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# ELASMOBRANCH LONGLINE CAPTURE: ECOLOGICAL APPLICATION, PHYSIOLOGICAL IMPACTS AND ALTERNATIVE TECHNIQUES

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**ELASMOBRANCH LONGLINE CAPTURE: ECOLOGICAL APPLICATION,  
PHYSIOLOGICAL IMPACTS AND ALTERNATIVE TECHNIQUES**

by

**Edward J. Brooks**

A thesis submitted to the University of Plymouth  
in partial fulfilment for the degree of

**DOCTOR OF PHILOSOPHY**

School of Marine Science and Engineering  
Faculty of Sciences

**December 2012**

*For Dad...*

# **ELASMOBRANCH LONGLINE CAPTURE: ECOLOGICAL APPLICATION, PHYSIOLOGICAL IMPACTS AND ALTERNATIVE TECHNIQUES**

**Edward J. Brooks**

## **ABSTRACT**

Longline fishing is the most common elasmobranch capture method in the world, both for commercial fishing, and to a lesser extent for scientific surveys. The capture of an animal on a longline initiates a series of physiological responses designed to promote survivorship in the short term, but if unchecked, can cause reduced individual fitness and/or mortality in the long term. Given widespread declines in shark populations, an improved understanding of the physiological costs of longline capture is needed. The aim of this thesis was to investigate the physiological response of sharks to capture and restraint, to assess novel, non-invasive alternatives to scientific longline surveys, and to generate scientific insight into poorly understood elasmobranch populations in The Bahamas. The results presented herein suggest that some species of shark are able to recover from the physiological stress of capture despite the presence of persistent negative stimuli. Tonic immobility was assessed as a means of generating baseline blood chemistry data, but was found to be inappropriate given that it increases the magnitude of physiological perturbation in the short term. To avoid the stress of capture altogether, Baited Remote Underwater Video Surveys (BRUVS) were considered as a non-invasive alternative to capture based surveys, however, it was concluded that they lack the resolution necessary to answer fine scale demographic questions. For the Caribbean reef shark, longline surveys yielded high resolution data allowing the identification of fine scale spatiotemporal shifts in demographic population structure with minimal cost (mortality). Nevertheless, the ethics of using capture based surveys on sensitive species are questionable when alternative techniques are available. Deep water sharks caught on longline surveys exhibited high mortality rates, however, for these very poorly understood species moribund specimens have great scientific value which in some cases can offset the high ecological costs of the surveys. The results presented in this thesis highlight the on-going need for improved biological and ecological research into the majority of elasmobranch populations, particularly with regards to anthropogenic interactions such as capture. Given the tenuous conservation status of many species, the acquisition of applied, management focused data should remain the priority of elasmobranch scientists.

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Relevant scientific conferences were attended at which work was often presented. All papers presented in this thesis have been submitted for publication, three (Chapters 2-4) have have been accepted, and two (Chapters 5 & 6) are currently 'In Review'.

### Publications:

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**Brooks EJ**, Sloman K, Liss S, Hassan-Hassanein L, Danylchuk A, Cooke S, Mandelman J, Skomal G, Sims D and Suski C. 2011. The stress physiology of extended duration tonic immobility in the juvenile lemon shark (*Negaprion brevirostris* Poey). *Journal of Experimental Marine Biology and Ecology*. 409:351 – 360.

**Brooks EJ**, Mandelman J, Sloman K, Liss S, Danylchuk A, Cooke S, Skomal G, Philipp D, Sims D and Suski C. 2011. The physiological response of the Caribbean reef shark (*Carcharhinus perezi*) to longline capture. *Comparative Biochemistry and Physiology Part A*. 162:94 – 100.

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**Brooks EJ**, Brooks A, Williams S, Howey-Jordan L, Jordan L, Chapman D, Abercrombie A and Grubbs D. 2012. Diversity, distribution and demographic population structure of deep water elasmobranchs in the north east Exuma Sound, The Bahamas. Ocean Sciences 2012, Salt Lake City, February 2012.

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**Brooks EJ**, Danylchuk A, Cooke S, Mandelman J, Skomal G, Philipp D, Sloman K, Sims D, Liss S and Suski C. 2010. The physiological consequences of longline capture in the Caribbean reef shark (*Carcharhinus perezii*). American Elasmobranch Society Meeting, Providence, Rhode Island, July 2010.

**Brooks EJ**, Sloman K, Sims D and Danylchuk A. 2009. Seasonal abundance, demographics and habitat use of the Caribbean reef shark (*Carcharhinus perezii*) in the north east Exuma Sound, The Bahamas. American Elasmobranch Society Meeting, Portland, Oregon, July 2009.

**Brooks EJ**, Oronti A, Wilchcombe J, Vellacott A, Berry C and Danylchuk A. 2009. Are baited remote underwater video surveys (BRUVS) an alternative to conventional longline surveys for determining the diversity and relative abundance of sharks? American Elasmobranch Society Meeting, Portland, Oregon, July 2009.

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A handwritten signature in black ink, appearing to read 'Brooks', with a long horizontal line underneath it.

Date: 15<sup>th</sup> December 2012

# Chapter One

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## 1. Introduction

Modern elasmobranchs represent one of the oldest extant lineages of fish, yet as a group, they are amongst the most endangered in the oceans (Baum and Myers 2004, Myers and Worm 2003). K-selected life history characteristics and chronic over fishing have caused precipitous declines of many species in every major ocean basin, affecting the stability and structure of marine ecosystems (Field et al. 2009). Despite these extensive declines, fundamental insights into the biology and ecology of most species are lacking, making the implementation of effective management and conservation measures virtually impossible (Simpfendorfer et al. 2011).

Commercial longline fishing is the single largest capture method for sharks around the world, both as a directed fishery, and those taken as by-catch when targeting other species (Beerkircher et al. 2002, Lewison et al. 2004a, Gilman et al. 2008). Despite its prevalence as an elasmobranch capture technique, the lethal and sub-lethal consequences of longline capture on the individual have received very little attention from the scientific community (Skomal and Mandelman 2012). Although the vast majority of sharks captured on longlines are harvested, many are also released alive due to low market value and/or fisheries legislation (Morgan et al. 2009), the latter of which is becoming more prevalent as many nations implement more conservative management measures. The current fisheries management paradigm maintains that sharks captured and released from commercial longlines not only survive, but return to normal behavioural functioning immediately post release (Skomal 2007). Based on the limited physiological and post-release telemetry data, this is an incorrect assumption (Moyes et al. 2006); however, far more research is needed before any in-depth conclusions can be made.



Longline fishing is also a commonly used survey technique by the scientific community (e.g. Holland et al. 2009, Pikitch et al. 2005). Whereas a commercial longline may be many miles in length, have many thousands of hooks and be fished for over 24 hours at a time (Lewison et al. 2004b), scientific longlines are generally less than 100 hooks, and are fished for only a few hours, all measures that are designed to promote survivorship. This type of survey yields high resolution data on the diversity, distribution and relative abundance of sharks in addition to acting as a vehicle for deploying numerous tag and electronic telemetry devices. Despite the value of the data they generate, longline surveys do carry an inherent level of mortality, and, as with commercial longline capture, the lethal and sub-lethal consequences of capture post release are poorly understood (Skomal 2007). An acceptable level of mortality remains an ethical decision based on cost and benefit. Given the applied and typically conservation based focus of modern elasmobranch research, surveys that cause excessive mortality in the target population are becoming less ethically acceptable.

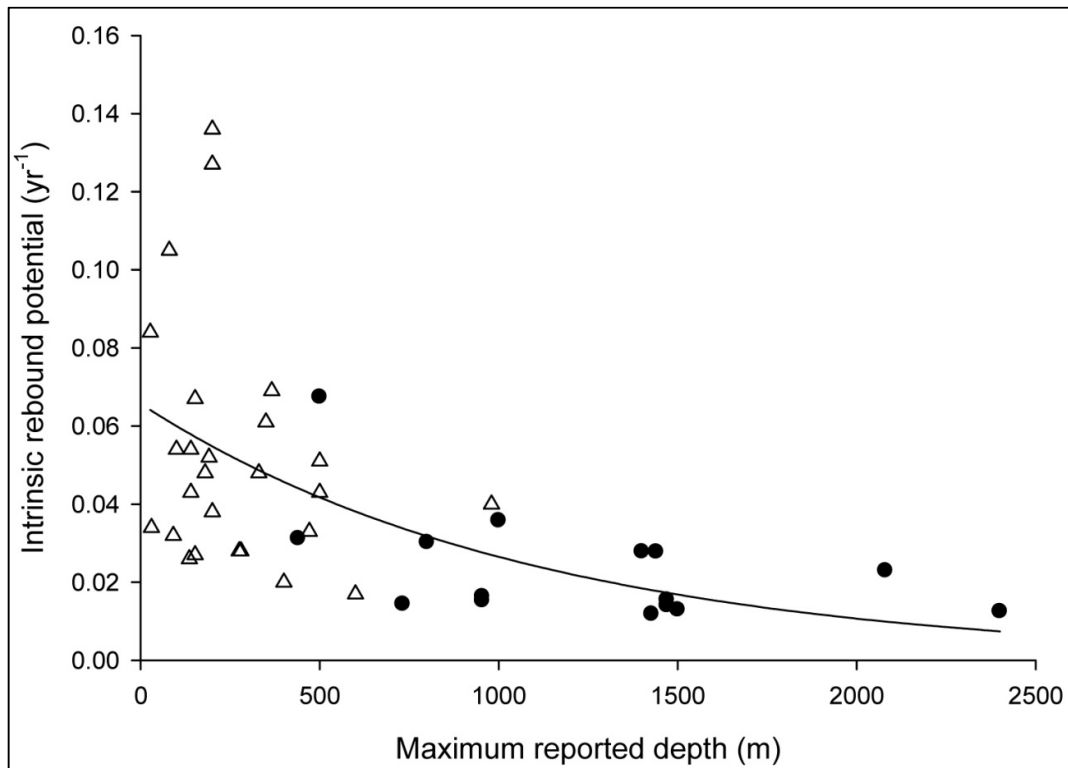
The effective management and conservation of elasmobranch resources is reliant on fundamental information pertaining to their biology and ecology, which at present is lacking for the majority of species (Simpfendorfer et al. 2011). Research into the effects of longline capture on the individual will yield benefits for both longline fisheries managers, and scientists who wish to make informed decisions on appropriate survey techniques. This literature review encompasses general background of elasmobranch fishes, their role within the marine ecosystem, and anthropogenic impacts on populations. It goes on to review common capture based survey techniques and types of data these surveys can generate directly or indirectly via the deployment of tags and telemetry devices. Finally, contemporary literature pertaining to effects of capture on sharks is reviewed and discussed.

## 1.1 The Elasmobranch Fishes

Over 400 million years ago the early ancestors of modern fishes diverged into two evolutionary successful lineages, those which evolved bony skeletons, the Osteichthyes, and those which retained an almost entirely cartilage skeleton, the Chondrichthyes (Compagno et al. 2004). Modern chondrichthyans are further subdivided into two sub classes, the Holocephali also known as the chimaeras, and the Elasmobranchii which includes all sharks, skates and rays. Species of elasmobranch inhabit every corner of the ocean and have become highly evolved to survive and thrive in extreme environmental conditions from shallow, high temperature, high salinity environments of tropical mangrove creeks (e.g. lemon shark, *Negaprion brevirostris*) to the dark, cold, high pressure environment of the deep ocean (e.g. gulper sharks, *Centrophorus sp.*).

Sharks exhibit predominantly K-selected life history traits which include slow growth rates, late ages of maturity, low fecundity and low levels of natural mortality (Bonfil 1994, Smith et al. 1998, Field et al. 2009). This combination of life history characteristics yield some of the lowest intrinsic rebound potentials ( $r$ ) of any fish, however, there are a diverse range of life histories and levels of productivity within the group (Smith et al. 1998). For example, the Australian sharpnose shark (*Rhizoprionodon taylori*) matures quickly (~1 year), is short lived (~7 years), and is relatively fecund producing litters of up to ten pups every year post maturity (Simpfendorfer 1999). These life history characteristics allow the population of this species to double in ~2.55 years assuming that population levels are below the carrying capacity of the ecosystem (Simpfendorfer 1999). At the other end of the spectrum is the Pacific spiny dogfish (*Squalus acanthias*) which matures late (~25 years), is long lived (~70 years), and has low fecundity producing litters of ~22 pups every three years post maturity (Saunders and McFarlane 1993). The estimated population doubling time for the Pacific spiny dogfish is 41.5 years (Smith et al. 1998). In general, deep water sharks

have far slower growth rates than their shallow water counterparts and as a result are far more vulnerable to anthropogenic sources of mortality (Figure 1).



**Figure 1 - Intrinsic rebound potential as a function of maximum observed depth for deep water (closed circles), coastal and pelagic (open triangles) chondrichthyan species (Kyne and Simpfendorfer (2007)).**

Spatiotemporal segregation by size and sex is a typical characteristic of shark populations (Springer 1967, Sims 2005, Wearmouth and Sims 2008, Mucientes et al. 2009). In many species demographically discrete sub-populations typically undertake complex, seasonal movements triggered by environmental (e.g. temperature), ecological (e.g. seasonal food sources), or biological (e.g. reproduction) stimuli (Speed et al. 2012). At present sexual segregation has been identified in only 10% (n = 38) of sharks, however, this is thought to be illustrative of the lack of ecological understanding of the majority of shark species, rather than it being a rare behaviour (Wearmouth and Sims 2008). Sexual segregation was identified in elasmobranch species as early as 1884 in the starry ray (*Amblyraja radiata*, Day 1884), and more recent examples include both

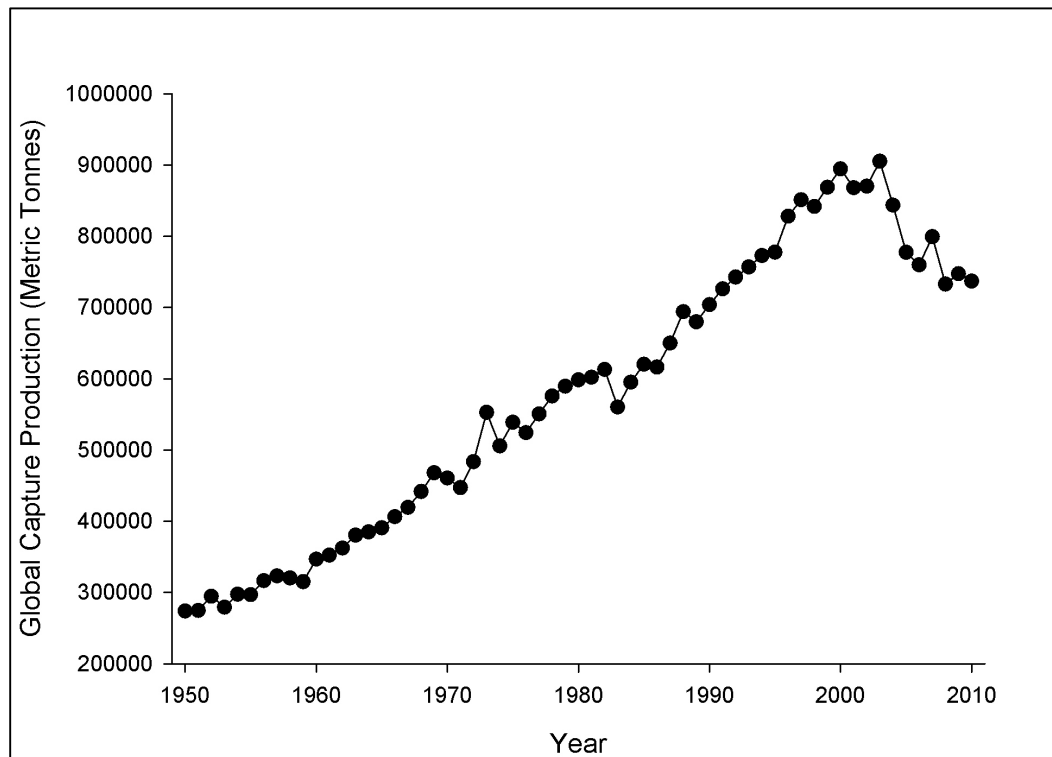
sex and size based segregation in the whale shark (*Rhincodon typus*, Borrell et al. 2011) and the scalloped hammerhead (*Sphyrna lewini*, Noriega et al. 2011). Sexual segregation is also apparent in deep water species including the Portuguese dogfish (*Centroscymnus coelolepis*) and the leafscale gulper shark (*Centrophorus squamosus*) (Girard and De Buit 1999). Identifying spatiotemporal patterns of movement and habitat association, in particular those that incorporate sexual or size based segregation, is critical when developing effective management and conservation strategies for sharks (Dingle 1996; Speed et al. 2012). These demographically stratified movements allow for the repeated harvesting of a single demographic within the stock, potentially skewing gender ratios and disproportionately reducing the overall reproductive potential of the population (Mucientes et al. 2009, Field et al. 2009)

## **1.2 Anthropogenic Impacts on Elasmobranchs**

Historical baselines of shark abundance are difficult to establish as human impact on the world's oceans occurred prior to structured ecological investigation (Jackson 2001). Consequently natural levels of abundance and true representations of decline are difficult to calculate (Jackson 2001, Baum and Myers 2004). Many studies rely on relatively recent data sets for accurate comparisons and still reveal precipitous declines; however, the following studies take into account longer term datasets and indicate more historical declines. Christensen et al. (2003) estimate a two third reduction of apex predators in the north Atlantic during the last fifty years and Myers and Worm (2003) suggest a global biomass of apex predators of just 10% of that prior to the industrialization of fishing. Baum and Myers (2004) show that oceanic white tip (*Carcharhinus longimanus*) and silky (*Carcharhinus falciformis*) sharks in the Gulf of Mexico have declined by 99% and 90% respectively since the 1950s. This level of over exploitation, coupled with K-selected life history characteristics, has led to 28.9% of all

chondrichthyans being listed as vulnerable, endangered or critically endangered on the IUCN Red List ([www.redlist.org](http://www.redlist.org)), and a further 46.0% as data deficient, meaning not enough fundamental data exists in order to formulate an assessment (IUCN 2012).

