to the fact that there was an inability to supply enough glucose to the tissues (Cliff and Thurman, 1984). By contrast, several studies on capture stress in different shark species have found very elevated glucose concentrations in stressed sharks and found hyperglycemia to reflect the level of the stressor and showed glucose to remain elevated for hours after the cessation of the stressor (Reviewed by Skomal and Mandelman 2012). These varied results may reflect species specific differences.

Hematocrit is the proportion, by volume, of the blood that consists of red blood cells. The oxygen carrying capacity of elasmobranch blood is between 1.9-2.1 mmol l<sup>-1</sup> when hematocrit ranges between 16-22% (Butler and Metcalf, 1988). Blood from unstressed sandbar sharks ranged from 18.1-22.4 (Arnold, 2005). Brill *et al.*, (2008) found significantly higher hematocrit in exercise-stressed sandbar sharks (21.4%) than control sharks (17.7%). On the other hand, Hight *et al.* (2007) found no clear patterns with capture stress in blue and mako sharks where hematocrit values ranged from 4-70%. In the current study, silky shark PCV ranged from 17 – 33% but hematocrit percentages (PCV) did not show a clear relationship with capture stress during the course of the fishing procedure. However, they do fall within published levels of other fishery-stressed shark species (Wells and Davie, 1985; Lai *et al.*, 1997; Hight *et al.*, 2007, Brill *et al.*, 2008). Our results suggest that glucose and hematocrit (PCV) are not good measures of stress in silky sharks.

### *Acid-Base and Blood-Gas Perturbations*

One of the physiological manifestations of stress is acidemia and is reflected by a depression in blood pH. Disruptions to acid-balance in fishes is a typical secondary stress response where changes in pH levels can be due to both respiratory (elevated  $p\text{CO}_2$  from hypoxia) and metabolic (production of lactate during anaerobic respiration) processes (e.g. Cliff and Thurman, 1984; Mandelman and Skomal 2009; Skomal and Bernal 2010). Several studies on capture stress and post release condition of elasmobranchs have focused on this aspect of the stress response (eg Skomal 2006; Mandelman and Skomal 2009; Heberer et al. 2010). Where the fishing interaction has typically resulted in increased  $p\text{CO}_2$  levels there is a concomitant increase in lactate and a decrease in pH. Indeed, this is what occurred in the silky sharks and the magnitude of these disturbances was exacerbated as the fishing procedure progressed. These differences were also highly correlated to the release conditions that were recorded for each animal.

pH values decreased dramatically as the fishing procedure progressed. These values should be interpreted with a degree of caution however, due to the fact that sharks have nucleated red blood cells that are metabolically active even after withdrawal, and our samples were not analyzed immediately but kept on ice until the end of each fishing set. To address this, I applied the time correction recommended by the analyzer manufacturer. However, a rate of decay has not been determined for sharks or other taxa that have nucleated red blood cells. Several studies on elasmobranchs and teleosts have validated the pH values determined through the use of this algorithm with standard laboratory techniques and found small margins of error (Gallagher et al. 2010; Harter et al., 2014; Malte et al. 2014). The silky shark pH data, therefore, was interpreted by comparing the magnitude of the differences in values between landing stages, release conditions and survivorship.

The main buffering systems in sharks are bicarbonate, non-bicarbonate proteins in the plasma and histidine imidazole and alpha and beta side chains in hemoglobin (Aschauer et al. 1985). In silky sharks, while  $p\text{CO}_2$  and lactate levels rose significantly between the relatively unstressed animals and those that had interacted with the net,  $\text{HCO}_3^-$  was depleted as the fishing procedure progressed. To determine the relative input that respiratory and metabolic responses had on acidosis during the fishing procedure I also measured base excess. The amount of base excess refers to the amount of base chemicals present (predominantly  $\text{HCO}_3^-$ ) in the blood and

assists in determining whether an acid/base disturbance is caused by a respiratory, metabolic, or a mixed metabolic/respiratory problem, but these determinations are noticeably absent in the literature (Mandelman and Skomal, 2012). While positive values indicate an excess of base, negative values indicate a deficit. While carbon dioxide defines the respiratory component of acid-base balance, base excess defines the metabolic component (i-STAT, 2013). In this study, base excess decreased significantly as the procedure progressed suggesting that lactic acid build-up could be the largest contributor to the acidemia observed in silky sharks. Total mortality rates estimated for silky sharks captured in purse seines in the Indian Ocean and the WCPO were estimated at 81 and 84% (Poisson et al. 2014, Hutchinson et al., 2014), while at-vessel mortality for silky sharks captured in longline fisheries is 66% (Beerkircher et al. 2002). Other studies in which sharks were captured in gill nets found the metabolic proton load to increase immediately after capture and the magnitude of the acid base disturbance to be greater in net capture than on a longline (Frick et al. 2012). This is probably because the sharks struggle violently to swim against a net while their ability to ram ventilate is hindered. On a longline, they can ventilate even after being hooked and this may underlie differences in fishing induced mortality reported for different gear types.

### *Osmotic Disturbance*

Osmoregulation in marine elasmobranchs differs from that of marine teleosts in that elasmobranchs maintain high concentrations of urea, trimethylamine oxide (TMAO), sodium and chlorine in the blood, which is isosmotic or hyperosmotic compared with the surrounding sea water (Shuttleworth, 1988). Where the typical osmotic concentration of the plasma is 1000-1100 mmol l<sup>-1</sup>, plasma osmolality concentrations increased significantly with stress in both dusky and sharpnose sharks (*Rhizoprionodon terraenovae*) (Cliff and Thurman, 1984; Hoffmayer and Parsons 2001 respectively). In this study, plasma osmolality measurements in silky sharks ranged from 989 to 1413 mmol l<sup>-1</sup> but did not appear to correlate with stress or condition.

The concentrations of sodium (Na<sup>+</sup>) found in silky blood ranged from 240 - 324 mmol l<sup>-1</sup> and there were no patterns of increasing or decreasing concentrations that emerged over the course of the fishing procedure, by release condition or in moribund sharks. Sodium concentrations were consistently around 280 mmol l<sup>-1</sup> in dusky sharks (*Carcharhinus obscurus*)

during periods of stress (Cliff and Thurman, 1984) whereas Brill et al. (2008) found significant rises in sodium concentrations by 8% in exercise stressed sandbar sharks. Increases in blood  $\text{Na}^+$  concentrations are thought to occur during acidosis from the environmental influx associated with branchial exchange ( $\text{Na}^+/\text{H}^+$  exchangers) at the gill epithelia (Shuttleworth 1988). Other shark species exposed to gill net capture did not show any changes in sodium concentrations with deteriorating condition, this was attributed to the restriction of water circulation through the gills thereby inhibiting ion exchange (Manire et al., 2001) and may apply to what was observed in this data set. The inability of silky sharks to ventilate when confined in the sack until they are brailed may be similar to the effects of gill net capture.

Potassium concentrations increased during the fishing operation and were highest in moribund sharks. Potassium is principally an intracellular ion. the presence in the plasma may be due to cellular leakage caused by lactic acidosis and may also be attributed to the depletion of blood glucose (Cliff and Thurman, 1984; Manire et al. 2001). A study on gummy sharks (*Mustelus antarcticus*) captured in a gill net found that sharks that died post-release had higher levels of lactate and potassium than surviving sharks (Frick et al. 2010). Elevated levels of extracellular potassium (hyperkalemia) may induce muscular tetany, caused by lowering the threshold for nerve impulse generation to the point where there is continual stimulation of the muscles (Cliff and Thurman, 1984). In mammals, myocardial function is impaired once potassium levels reach  $7 \text{ mmol l}^{-1}$  (Guyton 1971 cited in Cliff and Thurman, 1984). The mean potassium concentrations in relatively unstressed silky sharks sampled pre-set and after encirclement exceeded  $7 \text{ mmol l}^{-1}$  and are higher than studies on exercise stressed and gill net captured sharks (Brill et al., 2008; Manire et al. 2001; Cliff and Thurman, 1984). Silky sharks that were predicted to survive had potassium concentrations of  $10.23 \text{ mmol l}^{-1}$  on average, so bradycardia and muscular tetany must arise at higher concentrations in this species than in other sharks and vertebrate taxa.

Ionized calcium concentrations also increased as the fishing procedure progressed and were significantly higher in moribund sharks than survivors. Although most of the calcium in blood is bound to protein or complexed to smaller anionic species, the biologically active fraction of calcium is the free ionized form (i-STAT 2013). Through its role in a number of enzymatic reactions and in membrane transport mechanisms, ionized calcium is important in nerve conduction, neuromuscular transmission and in muscle contraction. White muscle fueled

by anaerobic glycolysis pathway shows adaptations for high ATP turnover where the activities of the rate limiting enzymes (phosphorylase and phosphofructokinase) are around five times higher in white muscle than in red and these enzymes are not regulated by circulating catecholamines as in mammals but by  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum (Bone, 1988). Very large increases in the anaerobic activity (increased lactate) were observed for silky sharks as they struggled against the net so increased  $\text{Ca}^{2+}$  concentrations may reflect the activation of this pathway. Increases in  $\text{iCa}^{2+}$  in the plasma may also be a natural consequence of intracellular acidosis that results in increased cell permeability (Cliff and Thurman, 1984).

## Conclusion

The degree of physiological responses to a stressor (e.g. purse seine capture) is a function of the magnitude and extent of the imposed stressor and the aerobic capacity and metabolic scope of a species. Elasmobranchs are capable of excreting  $\text{H}^+$  ions via the (non-adrenergic)  $\text{Na}^+/\text{H}^+$  exchanger and are able to increase  $\text{HCO}_3^-$  concentrations by passive diffusion of  $\text{Cl}^-$  to the environment at the gill epithelia (Shuttleworth, 1988) but this system is incapable of meeting the demands of silky sharks struggling and confined in a purse seine net. During the struggle against the net at the end of the net haul, lactate builds up in the cell and the intracellular pH levels become much lower than the extracellular fluid. Lactate is rapidly dissociated due to a low  $\text{pK}_a$  into  $\text{La}^-$  and  $\text{H}^+$  and diffuses out of the cells along the  $\text{H}^+$  gradient. Because silky sharks are unable to ventilate in the sack, the rapid exchange of protons for  $\text{Na}^+$  and increased  $\text{HCO}_3^-$  for  $\text{Cl}^-$  is not possible. Thus, once the sharks had been confined in the sack, high lactate concentrations in the plasma, elevated  $\text{pCO}_2$ , depleted bicarbonate and  $\text{pO}_2$  contribute to biologically detrimental decreases in pH levels from approximately 7.49 ( $[\text{H}^+] = 3.2 \times 10^{-8}$ ) to  $< 6.4$  ( $[\text{H}^+] = 3.98 \times 10^{-7}$ ).

No net change in plasma sodium concentrations or osmolality were found and red cell swelling following fluid shifts was not observed. Hyperkalemia and elevated  $\text{iCa}^{2+}$  indicated that cell lysis from lactic acidosis had likely occurred within the tissues. Essentially, there was a total physiological breakdown that occurred once the silky sharks were confined in the sack and most of the animals that are landed through the brails are dead before being discarded. I found that the effects of rapid increases in lactate and subsequent decrease in blood pH combined with the inability to move water across the gills to offload excess protons is what most likely leads to

mortality in this species. Elevated levels of lactate, partial pressures of CO<sub>2</sub>, potassium and ionized calcium and a depressed pH all correlated with mortality. These biomarkers could be used to get better estimates of fishing mortality in this and other fisheries where silky sharks are captured and subsequently discarded as bycatch.

The low levels of changes in stress parameters in encircled sharks indicates that the act of encirclement does not induce stress (it may not even be perceived by the sharks) and the comparatively modest changes and moderate survival levels of entangled sharks indicates that physical abrasion and struggle are not lethal *per se*. These results obtained through analysis of multiple types of blood chemistry assays indicate that the combination of struggle and the inability to ventilate are the basic causes of physiological collapse and death of the animals caught in the purse seine fishery.

Responses to stress and capture have been shown to be species specific even among closely related species (Wells 2009; Skomal and Mandelman 2012) and the stress response of silky sharks showed both similarities and differences with other species. Consequently, this data set is specifically pertinent to this very vulnerable and heavily impacted species and represents an important contribution to understanding and perhaps ameliorating fishery induced mortality.

### **Acknowledgements**

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**Table 3-1.** Release Condition Codes and Criteria

Condition	Code	Criteria
Excellent	4	Swam away rapidly without any obvious signs of distress or physical trauma.
Good	3	Swam away but appeared slower or disoriented
Fair	2	Swimming appeared laborious and/or they exhibited other visible signs of trauma
Poor	1	Sharks were able to right themselves and made efforts to swim
Dead	0	Sank upside down

**Table 3-2.** Parameters measured and the reportable ranges of the I-STAT cartridges used in this study.

Parameter	Units	Reportable Range
CG4+		
pH		6.5-8.2
PO <sub>2</sub>	mmHg	5-800
PCO <sub>2</sub>	mmHg	5-130
TCO <sub>2</sub> *	mmol/L	5-50
sO <sub>2</sub> <sup>*Δ</sup>	%	NA
HCO <sub>3</sub> <sup>*</sup>	mmol/L	1.0-85.0
Base Excess (BE) <sup>*</sup>	mmol/L	(-30) - (+30)
Lactate	mmol/L	0.3-20.0
CHEM 8+		
Sodium (Na)	mmol/L	100-180
Potassium (K)	mmol/L	2.0-9.0
Chloride (Cl) <sup>†</sup>	mmol/L	65-140
ionized Calcium (iCa)	mmol/L	0.25-2.50
TCO <sub>2</sub> <sup>†</sup>	mmol/L	5-50
Glucose (Glu)	mg/dL	20-700
Blood Urea Nitrogen <sup>†</sup>	mg/dL	3-140
Creatinine <sup>†</sup>	mg/dL	0.2-20.0
Hematocrit (% PCV) <sup>‡</sup>	%	10-75
Hemoglobin <sup>*Δ</sup>	g/dL	NA

\* Denotes values that are calculated by the machine and not measured directly. † Denotes a parameter that never returned a result due to values being outside the reportable ranges. ‡ In this study we use our measured PCV values and not the I-STAT values. Δ These values were not used in this study due to large error margins reported in other studies on elasmobranchs.

**Table 3-3A.** Mean values, ranges and standard deviation of the acid base parameters for each landing stage.

Landing Stage	Acid-Base Chemistry							
	pH	pH <sub>low</sub>	pH <sub>high</sub>	pO <sub>2</sub>	pCO <sub>2</sub>	HCO <sub>3</sub>	Base Excess	Lactate
	7.32	7.32	7.32	44.7	20.44	6.69	-19.125	1.004
Pre-Set	7.21-7.49	7.21 - 7.49	7.21-7.49	5.21-158.8	12.88-25.46	5.95-8.55	-21.41- -16.68	0.3 - 1.87
	± 0.096	± 0.1	±0.1	±52.3	±5.08	±0.84	± 1.62	± 0.556
	7.31	7.31	7.31	38	18.82	6.38	-19.81	0.73
Encircled	7.17 - 7.47	7.17 - 7.47	7.17-7.47	14.3-70.1	12.99-22.25	5.69-6.89	-22.8- -17.19	0.33 - 0.93
	± 0.1474	± 0.15	±0.15	±28.8	±15.17	±0.617	± 2.83	± 0.346
	6.98	6.99	7.06	46.52	22.22	3.78	-27.75	7.34
Tangled	6.41 - 7.43	6.40 -7.43	6.52-7.43	14.26-133.84	6.66-36.73	1.12-6.96	-39.65 - -17.7	1.19 - 14.92
	± 0.36	±0.36	±0.31	±7.59	±9.43	± 2.07	±7.59	± 5.33
	6.66	6.65	6.79	53.1	32.88	2.59	-34.23	13.96
First Brail	6.41 -7.27	6.40-7.27	6.40-7.27	9.77-180	16.83-52.19	0.797-5.695	-39.82 - -21.19	4.70 -19.93
	± 0.25	±0.26	±0.23	±28.8	±12.7	± 1.28	± 5.18	± 4.07
	6.56	6.56	6.69	25.71	34.13	2.04	-36.31	15.68
Brail	6.4-7.11	6.4-7.11	6.52-7.11	0-101.7	12.77-93.99	0.841 - 3.886	-40.03 - -26.6	10.09 - 20+
	± 0.18	±0.18	±0.15	±18.5	±15.2	± 0.698	± 3.17	± 2.18

**Table 3-3B.** Mean values, ranges and standard deviation of the acid base parameters by release conditions.

Acid Base Chemistry								
Release Condition	pH	pH <sub>low</sub>	pH <sub>high</sub>	pO <sub>2</sub>	pCO <sub>2</sub>	HCO <sub>3</sub>	Base Excess	Lactate
Excellent (4)	7.23	7.23	7.23	50.7	20.33	5.7	-37.86	3.525
	6.52 - 7.49	6.51 - 7.49	6.52 - 7.49	5.21 - 173.7	10.78 - 34.54	1.69 - 8.55	- 40.03 - -32.76	0.3 - 14.6
	± 0.223	± 0.223	± 0.223	± 47.4	± 6.93	± 1.59	± 1.7	± 4.06
Good (3)	6.85	6.85	6.9	49.65	21.49	2.57	-31.14	11.13
	6.41 - 7.21	6.4 -7.21	6.59 -7.21	16.56 - 101.68	6.66 - 34.69	1.12 - 6.54	-39.65 - -21.41	1.44 - 16.64
	± 0.172	± 0.25	± 0.21	± 27.25	± 9.18	± 1.53	± 5.05	± 5.31
Fair (2)	6.74	6.74	6.74	31.52	28.2	2.56	-32.917	13.19
	6.63 - 6.86	6.63 - 6.86	6.63 - 6.86	20.57 - 45.28	16.83 - 38.74	1.97 - 3.47	-34.878 - 31.56	9.79 - 16.72
	±0.09	± 0.09	± 0.09	± 8.2	± 7.71	± 0.50	± 1.47	± 2.39
Poor (1)	6.6	6.61	6.68	14.98	38.13	2.54	-35.01	15.22
	6.4 - 6.87	6.43 - 6.87	6.58 - 6.87	0 - 32.44	21.33 - 58.09	1.932 - 3.372	-38.4 - 30.57	13.1 - 19.09
	± 0.17	± 0.16	± 0.12	± 10.8	± 12.42	± 0.538	±2.87	± 1.96
Dead (0)	6.48	6.46	6.58	22.11	37.39	1.87	-37.858	16.11
	6.4-6.73	6.4 - 6.73	6.52 - 6.73	3.16 - 59.52	19.31 - 93.99	0.841 - 4.035	-40.03 - -32.76	10.35 - 20+
	±0.082	± 0.094	± 0.064	± 13.34	±14.96	± 0.706	± 1.7	± 2.004

**Table 3-4A.** Mean values, ranges and standard deviation of primary stress response parameters and electrolytes by landing stage.