In recent years elasmobranch fishing effort has increased dramatically due to global declines in fish stocks and an increased demand for shark fins on the Asian market (Rose 1996, Clarke 2004, Field et al. 2009). Global capture production is collated by the Food and Agriculture Organisation of the United Nations (FAO). FAO data describe a steady increase in the global capture of chondrichthyan species from 1950 to a peak of 905,346 tonnes in 2003 (Figure 2).



**Figure 2 - Global capture of chondrichthyan (Sharks, Skates Rays and Chimeras) species 1950 – 2010 (Source: FAO FisGIS).**

The FAO is the only international body to report global fisheries landings, but their database does not provide a comprehensive overview of shark catches, since discards are not reported and a large proportion of the total shark catch is thought to be

illegal, unreported or unregulated. Fisheries independent, scientifically robust estimates of global shark fisheries to which fisheries data can be compared are scarce given the scale and diversity of the fishery. Clarke et al. (2006a) used data derived from Hong Kong fin markets and auctions in 2000 to estimate the annual global catch of sharks to be between 26 - 73 million sharks or 1.37 – 1.91 million tonnes. These figures are over four times the catch reported to the FAO supporting the idea that much of the global shark fishery is illegal, unreported and unregulated (Clarke et al. 2006a).

### 1.2.1 Commercial Longline Fishing

Longline fisheries are diverse in form and scale given the variety of species and ecosystems the technique has been adapted to. Deep water demersal longlines target both teleost and elasmobranch fishes at thousands of meters of depth (e.g. Durán Muñoz et al. 2011), in-shore anchored demersal and surface set longlines are used to target coastal shark species (e.g. Morgan et al. 2009), and unanchored surface or mid-water pelagic lines drift with surface currents targeting pelagic sharks, tuna and billfish (Cortés et al. 2010). These types of longline vary in form but generally consist of a mainline with shorter lengths of line called gangions attached at regular intervals, each of which terminates in a single baited hook. Longline fisheries also vary in scale. The pelagic longline fishery in the north Atlantic routinely sets many thousands of hooks on mainlines up to 100 km in length from large, technologically advanced vessels (Brothers et al. 1999), to small scale artisanal fisheries settings short inshore lines (e.g. Bizzarro et al. 2009, Cartamil et al. 2011).

Commercial longlines, in various forms, are thought to be the single largest elasmobranch capture method in the world, both as a target species and as by-catch (Bonfil 1994, Beerkircher et al. 2002, Lewison et al. 2004a, Gilman et al. 2008). In 2000, the global pelagic longline fleet is thought to have deployed approximately 1.4

billion hooks; equivalent to 3.8 million each day of the year (Lewison et al. 2004b). Ocean basin specific mean catch rates (sharks per 1000 hooks) for all species combined, and estimated pelagic longline effort (total number of hooks) was used to coarsely estimate the total elasmobranch catch on longlines for the year 2000 (Ketteimer et al. 2012). This analysis generated estimates of 11,991,734, 10,967,330 and 667,439 individual sharks in the Pacific, Atlantic and Indian oceans respectively and a total global catch of 22,701,324 sharks. These estimates do not include inshore or deep-water shark longline fisheries.

### 1.2.2 Habitat Degradation

The degradation of key shark habitats can further reduce the resilience of shark stocks to anthropogenic mortality (Manire and Heuter 1995, Feldheim et al. 2002, Field et al. 2009). Coastal areas such as bays, estuaries and mangrove creeks provide protection from predators and an abundant source of prey for a variety of fish species, including many coastal and semi-pelagic sharks (Manire and Heuter 1995, Faunce and Serafy 2006). In the tropical and subtropical western Atlantic, the lemon shark (*Negaprion brevirostris*) uses tidal mangrove creeks as a nursery (Murchie et al. 2010), and the nurse shark (*Ginglymostoma cirratum*) utilises similar inshore habitat as a mating area (Pratt and Carrier 2001). The blacktip shark (*Carcharhinus limbatus*) utilises coastal embayments as nursery habitat (Keeney et al. 2005), and bull sharks (*Carcharhinus leucas*) use estuaries as for a similar purpose (Heupel and Simpfendorfer 2008). These same coastal areas have high levels of development pressure through land reclamation, sand mining, dredging and construction for a variety of industries, the major of which is tourism (Buchan 2000, Gruber and Parks 2002).

Elasmobranchs also associate with a variety of foraging areas dependent on species. Coastal feeding grounds can be subject to the same developmental pressures as

nursery grounds, and also suffer from the global problem of over fishing reducing the availability of prey items for many apex predators (Bearzi et al. 2006). Assessment of habitat degradation effects suffers from the same ‘shifting baseline’ problem as fisheries assessment. The development of the coastal environment occurred before any stratified assessment of habitat usage and shark abundance could be made, consequently the true ‘natural’ abundance of coastal sharks can never be known (Jackson 2001). However, the assessment of fishing and habitat degradation on coastal sharks, which generally have a restricted home range when compared to wide ranging pelagic species, may be possible by comparing impacted sites with areas of minimal human interference (Field et al. 2009).

### **1.3 The Ecosystem Effects of Apex Predator Removal**

The effects of widespread shark removal from marine food webs is not yet fully understood at the ecosystem level, however research suggests that the top-down control can explain variance in lower trophic level abundance seven to ten fold better than bottom-up control (Halpern et al. 2006). Stability of marine ecosystems largely depends on the interactions between predators and their prey, so consequently the effects of predator removal are likely to be felt community wide (Bascompte et al. 2005). Myers et al. (2007) directly linked the decline in the abundance of large sharks such as the blacktip shark (*Carcharhinus limbatus*) to the collapse of a 100 year old bay scallop (*Agropecten irradians*) fishery. Removal of blacktip sharks caused an increase in mid-level consumers (mesoconsumers), in this case the cownose ray (*Rhinoptera bonasus*), which led to the increased predation of the bay scallop and the collapse of the fishery. Recent work by Heithaus et al. (2008) suggests that direct predation, or the lack of, is an important mechanism in propagating trophic cascades; however, these effects can be exacerbated by the combined influence of predation risk. Many mesoconsumers forage



in sub-optimal areas where the risk of predation is reduced, consequently, reducing predation risk allows these mesoconsumers to expand into more optimal foraging areas where they previously were not found.

The effects of widespread apex predator removal on marine ecosystems have only recently become a focus of research, however it is generally accepted that the effects are far reaching, complex and difficult to predict. Given the current precipitous declines in shark abundance and the problematic trophic cascades that they produce, it is vital that marine predators, including sharks, are managed for both density and risk driven ecological processes and not just for demographic persistence (Heithaus 2008).

#### **1.4 Elasmobranch Survey Techniques**

Information on elasmobranchs is gathered from two distinct sources; commercial fisheries logbooks and observer programs (fisheries dependent data), or independent research cruises (fisheries independent data) (Jennings et al. 2001). Generally, fisheries independent data are preferred over fisheries dependent data as they lack the inherent bias associated with commercial or recreational fishers (e.g. changing gear type, methods and harvesting practices over time) (Simpfendorfer et al. 2002). However, fisheries dependent data can provide catch, landings and effort data over larger spatial and temporal scales (Jennings et al. 2001).

Relative abundance estimates incorporate catch and effort data generated by a series of identical fisheries independent surveys, or a single commercial fishing method, conducted over space, through time, or both (Henderson 2003). This allows for comparative measures of abundance between habitats or seasons from which information on species or demographic habitat preference and seasonal movements can be inferred (Chapman et al. 2007). The most common expression of relative abundance

comes in the form of catch-per-unit-effort (CPUE) which has seen considerable application in both the marine and terrestrial environments. For example, annual population trends in coyotes (*Canis latrans*) (Linheart and Knowlton 1975) and European red foxes (*Vulpes vulpes*) (Travaini et al. 1996), were generated from scent traps and individual track counts expressed in individuals per scent trap (foxes/coyotes trap<sup>-1</sup>); the relative abundance of twenty species of bats in relation to landscape variables was determined by standardized mist netting generating values of bats per meter of net per hour (bats m<sup>-1</sup> hr<sup>-1</sup>) (Jaberg and Guisan 2001); and variations in seasonal abundance were detected for four freshwater fish species by using recreational angler effort and catch data to generate CPUE estimates in fish per hour (fish hr<sup>-1</sup>) (Guy and Willis 1991).

Absolute population abundance estimates can be expressed as a number of individuals per unit area (Absolute Population), the number of individuals per unit of habitat (Population Intensity), and for larger animals that are easily observed and counted, (e.g. whales) as a total number of individuals (Southwood and Henderson 2000). Absolute abundance estimates can be generated in a number of ways. Distance sampling generates absolute abundance estimates by quantifying the area scanned by an observer and relating the area surveyed to the number of individuals observed. This technique has successfully generated absolute abundance estimates for a number of cetaceans species during boat based surveys (Buckland et al. 2001), and for angelfish (*Pomacanthidae* spp.) during diver based underwater visual census surveys (Kulbicki and Sarramégnia 1999). Sampling a known volume of habitat such as water, or sediment can provide absolute abundance estimates in individuals per unit volume. This method is commonly used to estimate the abundance of plankton in water whereby the volume of water passed through a plankton net is used in conjunction with the number of plankton caught to generate an estimate of plankton per unit volume (McCauley 1984).

The most common method of generating estimates of absolute abundance in marine species is through mark-and-recapture studies run in conjunction with either fishery dependent or independent capture methods (Henderson 2003). The underlying principle of all mark-recapture methods is as follows: if a sample of a population is captured, tagged and returned to the original population, which is then re-sampled, the ratio of marked individuals to the number of animals in the second sample will be the same as that of the number of animals in the first sample in relation to the total population size (Lincoln 1930). There are a number of variations on this fundamental principle, which assumes a closed population, and modern mark-recapture models have evolved to provide estimates of births, deaths and emigration rates in open populations (Southwood and Henderson 2000). Nearly all mark-recapture models have four underlying assumptions; 1) all marked animals mix completely throughout the population; 2) the probability of capturing a marked animal is the same as that of capturing any member of the population; 3) that sampling is undertaken at discrete time periods, and 4) animal behaviour or life expectancy is not affected by being marked and that marks are not lost over time (Southwood and Henderson 2000). This method has seen extensive application in a number of sharks including the whale shark (*Rhincodon typus*) (Meehan et al. 2006a), the dusky shark (*Carcharhinus obscurus*) (Govender and Birnie 1997), the lemon shark (*Negaprion brevirostris*) (Freitas et al. 2009) and the nurse shark (*Ginglymostoma cirratum*) (Castro and Rosa 2005).

Estimating the biodiversity of an ecosystem has been the subject of considerable research in recent years, largely due to the decreased diversity caused by extinctions (Mendes et al. 2008), and the resulting implications for ecosystem functioning and stability (e.g. Heithaus et al. 2008). The simplest representation of biodiversity is the number of unique species within a community or habitat, also known as species richness ( $S$ ) (Jennings et al. 2001). Species richness is a fundamental component for all

biodiversity estimates, however, in practice the number of unique species counted is a non-linear function of sampling effort and a true value of  $S$  is rarely reached (Southwood and Henderson 2000). Although this has implications for generating estimates of absolute species diversity, these estimates can be used to monitor changes in diversity over time or between habitat as long as sampling technique and effort remain consistent (Buckland et al. 2001). The two most commonly utilized measures of diversity are the Shannon Index ( $H$ ) derived from information theory (Shannon 1948), and the Simpson Index ( $D$ ) based on probability theory (Simpson 1949). Both summarize in a single number a description of the species richness, and species evenness or relative abundance of each species within the sample (Mendes et al. 2008).

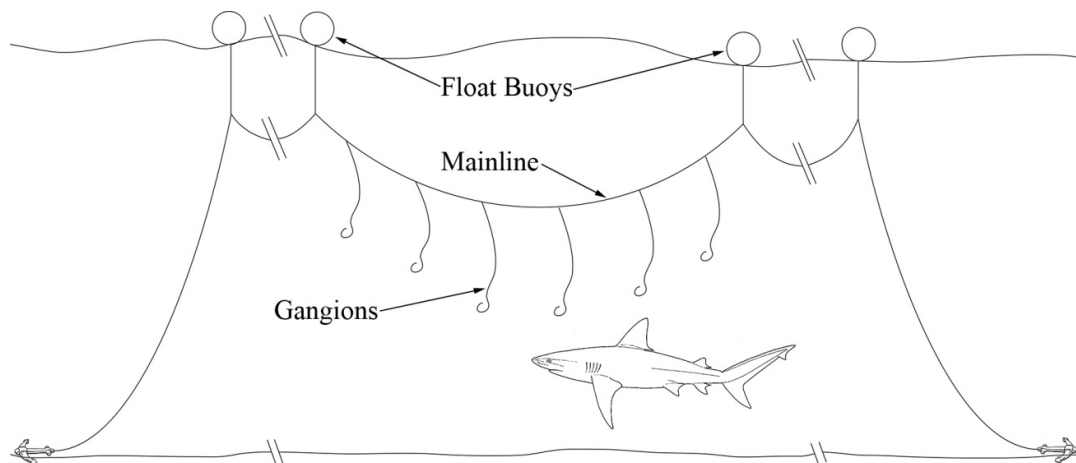
#### 1.4.1 Capture Based Surveys

##### *1.4.1.1 Scientific Longline Surveys*

Longline surveys are the most common way of quantifying relative abundance and diversity indices for sharks (e.g. Simpfendorfer et al. 2002, Chapman et al. 2007). In addition, they can yield data on species diversity and demographic population structure (Pikitch et al. 2005), while providing a vehicle for deploying a suite of telemetry and biologging devices. Longlines consist of a mainline, to which gangions (shorter lengths of line consisting of a clip at one end and a hook at the other), floats, and in some cases weights are attached (Figure 3). The total length of the line, the number of hooks deployed and the period of time the gear is fishing, known as the soak time, varies widely between studies, ranging from an 80 m mainline, 10 hooks deployed and a 60 min soak time (Garla et al. 2006a) to a 2500 m mainline, 140 hooks deployed and a 10 hr soak time (Simpfendorfer et al. 2002). Effort estimates for longlines are usually generated by multiplying the soak time of the gear by the number of hooks deployed. These measures of effort can be combined with catch data to generate

relative abundance estimates for a specific species, or a demographic within a species usually described in sharks per hook per hour (sharks hook<sup>-1</sup> hr<sup>-1</sup>) or multiples thereof (e.g. sharks hook<sup>-1</sup> 100hr<sup>-1</sup>).

Although scientific longline surveys are designed to be minimally invasive when compared to commercial counterparts, every capture event imposes a degree of physiological stress and physical trauma which can reduce individual fitness and induce mortality (Skomal and Mandelman 2012). For example, longline surveys in Belize had an at-vessel mortality of 5% - 7% (Pikitch et al. 2005, D. Chapman pers. comm.). The inherent level of mortality caused by longline surveys conflicts with the typically conservation based objectives of contemporary elasmobranch research. However, the conservation and scientific benefits are typically deemed to outweigh the inherent costs (mortality), and this survey technique is considered ethically acceptable by the scientific community.



**Figure 3 – A typical stationary, inshore, surface set scientific longline.**

#### *1.4.1.2 Drumline Surveys*

Drumlines consist of a central mooring point from which a long length of line or monofilament radiates, terminating in a single baited hook (Simpfendorfer et al. 2005). Drumlines were initially developed in South Africa as a less destructive alternative to

shark nets (Dudley et al. 1998), and have also found extensive use as a capture based survey technique for sharks (e.g. Heithaus 2001, Simpfendorfer et al. 2005, Wirsing et al. 2006). More recently, they have also been utilised in goliath grouper (*Epinephelus itajara*) surveys in Belize (Graham et al. 2009). The major benefit of drumlines over longlines is the vastly increased range of movement offered to the captured animal. It is not uncommon for the line length from the anchor point to the hook to be up to 30 m giving the shark a 60 m diameter circle in which it can swim (Simpfendorfer et al. 2005). Given this wider range of movement, drumlines are thought to cause less physiological impairment compared to longlines although this has never been directly assessed. As such they are a preferred, although not required method, of collecting viable candidate animals for telemetry or biologging tag attachment (Heithaus 2001). Drumlines also offer relative abundance estimates similar to longline surveys. Effort is calculated in hook hours by multiplying the number of drumlines set by the soak time (Wirsing et al. 2006). Catch and effort data are combined to generate CPUE estimates in a similar method to longline surveys.

#### *1.4.1.3 Baited Remote Underwater Video Surveys (BRUVS)*

Baited remote underwater video surveys (BRUVS) generate relative abundance and diversity data similar to longline and drumline surveys, but without actually capturing and physically restraining the animal and without the resulting risk of mortality. Under water imaging has been used to study the marine environment and its inhabitants for over 40 years (Bailey et al. 2007). Deep ocean research pioneered the use of this technology, deploying the first deep sea camera in the 1940s to study bottom topography (Ewing et al. 1967). The first baited camera was developed in 1967 at the Scripps Institute of Oceanography, University of California, San Diego (Issacs 1969), and the first elasmobranch surveyed in this manner was a Pacific sleeper shark

(*Somniosus pacificus*) (Heezen and Hollister 1971). In more recent years, underwater video surveys have become more prevalent in the literature following video's evolution into a cheap and accessible medium (Harvey and Mladenov 2001).

Baited video surveys can be classified by the orientation of the camera, either vertical, look down systems, or horizontal, look out systems (Cappo et al. 2006). Look down systems provide a fixed depth of field from the camera to the seabed, and this fixed distance, in conjunction with quadrats or scale bars, generates accurate length measurements for animals captured on tape (e.g. Willis et al. 2000). This technique does have significant drawbacks in that all animals must be identified by a dorsal view only, and when dealing with larger bodied animals such as sharks, many species cannot physically fit between the bait and the camera (Cappo et al. 2006). A comparative study showed that many species of fish, although attracted to the bait, were shy of entering the space between the camera and the seabed and as such would have avoided detection on the look down baited video system (Langlois et al. 2006). Look out baited video systems overcome many of the non-detection issues associated with the look down systems. Cappo et al. (2004) found that only 58% of the fauna detected on look-out baited video surveys approached the bait canister, and as such, would not have been detected on a look down system. This increased detection is at the expense of the accurate length measurements provided by the look down approach, however, there have been numerous innovations that overcome this deficiency in the form of stereo-video systems (Klimley and Brown 1983, Harvey et al. 2002b, Harvey et al. 2003).

Look-out, baited remote underwater videos surveys (BRUVS) have become the standard approach for larger bodied, more cautious reef fish including sharks (Meekan and Cappo 2004, Meekan et al. 2006a, Malcolm et al. 2007). BRUVS impart a number of major benefits. They are non-invasive and non-destructive and cause minimal

damage to the benthic environment. They include large, mobile animals that avoid divers and active fishing surveys such as trawls. All animals attracted to the vicinity of the bait are 'captured', which is not dependent on the effectiveness of the capture process. There is no size selection that can be prevalent in the choice of hook or mesh size in other capture based survey techniques, and the standardized surveys can be replicated at any depth, in any habitat at any time of day (Cappo et al. 2006).

A number of different relative abundance indices have been generated from BRUVS which include time of first arrival (Priede and Merrett 1996), maximum number of animals viewed on the tape at any one time (Willis et al. 2000, Malcolm et al. 2007), and standard catch-per-unit effort in sharks per hour (Meekan and Cappo 2004, Meekan et al. 2006b). Maximum number has become the most common relative abundance index for studying reef fish assemblages where the animals surveyed are numerous and constantly entering and leaving the video (Cappo et al. 2006). For larger, less abundant species such as sharks, where individuals are more easily identified by sex and size, catch-per-unit-effort is the more common choice. In some cases, absolute abundance has been estimated from baited video surveys, either by modelling the area covered by the bait plume (Sainte-Marie and Hargrave 1987, Priede and Merrett 1996), or by modelling the distribution of the scavenger and the distance travelled to reach the bait (Yau et al. 2001).

The use of BRUVS to quantify shark abundance and diversity has become more commonplace in recent years as it can generate datasets comparable to longline surveys but with no negative impact to the subject animals, a benefit that has become more of a necessity given global declines in shark abundance. To date, BRUVS have successfully been used to monitor sharks all over the world including Australia (e.g. Meekan and



Cappo 2004, Meekan et al. 2006), Florida (C Simpfendorfer pers. com.), The Cayman Islands (Gore et al. In Prep.) and Belize (Bond et al. 2012).

#### 1.4.2 Tagging and Telemetry

Large bodied, highly mobile marine animals such as sharks are logistically challenging to study. Sharks are typically elusive and dynamically distributed within a cryptic environment that is, by terrestrial standards, relatively inaccessible (Myrberg and Gruber 1974). Capture based survey techniques, in addition to generating a variety of data, can facilitate numerous other studies which rely upon affixing of either passive or electronic tags to an individual animal. These tags, in their various forms can provide insight into a myriad of biological and ecological functions over wide temporal scales depending on the device and/or tag that is deployed.

##### *1.4.2.1 Conventional Tagging*

The ability to positively identify an individual when recaptured can generate data on individual growth (e.g. Freitas et al. 2006), movement patterns (e.g. Awruch et al. 2012), survivorship (e.g. Gruber et al. 2001) and population size (e.g. Meekan et al. 2006a). Conventional tags, defined as those which can be identified visually and do not require any specialist equipment to detect, have been used in both fisheries dependent and independent research programs for centuries (Kohler and Turner 2001). As early as 1936, uniquely numbered tags have been deployed on elasmobranchs off the coast of the United Kingdom (Olsen 1953), and they continue to be used in modern research to elucidate life history parameters, stock size, behavioural and distribution patterns, and migration patterns in a variety of sharks (Kohler and Turner 2001). In many studies, a tagged animal is assumed to retain its tag permanently, but this assumption is rarely valid for certain tag types due to problems with bio-fouling and tissue necrosis (Dicken et al. 2006). In addition, certain external dart tags have been found to significantly

reduce growth rates in juvenile lemon sharks (*Negaprion brevirostris*) (Manire and Gruber 1991).

A modern version of the external tag is the Passive Integrated Transponder, or PIT tag, which was developed in the 1980s and has been used to approach many of the same research questions as conventional tags (Kohler and Turner 2001). PIT tags are composed of a microchip and antenna encapsulated in a ceramic or glass case and transmit a unique alphanumeric identification code, usually read by a hand-held scanner (Prentice et al. 1990). These internal transponders are thought to have near 100% retention rates with minimal impact on growth survivorship and behaviour in salmonids (Prentice et al. 1990). However, of 388 lemon sharks tagged a shed rate of 12.1% (n = 47) has been reported (Feldheim et al. 2002), but there were no discernible effects on growth rates in juveniles (Manire and Gruber 1991).

Photographic identification can be used to approach many of the same questions as conventional and PIT tags without actually needing to capture the shark. This technique relies on naturally occurring marks such as skin patterns, scars, tears, marks and notches in fins and tail flukes, to positively identify individuals from a larger population (Hammond 1986, Würsig and Jefferson 1990). Although primarily developed for cetaceans, the technique has been applied extensively to a number of species of sharks including nurse sharks (*Ginglymostoma cirratum*) (Castro and Rosa 2005), bonnethead sharks (*Sphyrna tiburo*) (Myrberg and Gruber 1974), great white sharks (*Carcharodon carcharias*) (Anderson and Goldman 1996, Klimley and Anderson 1996), whale sharks (*Rhincodon typus*) (Meekan et al. 2006a), and raggedtooth sharks (*Carcharhinus taurus*) (Van Tienhoven et al. 2007, Bansemmer and Bennett 2008).

#### 1.4.2.2 *Marine Biotelemetry and Biologging*

Innovation in the field of remote sensing has provided scientists with a suite of biotelemetry (radio telemetry, acoustic telemetry, satellite tracking) and biologging (archival loggers) techniques which can elucidate the daily activities and behaviour of sharks beyond the capture and recapture points offered by conventional tagging alone (Cooke 2008). These tools offer increasingly sophisticated means (e.g. large-scale telemetry arrays, fine-scale positioning, and use of physiological and environmental sensors) of evaluating the behaviour, spatial ecology, energetics, and physiology of free-living animals in their natural environment.

Biologging refers to the use of animal borne sensors which monitor and store data relating to the animal's movements, behaviour, physiology and physical environment (Rutz and Hays 2009). The 1980s saw the widespread development of miniaturised data-logging computers which, by the 1990s, were small enough to be attached to a variety of fish species without affecting swimming ability (Sims 2010). These loggers are capable of storing large amounts of data over extended deployments, and more recently have been able to incorporate a number of sensors which include the temperature, depth, and ambient light levels (Cooke 2008). The drawback to this technology is the need for the tag to be returned for data to be downloaded, except when linked with either a satellite or acoustic transmitters. Biologgers have been successfully applied to a number of questions relating to elasmobranch biology and ecology. The daily diary tags have been used to study the behaviour and metabolism of the lemon shark (*Negaprion brevirostris*) (Gleiss et al. 2009a), and have also been used to infer the magnitude and duration of a tagging response in the whale shark (Gleiss et al. 2009b). Less complicated data loggers have been used to identify diel activity patterns in captive whitetip reef sharks (*Triaenodon obesus*) (Whitney et al. 2007), and have also helped elucidate the bioenergetic strategy underlying diel vertical migration in the adult male

dogfish (*Scyliorhinus canicula*) (Sims et al. 2006). More recently, two biologgers, one logging temperature and depth and the other logging tri-axial acceleration, have been combined in a single tag package. The tag package was attached to several nurse sharks (*Ginglymostoma cirratum*) by way of a timed galvanic release in order to study mating behaviour (Whitney et al. 2010).

Acoustic telemetry, whereby an individual animal is fitted with an ultrasonic transmitter that can be tracked using a hydrophone, has been in use since the late 1950s (Trefethen et al. 1957). Ultrasonic sound waves of 30–100 kHz, which are above most animal auditory ranges and can be transmitted through seawater with minimal energy loss, have become the mainstay of aquatic acoustic telemetry systems (Voegeli et al. 2001). In its simplest form an acoustic transmitter, or pinger, identifies its presence by transmitting a unique identification based on the size of the intervals between a series of pings. More sophisticated tags can, in addition to the unique identification code, transmit data from a wide variety of on-board sensors. Such sensors include but are not limited to depth and temperature (Block et al. 1992), swimming speed (Sundström and Gruber 2002), heart rate (Scharold and Gruber 1991) and stomach pH (Papastamatiou et al. 2007). The sensor is able to modulate the interval between transmitter pings, the length of which usually has a linear relationship with the sensor value, and can be described with slope and intercept (Voegeli et al. 2001). In this way the time between pings can describe an accurate sensor value in almost any engineering unit (e.g. Block et al. 1992).

The pings emitted by the transmitter can be tracked in one of two ways, either by a single boat mounted hydrophone (active tracking), or through an array of stationary acoustic hydrophones (passive tracking), or a combination of the two (Sundström et al. 2001, Cooke 2008). Active tracking requires the use of a portable receiver with a

directional hydrophone and a vessel with which to follow the transmitter and consequently the subject animal. As the animal moves the boat follows, logging the animal's track through the use of a GPS and recording any sensor data from the transmitter via the hydrophone (Cooke 2008). This technique has severe limitations for highly mobile animals like sharks which can travel large distances in a single day and range far from land making long term tracks logistically difficult and prohibitively expensive (Sundström et al. 2001, Voegeli et al. 2001). Active tracking provides excellent data for a single individual over a short period of time, however it is possible to infer long term behavioural or movement patterns using this technique (Sims et al. 2006). Passive acoustic telemetry uses stationary, geo-located, omni-directional acoustic hydrophones to monitor the movements of animals carrying transmitters (Voegeli et al. 2001). These receivers can detect acoustic transmitters within a 500 m radius, archiving location and sensor data until the unit is retrieved and downloaded (Heupel and Hueter, 2002). Detection range varies with a number of environmental parameters such as water depth, surface sea state and salinity (Sims 2010)

Since the 1970s and the launch of the U.S. National Oceanic and Atmospheric Administration (NOAA) polar orbiting ARGOS system, it has been possible to track the movements of animals by satellite (Sims 2010). The potential of satellite telemetry for elucidating the movements of sharks was demonstrated in 1984 when an ARGOS transmitter was moulded into a large buoyant float and attached to a 7 m long basking shark (*Cetorhinus maximus*) (Priede 1984). This resulted in a 17 day track delineated by position fixes generated whenever the tag was able to break the surface and communicate with the ARGOS system (Priede 1984; Priede and Miller 2009). Technological evolution has seen the miniaturization of satellite transmitters, changes in attachment methods, and the incorporation of environmental sensing and archival capabilities into a single tag package (Arnold and Dewar 2001, Rowat and Gore 2007).

These tags have successfully tracked the movements of tiger sharks (*Galeocerdo cuvier*; Heithaus et al. 2007), juvenile whale sharks (*Rhincodon typus*; Rowat et al. 2007), white sharks (*Carcharodon carcharias*; Bruce and Bradford 2008) and salmon sharks (*Lamna ditropis*; Weng et al. 2005).

The reliance of satellite tracking on radio waves for geolocation and data collection via the ARGOS system limits the application of this technology to sharks which exhibit surfacing behaviour (Sims 2010). This led to the development of a hybrid tag, known as a pop-off archival satellite transmitter (PSAT), which incorporates an ARGOS transmitter coupled with a biologger capable of sensing depth, temperature and ambient light (Arnold and Dewar 2001). The PSAT is attached to the subject animal and continually logs water temperature, animal depth and light levels at pre-determined intervals for a pre-determined period of time, after which the tag detaches, floats to the surface, and transmits the archived data via the ARGOS system (Sims 2010). Estimates of the tagged animal's location can be generated from the archived ambient light data by calculation of the local apparent noon and the day length to derive longitude and latitude respectively (Hill and Braun 2001; Musyl et al. 2001). PSATs have become one of the most common methods for answering a variety of ecological and behavioural questions pertaining to elasmobranchs. For example, PSATs were used to identify large scale, trans-Atlantic (Gore et al. 2008) and trans-equatorial (Skomal et al. 2009) movements of basking sharks. Transoceanic migration was identified in white sharks (*Carcharodon carcharias*) when a PSAT deployed in South Africa popped-off in western Australia, a journey of 11,100 km in 99 days (Bonfil et al. 2005). PSATs were also used to identify diel variation in vertical habitat usage and corresponding temperature variation for the Pacific sleeper shark (*Somniosus pacificus*) (Hulbert et al. 2006), and the Greenland shark (*Somniosus microcephalus*) (Stokesbury et al. 2005), and plasticity in depth and temperature preferences in the porbeagle shark (*Lamna nasus*) (Pade et al. 2009).