Landing Stage	Primary Stress Response			Electrolyte Concentrations			
	Adrenaline ng/mL	Glucose mg/dL	%PCV	Osmolality mmol/L	Sodium mmol/L	Potassium mmol/L	ionized Calcium mmol/L
Pre-Set	6.4	109.3	24.57	1161	282.4	9.28	2.788
		78 - 206 ± 44	21 - 30 ± 2.94	1060-1413 ± 146.4	264-302 ± 15.32	7.4-12 ± 1.781	2.58 - 3.02 ±0.181
Encircled		105.33	20.67	1043	276.7	9	2.66
		94 - 124 ± 16.3	17 - 24 ± 3.51	996-1094 ± 49.1	250 - 300 ± 25.2	7-12.4 ± 2.96	2.18 - 2.98 ± 0.423
Tangled	120	98.85	25.85	1121	275.08	11.72	2.825
	93- 138 ± 23.8	68 - 137 ± 20.1	17 - 33 ± 3.72	1060-1364 ± 82.4	240-294 ± 15.18	7.6 - 18+ ± 3.15	1.68 - 3.14 ± 0.392
First Brail	315	96.4	25.1	1126.3	283.56	11.7	3.238
	49 - 581 ± 376	80 - 132 ± 17.4	20 - 28 ± 2.34	1030 - 1259 ± 75.1	266 - 292 ± 7.47	9.2 - 18+ ± 2.27	2.94 - 3.58 ± 0.213
Brail	819	93.5	25.64	1127.8	276.83	12.76	3.1689
	590-1065 ± 238	42 - 142 ± 19.9	17 - 30 ± 2.53	989-1358 ± 74.8	256-324 ± 11.69	6.4 - 18+ ± 2.07	1.14 - 3.88 ± 0.451

**Table 3-4B.**

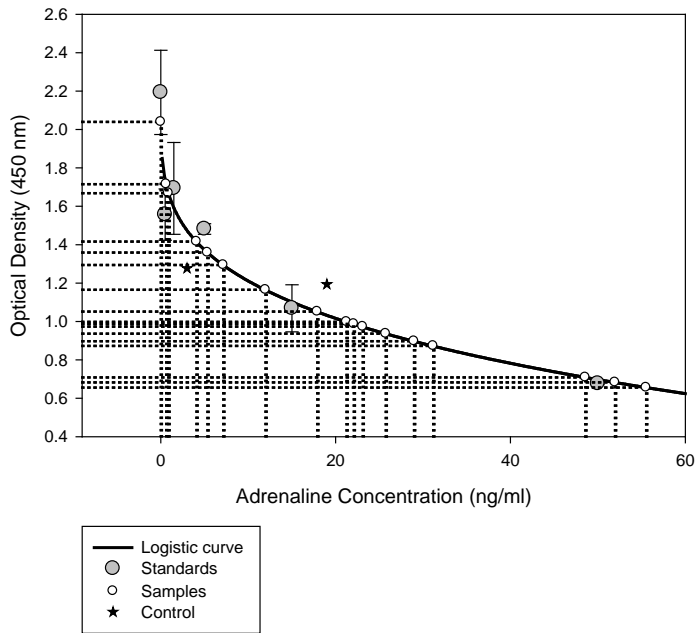
Mean values, ranges and standard deviation of primary stress response parameters and electrolytes by release condition.

Release Condition	Primary Stress Response			Electrolyte Concentrations			
	Adrenaline ng/mL	Glucose mg/dL	% PCV	Osmolality mmol/L	Sodium mmol/L	Potassium mmol/L	ionized Calcium mmol/L
Excellent (4)	83.1	100.87	24.3	1129	276.25	10.56	2.74
	6.4- 138	76 - 206	17 - 33	996 - 1413	256 - 294	7 - 20	1.68 - 3.14
	± 55.4	± 26.8	± 3.95	± 108.4	± 17.45	± 3.4	± 0.36
Good (3)	1065	107.7	26.3	1113.7	282	13.43	3.05
		76 - 137	21 - 31	989 - 1358	266 - 324	10.0 - 17.8	2.58 - 3.6
		± 22.88	± 3.13	± 127.4	± 21.17	± 2.59	± 0.336
Fair (2)	803	98.57	25.29	1073.4	278.67	11	3.11
		76 - 126	22 - 28	1030 - 1116	266 - 294	7.6 - 18+	2.94 - 3.26
		± 20.93	± 2.43	± 31.1	± 10.41	± 2.59	± 0.119
Poor (1)	590	96.86	25.57	1148.8	284	12.33	3.25
		86 - 114	23 - 30	1077 - 1257	278-288	10 - 14	3.1 - 3.48
		± 9.49	± 2.5	± 80.2	± 4.2	± 1.44	± 0.15
Dead (0)	581	91.47	25.75	1134	276.73	13.61	3.19
		42 - 132	17 - 30	1058 - 1340	256-294	6.4 - 18+	1.14 - 3.88
		± 21.13	± 2.38	± 65.2	± 9.89	± 2.29	± 0.49

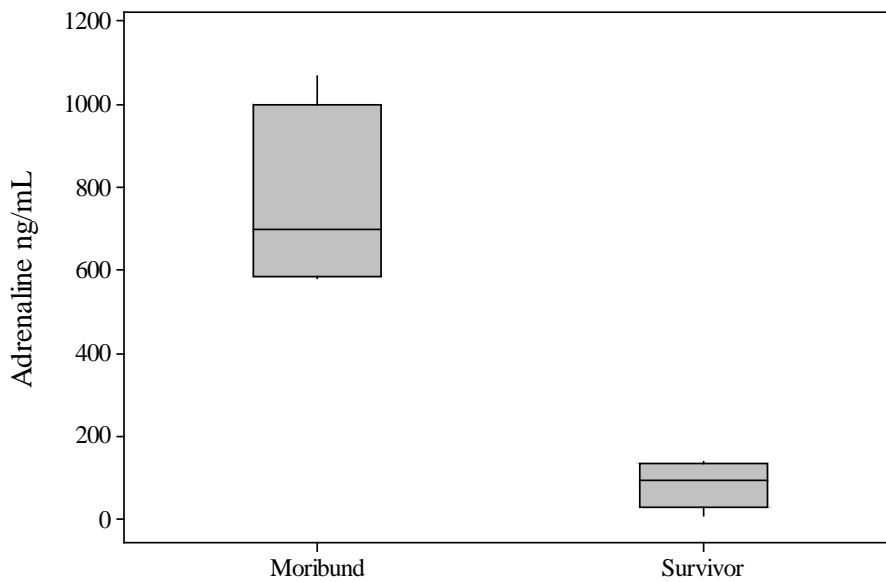
**Table 3-5.** Results of the two sample comparisons of blood parameters between sharks predicted to survive and those that were moribund.

Parameter	Test	Statistic	Survivor	Moribund	P-Value
Primary Stress Response					
Adrenaline	T-test	5.8	83.1	760	0.01
Glucose	Mann-Whitney	2352.5	96	90.5	0.41
Hematocrit (% PCV)	T-test	1.91	24.24	25.72	0.064
Acid Base Chemistry					
pH	T-test	-10.27	7.21	6.69	0
PO <sub>2</sub>	T-test	-2.15	48.43	30.1	0.039
PCO <sub>2</sub>	T-test	6.3	19.6	33	0
HCO <sub>3</sub> <sup>*</sup>	T-test	-8.59	5.37	2.032	0
Base Excess <sup>*</sup> (BE)	T-test	-12.39	-22.5	-36.27	0
Electrolytes					
Osmolality	T-test	0.37	1119	1129.5	0.718
Sodium (Na)	T-test	-1.64	278.9	277.2	0.693
Potassium (K)	T-test	3.44	10.23	13.41	0.002
ionized Calcium (iCa)	Mann-Whitney	1874.5	2.85	3.18	0.0001

**Figure 3-1. Adrenaline Standard Curve**

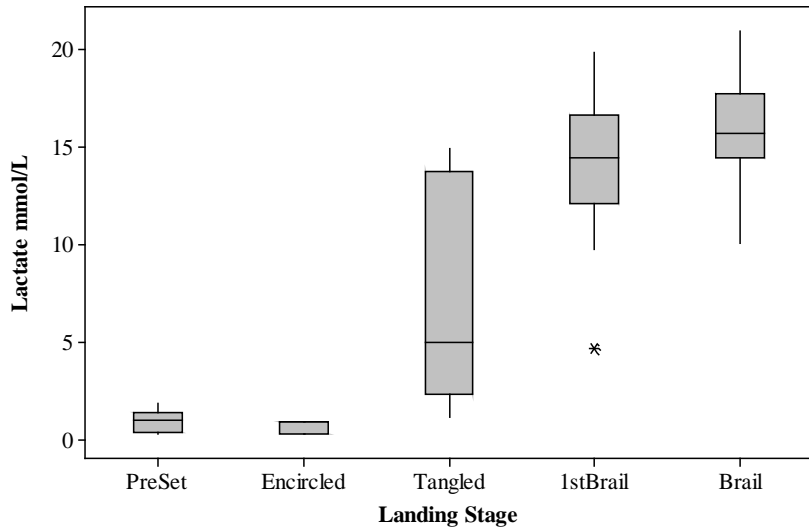


**Figure 3-2. Adrenaline concentrations in sharks predicted to survive and those deemed moribund. Boxplots encompass the first and third quartiles, whiskers extend to the upper and lower data limits, the median value is displayed by the line in the box (outliers, not pictured here are shown as asterisks).**

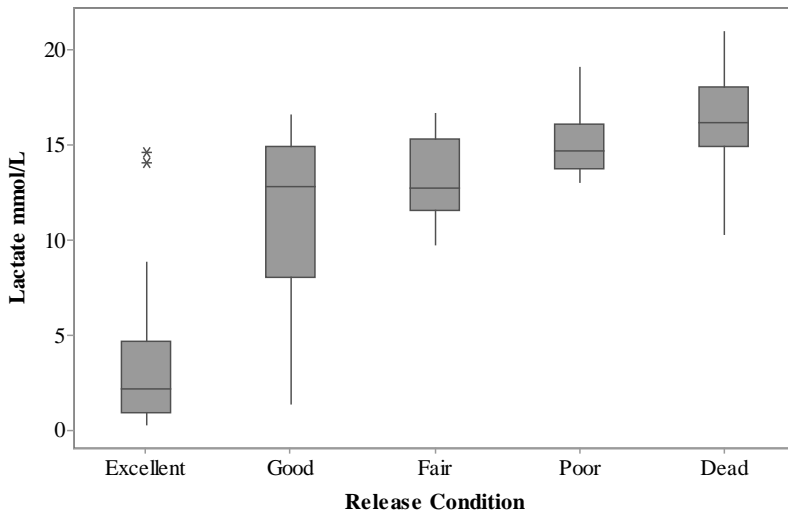


**Figure 3-3.** Lactate concentrations by landing stage (A) and release condition (B). Boxplot values are as described in Fig 2.

**A.**

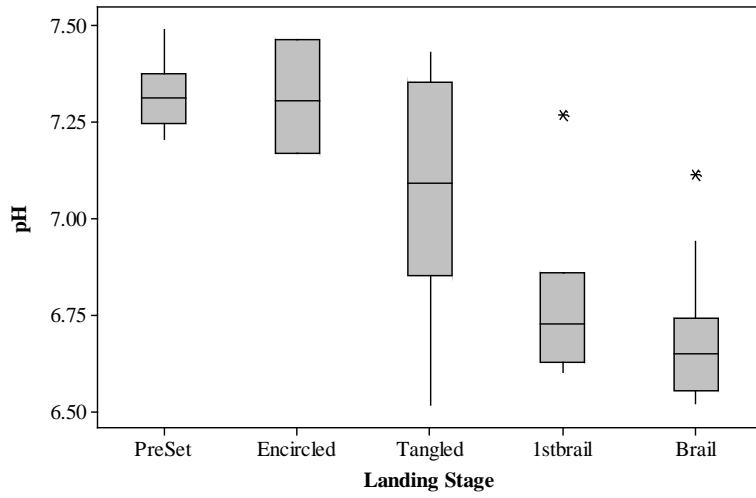


**B.**

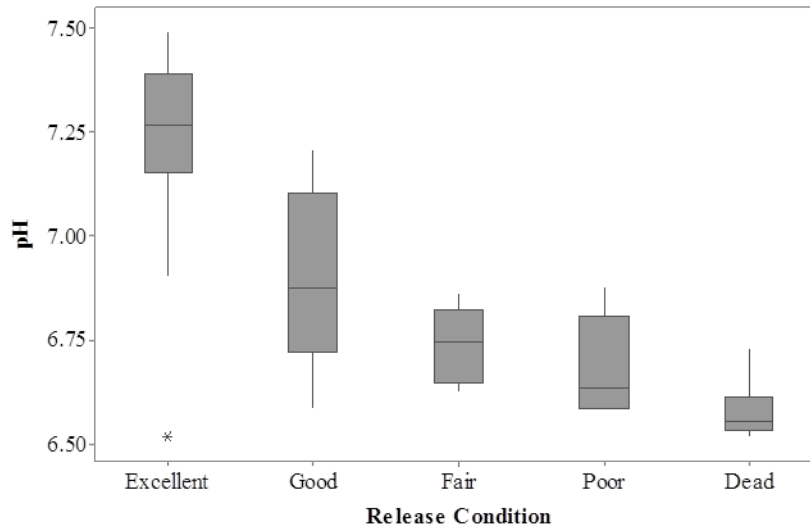


**Figure 3-4.** pH by landing stage (A), release condition (B) and survivorship (C). Boxplot values are as described in Fig 2.

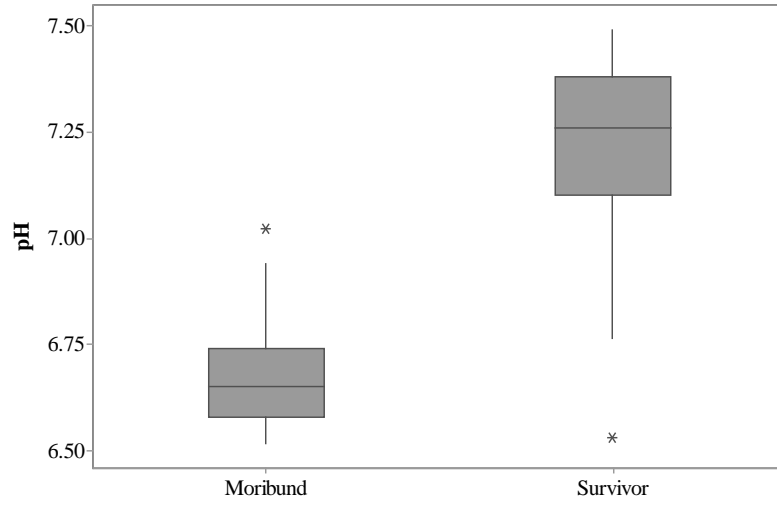
**A.**



**B.**

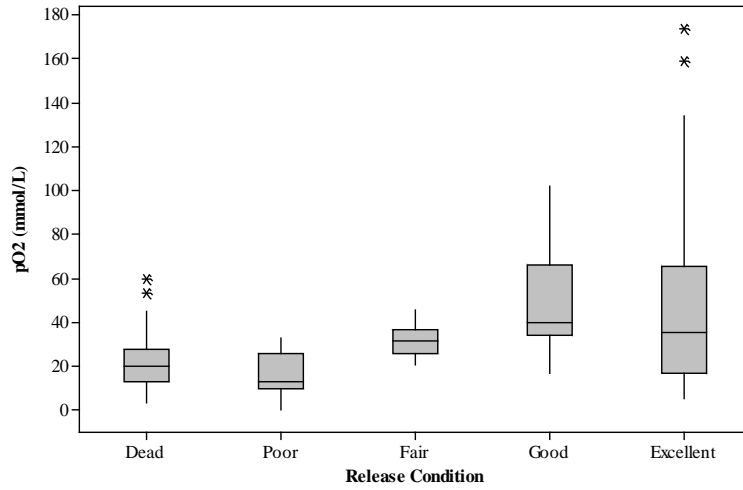


C.

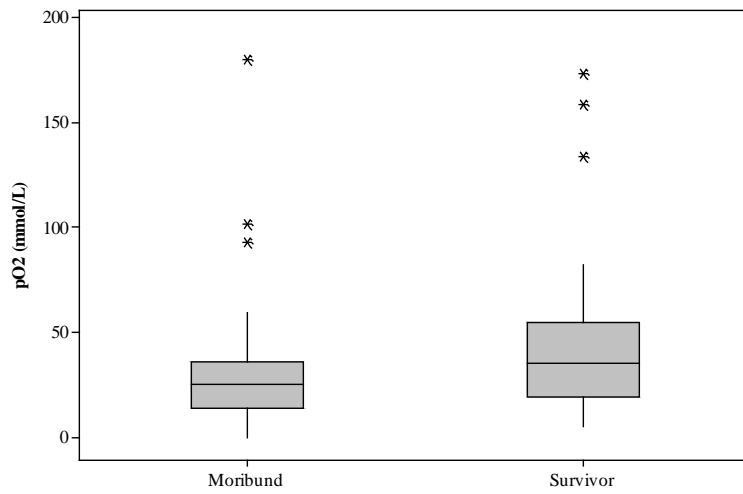


**Figure 3- 5.** Oxygen partial pressures found in sharks by release condition (A) and predicted survivorship (B). Boxplot values are as described in Fig 2.