Caribbean reef sharks (*Carcharhinus perezii*), previously thought to be restricted to the top 40 m of the water column, were found to travel to depths in excess of 350 m experiencing temperatures of as low as 12.4°C, far below those expected for a tropical, coral reef associated species (Chapman et al. 2007).

### 1.5 The Physical and Physiological Effects of Capture

A capture event, such as those incurred on commercial or scientific longlines, imposes various degrees of physical trauma and physiological stress; the magnitude of which is dependent on the capture method and handling time (Skomal 2006, Skomal 2007). If either physiological stress or physical trauma, or a combination of the two, is excessive, then immediate or delayed (post-release) mortality is possible (Figure 4).

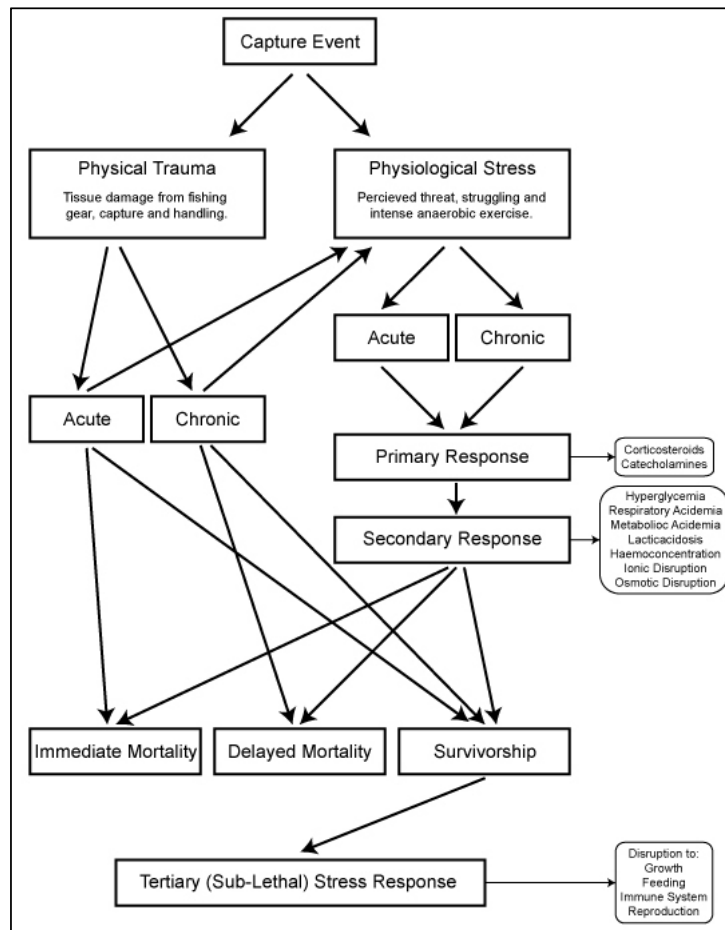


Figure 4 – Conceptual model summarizing the cumulative impacts of capture stress in fish (Adapted from Skomal 2006).

### 1.5.1 Physiological Stress

Elasmobranchs, as with all other vertebrates, initiate the ‘fight or flight’ response to cope with extreme negative stimuli that are likely to cause injury or death (Bradford Cannon 1915). This response instigates physiological changes which prepare the body to either fight or flee thus maximising the chances of survival and minimising the chances of injury (Wendelaar-Bonga 1997). Situations which initiate this response in elasmobranchs can be either natural (e.g. a predation attempt), or anthropogenic (e.g. a capture event) in origin, and either acute (short term high level stressor) or chronic (long term low level stressor) in type (Skomal and Mandelman 2012). The perception of a stressor elicits a primary neuroendocrine/endocrine response in the form of catecholamines and corticosteroids, which cascades into a suite of secondary responses which include increased circulation and the mobilisation of energy resources (Skomal and Mandelman 2012). The primary and secondary stress responses increase the capacity for fight-or-flight and increase survivorship in the short term, however, they are metabolically costly to maintain and in the long term can decrease individual fitness resulting in tertiary, population level consequences (Skomal and Bernal 2010).

#### *1.5.1.1 The Primary Stress Response*

The perception of a stressor triggers a neuroendocrine and endocrine response which rapidly increases the levels of catecholamine and glucocorticoid stress hormones circulating within the blood stream (Skomal and Mandelman 2012). The primary catecholamines found in elasmobranchs are adrenaline and noradrenaline which are secreted from chromaffin tissue located on the surface of the kidney (Randall and Perry 1992). Very few studies have assessed the effect of capture on catecholamine levels. Hight et al. (2007) found that catecholamine levels increased from 100 to as much as



1600 fold above presumed baseline levels in blue and shortfin mako (*Isurus oxyrinchus*) sharks subsequent to capture by pelagic longline fishing gear.

In teleosts, the primary glucocorticoid associated with the stress response is cortisol (Romero 2004), however, in elasmobranchs this is replaced by 1 $\alpha$ -hydroxycorticosterone (1 $\alpha$ -OHB) (Hazon and Balment, 1998, Anderson 2012). The release of 1 $\alpha$ -OHB is triggered as the final phase of the hypothalamus–pituitary–interrenal (HPI) axis which is initiated by the release of corticotropin-releasing factor (CRF) from the hypothalamus, which in turn triggers the release of adrenocorticotrophic hormone (ACTH) from the pituitary gland, triggering the release of 1 $\alpha$ -OHB from the adrenocorticoid gland, also located in the kidney (Gelsleichter 2004). Challenges associated with the measurement of 1 $\alpha$ -OHB have limited its use in assessing the primary stress response of elasmobranchs (Pankhurst 2011, Anderson 2012). Until the corticosteroid is commercially available, and an immunoassay has been validated, progress in this field of research is likely to be limited (Anderson 2012, Skomal and Mandelman 2012).

#### *1.5.1.2 The Secondary Stress Response*

The myotomal muscle mass of fish is dominated (80%-95%) by white, fast twitch fibres capable of high work output in short bursts (Driedzic and Hochachka 1978). This comprises approximately 30% of the total body mass of the fish (Wells et al. 1986). As blood represents only 3%-6% of body mass, changes in muscle biochemistry are strongly reflected in the blood (Wells et al. 1986). By monitoring changes in blood chemistry relative to the degree of physical exhaustion, quantitative data relating to the magnitude of physiological stress can be generated (Skomal and Mandelman 2012).

Secondary stress responses are triggered by the hormonal cascade of the primary stress response, the exhaustive exercise associated with either fighting or fleeing, or a

combination of the two. In general exhaustive anaerobic exercise causes a marked decrease in elasmobranch blood pH also known as acidemia (Mandelman and Skomal 2009). This acidemia can be either respiratory or metabolic in origin, the former due to elevated pCO<sub>2</sub> levels, and the latter to an abundance of H<sup>+</sup> (Skomal and Bernal 2010, Skomal and Mandelman 2012), although the specific metabolic source of this is at present uncertain (Lindinger, 2004; Robergs et al., 2004). Wells et al. (1986) studied blood taken post-mortem from tournament caught game fish and sharks and found that elevated levels of glucose, lactate osmolality, plasma electrolytes, haematocrit and plasma enzymes were useful indicators of stress. Hoffmayer and Parsons (2001) recorded significant increases in glucose, lactate, and osmolality, and decreases in blood pH in Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*) caught on recreational rod and line gear. Manire et al. (2001) studied serological changes in bonnethead sharks (*Sphyrna tiburo*), blacktip sharks and bull sharks (*Carcharhinus leucas*) following capture in a gill net. It was concluded that species specific differences in mortality were likely due to differences in respiratory physiology and the degree of struggling. Skomal (2006) found significant changes in blood pH, glucose, lactate, electrolytes and proteins in rod-and-line caught game fish. It was also confirmed that the magnitude of change correlated with the magnitude of the stressor, in this case angling time.

#### 1.5.1.3 *The Tertiary Stress Response*

Physiological stress and physical trauma can be detrimental to individuals and populations even if they are not of a sufficient magnitude to induce mortality (Skomal 2007). Acute homeostatic disruptions can potentially impact growth, feeding, movement patterns, behaviour and the immune system leading to population-level consequences (Cooke et al. 2002, Skomal 2006). Some behavioural life history aspects can be monitored remotely, post release with existing technology (see Cooke et al.

2002, Donaldson et al. 2008 for reviews). These include location data such as habitat association and movement patterns, environmental data such as depth and temperature selection, and physiological data such as heart rate, activity level and ventilation rate (Cooke et al. 2002). To date very few of these parameters can be measured in large, highly mobile species such as sharks given the limits of technological evolution (Skomal 2007).

Examples of behavioural disruption in large, mobile species include variation in swimming behaviour for black marlin (*Makaira indica*) (Pepperell and Davis 1999), habitat association in white marlin (Horodysky et al. 2008) and disrupted dive profiles in shortfin mako sharks (Holts and Bedford 1993) and blue sharks (Sciarrotta and Nelson 1977, Tricas 1979). Gurshin and Szedlmayer (2004) used active acoustic tracking to study swimming speeds in Atlantic sharpnose sharks following release from standard recreational fishing gear. No significant difference in post release swimming speeds were noted between jaw and gut hooked animals however it is unlikely the internal trauma of gut hooking would have manifested given the short tracking durations (45 - 309 minutes).

Behavioural disruptions for released individuals have been attributed to recovery from physiological stress such as repaying an oxygen debt or post exercise temperature regulation (Holts and Bedford 1993). Skomal (2006) noted that four blue sharks made post release dives below the thermocline and displayed limited vertical movements for a 2-3.5 hour period. Following this period these animals returned to 'normal' behaviour as described in previously published studies and as such it was hypothesized that 2-3.5 hours is the recovery period for blue sharks following exhaustive exercise.

#### 1.5.1.4 *Generating Baseline Physiology Estimates*

A problem for physiological studies targeting large bodied species of fish is the generation of baseline levels of blood chemistry, a fundamental requirement for most piscivorous studies of stress physiology. For smaller, easily confined species this is generally achieved by sensory deprivation for an extended period of time followed by euthanasia and rapid blood sampling (e.g. Suski et al. 2007). This is a technique that cannot be applied to large, free ranging species such as sharks, tuna, and billfish (Skomal 2007, Mandelman and Skomal 2009). For these species past research has used techniques such as the electro-harpooning of bluefin tuna (*Thunnus thynnus*; Skomal 2006) and the tail roping of free swimming blue sharks (Skomal 2006) but there is currently no reliable means for generating baseline blood chemistry estimates for large marine fish (Skomal 2007).

#### 1.5.2 Physical Trauma

The physical trauma of capture is manifested in two different ways including external lacerations and internal trauma. Hook location determines the degree of trauma inflicted and generally jaw hooked fish experience less physical trauma and a higher chance of survival than deep hooked fish (Skomal 2006). Deep hooking in the oesophagus and stomach was found to cause chronic systemic disease (oesophagitis and peritonitis) and hepatic laceration in blue sharks (Borucinska et al. 2002). External lacerations caused by fishing gear, contact with the substratum or rough handling practices are unlikely to be lethal, however can have metabolic costs of repair (Skomal et al. 2002).

#### 1.5.3 Capture Induced Mortality

Survivorship following a capture event can largely be split into two categories, immediate or at-vessel mortality, and post-release or delayed mortality. The latter is

more difficult to quantify for sharks given the technological limitations of tracking large highly mobile animals for extended periods post release (Skomal 2007).

In general, very little research has linked mortality with capture stress. Species specific variation in at-vessel-mortality (i.e. immediate mortality) were reported for the demersal longline shark fishery on the east coast of the United States, ranging from 9% for the tiger shark and 94% for the greater hammerhead shark (*Sphyrna morkarran*) (Morgan and Burgess 2007). Mandelman and Skomal (2008) explained this variation by monitoring the magnitude of acid-base disruption in demersal longline captured sharks and correlating it with at-vessel-mortality rates (Table 1). They also note that the recapture rates from the NMFS Cooperative Shark Tagging Program mirror the at-vessel-mortality rates reflecting species-specific ability to recover from physiological stress.

**Table 1 - Ranked (in ascending order) degree of blood acid-base disturbance in five carcharhinid shark species caught by demersal longline, and two dogfish control species. Degree of disturbance based on highest lactate and pCO<sub>2</sub> values, and most depressed pH (Mandelman and Skomal 2009).**

Species	Median Rank	Physiological Disturbance	At-Vessel-Mortality Rate (%)
Dogfish spp.	1	Least	n/a
Tiger	2		9
Sandbar	3		36
Dusky	5		81
Atlantic Sharpnose	5		n/a
Blacktip	6	Most	88

A survival rate of 100% was noted by Holts and Bedford (1993) for three shortfin mako sharks also using short term, active acoustic tracking and also following release from recreational fishing gear. It should be noted that this study did not attempt to link survivorship to physical or physiological trauma. Short term survivorship in blue sharks was estimated by Skomal (2006). Four blue sharks were tracked for between 4.0-9.2 hours and no mortalities occurred. Sharks had been caught on recreational fishing gear

and angled for 24-64 minutes and survived despite acutely disrupted blood chemistry and in some cases gut hooking. Gurshin and Szedlmayer (2004) used active acoustic tracking to estimate a short term survival rate of 90% for the Atlantic sharpnose shark following capture on recreational fishing gear. They found no significant difference in survivorship between gut and jaw hooked animals, however the short tracking periods were unlikely to reveal the types of internal trauma described in blue sharks by Borucinska et al. (2001).

Pop-up archival satellite tags (PSAT) have been successfully employed to study post release survivorship in blue marlin (*Makaira nigricans*) and white marlin (*Tetrapturus albidus*) by Graves et al. (2002) and Horodysky and Graves (2005) respectively. Neither of the above studies tried to link physical or physiological trauma to survivorship. Many ecologically focused studies exist which apply PSAT technology to sharks (e.g. Weng and Block 2004, Bonfil et al. 2005), however these studies usually select healthy animals for transmitter application and as such conclusions on post release survivorship cannot be drawn. PSAT technology was used to estimate post release survivorship in two blue sharks caught on recreational fishing gear (Skomal 2006). Both sharks survived for the duration of the tag deployment of 121 days. Blood chemistry parameters were commensurate with angling time and one shark was hooked in the gut and one on the jaw, however, given the small sample size only limited conclusions can be drawn.

## **1.6 Summary**

Sharks are arguably the most endangered group of marine animals in the world given their k-selected life histories and severe over exploitation (Field et al. 2009). Conservation and management plans are hindered by lack of fundamental ecological and biological understanding for these important apex predators, and as such primary

research on severely depleted and/or commercially important species must be prioritised (Bonfil 1994, Musick et al. 2000, Baum et al. 2003, Field et al. 2009).

Studying sharks is logistically difficult as they are generally, large highly mobile predators which in many cases undertake large migrations many of which are trans-boundary in nature (Sims 2010, Field et al. 2009, Speed et al. 2012). As such, elucidating behavioural and ecological trends is highly reliant on capture based survey techniques, either to produce abundance and diversity indices between habitats and seasons, or to deploy tagging and electronic tracking devices on which long term behavioural and movement studies are dependent.

The lethal and sub-lethal effects of catch and release events are still very poorly understood for most elasmobranchs (Skomal 2007). The limited research that has been carried out suggests that sharks are able to recover from acute physiological stress (Skomal and Bernal 2010, Skomal and Mandelman 2012), and in some cases severe physical trauma (Skomal 2006). An additional confounding issue is the highly variable respiratory capacities of even closely related species of sharks which ensures that each species will experience varying degrees of physiological stress over the same hooking durations and as such broad conclusions cannot be drawn across species (Mandelman and Skomal 2009).

There is likely a correlation between the magnitude of the stress and both immediate and post-release mortality, however current research has not displayed this conclusively as yet. Additionally, a suite of sub-lethal impacts are expected that can impact behaviour and individual fitness of the captured animal (Cooke et al. 2002), however these questions have not yet been approached. This limited understanding of post-release behaviour and survivorship is largely due to the lack of application of modern telemetry techniques to these specific questions. For example, PSATs have

been shown to provide quantitative survivorship and behavioural data post release for a number of species of marlin (Graves et al. 2002, Horodysky and Graves 2005), however they have seen limited application to a single species of shark (see Skomal 2006, Moyes et al. 2006). In addition, tag packages which incorporate biologgers and VHF transmitters which are pre-programmed to release from the animal after a few days (Whitney et al. 2010), could provide ultra-high resolution data for up to a week post release for species that are likely to remain in a fairly small geographic range (e.g. Caribbean reef shark; Chapman et al. 2007).

The benefits of understanding the true effects of capture are twofold. Firstly capture based survey techniques can be adapted to ensure maximum survivorship and minimum disruption to post release behaviour, which have overarching conservation benefits, but also will ensure the viability of animals carrying increasingly expensive and technologically advanced telemetry packages. Secondly, most fisheries management relies on the assumption that unwanted bycatch or protected species are released from the fishing gear alive with no mortality or impact on individual fitness (Morgan and Burgess 2007). This is unlikely to be the case, and an improved understanding of the true effects of capture will allow managers to make more informed fisheries management decisions.

Despite the evidence suggesting that most sharks do survive post release, every capture technique carries an inherent risk of mortality. Given the conservation based objectives of most shark research programs, ethical decisions need to be made with regards to the impact of the survey technique. If the objective of the research is purely the generation of relative abundance and diversity indices then baited remote underwater video surveys are possibly a less invasive alternative to more traditional



capture based techniques, however this technology is still very new and further validation and testing is required.

Given the above, it is clear that there is an urgent need to gather fundamental ecological data on a wide variety of shark species, in addition to gaining a clearer insight of the impacts of more traditional, capture based, survey techniques. Therefore, the aims of my thesis are to:

- Test a novel method of generating baseline blood chemistry values for elasmobranchs (Chapter Two).
- Examine the physiological response of elasmobranchs to longline capture (Chapter Three).
- Assess the performance of baited remote underwater video surveys (BRUVS) as a non-invasive alternative to traditional capture based survey techniques (Chapter Four).
- Describe the philopatry, seasonal abundance, habitat use and demographic population structure of Caribbean reef sharks (*Carcharhinus perezi*) shark assemblages in the north east Exuma Sound, The Bahamas using stratified longline surveys (Chapter Five).
- Describe the diversity, distribution and relative abundance of deep water elasmobranchs inhabiting the north east Exuma Sound, The Bahamas, using stratified deep water demersal longline surveys (Chapter Six).

## Chapter Two

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### 2. The stress physiology of extended duration tonic immobility in the juvenile lemon shark, *Negaprion brevirostris* (Poey 1868)

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#### ABSTRACT

Tonic immobility (TI) is a reversible coma-like stasis inherent to a variety of terrestrial and aquatic taxa, including elasmobranchs, yet virtually nothing is known about its underlying neurological and physiological processes in any taxa. The purpose of this research was to investigate the physiological effects of TI on the juvenile lemon shark (*Negaprion brevirostris*). Eight juvenile lemon sharks were subjected to four, three-hour treatments during which blood was sampled at 0, 30, 90 and 180 minutes, over a 6 week period. Treatments were differentiated by the method of maintaining the shark, either in TI, or allowed to swim freely between blood samples and the presence or absence of a pre-treatment exercise period designed to simulate the capture induced exhaustion that usually precedes the use TI in the field. The results suggest that TI is an inherently stressful experience, which magnifies the degree of perturbation observed in a number of blood chemistry parameters. It is thought that TI induced a short term reduction in ventilatory efficiency, which appeared to be countered by a series of compensatory mechanisms that include increased ventilation rates, and maintenance of the primary stress response. TI remains one of the most enigmatic areas of biology for all taxa and further research into its underlying psychological, physiological and neurological processes is recommended.

## 2.1 Introduction

Tonic immobility (TI) is an unlearned, reversible, coma-like stasis displayed by a large number of taxa (Gallup 1974). In general, TI is thought to be the final stage of a ‘defensive cascade’ of behaviours initiated in response to the presence of a predator (Ratner 1967). This cascade, which begins with a period of voluntary immobility intended to decrease the probability of detection and heighten responsiveness, then transitions through the ‘flight or fight’ response, and if escape is unsuccessful resulting in capture and restraint (i.e. by a predator), terminates with the onset of TI (Marx et al. 2008). TI is characterised by a catatonic motionless posture and a profound but reversible physical immobility which, in terrestrial vertebrates, is caused by muscle rigidity and unresponsiveness to painful stimulation (Marx et. al. 2008. Ratner 1967).

Nearly all research into TI has focused on terrestrial vertebrates such as lizards (e.g. Edson and Gallup 1972), chickens (e.g. Gallup et al. 1976), guinea pigs (e.g. Bis Vieira et al. 2011) and humans (e.g. Marx et al. 2008). However, this phenomenon is also exhibited by a large number of elasmobranchs (Henningsen 1994, Watsky and Gruber 1990; Whitman et al. 1986). Like many terrestrial vertebrates, TI in sharks is characterised by a state of immobility (Henningsen 1994 Watsky and Gruber 1990), yet in contrast to their terrestrial counterparts, sharks exhibit relaxed muscle tone (the “limp” response; Whitman et al. 1986). In addition, for species with the ability to self-ventilate via buccal pumping, individuals in TI exhibit deep rhythmical ventilations (Watsky and Gruber 1990). In sharks, TI is typically induced by rapid dorsoventral inversion (Watsky and Gruber 1990, Whitman et al. 1986) and its onset is relatively rapid (<1 min – Henningsen 1994, Whitman et al. 1986), lasting for less than a minute to several hours in unrestrained individuals (Henningsen 1994, Watsky and Gruber 1990).

TI is commonly used to safely restrain and handle sharks following capture for both scientific research (e.g. Brooks et al. 2011b, Holland et al. 1999, Murchie et al. 2010) and aquarium husbandry (e.g. Gruber 1980, Henningsen 1994). However, at present, the effects of TI on the physiological homeostasis of elasmobranchs is unknown, especially when coupled with the exhaustive anaerobic exercise and acute physiological disruption associated with most elasmobranch capture events (e.g. Mandelman and Skomal 2009, Skomal 2007). The little research that has been conducted to date suggests that TI is relatively benign, given the limp muscle tone and deep rhythmical ventilations exhibited (Watsky and Gruber 1990). In addition, heart rate and blood pressure have been found to remain stable in blacktip reef sharks (*Carcharhinus melanopterus*) maintained in TI and provided with branchial irrigation (Davie et al. 1993).

The purpose of this project was to investigate the physiological and behavioural effects of extended duration tonic immobility in juvenile lemon sharks. Lemon sharks are members of the largest of the shark families, the carcharhinids, and are widely distributed throughout the tropical and sub-tropical western Atlantic and Caribbean (Compagno 1984). Juvenile lemon sharks are easily captured and maintained in captivity (Dallas et al. 2010, Gruber 1980), and are commonly found in the mangrove creeks surrounding Cape Eleuthera, making it an ideal subject animal for this study. To the authors' knowledge, this study represents the first investigation into the physiological effects of TI in any species to date.

## **2.2 Methods**

This study was conducted between June 9th and October 1st 2009, at the Cape Eleuthera Institute (CEI), Eleuthera, The Bahamas (24.54° N 76.12° W). All research was carried out under research permits MAF/FIS/17 and MAF/FIS/34 issued by the

Bahamian Department of Marine Resources and in accordance with CEI animal care protocols developed within the guidelines of the Association for the Study of Animal Behaviour and the Animal Behaviour Society (Rollin and Kessel 1998).

### 2.2.1 Animal Collection, Transport and Husbandry

Juvenile lemon sharks were collected from local mangrove creeks using conventional hook-and-line angling gear that consisted of a standard spinning rod, a steel leader and a 9/0 circle hook. Upon capture, lemon sharks were transferred to a 200 l cooler of seawater, the hook was removed, the total length (cm) measured and the sex identified. The cooler was transferred to a boat for the journey back to the CEI laboratory, typically taking between 8 - 16 min. To ensure adequate oxygenation during transport, ~50 % of the water in the cooler was exchanged with fresh seawater every 5 min during the journey. Upon arrival at the laboratory, sharks were housed individually in 13,000 l (3.7 m diameter × 1.25 m depth) circular tanks continuously supplied with fresh seawater at a rate of approximately 120 l hr<sup>-1</sup> (Dallas et al. 2010). Individual sharks remained in captivity for 4 - 6 weeks during the experimental period prior to being released back into the creek from which they were caught. Sharks were offered food daily in the form of chunks of bonito tuna (*Euthynnus alletteratus*) or Spanish sardines (*Sardinella aurita*) (Gruber 1980). Dissolved oxygen, temperature and salinity were measured twice daily with a YSI 85 oxygen, salinity and temperature probe (YSI Inc, Yellow Springs, Ohio, USA). Between June and October 2009, four male and four female juvenile lemon sharks ( $\bar{x}$  Total Length = 679 mm,  $\pm$  21.8 S.E.) were captured and maintained according to these protocols. During the course of the experiments the ambient water temperature ranged from 19.2 - 37.1 °C ( $\bar{x}$  = 25.8,  $\pm$  0.15 SE), dissolved oxygen ranged from 5.12 - 9.46 mg l<sup>-1</sup> ( $\bar{x}$  = 6.58,  $\pm$  0.02 SE) and salinity 34.1 - 39.8 ppt ( $\bar{x}$  = 36.6,  $\pm$  0.19 SE).

### 2.2.2 Experimental Design

Juvenile lemon sharks were subjected to a series of four treatments with a minimum rest period of four days between trials. In all cases the subject animal resumed feeding the same day, or the day following a trial. Prior to a trial the water level in the tank was lowered to a depth of approximately 60 cm to facilitate the capture and handling of the animals. During previous laboratory experiments it was observed that shark swimming speed was temporarily elevated immediately following a change in water level; as such, a 24 hr acclimation period was established between the lowering of the water level and the trial. All eight sharks were subjected to four treatments in a random order and each shark was subjected to an individual treatment only once.

During all treatments, blood samples were taken 0, 30, 90 and 180 minutes from the commencement of the trial. Treatments were differentiated by the method of maintaining the subject animal during the course of the three hours, either held in TI by a field assistant, or allowed to swim freely between blood samples, both coupled with the presence or absence of an initial exercise period. Exhaustive exercise, in the form of three minutes of chasing and tail grabbing, was incorporated into the study design to simulate the physiological stress that typically precedes the use of TI. Chasing and tail grabbing have been shown to produce physiological responses similar to those imposed by angling (Kieffer 2000, Suski et al. 2006, Suski et al. 2007, Wood 1991). The four treatments consisted of all possible interactions of these two variables, consisting of two treatments whereby a subject animal was maintained in TI for three hours, one of which had an initial exercise period and one which did not, and two treatments where the subject animal was allowed to swim freely between blood samples, one of which had an initial exercise period and one which did not. Across all treatments, sharks were netted in under 25 seconds ( $\bar{x} = 11.6, \pm 2.3$  SE), inverted until the onset of tonic immobility, which took less than 100 seconds ( $\bar{x} = 60.2, \pm 4.8$  SE), and immediately blood sampled.

The time elapsed from netting to blood draw was typically less than 120 s ( $\bar{x} = 104.7, \pm 7.4$  SE).

### 2.2.3 Behavioural Observations

Lemon sharks supplement ram ventilation by buccal pumping, which allows them to remain stationary on the sea bed for long periods of time (Kessel et al. 2009). Depending on the treatment, one of two behaviours important for respiratory regulation was quantified. For treatments that required the maintenance of animals in TI for an extended period, preventing the use of ram ventilation, ventilation rates were determined by counting the number of contractions of the buccal chamber in one minute ( $v \text{ m}^{-1}$ ; Barreto and Volpato 2004, Chapman et al. 2011, Shultz et al. 2011). Ventilation rates were measured six times over the course of a 15 minute observation period immediately after blood sampling at 0, 30, 90 and 180 minutes. Swimming speed, which is an important behaviour for respiratory regulation in ram ventilating sharks (Parsons and Carlson 1998), was quantified for treatments where the subject animal was allowed to swim freely between blood samples. Relative swimming speed was determined by counting the number of tail beat cycles per minute ( $tbc \text{ m}^{-1}$ ), defined by the complete movement of the tail through the cycle returning to the start position (Graham et al. 1990). This measure was not designed to give absolute swimming speeds, but rather to identify relative changes in swimming speed both within and between treatments. Relative swimming speed was quantified on a similar schedule to ventilation rates, measured six times over the course of a 15 minute observation period immediately after blood sampling at 0, 30, 90 and 180 minutes. Control values for ventilation rates and tail beat frequencies were taken during the 4 – 6 day rest periods between treatments, but not within 24 hrs of the completion of a treatment. Sharks were observed for a minimum of five, 15 minute observation periods during which either

ventilation rates, or tail beat frequencies were quantified every third minute depending on the behaviour of the shark at the time. Behavioural observations were only conducted on four of the eight sharks ( $M = 2$ ,  $F = 2$ ;  $\bar{x}$  Total Length = 680 mm,  $\pm 21.7$  S.E.).

#### 2.2.4 Blood Sampling and Analyses

Blood (~1 ml) was drawn by caudal venipuncture using a 38 mm, 20 gauge needle and a 3 ml syringe (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). All syringes were washed with the anticoagulant sodium heparin prior to drawing blood. A portion of the blood sample was immediately transferred from the syringe to a 0.5 ml microcentrifuge vial. From this vial, approximately 100  $\mu$ l of whole blood was immediately transferred from the syringe into an iStat point of care device (Heska Corporation, Fort Collins, CO, USA) for immediate analysis of pCO<sub>2</sub>, pH, and lactate (Mandelman and Farrington 2007, Mandelman and Skomal 2009, Gallagher et al. 2010, Brooks et al., 2011a). The time from blood draw to the insertion of the cartridge into the iStat was < 4 min. To confirm there was no significant change in blood chemistry parameters during this period a series of six samples were run in duplicate, one using the standard protocol and one using blood transferred directly from the syringe to the iStat cartridge <1 minutes post blood draw. A pooled t-test showed no significant differences between the standard protocols and the immediate analysis of the blood for pH ( $p = 0.885$ ), pCO<sub>2</sub> ( $p = 0.759$ ), HCO<sub>3</sub> ( $p = 0.676$ ) and lactate ( $p = 0.964$ ). Glucose was measured by adding 10  $\mu$ l of whole blood to an Accu-Chek glucose meter (Roche Diagnostics, Basel, Switzerland) which has previously been validated for use on fish (Cooke et al., 2008). The balance of the sample was transferred to a 3 ml vacutainer containing lithium heparin (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and spun in a centrifuge (Clay Adams Compact II Centrifuge) for 5 min at 10,000



g to separate plasma from red blood cells. Plasma was transferred to a microcentrifuge tube using a pipette and stored at -20 °C.

Plasma samples were subsequently transported in liquid nitrogen to a laboratory at the University of Illinois where they were stored in an ultracold freezer (-80 °C) prior to analysis of ions and urea concentrations. Plasma sodium and potassium levels were quantified using a flame photometer (Cole-Parmer Single-Channel Digital Flame Photometer, model WU-02655-00, Vernon Hills, IL, USA). Plasma urea, chloride, magnesium, and calcium levels were quantified using commercially available kits (BioAssay Systems, Hayward, CA, USA – Urea DIUR-500, Chloride DICL-250, Magnesium DIMG-250; Calcium DICA-500).

#### 2.2.5 Conversions

The iStat point of care device is designed for use on endothermic animals, and, as such, is thermostatted to 37 °C. To accurately quantify *in vivo* blood gas and pH values for exothermic species, it is necessary to correct for temperature (Reeves 1977). Each pH and pCO<sub>2</sub> value generated was therefore adjusted using in tank seawater temperature taken at the beginning of each treatment (Mandelman and Skomal 2009, Gallagher et al. 2010). Recent research has suggested that species-specific conversions are required to generate absolute blood gas and pH values from raw iStat data (Gallagher et al. 2010). However, given the present lack of conversion values specifically for the lemon shark, established protocols described by Mandelman and Skomal (2009) were used, which provide relative differences in pH and pCO<sub>2</sub> whilst taking into account variation in water temperature over the course of the study. Bicarbonate (HCO<sub>3</sub><sup>-</sup>) was calculated via the Henderson-Hasselbalch equation using temperature corrected pCO<sub>2</sub> and pH values (Brooks et al. 2011a, Mandelman and Skomal 2009).