**A.**

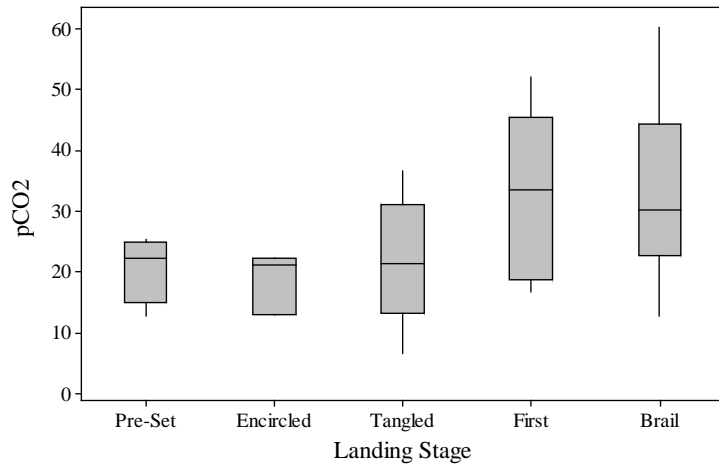


**B.**

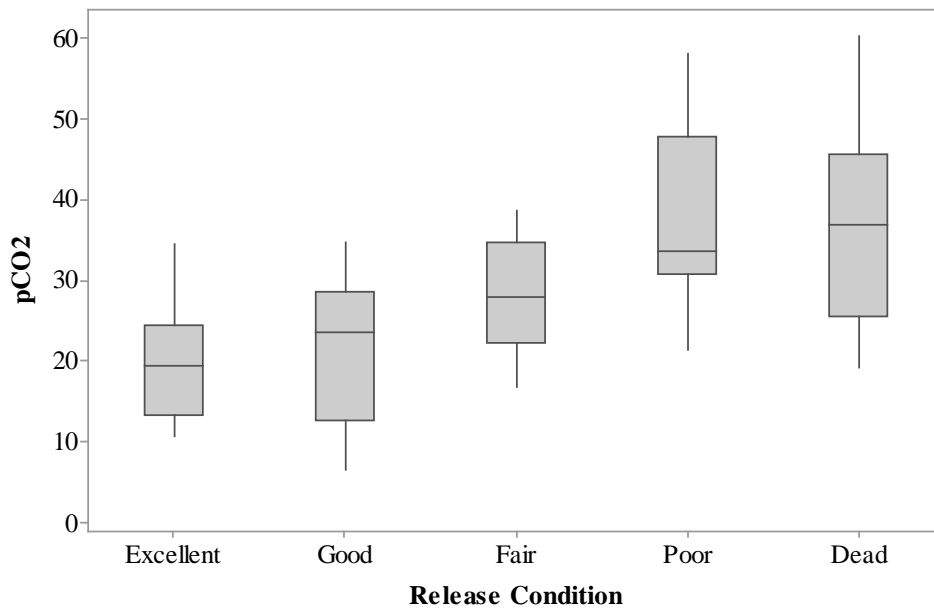


**Figure 3-6.** Carbon dioxide partial pressures by landing stage (A), release condition (B) and survivorship (C). Boxplot values are as described in Fig 2.

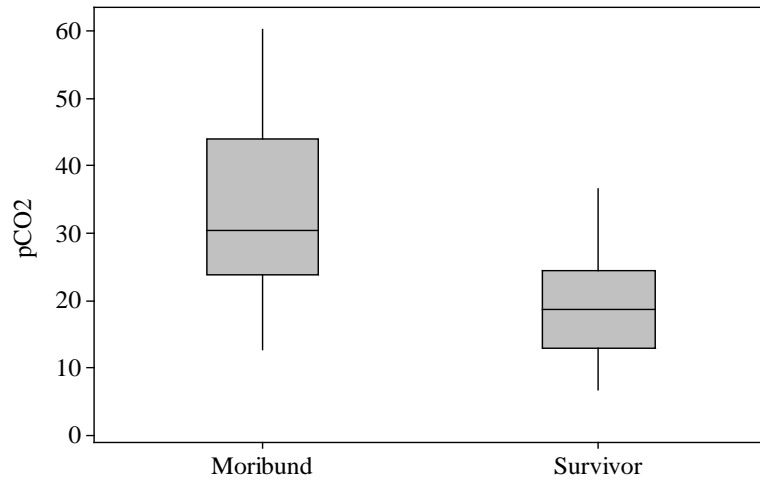
**A.**



**B.**

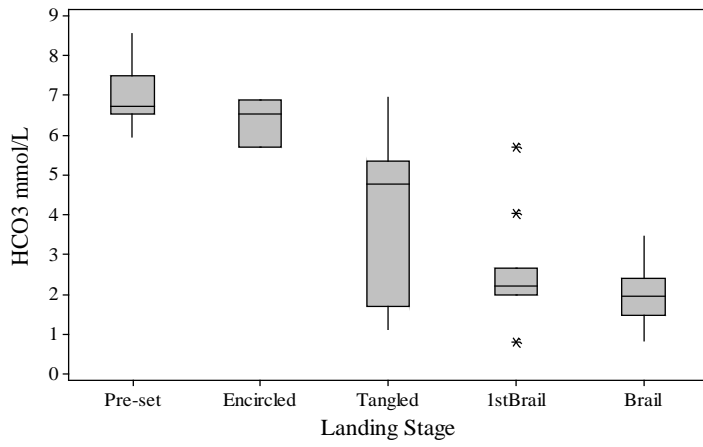


C.

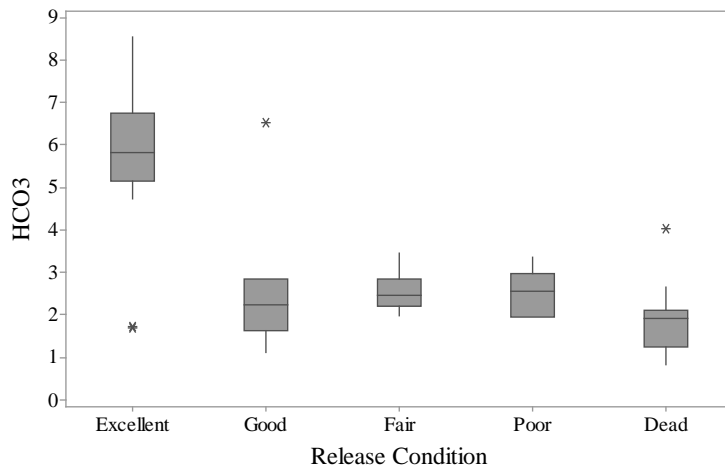


**Figure 3-7.** Bicarbonate concentrations by landing stage (A), release condition (B) and survivorship (C). Boxplot values are as described in Fig 2.

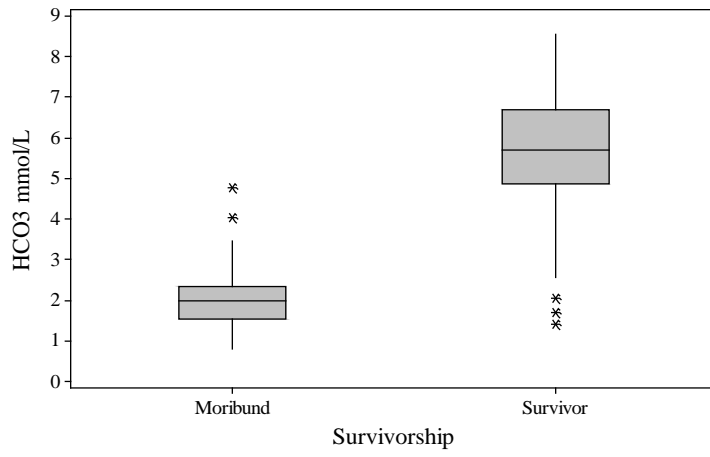
**A.**



**B.**

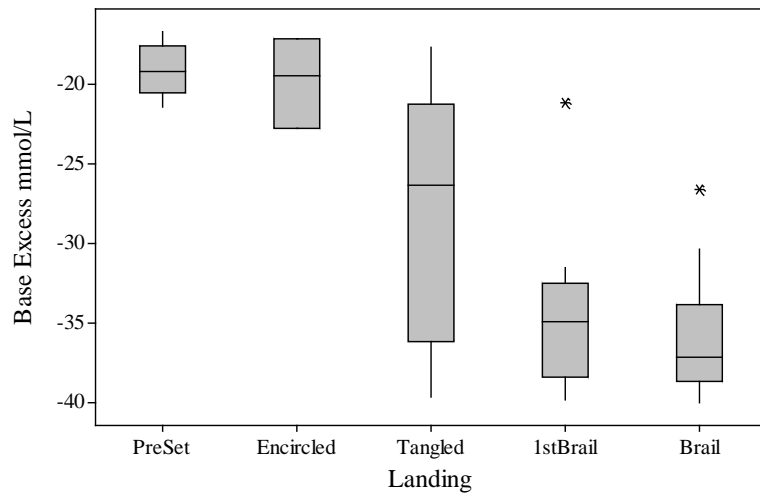


C.

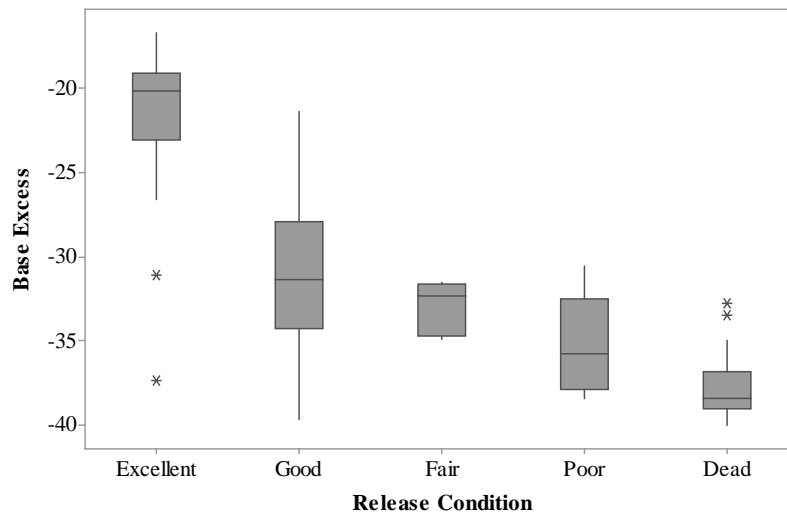


**Figure 3-8.** Base excess values by landing stage (A), release condition (B) and survivorship (C). Boxplot values are as described in Fig 2.

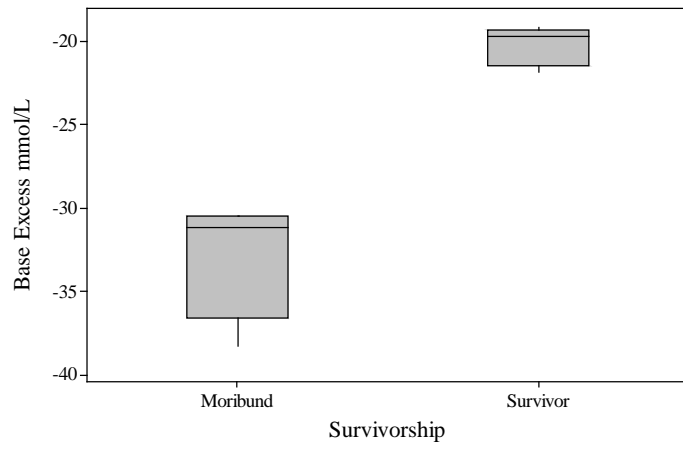
**A.**



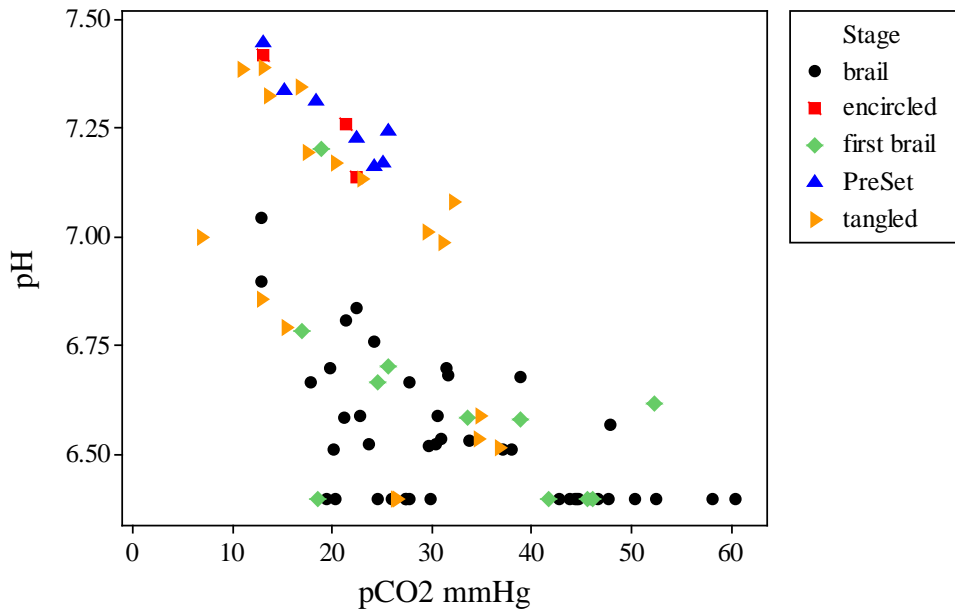
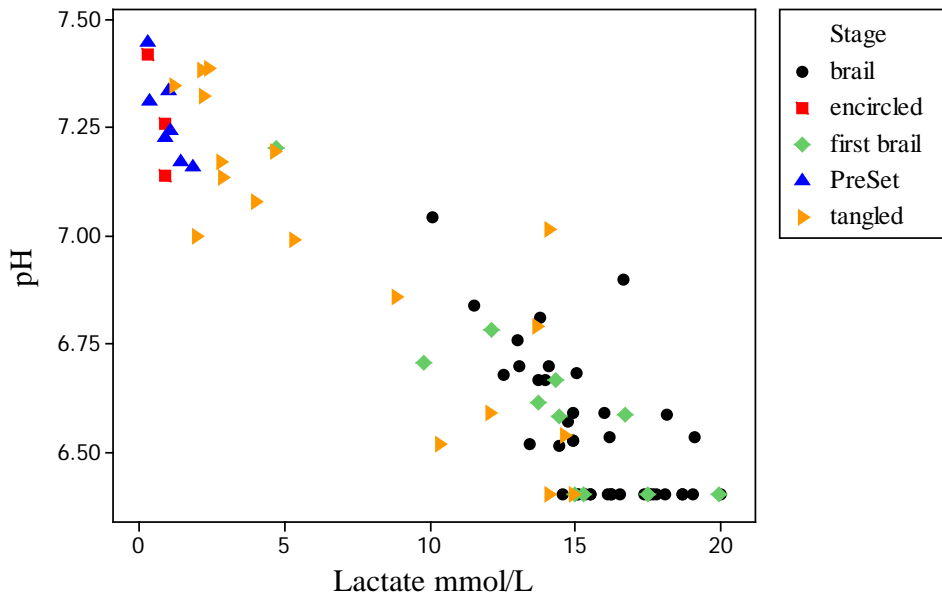
**B.**



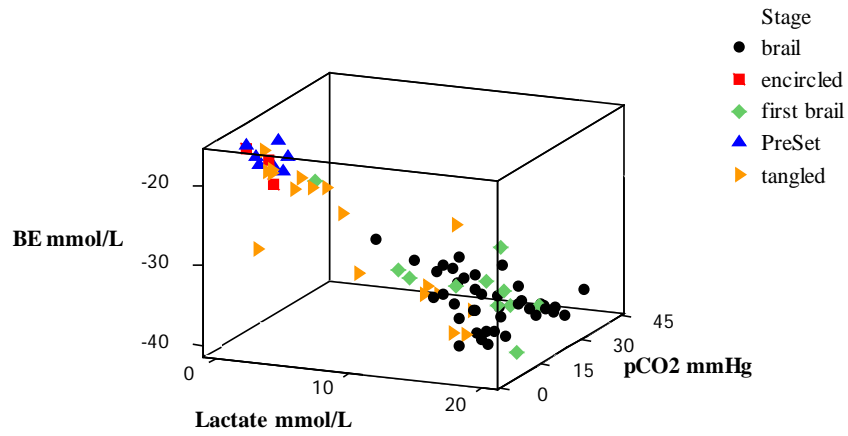
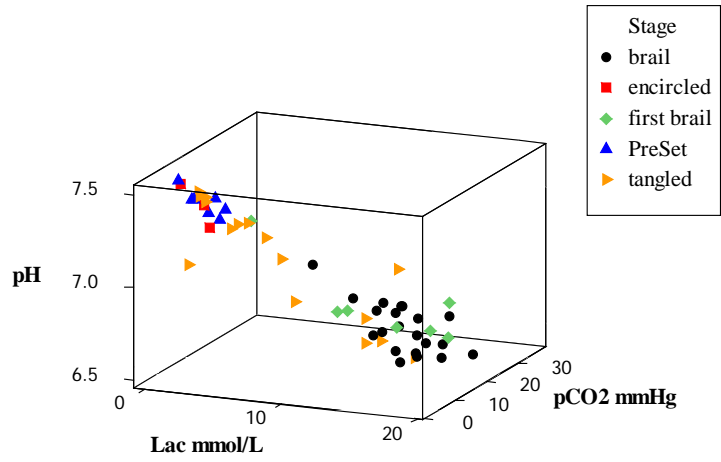
C.



**Figures 3-9.** Silky shark acid-base disturbances. The relationship of pH to lactate (A) and pCO<sub>2</sub> (B) by landing stage.

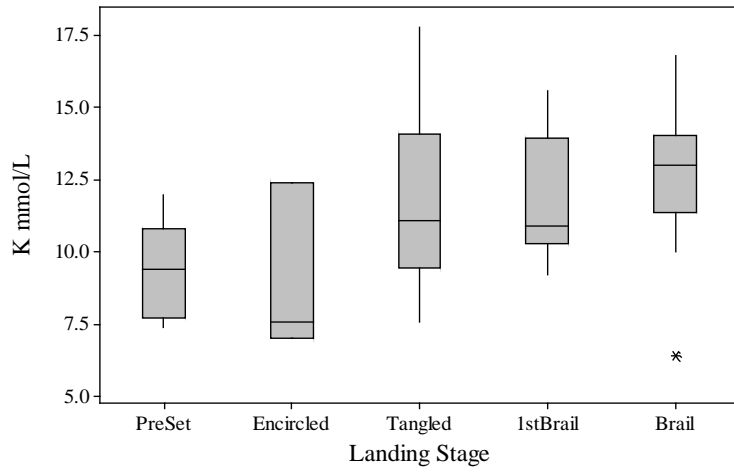


**Figure 3-10.** Visualizations of the acid base perturbations and the relative contributions (metabolic or respiratory) to acidosis by landing stage.