### 2.2.6 Data Analysis

All analyses were performed using JMP 7.0.1 (SAS Institute, Cary, NC, USA) and the level of significance ( $\alpha$ ) for all tests was 0.05. Data were analysed using a repeated measures multivariate analysis of variance (RM-MANOVA) (O'Brien and Kaiser 1985), which incorporated the presence and absence of TI (TI/NoTI) and exercise (Ex/NoEx) and the interaction between the two (TI/NoTI\*Ex/NoEx) as fixed effects. In addition, individual sharks were incorporated into models as fixed effects to account for individual variation both within and between treatments. Where RM-MANOVAs indicated significance between treatment groups, post-hoc pooled *t*-tests were used to compare means at specific sampling points. The threshold of significance ( $\alpha$ ) for the post-hoc pooled *t*-tests was not subjected to Bonferroni corrections when performing multiple comparisons. The use of Bonferroni corrections has been strongly contested as it reduces the probability of Type I error at the cost of inflating the probability of the equally deleterious Type II error (Nakagowa 2004, Perneger 1998, Rothman 1990).

### 2.3 Results

Independent of each other, both TI (TI/NoTI) and exhaustive exercise (Ex/NoEx) had significant effects on a number of blood parameters, as did individual variation between subject animals. However, the interaction between TI and exercise (TI/NoTI\*Ex/NoEx) had no statistical effect on any blood chemistry parameter and are not discussed further. Furthermore, given the lack of a combined effect, the effects of TI and exercise are presented separately to facilitate visualisation of the data.

Across all treatments, lemon sharks responded to the stress of capture and restraint with significant disruption to their physiological homeostasis over time (Table 2 and 3). Whole blood pH was significantly depressed at the 30 min sampling point but

recovered to a less perturbed state through the 90 and 180 minute sampling points (Figures 5a and 7a). Carbon dioxide followed a pattern directly inverse to that of pH, increasing between the 0 -30 minutes, but became significantly depressed after 180 minutes (Figures 5b and 7b). There was a significant increase in lactate values through the 0, 30 and 90 minute sampling points, however there was no significant rise between 90 and 180 minutes (Figures 5c and 7c). Variation in bicarbonate concentration mirrored that of pH, becoming significantly depressed at the 30 minute sampling point when compared to both the 0 and 180 minute sampling points (Figures 5d and 7d). Glucose concentrations were significantly higher at the 30, 90 and 180 minute sampling point compared to the 0 minute sampling point (Figures 5e and 7e). Plasma magnesium (Figures 6a and 8a) and sodium (Figures 6b and 8b) became significantly elevated by the 180 minute sampling point compared to the 0 minute point, and there was a significant decrease in plasma potassium concentrations across sampling points (Figures 6c and 8c). Plasma chloride, calcium and urea did not vary across sampling points (Figures 6d-f and 8d-f).

**Table 2 - RM-MANOVA results for effects of tonic immobility and exercise on whole blood chemistry in the juvenile lemon shark (*Negaprion brevirostris*). Bold font indicates significance.**

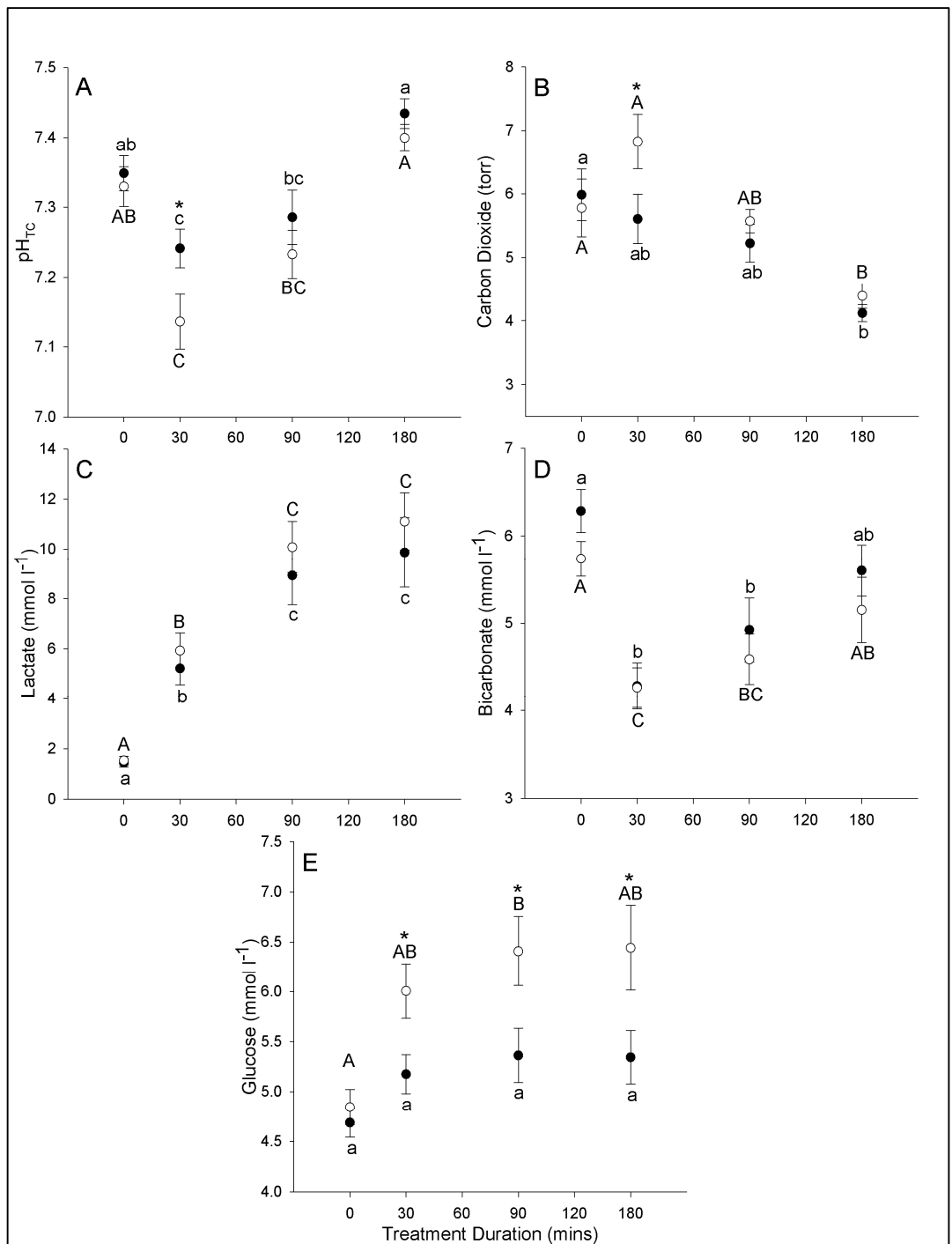
Factor	pH	Carbon Dioxide	Lactate	Bicarbonate	Glucose
Time	<b>p = &lt;0.001, F<sub>3,16</sub> = 60.03</b>	<b>p = &lt;0.001, F<sub>3,18</sub> = 37.33</b>	<b>p = &lt;0.001, F<sub>3,17</sub> = 88.89</b>	<b>p = &lt;0.001, F<sub>3,18</sub> = 41.63</b>	<b>p = &lt;0.001, F<sub>3,17</sub> = 27.86</b>
TI/NoTI	<b>p = 0.018, F<sub>1,18</sub> = 6.75</b>	p = 0.124, F <sub>1,20</sub> = 2.58	p = 0.389, F <sub>1,19</sub> = 1.09	p = 0.353, F <sub>1,20</sub> = 0.9	<b>p = 0.011, F<sub>1,19</sub> = 8.05</b>
TI/NoTI*Time	p = 0.091, F <sub>3,16</sub> = 2.57	<b>p = 0.049, F<sub>3,18</sub> = 3.12</b>	p = 0.634, F <sub>3,17</sub> = 0.58	p = 0.238, F <sub>3,18</sub> = 1.54	<b>p = 0.027, F<sub>3,17</sub> = 3.91</b>
Ex/NoEx	<b>p = &lt;0.001, F<sub>1,18</sub> = 19.76</b>	<b>p = 0.024, F<sub>1,20</sub> = 5.99</b>	<b>p = 0.003, F<sub>1,19</sub> = 11.76</b>	<b>p = 0.002, F<sub>1,20</sub> = 12.08</b>	p = 0.866, F <sub>1,19</sub> = 0.03
Ex/NoEx*Time	p = 0.071, F <sub>3,16</sub> = 2.8337	p = 0.222, F <sub>3,18</sub> = 1.61	<b>p = 0.036, F<sub>3,17</sub> = 3.89</b>	<b>p = 0.006, F<sub>3,18</sub> = 5.85</b>	p = 0.569, F <sub>3,17</sub> = 0.69
TI/NoTI*Ex/NoEx	p = 0.117, F <sub>1,18</sub> = 2.71	p = 0.101, F <sub>1,20</sub> = 2.96	p = 0.559, F <sub>1,19</sub> = 0.354	p = 0.858, F <sub>1,20</sub> = 0.03	p = 0.489, F <sub>1,19</sub> = 0.49
TI/NoTI*Ex/NoEx*Time	p = 0.754, F <sub>3,16</sub> = 0.4	p = 0.591, F <sub>3,18</sub> = 0.65	p = 0.205, F <sub>3,17</sub> = 1.69	p = 0.451, F <sub>3,18</sub> = 0.92	p = 0.053, F <sub>3,17</sub> = 0.312
Individual Sharks	<b>p = 0.026, F<sub>7,18</sub> = 3.07</b>	<b>p = 0.026, F<sub>7,20</sub> = 2.98</b>	<b>p = 0.021, F<sub>7,19</sub> = 3.2</b>	<b>p = 0.032, F<sub>7,20</sub> = 2.84</b>	<b>p = &lt;0.001, F<sub>7,19</sub> = 6.54</b>

**Table 3 - RM-MANOVA results for effects of tonic immobility and exercise on blood plasma ions and urea in the juvenile lemon shark (*Negaprion brevirostris*). Bold font indicates significance.**

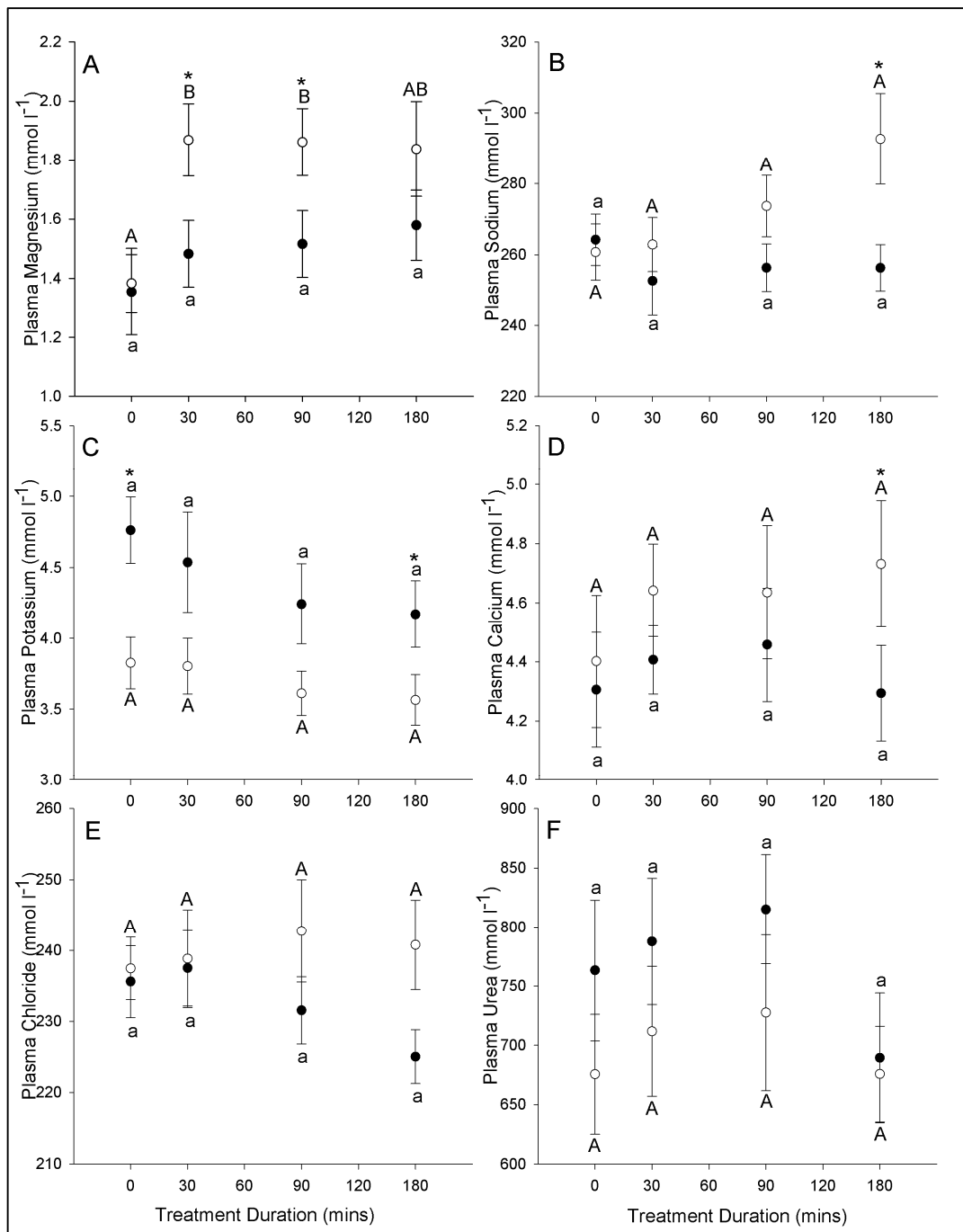
Factor	Plasma Magnesium	Plasma Sodium	Plasma Potassium	Plasma Calcium	Plasma Chloride	Plasma Urea
Time	<b>p = &lt;0.001, F<sub>3,18</sub> = 16.98</b>	<b>p = 0.045, F<sub>3,16</sub> = 3.37</b>	<b>p = 0.048, F<sub>3,17</sub> = 3.25</b>	p = 0.226, F <sub>3,14</sub> = 1.61	p = 0.226, F <sub>3,16</sub> = 1.61	p = 0.099, F <sub>3,18</sub> = 2.42
TI/NoTI	<b>p = 0.004, F<sub>1,20</sub> = 10.37</b>	<b>p = 0.01, F<sub>1,18</sub> = 8.24</b>	<b>p = 0.010, F<sub>1,19</sub> = 8.09</b>	<b>p = 0.036, F<sub>1,16</sub> = 5.25</b>	p = 0.406, F <sub>1,18</sub> = 0.72	p = 0.132, F <sub>1,20</sub> = 2.47
TI/NoTI*Time	p = 0.247, F <sub>3,18</sub> = 2.15	<b>p = 0.025, F<sub>3,16</sub> = 4.07</b>	p = 0.515, F <sub>3,17</sub> = 0.79	p = 0.383, F <sub>3,14</sub> = 1.09	p = 0.383, F <sub>3,16</sub> = 1.09	p = 0.636, F <sub>3,18</sub> = 0.58
Ex/NoEx	p = 0.888, F <sub>1,20</sub> = 0.02	p = 0.226, F <sub>1,18</sub> = 1.57	p = 0.312, F <sub>1,19</sub> = 1.08	p = 0.824, F <sub>1,16</sub> = 0.05	p = 0.505, F <sub>1,18</sub> = 0.46	p = 0.483, F <sub>1,20</sub> = 0.51
Ex/NoEx*Time	p = 0.686, F <sub>3,18</sub> = 0.5	p = 0.457, F <sub>3,16</sub> = 0.91	p = 0.878, F <sub>3,17</sub> = 0.22	p = 0.532, F <sub>3,14</sub> = 0.76	p = 0.769, F <sub>3,16</sub> = 0.379	p = 0.91, F <sub>3,18</sub> = 0.18
TI/NoTI*Ex/NoEx	p = 0.381, F <sub>1,20</sub> = 0.8	p = 0.861, F <sub>1,18</sub> = 0.03	p = 0.697, F <sub>1,19</sub> = 0.16	p = 0.364, F <sub>1,16</sub> = 0.88	p = 0.371, F <sub>1,18</sub> = 0.84	p = 0.837, F <sub>1,20</sub> = 0.04
TI/NoTI*Ex/NoEx*Time	p = 0.129, F <sub>3,18</sub> = 2.15	p = 0.245, F <sub>3,16</sub> = 1.53	p = 0.930, F <sub>3,17</sub> = 0.15	p = 0.519, F <sub>3,14</sub> = 0.79	p = 0.769, F <sub>3,16</sub> = 0.38	p = 0.486, F <sub>3,18</sub> = 0.85
Individual Sharks	<b>p = &lt;0.001, F<sub>7,20</sub> = 6.13</b>	<b>p = &lt;0.001, F<sub>7,18</sub> = 10.89</b>	p = 0.109, F <sub>7,19</sub> = 1.2	<b>p = &lt;0.001, F<sub>7,16</sub> = 6.83</b>	<b>p = 0.025, F<sub>7,18</sub> = 3.09</b>	<b>p = 0.002, F<sub>7,20</sub> = 5.27</b>