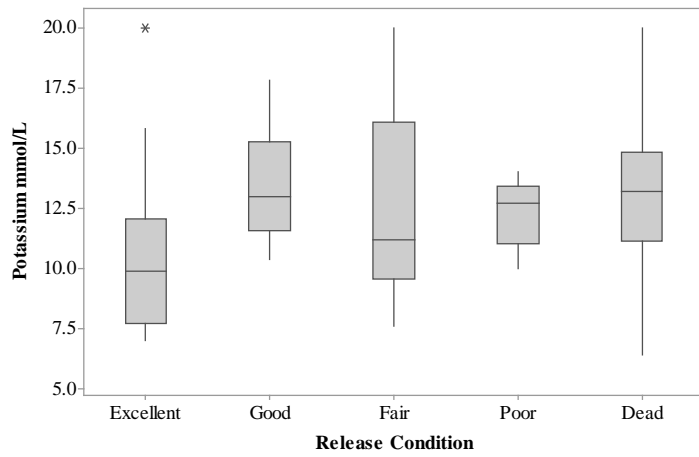


**Figure 3-11.** Potassium concentrations by landing stage (A), release condition (B) and survivorship (C). Boxplot values are as described in Fig 2.

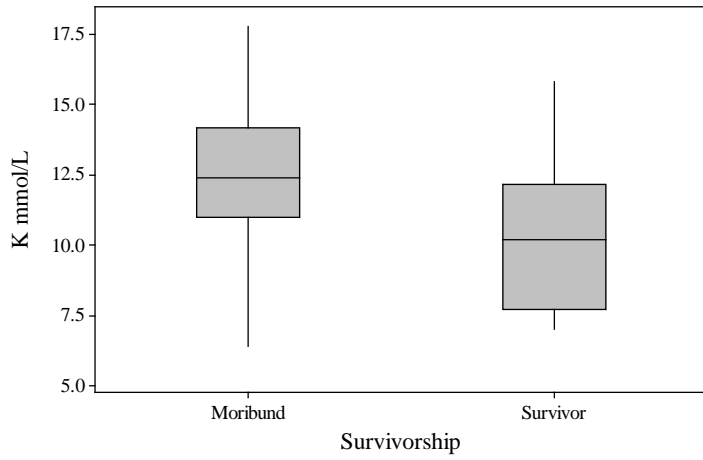
**A.**



**B.**

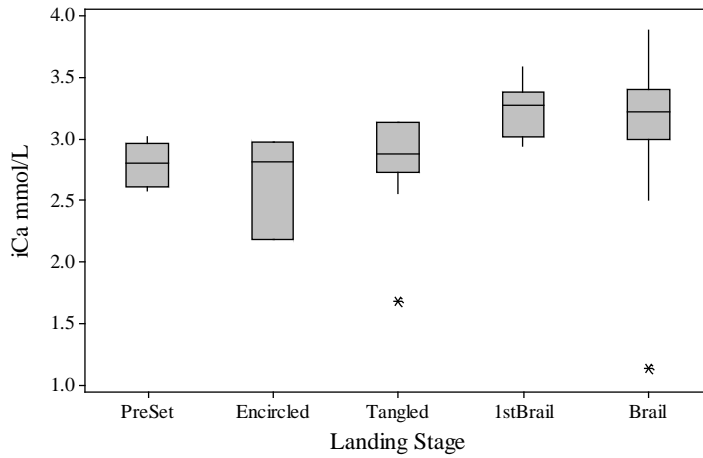


C.

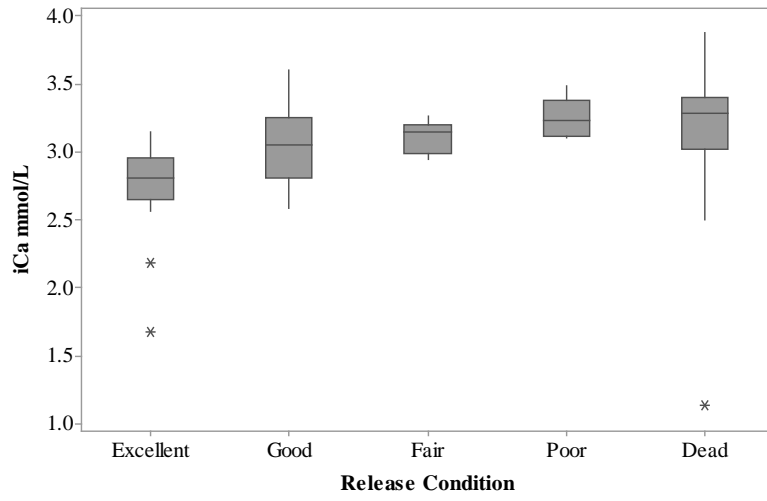


**Figure 3-12.** Ionized calcium concentrations by landing stage (A), release condition (B) and survivorship (C). Boxplot values are as described in Fig 2.

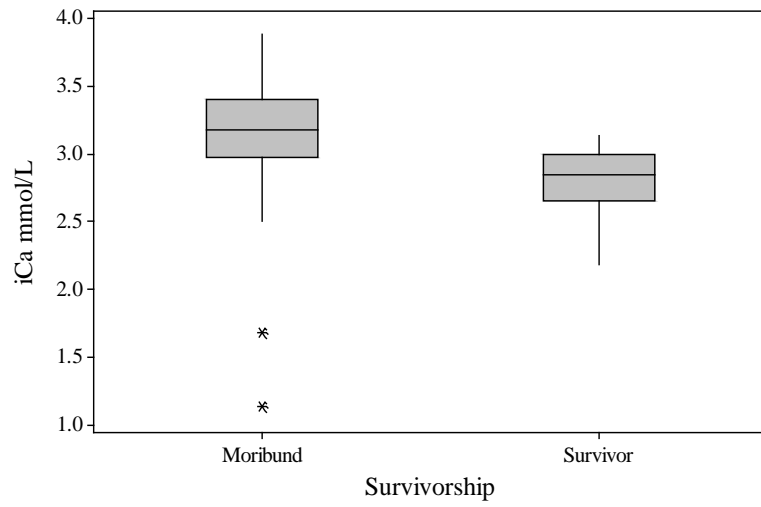
A.



**B.**



**C.**



## CHAPTER 4

# THE VERTICAL AND HORIZONTAL HABITAT USE OF JUVENILE SILKY SHARKS IN THE WESTERN AND CENTRAL PACIFIC OCEAN: UNDERSTANDING BEHAVIOR TO IDENTIFY BYCATCH MITIGATION STRATEGIES.

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## **Abstract**

Understanding the habitat use and behavior of commercially exploited species throughout ontogeny is critical for devising effective management and conservation strategies. In tropical tuna purse seine fisheries in the western central Pacific Ocean juvenile silky sharks, *Carcharhinus falciformis* compose greater than 95% of the total elasmobranch bycatch. There is now growing recognition of declines in silky shark populations and the need for international collaboration in conservation efforts. Yet very little is known about this species' movement behavior or habitat use in this region. In this study I investigated the movement behavior of juvenile silky sharks through the use of pop-up satellite archival tags placed on sharks that were captured during a chartered cruise on a commercial tuna purse vessel using drifting Fish Aggregating Devices (FAD). Analysis of the horizontal and vertical movement behavior revealed silky sharks spend nearly 100% of their in shallow thermally structured, epipelagic regions of the water column with depth preferences in the upper 30 m, making them vulnerable to capture in both purse seine and longline fisheries. Reconstruction of their horizontal movements showed they frequently move into and out of adjacent national waters, highlighting the importance of regional conservation measures.

**Key Words:** Satellite tag, *Carcharhinus falciformis*, Fish Aggregating Device, purse seine fishery

## Introduction

The silky shark, *Carcharhinus falciformis* is a cosmopolitan, circumtropical species inhabiting both coastal and pelagic waters (Compagno, 1984), and is found in all tropical oceans where water temperatures are warmer than 23° C (Last and Stevens, 1994). This results in a narrow distribution between 20° N and S latitude (Clarke et al., 2011), and tends to overlap with most of the commercial fishing effort targeting tuna. As such, silky sharks make up a large component of the elasmobranch bycatch in both purse seine and longline fisheries worldwide (e.g. Watson et al., 2008; Clarke et al., 2011). In the western and central Pacific Ocean (WCPO) tuna purse seine fishery, silky sharks comprise greater than 90% of the shark bycatch, where median catch estimates for 2006 range from 200,000 to 500,000 individuals (Lawson, 2011). These numbers were not this high until recently, when in the 1980s demand for dolphin safe canned tuna from the market increased and purse seine fishing effort switched from fishing on porpoise schools in the eastern tropical Pacific Ocean (ETP) to fishing on drifting fish aggregating devices (FADs) in the western and central Pacific Ocean (WCPO; Hall 1998). Shark catch rates are typically twice as high in FAD-associated sets versus unassociated or 'free school' fishing sets in the WCPO (Clarke et al. 2011). Juvenile silky sharks aggregate in large numbers around these drifting objects and become incidentally caught in purse seines targeting skipjack tuna, *Katsuwonus pelamis* for the cannery (Watson et al., 2009; Filmalter et al., 2011). Recent studies on the post release condition of silky sharks captured in tropical tuna purse seine fisheries showed relatively high total mortality rates: 84.3% in the WCPO and 81% in the Indian Ocean (Hutchinson et al., 2014; Poisson et al., 2014). Most animals that are landed do not survive the interaction due to the nature of the fishing methods, thus efforts to mitigate fishery impacts on the population need to be focused on avoidance strategies.

There is growing international concern over the population status of this species. A stock assessment conducted by the Secretariat to the Pacific Communities (SPC) concluded the silky shark population(s) in the Western Central Pacific Fisheries Commission (WCPFC) statistical area is in decline, overfished and that overfishing is currently occurring (Rice and Harley, 2013). More recently, silky sharks have been listed in Appendix II of the Convention on the Conservation of Migratory Species of Wild Animals (CMS). Appendix II listing commits countries to coordinate trans-boundary conservation measures throughout the species' range.

Despite the growing recognition of population declines and the need for international collaboration in conservation very little is known about this species' movement behavior, habitat use or FAD associative behaviors. The objective of this study is to identify potential patterns in silky shark behavior in time and space using a combination of pop-off archival satellite tags (PATs) and acoustic tags to devise effective bycatch mitigation strategies.

Some of the early work on the biology of this species found neonates and juveniles to occupy nursery grounds and have a demersal lifestyle (Branstetter 1987; Bonfil 1997). While in the Eastern Tropical Pacific (ETP) a study on catches of silky sharks in purse seines identified persistent spatio-temporal hotspots in oceanic and neritic waters, where increased catch rates were due to aggregations of juveniles at latitudes North of 5° (Watson et al. 2008). Off the western coast of Baja California Sur, the presence of silky sharks in catches sampled from fishing camps were also seasonal (June- November: Sanchez-de Ita et al., 2011). A mark-recapture study in the Gulf of Mexico and Atlantic Ocean (part of the National Marine Fisheries Service Cooperative Shark Tagging Program), tagged 819 silky sharks 6.6% were recaptured resulting in a max distance between the tagging location and recapture of 723 nm where their maximum speed was found to be 2.5 km/h (Kohler et al., 1988). Where mark-recapture studies may reveal ranges of sharks the use of electronic tag technologies can give rise to fine scale information regarding vertical habitat use as well as horizontal movement behavior of migratory marine species. The only published satellite tagging study conducted on silky sharks to date comes from 10 sharks tagged in the Pacific Ocean near Hawaii (Musyl et al. 2011). Musyl et al. (2011) found a linear displacement of up to 687 km over 132 days and concluded silky sharks are epipelagic and remain within the upper mixed layers of the water column.

Here I report the movement behavior of juvenile silky sharks that were captured at FADs in a tuna purse seine in the western and central Pacific Ocean (WCPO). My objective was to characterize the vertical and horizontal habitat use and to investigate time spent on and off FADs, to elucidate the underlying behaviors that lend this species vulnerable to fisheries capture. Through the use of electronic archival tags I reveal the thermal and vertical niche of juvenile silky sharks and expand on their use of horizontal space.

## **Materials and Methods**

### *Shark capture and tag details*

Sharks were captured and tagged during a chartered cruise on the commercial purse seine vessel MV Cape Finisterre in the WCPO during May - June 2012 (see Itano et al. 2012 for vessel and cruise details). A total of 295 juvenile silky sharks aged 0 - 4 ( $115.2 \pm 17.5$  cm mean total length) were captured as part of a larger shark bycatch post-release survival study (Hutchinson et al., 2014). Age at length information was derived from Joung et al. (2006). Twenty-six of the juvenile silky sharks landed during different stages of the fishing operation were selected for telemetry and fitted with miniPAT, pop-off satellite archival tags (Wildlife Computers Inc., Redmond, WA.). MiniPATs measure depth, temperature and light-levels and were programmed to sample and archive data at 75 second, 5 minute or 10 minute intervals. Tags were programmed to release after 30, 100 and 360 day deployments or to release from the tether if they were at a constant depth for two days or at depths that exceeded 1600 m, which are consistent with an animal that had died and was either sitting on the bottom or was sinking out of the water column. Data were compressed into 1, 8, 12 or 24 hour bins and summary intervals of time series information for transmission to Argos satellites. Satellite tags were attached intramuscularly, under the first dorsal fin with a stainless steel tether and nylon anchor. Acoustic transmitters, Vemco v13p R-coded 69 KHz acoustic tags (Amirix Systems Inc. Nova Scotia, Canada) were surgically implanted into the ventral cavity of three sharks at two different FADs to investigate fidelity and residence times at the FADs. VR2W receivers were attached to the drifting FADs and retrieved after ten and five days respectively.

#### *Data treatment and statistical analysis*

Light based geolocation estimates were approximated using the tag manufacturer's proprietary software WC-GPE2 (Wildlife Computers) from the transmitted light intensity curves after pop-up or from the physical archives of recovered tags ( $n = 2$ ). The GPE estimates were post-processed using the IKNOS-WALK movement model (Tremblay et al. 2009) in MatLab (Mathworks). In this approach, as in most state space models, estimates of spatial accuracy are used to infer animal positions but with this model there is no speculation regarding the unknown or posterior behavioral states to calculate subsequent positions. Positions are estimated as the geographic average of a cloud of possible positions that can be weighted or constrained using a speed filter, the probability of being on land or matching sea surface temperature (Tremblay et al. 2009). The tracks were interpolated to obtain two positions per day using a speed filter of 4

km/h. Euclidean distances between release and pop-off locations and maximum speed between IKNOS points were found using the Tracking Analyst Toolbox in ArcGIS v. 10.2.2 ESRI (Table 1). The reconstruction of the tracks was plotted in ArcGIS and viewed on a base map with the exclusive economic zones (EEZ) of the region to identify how often silky sharks migrate in and out of the EEZs of neighboring island nations (Figures 1 & 2, Table 1). Shark 54270 had the only deployment that spanned four months (129 days) so its horizontal movement was investigated for evidence of 'resident' or 'migrating' behavioral states using Track-project.org. This interface uses a hidden markov model (HMM) to reveal the hidden behavioral states (posterior process) underlying the horizontal displacement of this animal with the light based position estimates generated by the GPE (Pedersen et al. 2011, Jonsen et al. 2013; Figure 3).

Vertical and thermal habitat use was quantified using the transmitted (and archival record from tag 62936) pressure and temperature time series data in MatLab. Diel differences in vertical habitat was investigated to infer day and night time depth and temperature preferences. Pressure data was partitioned into 10 meter depth bins between 0-100 m, 50 meter bins between 100-300 m, and a 300-500 m depth bin. The mean percent time spent in each depth bin was quantified by day and at night (Table 2; Fig 4). The temperature data were analyzed for percent time spent in 1 °C increments between 25-30 °C and binned for temperatures < 25 or >30 °C. The mean time spent during the day and at night within each bin was also quantified (Table 3; Fig 5). The depth of the thermocline or 'mixed layer' and the amount of time spent within the mixed layer was measured and reported by each tag.

## **Results**

Of the 26 tagged silky sharks, eight sharks died upon release while three tags were shed within five days of deployment and were not used in the horizontal movement model. The 15 tags (of 26 total) where deployments went beyond five days resulted in 558 days of data (Table 1). Seven of the 15 tags reached their pre-programmed pop-off dates. The distance between the deployment location and pop-off locations are given in Table 1. Two of the tags were physically recovered allowing access to the entire archival record (tags 54247 & 62936). Using the transmitted (and archival) data from all tags combined; the mean sea surface temperature was found to be 29.1 °C ranging from 27.95 - 29.85 °C. The mean depth of the mixed layer

(thermocline) in the region was 84.7 m ( $\pm 27.5$ ). For practical purposes the depth of the thermocline is denoted as 85 m in all further analyses.

### *Horizontal movements*

Horizontal movement tracks were reconstructed using the IKNOS-Walk method to estimate two positions per day and are illustrated in Figure 1 for all tags combined and each individual track in Figure 2. The distance travelled and speed summaries are given in Table 1. All sharks had mean speeds ranging between 2.0 - 2.67 km h<sup>-1</sup>. Total track lengths ranged from 576 to over 6700 km, where the actual distance between the tagging location and where the tags came off was at least an order of magnitude shorter in most cases (98 - 1011 km). Most of the deployments were too short to get reliable estimates of core habitat use or to identify regions of residency and conversely, areas that might be considered migration corridors. All animals were however, found in at least two different EEZs during the course of the tag deployments. Many also moved through international waters (Table 1, Figs. 1 & 2). The reconstruction of the movement data for shark 54270, that went to 129 days, did show evidence of slower and more tortuous movement patterns consistent with resident behavior for the greatest proportion of the 129 day track using a HMM (Fig. 3). The total track length for this shark was 6766 km and there were straight line movements between regions of residency. The tag popped off between two of these regions and it appears as if this animal was headed back to area where it was originally tagged (Fig 1 & 2). Shark 62936 (100 day deployment, 5652 km travelled) also exhibited straight line paths after periods of tortuous movements and dense location estimates (Figs. 1 & 2). The IKNOS-WALK method could not converge the later location estimates with the site of tag detachment. But the tag began transmitting after it initiated release closer to the original tagging location than was predicted by the model, indicating this animal may have been heading back to the region where it was tagged. The movement of 62936 closely resembled the movement behavior of shark 54270, where there appeared to be areas of residency interspersed with directed movement to different regions and returns to areas that may be core habitats.

### *Vertical movement behavior and diel patterns*

Juvenile silky sharks spent the largest proportion of their time (93.6%  $\pm$  9.6) in the epipelagic zone, above the thermocline, occupying a very narrow temperature range around 29.1

°C in depths less than 90 m (Table 2 & 3; Figures 4 & 5). Vertical data shows the preferred habitat is in the upper 30 m of the water column, where 52% (day) and 45% (night) of their time is spent within these shallow depths with infrequent shallow dives beyond the thermocline (Figs.5, 8, 9). Dive behavior is virtually absent in this species, there were no deeper dives that occurred with any regularity. There was not any evidence of diel shifts in depth or temperature preferences (Tables 2 & 3, Figs. 5 & 7). The reconstruction of the habitat use of silky shark 62936 with the tag's archive record shows a detailed illustration of a juvenile silky shark's vertical and thermal range (Fig 8 & 9). In this region silky sharks remain in the mixed layer and well within the vertical range of a purse seine net at all times of the day (Fig 8). The fine scale vertical movements of this silky shark is illustrated over two different seven day periods where one week shows typical depths and thermal ranges and the second encapsulates two of the deepest dives found in this archival record (Fig. 9). Overall, the tagged juvenile silky sharks spent 99.98% of their time in water shallower than 150 meters which is the mean maximum depth of a purse seine net (Itano et al., 2012). Therefore silky sharks do not exhibit any vertical movement behavior that might signify an escape from a purse seine net in time or space.

#### *FAD residency*

The acoustic data set revealed short term FAD fidelity during the duration of the receiver deployments with repeated evening departures from two of the three silky sharks tagged at two different FADs (Fig. 10). Silky shark 54247 was tagged at FAD object 33 and a VR2W receiver was attached to the FAD for 10 days until the vessel returned to the FAD and the receiver was retrieved and downloaded. CF 54247 had both a miniPAT and an acoustic tag. The fate of this animal is relatively mysterious as both the acoustic and satellite tag data reveal typical vertical behavior for 9 hours post release with consistent acoustic transmissions until it disappeared from the detection range of the receiver (~800 m). At the same time the depth record from the miniPAT shows a constant depth of 35.5 m for one hour until the tag floats to the surface. CF 54249 (also tagged with a miniPAT) and CF 110425 (no miniPAT) were both tagged at FAD object 30. The receiver was retrieved from this FAD after 5.5 days. CF 54249 and 110425 were present at the FAD during the entire deployment. There were regular pre-sunset departures from the FAD every evening at ~ 5:30 pm local time, lasting 3.5 - 6 hours (Fig 10).

## **Discussion**

This dataset is the first satellite telemetry study conducted on juvenile silky sharks captured in a purse seine fishery in the western central Pacific Ocean, a region that is heavily exploited by commercial fisheries targeting tuna. At present the population(s) in the WCPO has been identified as depleted and declining due to over-fishing (Rice and Harley 2013). There are two components to this study that provide essential pieces of information for managers to implement effective conservation strategies. First, I identify horizontal ranges and movement through national and international jurisdictional waters which can be used to facilitate cooperative agreements between nations as mandated by the recent Appendix II listing by the Convention on the Conservation of Migratory Species of Wild Animals. Furthermore, data of this nature can inform stock assessments of potential discrete populations or genetic stocks that have become isolated in time or space due to restricted ranges or movements. Second, an understanding of where juvenile silky sharks are in the water column and how they move through their environment may provide some insight into their ecological role and give rise to different bycatch mitigation strategies. This dataset is small and only represents the movement behavior of the juvenile life stage. But it is extremely relevant for silky shark conservation measures because it is predominantly juvenile silky sharks that are captured in high numbers in both longline and purse seine fisheries in the region (Lawson 2011, Rice and Harley 2013) and because reducing mortality on the juvenile life stages of silky sharks has the most profound effect on future population growth (Beerkircher et al., 2003; Roman-Verdesoto 2014).

### *Horizontal movement*

The most pertinent result that came out of this dataset is that silky sharks may be wider ranging than previously thought and were shown to consistently cross jurisdictional boundaries with intermittent passage through international waters. All of the juvenile silky shark tracks in this study went through at least two exclusive economic zones (EEZ) in a relatively short amount of time (< 30 days), despite exhibiting diffusive and somewhat meandering movements. Thereby highlighting the need for collaborative international management efforts in this region. The reconstructed tracks in this dataset all appeared to have areas of higher density locations interspersed with directed movements into other focal areas. This behavior was particularly evident in the longer deployments seen in sharks 62936 and 52740. Musyl et al. (2011) also

found both modes of movement behavior where seven of their tagged sharks showed diffuse meandering movements and three of their tracks appeared to have directed north south headings. The inability to resolve any true patterns in both of these datasets is primarily due to relatively short deployments where we cannot determine any true seasonality or persistent movements that may occur over the long term (although seasonality is less of a factor in tropical latitudes). Additionally, many shark species exhibit ontogenetic shifts in their habitat requirements and it is reflected in their movement behaviors (Grubbs 2010). The sharks tagged in this data set are all juveniles so shifts in habitat use due to sexual segregation or movements into and out of mating grounds or areas for parturition would not be detected.

I did find evidence of residency however in silky shark 54270, the only deployment that went beyond four months. Silky shark 54270 had very tortuous and restricted movements in two focal areas that was interspersed by straight line movement between the two sites, consistent with 'resident' and 'migrational' behavior state switching (Pedersen et al., 2011). It also appeared that this animal was returning from the second focal area back towards the first when the tag popped off. This was also observed in oceanic whitetip sharks tagged in the Atlantic Ocean (Howey-Jordan et al. 2013). Where several individuals occupied restricted areas of higher use in the first 30 days of the deployments (within 500 km of the tagging area), then made long range movements away from these sites and returned (Howey-Jordan et al. 2013). In this dataset juvenile silky sharks remained within 400 km of the original tagging locations in this same period of time. Silky sharks and oceanic whitetips occupy very similar niches where both are epipelagic with similar vertical ranges that are restricted to the upper mixed layers (Musyl et al. 2011). Incidentally, both species were once considered to be among the world's most abundant shark species but are now depleted mostly due to overfishing (Bonfil et al., 2008).

Furthermore, in the absence of genetic studies which have identified distinct genetic stocks, telemetry datasets such as this one may help scientists reconcile the effects of reproductive isolation which gives rise to differences in recruitment dynamics and a population's ability to recover from fishing pressure. Several life history papers conducted on silky sharks in different regions have shown contrasting vital rates (Branstetter 1987; Bonfil et al. 1993; Oshitani et al. 2003; Joung et al. 2008; Hall et al., 2012). Thus understanding the level and direction of population connectivity is important in marine fisheries management because large differences in natural mortality and growth between populations will have large influences on

productivity (von der Heyden et al., 2014). Identification of the extent of the species' range and oceanographic or geographic barriers to dispersal are necessary to inform managers on the appropriate scale of fisheries management and conservation. This dataset did not result in any tracks that were long enough in duration to draw any conclusions regarding the extent of the range for juvenile silky sharks but it begins to illustrate the nature of their horizontal behavior. Where juvenile sharks are capable of directed long distance migrations in and out of regional waters, they appear to have focal areas of core habitat use at least over the short term.

### *Vertical movement*

The factors that affect the vertical position of pelagic predators within the water column may be species specific, abiotic (e.g. temperature and light) or biotic where the animal is following movements of prey, exhibiting behavioral thermoregulation or there is sexual segregation by depth (Holland and Sibert, 1994; Meyer et al., 2009; Gleiss et al. 2013). Diel vertical movements of ectothermic sharks are often attributed to foraging excursions to the deep scattering layer at night. Diet studies have confirmed the presence of several deepwater or diel vertical migrating (DVM) layer species in the stomachs of pelagic sharks (e.g. blue sharks - Carey et al., 1993; porbeagle sharks- Joyce et al. 2002; white sharks- Weng et al. 2007). In this study juvenile silky sharks did not exhibit diel diving behavior but infrequent and irregular dives beyond 300 m were found in a small proportion of the tracks. Nor did I find large differences in vertical ranges between day and night time depths, nighttime vertical ranges were a bit larger but the differences were typically within 15 meters. One reason could be that juvenile silky shark behavior at FADs is altered and they are not leaving the FAD to go on foraging excursions. This behavior was observed in satellite tagged dolphinfish, *Coryphaena hippurus* (Merten et al. 2014). As dolphinfish became associated with a drifting FAD the presence of FAD associated prey items was influencing their patterns of surface oriented behavior. Alternatively, juvenile silky sharks simply forage in the warm surface layers of the water column. A diet study conducted off the west coast of Baja California found the pelagic red crab *Pleuroncodes planipes* dominated the diet followed by chub mackerel and giant squid (Cabrera-Chavez-Costa et al., 2010). The red crab has a pelagic phase but is abundant over upwelling zones and may be restricted to the California current so it is not likely to be a large component of the diet in silky sharks in the WCPO, but a comparable diet study has not been completed for animals captured in

this region. A telemetry study on 10 juvenile and sub-adult silky sharks that were not captured at FADs near Hawaii found vertical behaviors similar to those in this study (Musyl et al. 2011). Silky sharks north of the equator spent nearly 100% of their time above 100 m in the warmer surface waters and there weren't large diel shifts in vertical structure. Interestingly, despite the silky shark proclivity for surface waters in both higher and lower latitudes silky sharks are captured almost exclusively in the deep-set sector (as opposed to the shallow set or swordfish sector) of the Hawaii based longline fishery targeting bigeye tuna (Walsh et al. 2009). Probably due in part to most swordfish fishing effort occurring in cooler northern latitudes falling outside the silky shark's warmer thermal habitat requirements. Nevertheless longline fishing effort at lower latitudes is responsible for the greatest amount of fishing pressure in the Pacific Ocean (Lawson, 2011; Rice and Harley, 2013).

#### *FAD residency*

Silky shark bycatch at FADs in the tropical tuna purse seine fishery in the WCPO (and elsewhere) contributes a significant component to the total fishing mortality (Rice and Harley 2013). Thus understanding FAD associative behaviors of silky sharks is a key element to mitigating the interactions of this species with the purse seine fishery. It is not well understood why some species are attracted to, and aggregate at, FADs. Scientists believe that FADs may act as ecological traps where fish behavior is altered and may ultimately affect fitness (e.g. Marsac et al. 2000; Dagorn et al. 2010). There is a general lack of information on the associative behavior of juvenile silky sharks at FADs (Filmlalter et al. 2011). Yet in the WCPO purse seine fishery twice as many silky sharks are captured in FAD associated sets than unassociated sets (Clarke et al. ) and aggregations of juvenile silky sharks at FADs has been documented in several studies (e.g. Lennert-Cody et al. 2000; Watson et al. 2008; Filmlalter et al. 2011). A study of ten juvenile silky sharks acoustically tagged in the Indian Ocean at drifting FADs (Filmlalter et al. 2011) found continuous residence times (CRT = periods of absence not exceeding 24 hours) of 0.42 to 10.7 days. Our small acoustic data set of three sharks tagged at two different FADs proved inconclusive in elucidating FAD residence times in the WCPO. This was due in part to low retention and mysterious depth data from shark 54247 tagged at FAD object 33 who disappeared from the detection range of the receiver after only nine hours. Sharks 54249 and 110425 were continuously present at FAD object 30 but the residency study was artificially

terminated when the receiver was removed from the FAD after only 5.5 days. Sharks tagged at FAD object 30 did exhibit repeated evening departures that occurred pre-sunset and lasted 1-6 hours throughout the night. This phenomenon was also seen in the Indian Ocean by Filmalter et al. (2011) where all departures longer than three hours only occurred at night. These excursions beyond the range of the receiver would have to have been horizontal movements away from the FAD because the PSAT tag data did not reveal any diel dive behavior. Clearly, the times and length of residency at FADs needs further investigation. Particularly because this associative behavior at FADs not only lends them vulnerable to purse seine capture but also presents an entanglement hazard, where entanglement in the subsurface structures of the FADs contribute to a largely unknown quantity of additional mortality. An estimation of juvenile silky shark FAD entanglement rates in the Indian Ocean postulates that this source of mortality far exceeds mortality rates from purse seine capture (Filmalter et al., 2013).

### **Conclusions and future directions**

Juvenile silky sharks in the WCPO are wide ranging, frequently cross national boundaries and are found almost exclusively in the upper thermally structured layer of the water column. This thin surface distribution coincides with the ecological niche of tropical tuna species that are the target of extensive fishing effort. Thus if vulnerability to capture is a function of the degree of overlap with fishing gear, baited hooks and purse seine nets set within the thermocline (which is almost all fishing gear targeting tuna) will increase the potential for interactions with silky sharks. Essentially, the ecological niche that silky sharks occupy is what lends this species vulnerable to fisheries capture so interaction mitigation measures need to be focused on reducing capacity, restrictions on FAD use and/or shark deterrents.

In response to these declines in median sizes and CPUE trends (Clarke, 2011) there has been a slow response to reduce the fishing mortality to silky sharks from two out of five of the world's regional fishery management organizations (RFMO). The International Commission for the Conservation of Atlantic Tunas (ICCAT) and the Western Central Pacific Fishery Commission (WCPFC) have banned retention of silky sharks and the WCPFC has issued temporal bans on FAD usage in the purse seine fishery. While several ICCAT and WCPFC parties, including the United States and the European Union, have prohibited retention of silky sharks in pelagic Atlantic fisheries other regional bodies governing vast sections of the silky

shark range have not yet adopted conservation measures for the species. Several silky shark range states, including French Polynesia, Palau, Cook Islands, Honduras, and Bahamas, have banned commercial shark fishing but complementary actions for adjacent waters through which silky sharks migrate are still necessary (Camhi et al.2008; Camhi et al. 2009). The recent listing of silky sharks with Appendix II of the CMS will hopefully encourage the cooperation between these range states to implement more effective conservation strategies.

This statement perfectly encapsulates the fundamental issue, "In the context of declining shark populations and their deleterious ecological effects, accurately identifying spatial organization is key to developing appropriate management plans for the protection of the most vulnerable life stage" (Vandeperre et al. 2014). Quantitative assessment of silky shark movement throughout ontogeny is urgently needed to identify hot spots and the environmental drivers that define these conditions within the WCPO.

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Satellite tags were provided by the International Seafood Sustainability Foundation and the Secretariat of the Pacific Communities. Acoustic tags and receivers were purchased through a generous contribution from WestPac. I would like to thank and acknowledge Frank Mancini for his time and assistance with ArcGIS, JD Filmler and Robert Bauer at IRD for their help with the movement models.

**Table 4-1.** Summary of silky shark biological data and tag deployment details. †denotes a shark that was also acoustically tagged. ø Represents a tag that was recovered. EEZs traversed refers to the number of EEZs a shark visited during the deployment and the \* indicates the animal was also found in a high seas pocket (international waters). For sharks whose tags were shed in 5 days or less ND refers to not enough data to run the movement models.

Shark ID	Sex	Total Length (cm)	Date tagged	Deploy latitude	Deploy longitude	Days at liberty	EEZs traversed	Track length (km)	Mean speed (km hour <sup>-1</sup> )	Distance between tagging and pop-off locations (km)
54245	M	105	05/30/2012	-8.65	-177.35	21	2*	1018.03	2.02	158
54246	M	104	06/03/2012	-9.83	-176.38	32	2*	1584.47	2.20	409
54247 <sup>†ø</sup>	M	104	06/15/2012	-8.27	-169.2	2	ND	ND	ND	ND
54249 <sup>†</sup>	M	93	06/24/2012	-8.45	-169.18	13	2	666.72	2.32	103
54267	F	116	06/28/2012	-8.12	-169.15	2	ND	ND	ND	ND
54270	M	145	06/30/2012	-8.23	-171.08	129	4*	6766.32	2.19	1011
54274	M	144	06/21/2012	-8.55	-168.37	30	3	1437.94	2.00	98
54305	M	127	06/03/2012	-9.83	-176.38	5	ND	ND	ND	ND
62936 <sup>ø</sup>	M	133	06/25/2012	-7.95	-169.58	100	4*	5651.64	2.37	300
62937	M	122.5	06/30/2012	-8.23	-171.08	9	2	576.50	2.67	245
117916	M	123	05/25/2012	-9.65	-175.7	25	3*	1407.11	2.39	207
117919	U	110	06/04/2012	-7.93	-174.35	30	3*	1480.66	2.06	183
117921	M	116	07/07/2012	-8.42	-170.3	30	3	1862.50	2.59	193
117922	M	137	06/23/2012	-8.65	-168.75	30	2	1710.63	2.38	121
117923	M	125	05/24/2012	-10.53	-174.55	15	2	959.51	2.67	153
117925	F	104	06/03/2012	-9.83	-176.38	30	2*	1758.03	2.48	153
117926	F	119	05/30/2012	-8.65	-177.35	30	2	1598.30	2.3	145
117929	M	93	06/04/2012	-7.93	-174.35	24	2	1467.13	2.66	192
1104025 <sup>†</sup>	F	101.5	06/24/2012	-8.45	-169.18	NA	NA	NA	NA	NA

**Table 4-2.** Vertical depth preferences. The mean proportion of time spent in each depth bin by day and at night.

Depth Bins (m)	Day (Mean)	Day (SD)	Night (Mean)	Night (SD)
'0-10'	0.2486	0.2409	0.1813	0.0920
'10-20'	0.1568	0.0874	0.1331	0.0681
'20-30'	0.1131	0.0641	0.1340	0.0479
'30-40'	0.0909	0.0539	0.1141	0.0197
'40-50'	0.0833	0.0473	0.1143	0.0280
'50-60'	0.0844	0.0516	0.1032	0.0423
'60-70'	0.0938	0.0852	0.0960	0.0641
'70-80'	0.0625	0.0730	0.0562	0.0387
'80-90'	0.0348	0.0432	0.0371	0.0344
'90-100'	0.0272	0.0410	0.0253	0.0312
'100-150'	0.0044	0.0086	0.0038	0.0042
'150-200'	0.0002	0.0003	0.0010	0.0010
'200-250'	0.0000	0.0000	0.0004	0.0005
'250-300'	0.0000	0.0001	0.0001	0.0003
'300-500'	0.0000	0.0000	0.0002	0.0008

**Table 4-3.** Thermal habitat preference. The mean proportion of time spent at temperature by day and at night.

Temp Bins (°C)	Day (Mean)	Day (SD)	Night (Mean)	Night (SD)
'< 25'	0.0036	0.0045	0.0051	0.0050
'25-26'	0.0060	0.0054	0.0103	0.0053
'26-27'	0.0200	0.0225	0.0306	0.0295
'27-28'	0.1102	0.1256	0.1579	0.1427
'28-29'	0.6780	0.8017	0.1505	0.1511
'29-30'	0.1822	0.0403	0.2211	0.0563
'> 30'	0.0000	0.0000	0.0000	0.0000

**Figure 4-1.** Juvenile silky shark movements in the WCPO. The exclusive economic zones for each island nation is outlined in black.