### 2.3.1 The Effects of Tonic Immobility

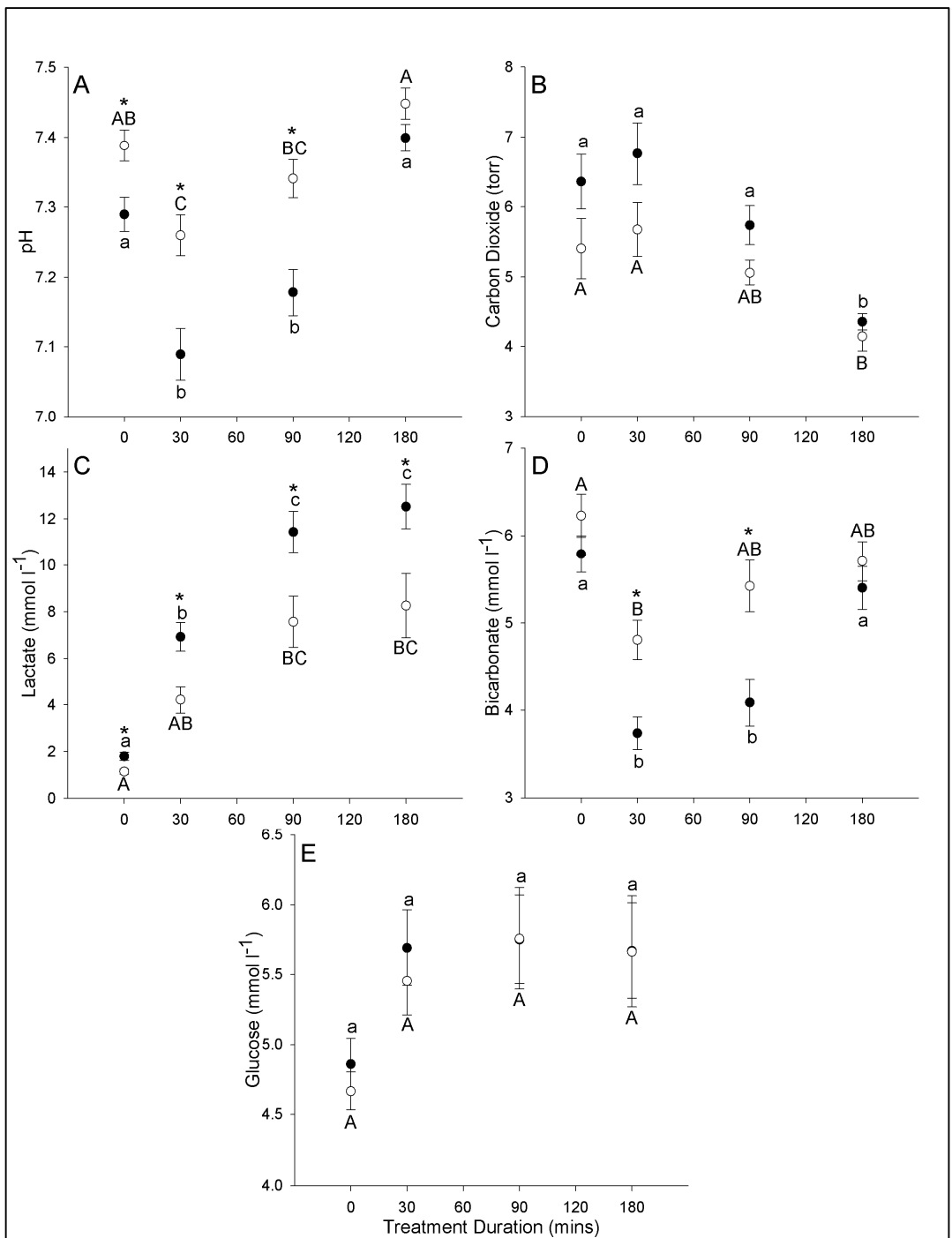
Tonic immobility significantly affected a number of blood chemistry parameters (Table 2 and 3). The magnitude of the blood acidosis observed in animals maintained in TI between the 0 and 30 minute sampling points was double that for those sharks that were allowed to swim freely between blood samples ( $\bar{x} \Delta \text{pH TI} = 0.22$ ;  $\bar{x} \Delta \text{pH NoTI} = 0.11$ ) (Figure 5a). This acidosis was mirrored by a significant increase in carbon dioxide at the 30 minute sampling point for animals maintained in TI, mean values of which were over double those of animals allowed to swim freely between blood samples (Figure 5b). Neither lactate nor bicarbonate concentrations were significantly affected by tonic immobility despite significant perturbation following capture and restraint (Figure 5c-d). Animals maintained in TI were significantly hyperglycemic compared to those that were not (Figure 5e). Furthermore, glucose concentrations became significantly elevated between the 0 and 90 minute sampling points for animals maintained in TI, however, there was no significant variation across sampling points for animals allowed to swim freely between blood samples (Figure 5e). Animals maintained in TI presented significantly greater disruption to their electrolyte balance compared to those allowed to swim freely between blood samples. Plasma magnesium, sodium and calcium were significantly elevated, and plasma potassium significantly depressed for animals maintained in TI (Figure 6 a-d). The time scales over which these perturbations were presented varied, with magnesium becoming maximally elevated at the 30 minute sampling point (Figure 6a), in contrast to plasma sodium and calcium, which did not reach maximum perturbation until the 180 minute sampling point (Figure 6b and 6d). The presence or absence of TI had no significant effect on plasma chloride or urea.



**Figure 5 - Variation in blood gas and metabolite concentrations of juvenile lemon sharks (*Negaprion brevirostris*) in response to capture and restraint. Filled points (●) represent animals allowed to free swim between blood samples and clear points (○) represent animals maintained in tonic immobility. Data represent mean blood chemistry values of whole blood (A) pHTC, (B) carbon dioxide, (C) lactate, (D) bicarbonate and (E) glucose ( $\pm 1$  SE). Asterisks (\*) indicate a statistically significant difference between TI and non-TI treatments at a given sampling point (Pooled t-test,  $\alpha < 0.05$ ). Dissimilar capital letters indicate statistically significant differences between sampling points for treatments involving extended TI, and dissimilar small letters for those treatments that did not involve TI (ANOVA,  $\alpha < 0.05$ ).**

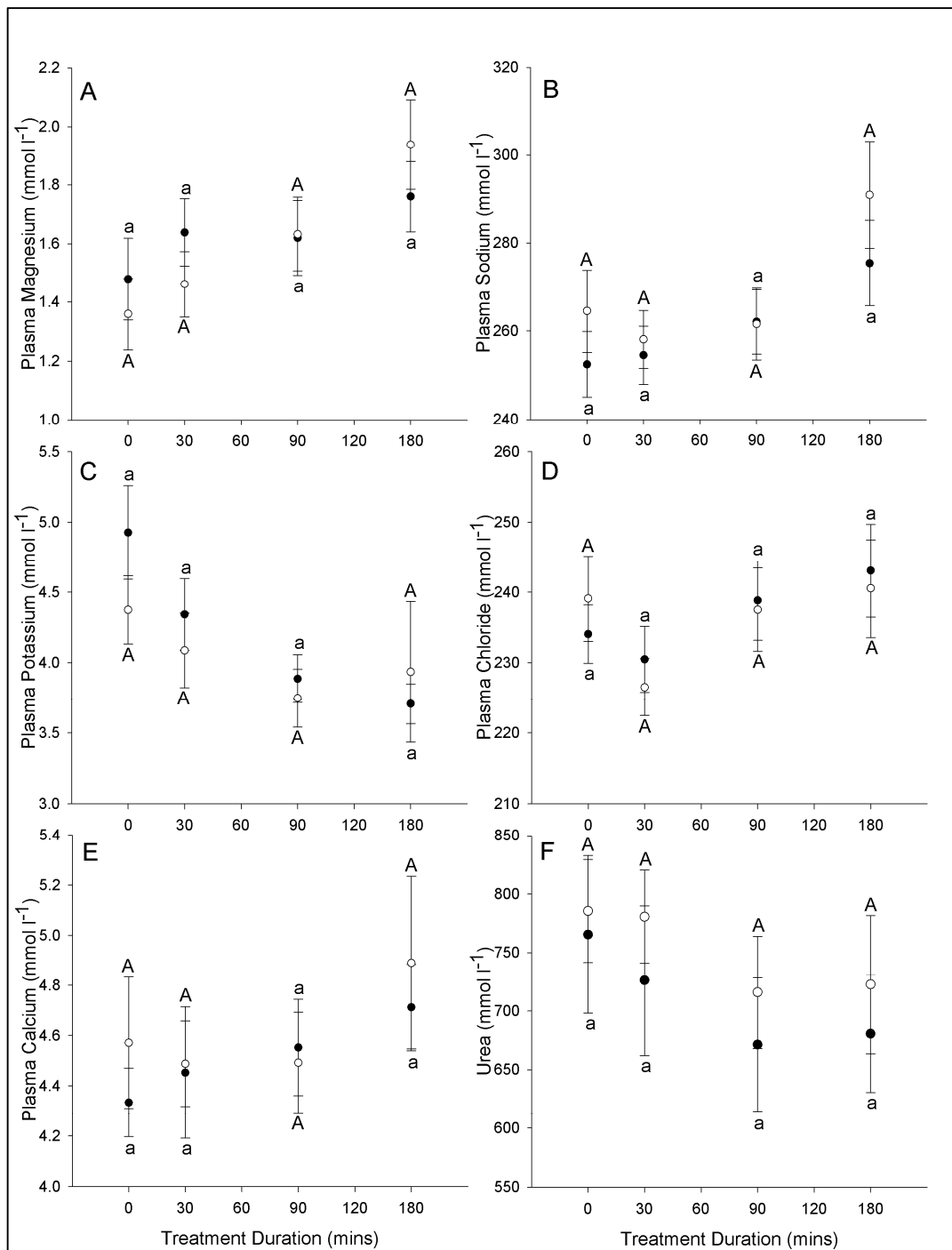


**Figure 6 - Variation in plasma ion and urea concentrations of juvenile lemon sharks (*Negaprion brevirostris*) in response to capture and restraint. Filled points (●) represent animals allowed to free swim between blood samples and clear points (○) represent animals maintained in tonic immobility. Data represent mean blood chemistry values of whole blood (A) magnesium, (B) sodium, (C) potassium, (D) chloride, (E) calcium and (F) urea ( $\pm$  1 SE). Asterisks (\*) indicate a statistically significant difference between TI and non-TI treatments at a given sampling point (Pooled *t*-test,  $\alpha$  = <0.05). Dissimilar capital letters indicate statistically significant differences between sampling points for treatments involving extended TI, and dissimilar small letters for those treatments that did not involve TI (ANOVA,  $\alpha$  = <0.05).**



**Figure 7 - Variation in blood gas and metabolite concentrations of juvenile lemon sharks (*Negaprion brevirostris*) in response to exercise. Filled points (●) represent animals subjected to exhaustive exercise and clear points (○) represent animals not exercised. Data represent mean blood chemistry values of whole blood (A) pHTC, (B) carbon dioxide, (C) lactate., (D) bicarbonate and (E) glucose ( $\pm 1$  SE). Asterisks (\*) indicate a statistically significant difference between exercise and non-exercise treatments at a given sampling point (Pooled t-test,  $\alpha = <0.05$ ). Dissimilar capital letters indicate statistically significant differences between sampling points for treatments involving exercise, and dissimilar small letters for those treatments that did not involve exercise. (ANOVA,  $\alpha = <0.05$ ).**





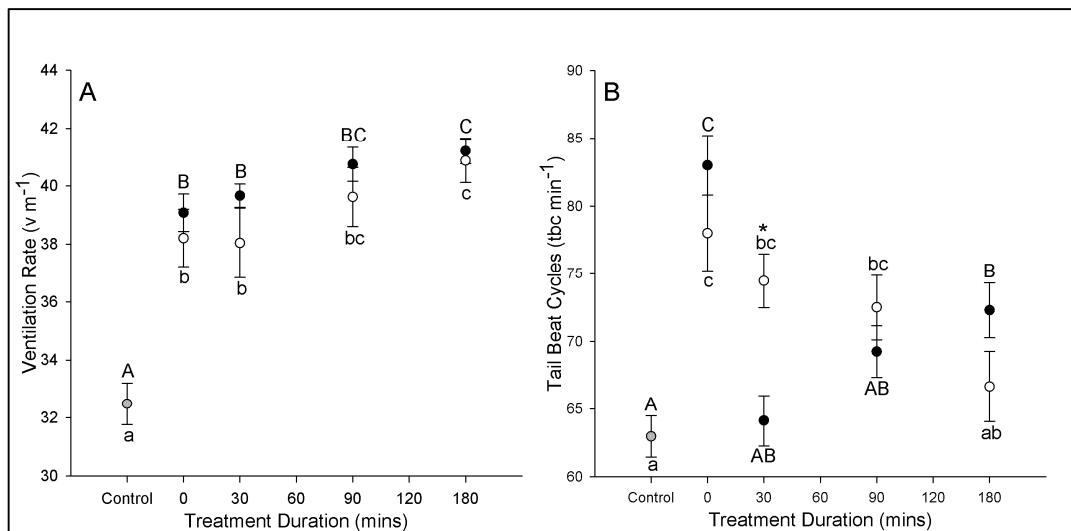
**Figure 8 - Variation in plasma ion and urea concentrations of juvenile lemon sharks (*Negaprion brevirostris*) in response to exercise. Filled points (●) represent animals subjected to exhaustive exercise and clear points (○) represent animals not exercised. Data represent mean blood chemistry values of whole blood (A) magnesium, (B) sodium, (C) potassium, (D) chloride, (E) calcium and (F) urea ( $\pm 1$  SE). Asterisks (\*) indicate a statistically significant difference between exercise and non-exercise treatments at a given sampling point (Pooled *t*-test,  $\alpha = <0.05$ ). Dissimilar capital letters indicate statistically significant differences between sampling points for treatments involving exercise, and dissimilar small letters for those treatments that did not involve exercise (ANOVA,  $\alpha = <0.05$ ).**

### 2.3.2 The Effects of Exhaustive Exercise

Exhaustive exercise increased the magnitude of homeostatic disruption in a number of blood chemistry parameters (Table 2 and 3). Whole blood pH was significantly more depressed in animals subjected to exhaustive exercise when, compared to those that were not (Figure 7a). This acidosis was accompanied by significantly elevated levels of carbon dioxide (Figure 7b), lactate (Figure 7c), and significantly depressed levels of bicarbonate (Figure 7d) in exercised animals. Exhaustive exercise did not affect the hyperglycemic response (Figure 7e), nor did it have any significant effect on any blood plasma constituents (Figure 8 a-f).

### 2.3.3 Behavioural Observations

For treatments involving extended durations of TI, ventilation rates were significantly elevated when compared to control values at all sampling points for both treatments, and increased significantly from the 0 and 30 minute sampling points through to the 180 minute sampling point (Figure 9a). Exhaustive exercise caused a small but significant increase in ventilation rates (Figure 9a). For treatments not involving extended durations of TI, tail-beat-cycles were significantly elevated when compared to control values at all sampling points, particularly at the 0 minute sampling point (Figure 9b). Exhaustive exercise had no significant effect on tail-beat-cycles, however, there was a significant interaction between exercise and sampling point; further analysis indicated fewer tail-beat-cycles at the 30 minute sampling point in animals subjected to exhaustive exercise (Figure 9b).