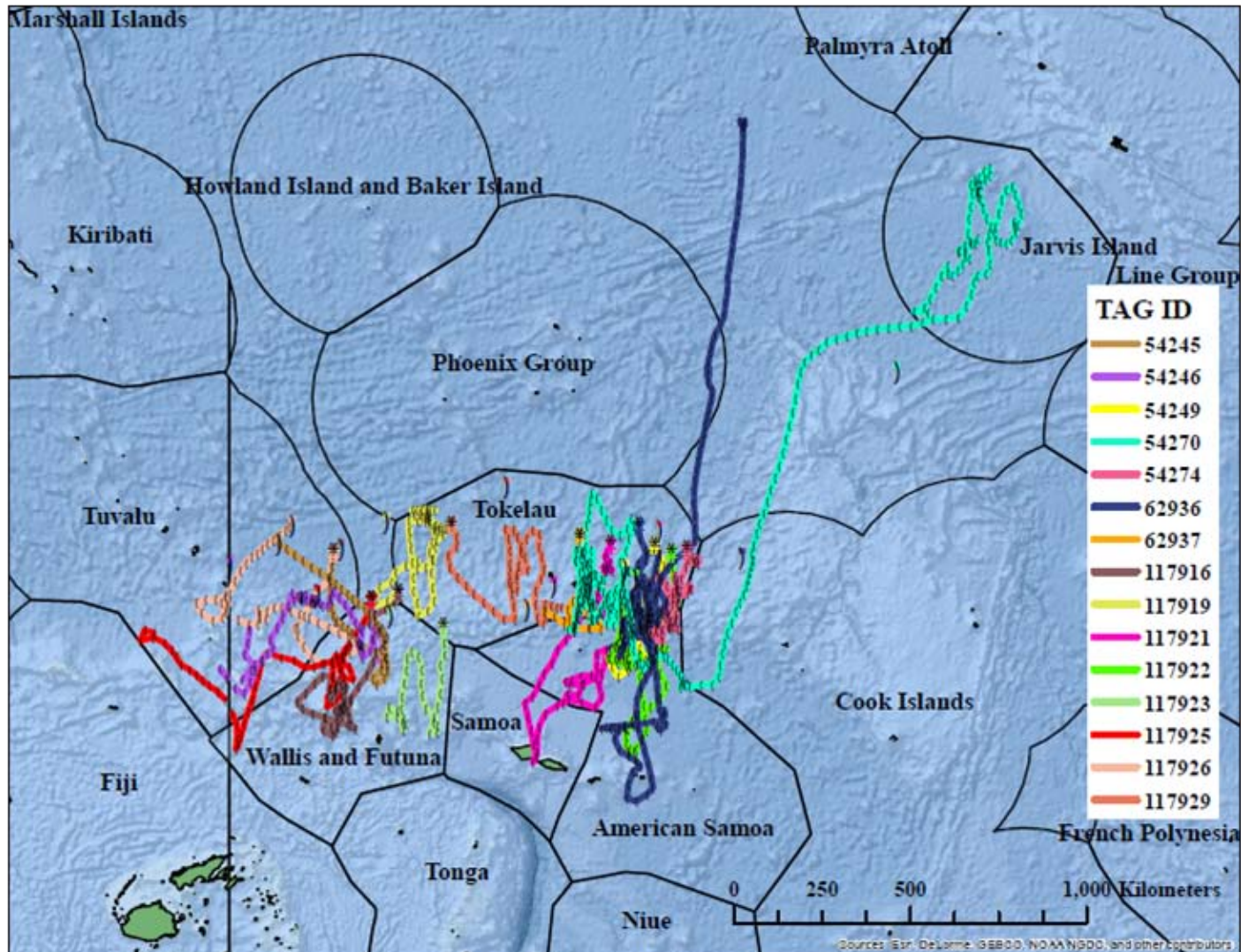


Figure 4-2. Individual tracks.

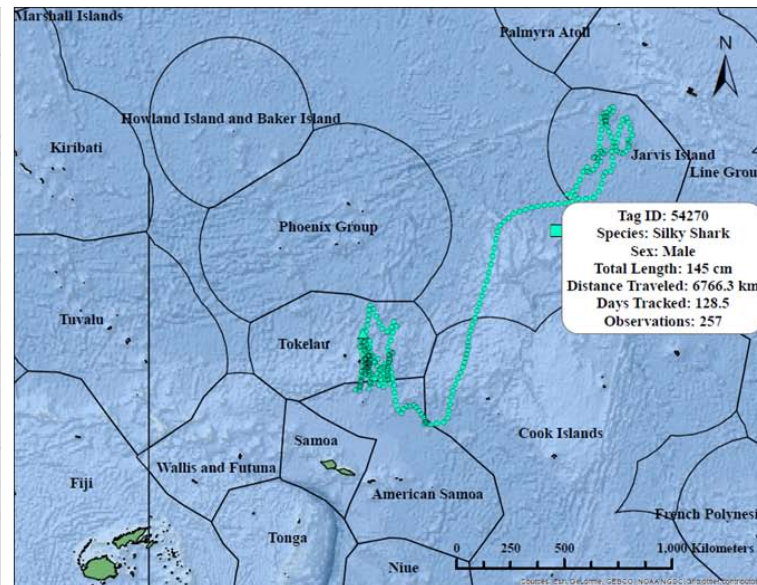
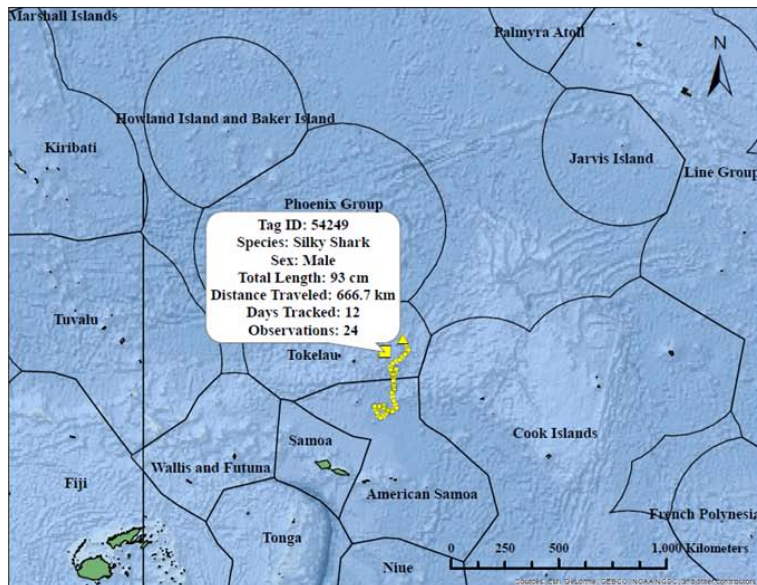
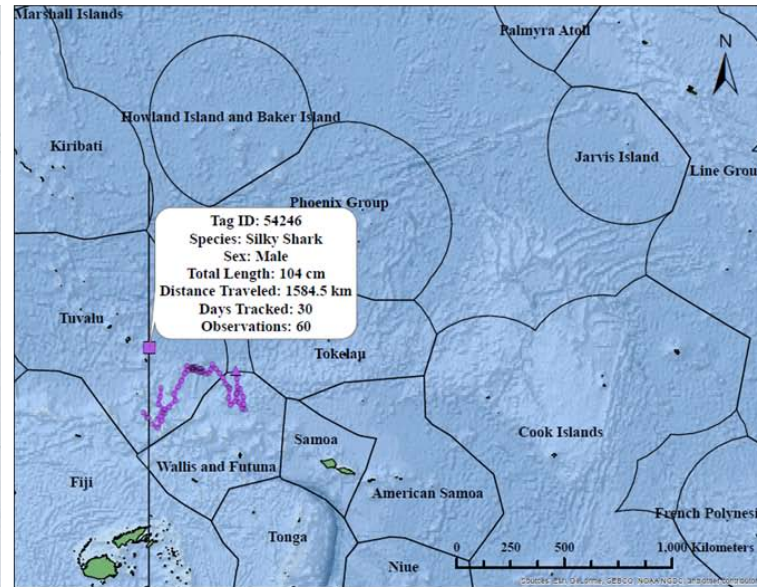
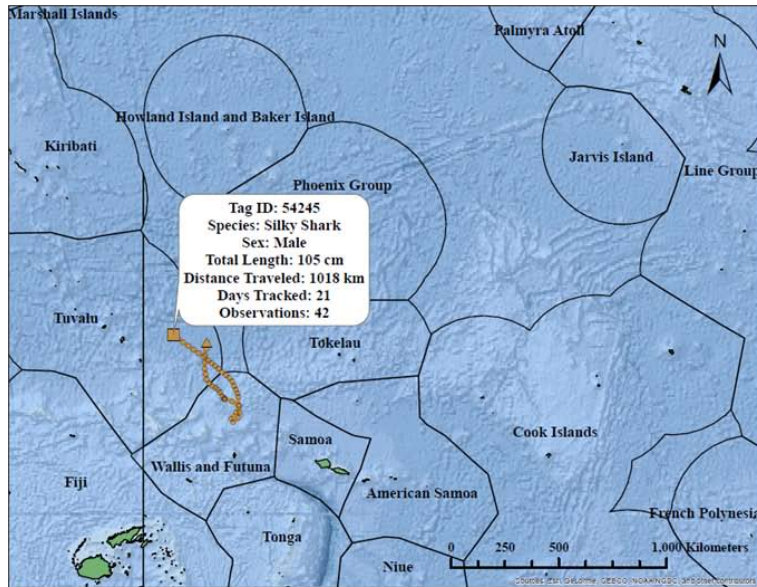


Figure 4-2 (continued). Individual tracks.

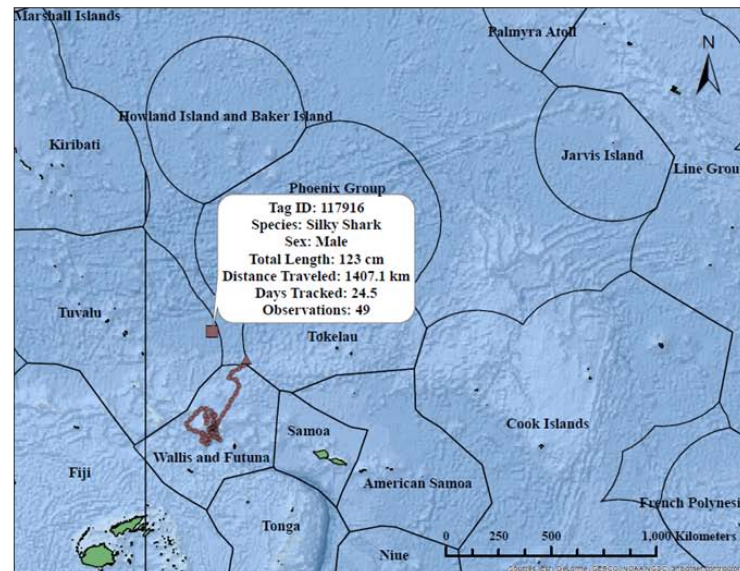
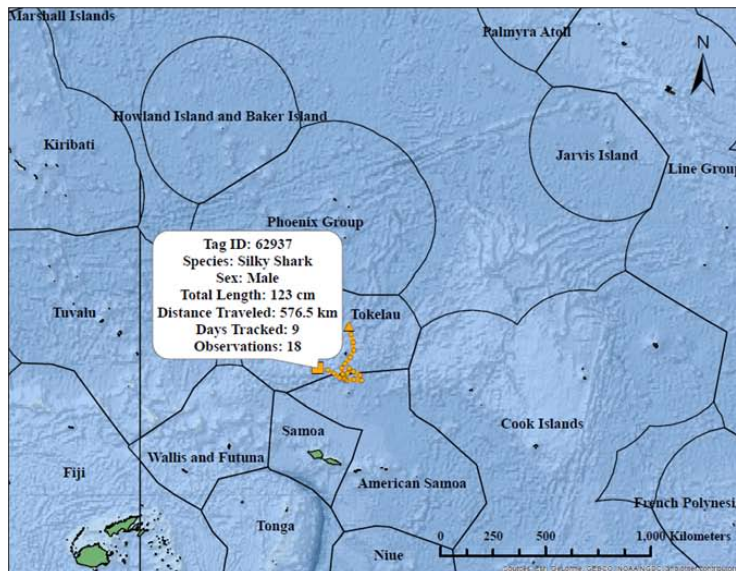
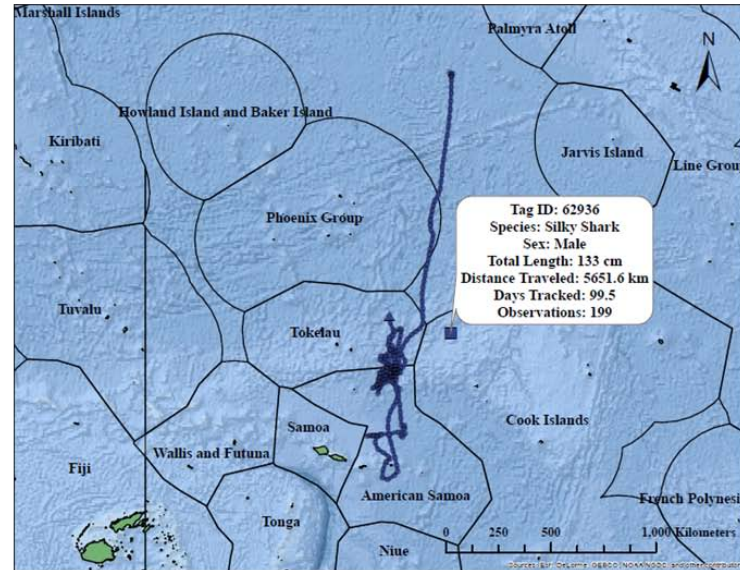
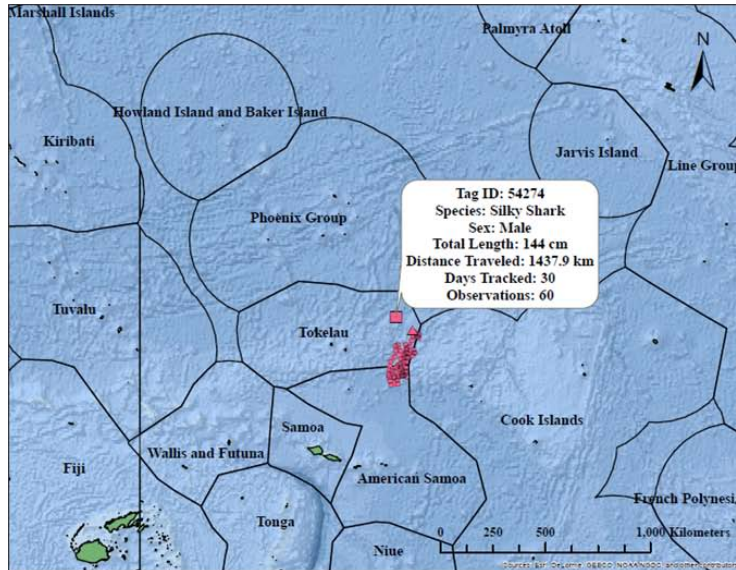


Figure 4-2 (continued). Individual tracks.

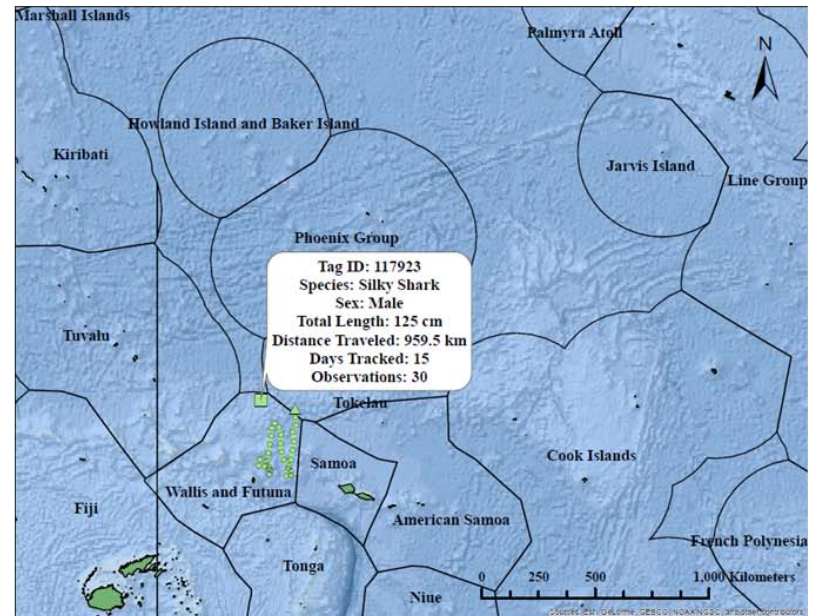
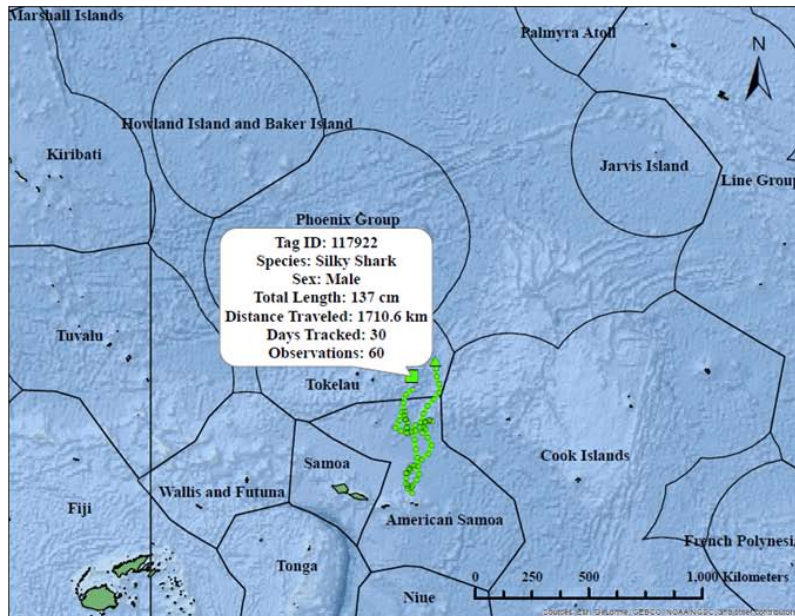
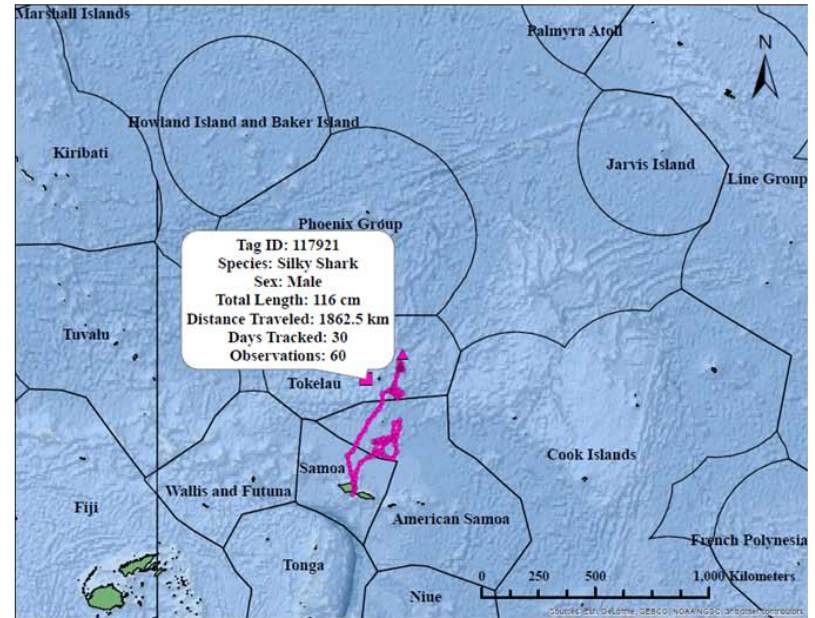
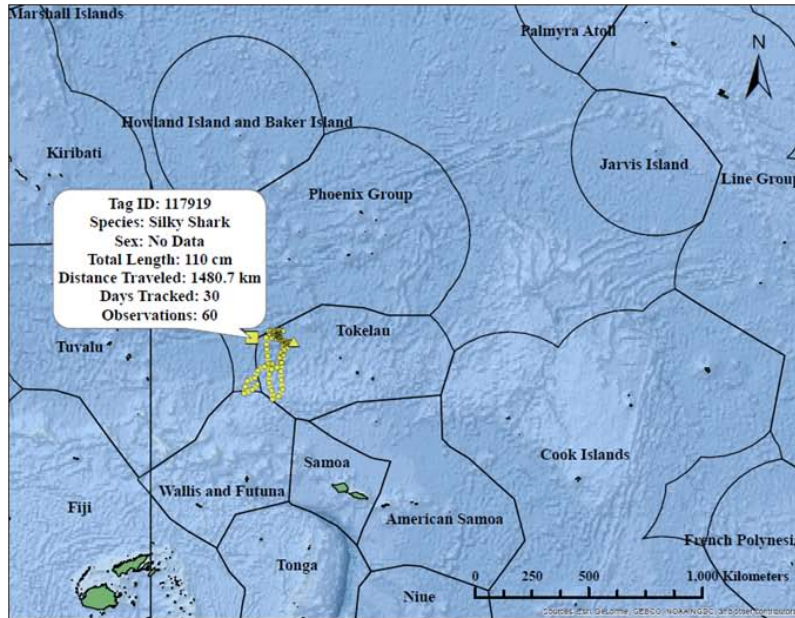
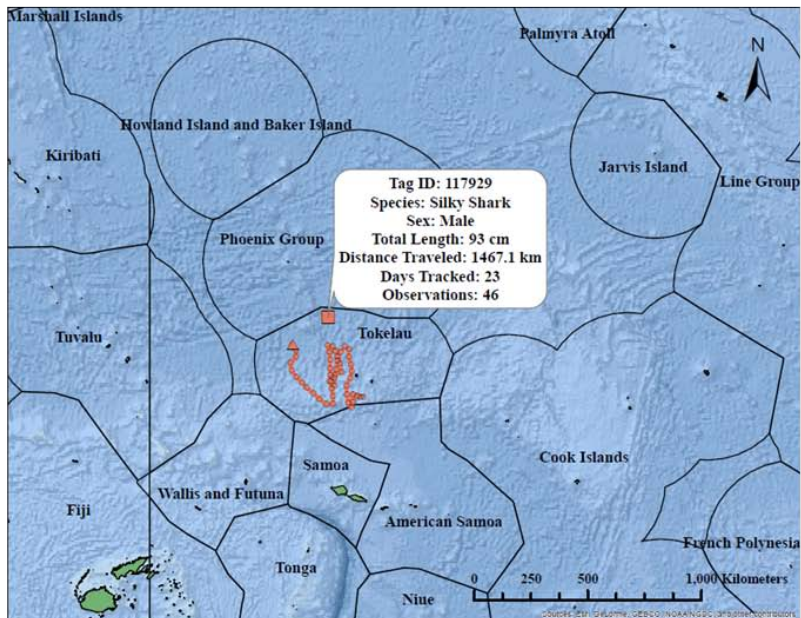
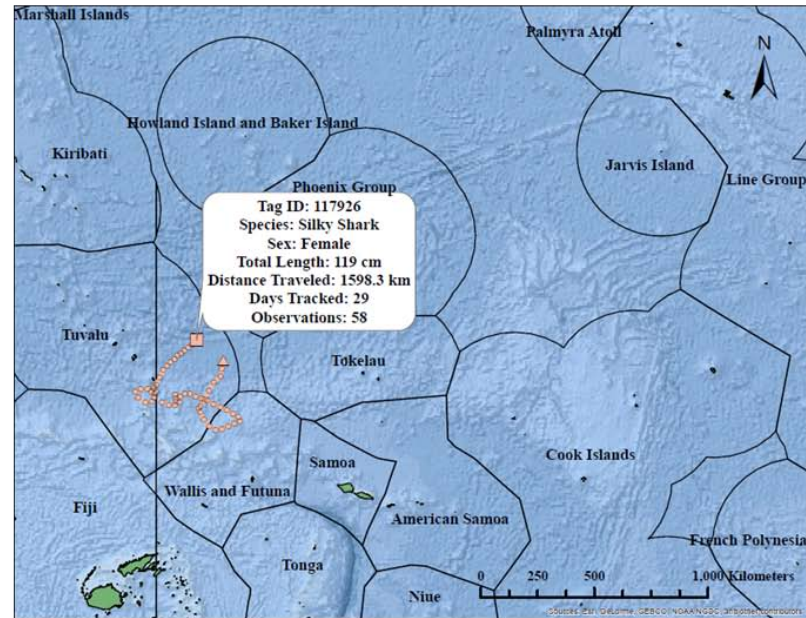
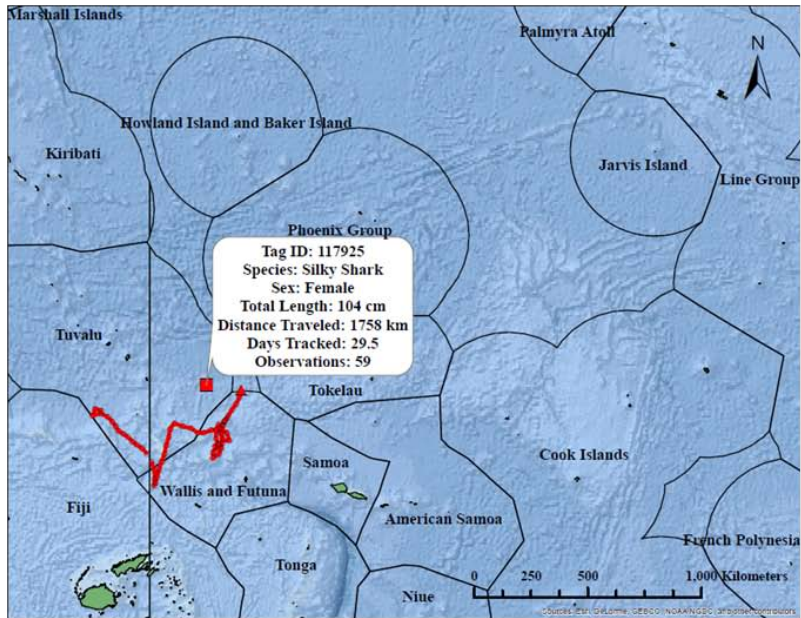
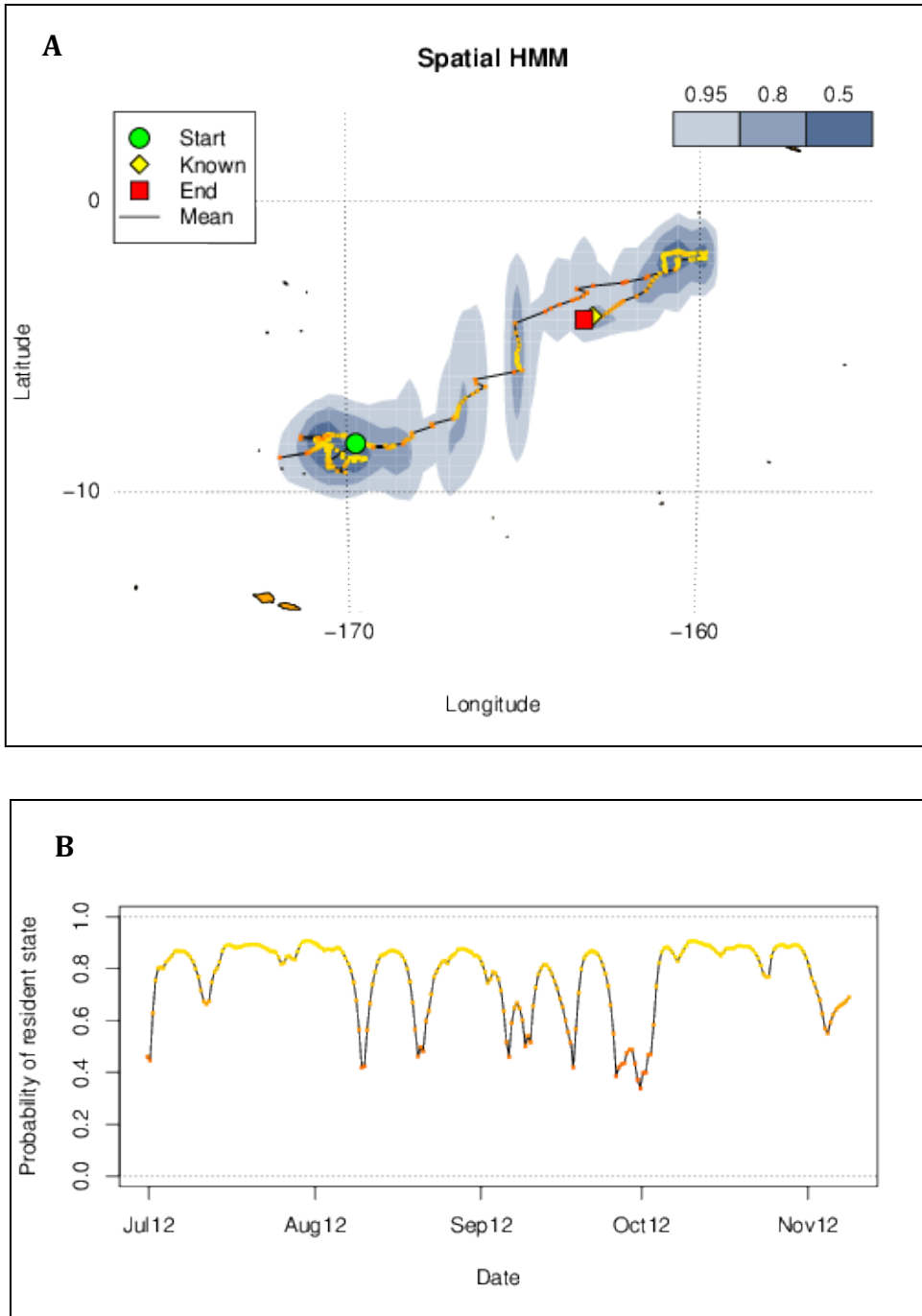


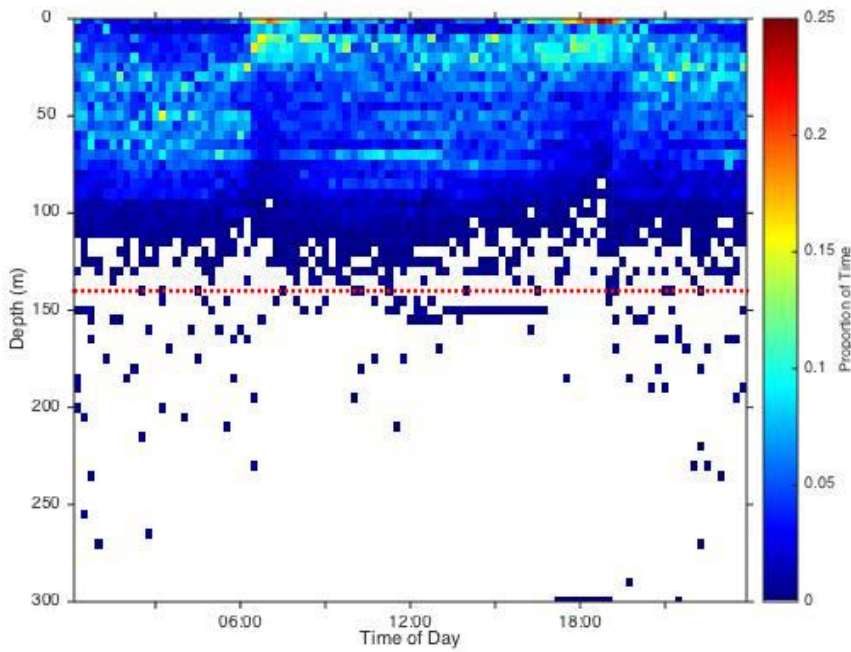
Figure 4-2 (continued). Individual tracks.



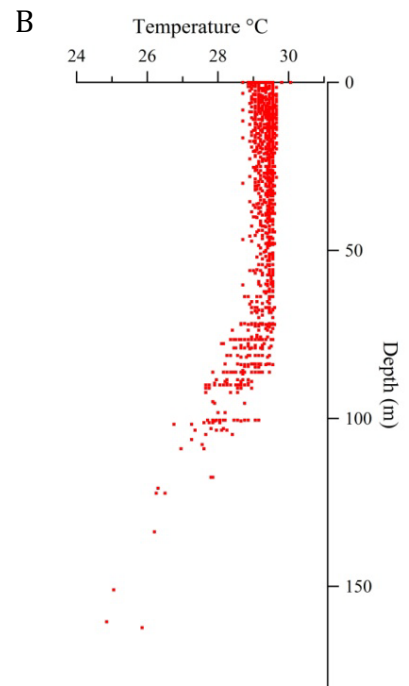
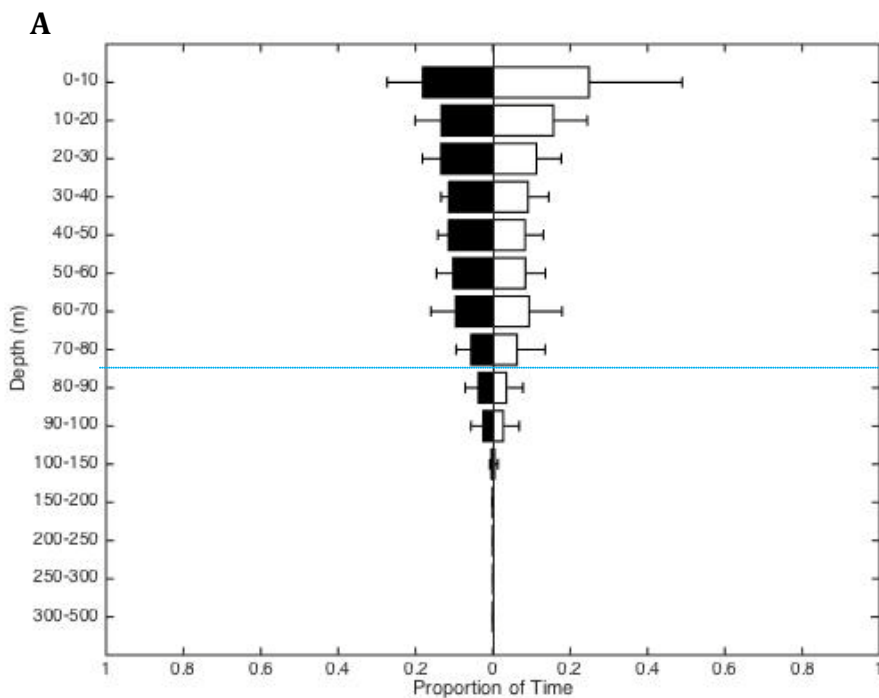
**Figure 4-3.** Spatial HMM for tag 54270 (A), behavioral state probabilities (resident or migrating) heat map in (B). The colors of the points in A and B correspond to the probability of being in a resident state. Yellow points equate to slower distance over ground movements while orange indicates migratory behavior and faster horizontal movements.



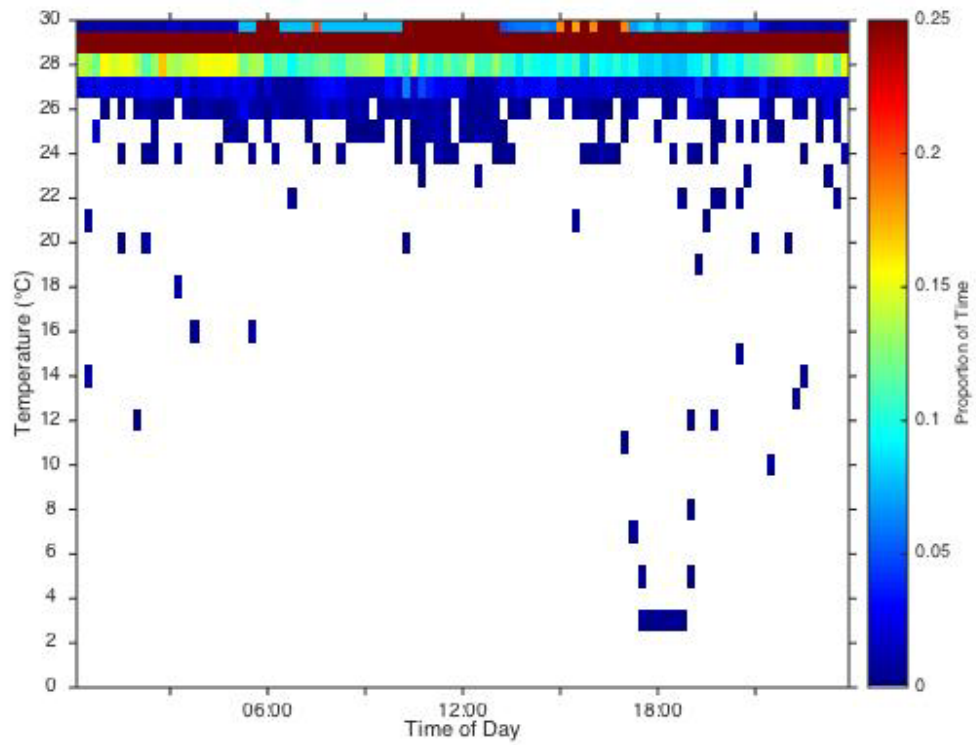
**Figure 4-4.** The proportion of time spent at depth. The dotted red line denotes the mean maximum depth of the purse seine net.



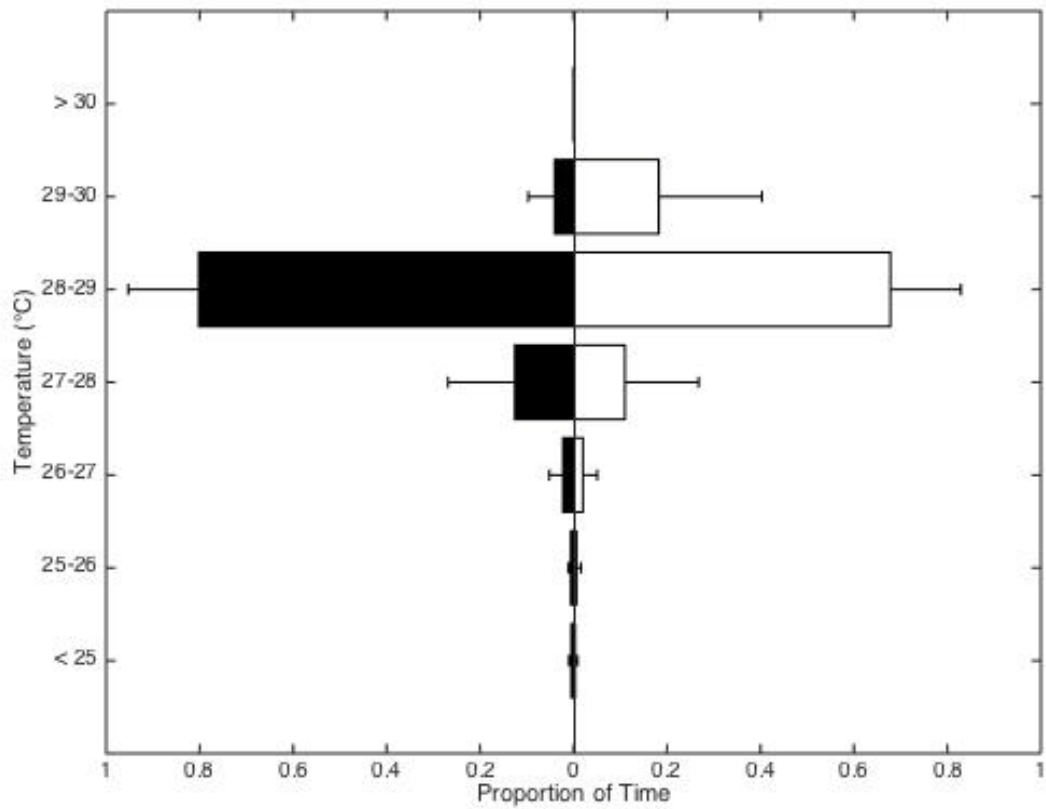
**Figure 4-5.** Proportion of time spent in each 10 m depth bin, at night (black bars) and during the day (white bars). The blue dotted line indicates the depth of the thermocline (85 m). Water column temperature profile as recorded by tag 54246 (B).



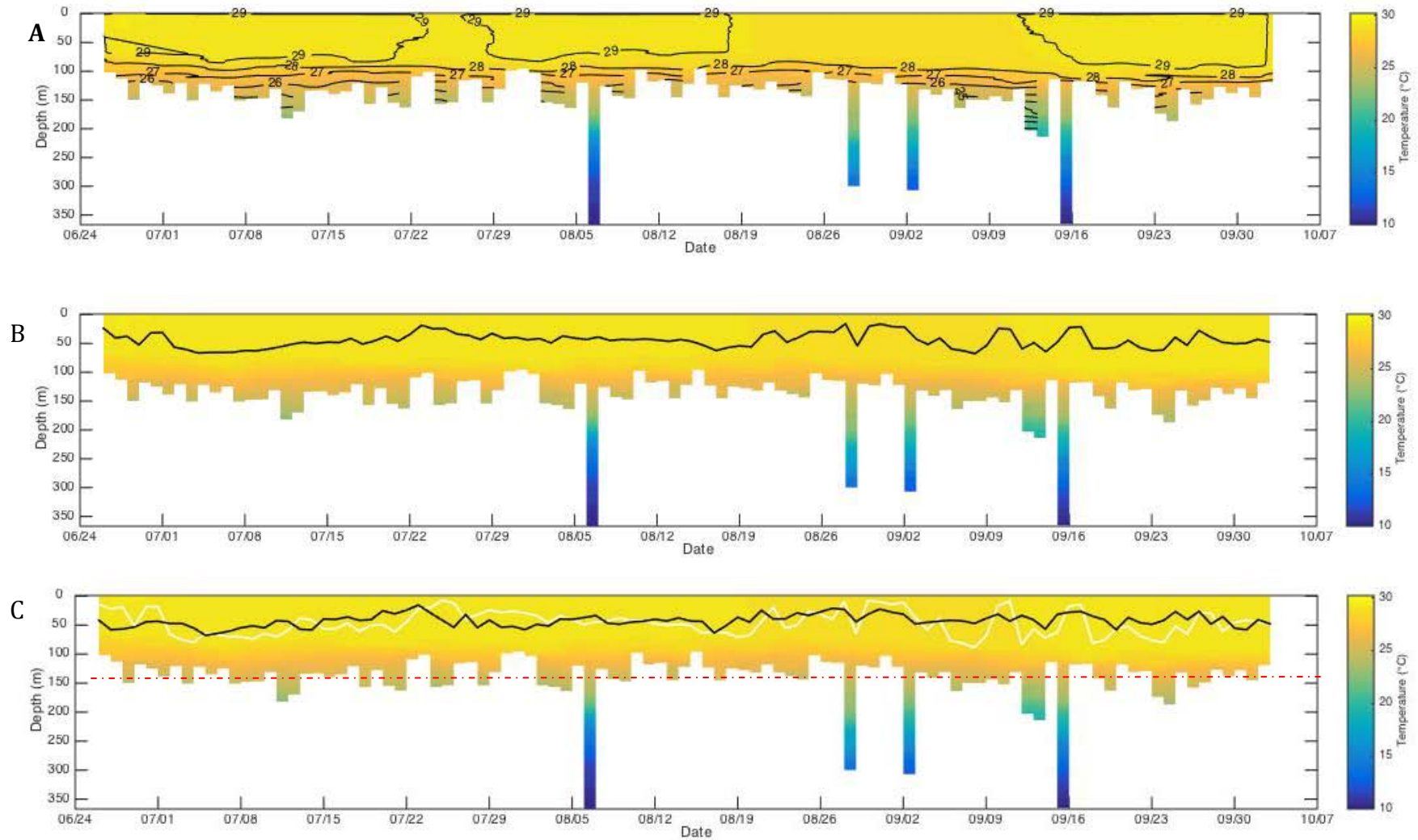
**Figure 4-6.** Temperature preferences. Color coding corresponds to the proportion of time spent at each temperature (A).



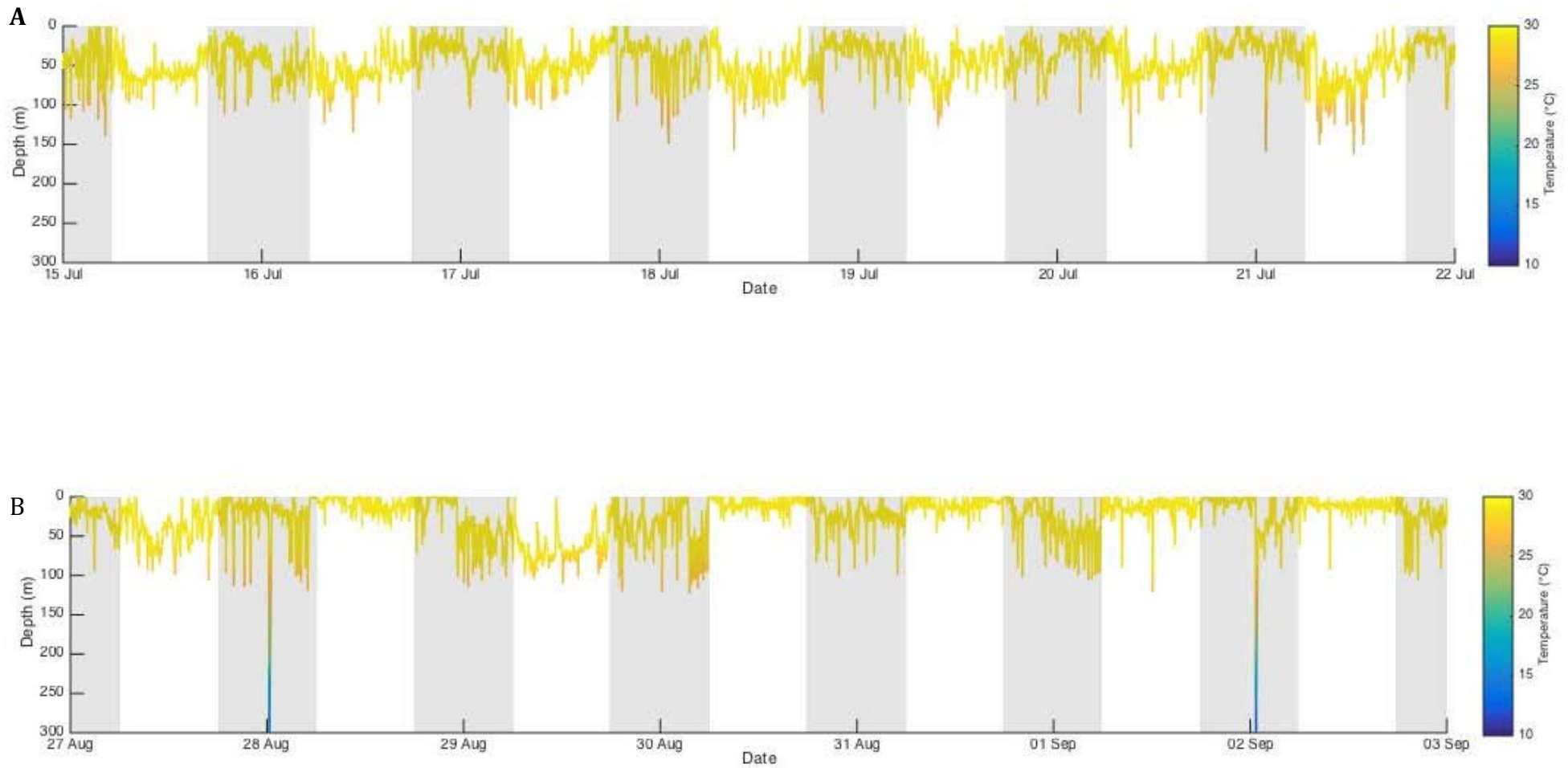
**Figure 4- 7.** Diel temperature preferences, black bars indicate proportion of time spent at temp at night, white bars are daytime proportions.



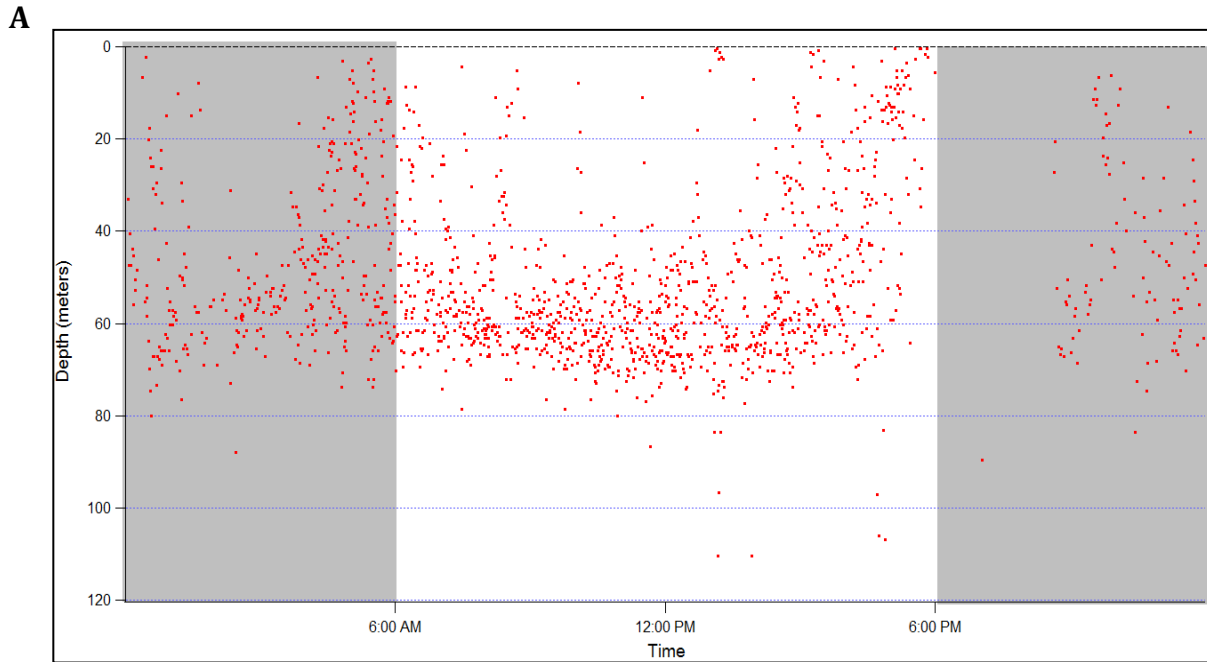
**Figure 4- 8.** Time series plots from the recovered tag 62936 archival record. This tag completed the full deployment period of 100 days. The top plot (A) has contour lines corresponding to temperature gradients. The second (B) plot has a black line denoting overall mean daily depth. The third (C) plot has a black line denoting daily mean depth at night and a white line denoting daily mean depth during the day. The dashed red line in this figure represents the mean maximum depth of the purse seine net.



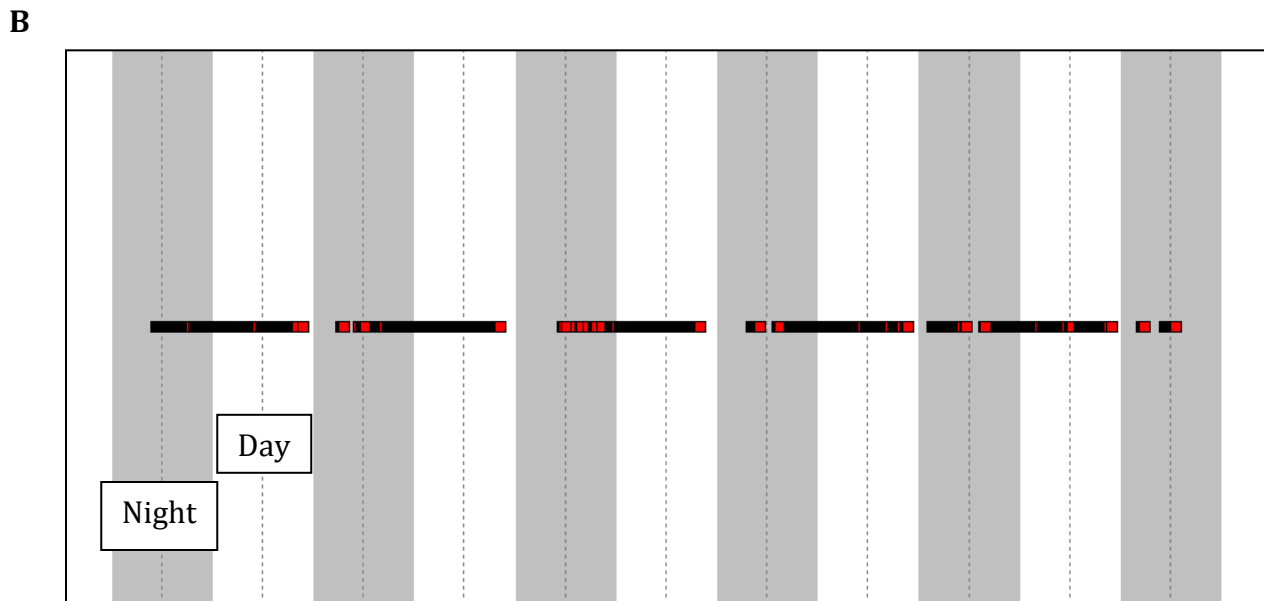
**Figure 4-9.** Fine scale resolution of the diel vertical movements and thermal ranges from shark 62936 archive record. A shows a typical week in the life of a juvenile silky shark, in B two of the infrequent deepest dives are reconstructed.



**Figure 4-10.** Acoustic detections at drifting FADs. All three acoustic tag detections are plotted over a 24 hour cycle in (A). Where the grey shading depicts night and the white background is day.



In (B) acoustic detections for shark 1104025 are plotted over the 5.5 day receiver deployment as red squares. The repeated pre-sunset departures from the FAD are readily visible.



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