**Figure 9 - Behavioural response of juvenile lemon sharks (*Negaprion brevirostris*) to exhaustive exercise, capture and restraint. Behaviour was quantified by ventilation rates for animals maintained in TI (A), and by tail-beat-cycles for those that were not (B). Filled points (●) represent animals subjected to exhaustive exercise and clear points (○) represent animals not exercised. Data represent mean values of each metric ( $\pm 1$  SE). Asterisks (\*) denote a statistically significant difference between exercise and no-exercise treatments at a given sampling point (Pooled  $t$ -test,  $\alpha = <0.05$ ). Dissimilar capital letters indicate statistically significant differences between sampling points for treatments involving exhaustive exercise, and dissimilar small letters for those treatments that did not. (ANOVA,  $\alpha = <0.05$ ).**

#### 2.3.4 Individual Variation in the Stress Response

Lemon sharks exhibited considerable individual variation in the stress response (Tables 2 and 3). Across all treatments, significant differences in blood chemistry between individual sharks were established for ten of the eleven parameters analysed including whole blood pH, carbon dioxide, lactate, bicarbonate, glucose, magnesium, sodium, chloride, calcium and urea. There was no significant variation in plasma potassium between sharks. Furthermore, there was significant variation in both ventilation rates, and tail beat cycles between individual sharks.

## 2.4 Discussion

The purpose of this study was to investigate the physiological and behavioural effects of TI on juvenile lemon sharks. The results suggest that TI is an inherently

stressful experience, which magnifies the degree of perturbation observed in a number of blood chemistry parameters.

Tonic immobility appears to disrupt the short term ventilation efficiency of juvenile lemon sharks as indicated by significant elevation of carbon dioxide at the 30 minute sampling point, which is the likely cause of the concomitant drop in blood pH at the same point (Mandelman and Skomal 2009). This is potentially due to the restriction of lemon sharks to the apparently less efficient ventilation method of buccal pumping, in contrast to the ram ventilation conducted during free swimming treatments. By the end of both TI and non-TI treatments all animals had reduced carbon dioxide concentrations to a similar point suggesting that, in response to this reduction in ventilatory efficiency, lemon sharks initiated a number of compensatory mechanisms that successfully improved gas exchange. One such compensatory mechanism is suggested by the significant increase in ventilation rates compared to control values observed during TI treatments. Increases in ventilation rates are known to increase the capacity for gas exchange by increasing the volume of water passing over the gill lamellae (Butler and Metcalf 1989, Carlson and Parsons 2003, Hawkins et al. 2004). Furthermore, it is postulated that physical adaptations to the gills and circulatory system typically associated with the primary and secondary stress response (McDonald and Milligan 1997), were sustained during TI, which, in combination with elevated ventilation rates, facilitated the decline of carbon dioxide concentrations.

The primary stress response is characterised by a hormonal cascade of catecholamines and corticosteroids which in turn trigger a number of physiological and physical adaptations designed to promote the capacity for 'flight or fight', and promote survivorship in the short term (Busch and Hayward 2009, Romero 2004, Skomal and Bernal 2010). Some of these adaptations (e.g. the recruitment of additional gill lamellae

and the vasodilatation of branchial blood vessels) are designed to promote gill perfusion and the capacity for gas exchange (Randall 1982, Skomal and Bernal 2010); however, these adaptations also increase the permeability of the gills to ions, resulting in disruption to the electrolyte balance of the blood (Gonzalez and McDonald 1992, Randall 1982). Unlike marine teleosts, marine elasmobranchs maintain themselves hyper-osmotic to their surrounding environment by retaining nitrogenous organic compounds such as urea and trimethylamine oxide (Hazon et al. 2003, Pang et al. 1977). The balance of osmolarity is derived from inorganic ions maintained at concentrations below that of the surrounding water, resulting in a continuous diffusion of ions across the gills into the blood, which is balanced by salt excretion from the rectal gland and kidneys (Shuttleworth 1988). Lemon sharks maintained in TI presented significantly greater perturbation to their electrolyte balance than those allowed to swim freely between blood samples, suggesting that the increased permeability of the gill epithelium, and the hormonal cascade that triggers it, was more acute during TI treatments. Further evidence of a sustained primary stress response, though not directly related to promoting gas exchange, is found in the significantly elevated glucose levels at all time points of animals maintained in TI. Glucocorticoid hormones are a key component of the primary stress response, ensuring an adequate supply of energetic substrates by controlling the degree of hepatic glycogen mobilisation (Barton 2002, Busch and Hayward 2009, Skomal and Bernal 2010). The significantly elevated levels of glucose found in animals maintained in TI suggest that a chronic level of stress persisted for the duration of the experiment.

To the authors' knowledge, this study represents the first investigation into the physiological response to tonic immobility of any taxa to date. Our results demonstrate that juvenile lemon sharks display physiological perturbations in response to being in tonic immobility, over and above those associated with the capture and taking of blood

samples. The increased physiological perturbation associated with TI is thought to be in response to a respiratory challenge induced by confinement to buccal pumping. In response to this, lemon sharks were able to implement a number of compensatory mechanisms; which suggests that central neural processing remains intact during TI, a phenomenon which has been shown to exist in chickens (Gallup et al. 1980). In the short term TI increased the magnitude of physiological stress experienced by the animal, and care should be taken when using the technique for extended periods on less robust elasmobranch species, in particular those which are unable to supplement ram ventilation with buccal pumping. Furthermore, these findings have implications for all conservation physiology studies of elasmobranchs which commonly utilise TI as a means of facilitating blood sampling. It should be noted that this experiment was conducted in static water with no supplemental ventilation provided by pumps, a common practice in a number of research endeavours, and as such, further investigation into the potential mitigating effects of artificial ventilation are necessary. Despite its widespread use, TI remains one of the least studied and enigmatic areas of elasmobranch biology and further interdisciplinary research into the underlying psychological, physiological and neurological processes associated with it is highly recommended.

## Chapter Three

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### 3. The physiological response of the Caribbean reef shark (*Carcharhinus perezii*) to longline capture.

Originally published as: Brooks EJ, Mandelman J, Sloman K, Liss S, Danylchuk A, Cooke S, Skomal G, Philipp D, Sims D and Suski C. 2011. The physiological response of the Caribbean reef shark (*Carcharhinus perezii*) to longline capture. *Comparative Biochemistry and Physiology Part A*. 162:94 – 100.

#### ABSTRACT

Longline fishing is the most common elasmobranch capture method around the world, yet the physiological consequences of this method are poorly understood. To quantify the sub-lethal effects of longline capture in the commonly exploited Caribbean reef shark (*Carcharhinus perezii*), 37 individuals were captured using standard, mid-water longlines. Hook timers provided hooking duration to the nearest minute. Once sharks were landed, blood samples were taken and used to measure a suite of physiological parameters. Control data were obtained by sampling an additional three unrestrained Caribbean reef sharks underwater at an established shark feeding site. The greatest level of physiological alteration occurred after 120-180 min of hooking, whereas sharks exposed to minimal and maximal hook durations exhibited the least disturbed blood chemistry. Significant second-order quadratic relationships were established between hooking duration and blood pCO<sub>2</sub>, lactate, glucose, and plasma calcium and potassium. Longline capture appears more benign than other methods assessed to date, causing a shift in the stress response from acute at the onset of capture to a sub-acute regime as the capture event progresses, which facilitates physiological recovery. The continued investigation into the physiological response of elasmobranchs to longline capture is vital for the effective management of such fisheries.

### **3.1 Introduction**

Longline fishing is thought to be the predominant method of commercial capture for sharks (Beerkircher et al. 2002, Lewison et al. 2004a, Gilman et al. 2008). In the majority of longline fisheries all or part of the shark is harvested, however, in some areas large numbers of sharks are released alive, due to low species-specific commercial value (Beerkircher et al. 2002) and/or to comply with fisheries regulations (Morgan et al. 2009). The emerging discipline of conservation physiology is focused on using physiological tools and knowledge to understand and address conservation problems (Wikelski and Cooke 2006, Cooke and O'Connor 2010). Documenting the physiological consequences of longline capture in sharks is a vital undertaking for effective fisheries management because it can provide insights into the underlying causes of at-vessel mortality and into the viability of animals post release (Skomal 2007).

The capture and release of a shark induces various degrees of physical trauma and physiological stress, the magnitude of which is thought to be dependent on the capture method, capture duration, and the specific metabolic capacity of the species (Skomal 2006, Skomal 2007, Mandelman and Skomal 2009). If physiological stress, physical trauma, or a combination of the two is excessive, then immediate or delayed (post-release) mortality is possible (Skomal 2007). In cases where sharks survive the capture event, a suite of homeostatic disruptions can potentially impact growth, feeding, swimming behaviour, and the immune system, leading to population-level consequences (Cooke et al. 2002, Skomal 2007).

Although the study of elasmobranch stress physiology is very much in its infancy, the physiological effects of capture have been quantified for a number of different species and gear types. The majority of research to date has focused on the



physiological effects of recreational rod and reel capture (e.g. Heberer et al. 2010), however, the effects of both gillnet (e.g. Frick et al. 2010) and trawl capture (Mandelman and Farrington 2007) have also been quantified. In those studies that quantified capture duration, the magnitude of the stress response was found to be proportional to the magnitude of the stressor, which in the case of most capture events is determined by capture duration and gear type (Skomal and Bernal 2010). Despite being the most common capture method for sharks, the physiological consequences of longline capture have received very little attention, with only four published studies to date (Moyes et al. 2006, Hight et al. 2007, Mandelman and Skomal 2009, Frick et al. 2010); none of these studies quantified the magnitude of the stress response relative to capture duration. It has been suggested that there is considerable inter-specific variation in the stress response in sharks, likely associated with differences in metabolic scope (Mandelman and Skomal 2009). However, the response to capture has yet to be assessed for the majority of species commonly captured on longlines. This information is vital to understanding the causative factors of at-vessel-mortality and the viability of sharks post release, both of which have implications for elasmobranch fisheries management (Moyes et al. 2006).

The Caribbean reef shark (*Carcharhinus perezii*) is an abundant, large bodied, reef-associated, apex predator distributed throughout the tropical and sub-tropical western Atlantic and greater Caribbean (Compagno 1984). Although fisheries data pertaining to the Caribbean reef shark are sparse, there are indications that it is commonly captured by longline fisheries throughout its range (Amorim et al. 1998, Arocha et al. 2002, Rosa et al. 2006). In the United States, it is prohibited to land *C. perezii*, which is commonly captured and subsequently released in the bottom set longline fishery off the Florida Keys (Morgan et al. 2009). Based on the limited data available, the IUCN currently lists the Caribbean reef shark as near threatened (Rosa et

al. 2006). Despite its common interactions with fisheries, there has been no investigation into the capture driven stress physiology of this species to date. Thus, the aim of this project was to quantify and characterize physiological disturbances induced by varying duration of longline capture in the Caribbean reef shark.

### **3.2 Methods**

This study was conducted between 9<sup>th</sup> June and 1<sup>st</sup> October 2009, at the Cape Eleuthera Institute (CEI), Eleuthera, The Bahamas (24.54° N 76.12° W). All research was carried out under research permits MAF/FIS/17 and MAF/FIS/34 issued by the Bahamian Department of Marine Resources and in accordance with CEI animal care protocols developed within the guidelines of the Association for the Study of Animal Behaviour and the Animal Behaviour Society (Rollin and Kessel 1998).

#### **3.2.1 Animal Collection and Sampling Structure**

Stationary, mid-water longlines, 500 m in length with 25 baited gangions, were set in 15 – 30 m of water for up to 8 hours. Gangions were 2.5 m in length and spaced ~15 m apart along the mainline with a support buoy every five hooks. Each gangion was connected to the mainline via a tuna clip and hook timer (Lindgren Pitman HT600 Hook Timer, Pompano Beach, Florida, USA) rigged with monofilament line. The gangion itself consisted of 2 m of braided polyester cord, 0.5 m of monofilament line, 0.5 m of steel leader, and a 16/0 circle hook. Gangion materials were connected with 8/0 swivels and each hook was baited with a 100 g chunk of bonito tuna (*Euthynnus alletteratus*). Line checks were performed every hour to identify candidate sharks and to release non-candidate sharks and by-catch.

Once a candidate shark was identified, its position on the line was marked with a numbered and coloured marker and the time of hooking back-calculated from the

elapsed time on the hook timer. To quantify the physiological stress response over a broad time range, the hooking duration of each candidate animal was manipulated to encompass up to four hours on the line. The shark was then retrieved from the line, secured alongside the boat, and placed in tonic immobility (Henningesen 1994) for blood sampling by caudal venipuncture. Hook duration was defined as the time between hooking and phlebotomy. Following blood sampling, the hook was removed and the animal released.

### 3.2.2 Blood Collection and Analyses

Blood (~ 3 ml) was drawn by caudal venipuncture using a 38 mm, 20 gauge needle and a 3 ml syringe (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). All syringes were washed with the anticoagulant sodium heparin prior to drawing blood. Approximately 95  $\mu$ l of whole blood was immediately transferred from the syringe into an iStat CG4+ cartridge, which was in turn inserted into an iStat point of care device (Heska Corporation, Fort Collins, CO, USA) for immediate analysis of pCO<sub>2</sub>, pH, and lactate levels (Mandelman and Farrington 2007, Mandelman and Skomal 2009, Gallagher et al. 2010). The time from blood draw to the insertion of the cartridge into the iStat was <30 s. Glucose was measured by adding 10  $\mu$ l of whole blood directly from the syringe to an Accu-Chek glucose meter (Roche Diagnostics, Basel, Switzerland), which had previously been validated for use on fish (Cooke et al. 2008). To calculate haematocrit, a small volume of whole blood was transferred to a 75 mm micro-hematocrit tube (Drummond Scientific, Broomall, PA, USA), the base of which was sealed with Critoseal putty (McCormick Scientific, St Louis, MO, USA). The sample was spun in a micro-hematocrit centrifuge (LW Scientific Zippocrit, Atlanta, Georgia, USA) at 4,400 g for 5 min. The balance of the sample was transferred to a 3 ml vacutainer containing lithium heparin (Becton, Dickinson and Company, Franklin

Lakes, NJ, USA) and spun in a centrifuge (Clay Adams Compact II Centrifuge) for 5 min at 10,000 g to separate plasma from red blood cells. Plasma was then transferred to three microcentrifuge tubes using a pipette and stored in crushed ice until it could be frozen at -20 °C upon returning to the laboratory.

Plasma samples were subsequently transported in liquid nitrogen to a laboratory at the University of Illinois where they were stored in an ultra-cold freezer (-80 °C) prior to analysis of ions and urea concentrations. Plasma sodium and potassium levels were quantified using a flame photometer (Cole-Parmer Single-Channel Digital Flame Photometer, model WU-02655-00, Vernon Hills, IL, USA). Plasma urea, chloride, magnesium, and calcium levels were quantified using commercially available kits (BioAssay Systems, Hayward, CA, USA – Urea DIUR-500, Chloride DICL-250, Magnesium DIMG-250; Calcium DICA-500).

### 3.2.3 Conversions

The iStat point of care device is designed for use on homeothermic animals, and, as such, is thermostatted to 37 °C. To accurately quantify *in vivo* blood gas and pH values for ectothermic species, it is necessary to correct for temperature (Reeves 1977). Each pH and pCO<sub>2</sub> value generated was, therefore, adjusted using sea surface water temperature taken at the time of capture using equations (1) and (2) respectively, based on research conducted on the larger spotted dogfish (*Scyliorhinus stellaris*), where  $pH_M$  and  $pCO_{2M}$  represent the values derived directly from the iStat, and where  $pH_{TC}$  and  $pCO_{2TC}$  represent the temperature corrected values (Mandelman and Skomal 2009).

$$(1) \quad pH_{TC} = pH_M - 0.011(T - 37)$$

$$(2) \quad pCO_{2TC} = pCO_{2M} (10^{-0.019\Delta T})$$

More recent research has suggested that species-specific conversions are required to generate absolute blood gas and pH values from raw iStat data (Gallagher et al. 2010). Given the present lack of specific conversion values for the Caribbean reef shark, established protocols described by Mandelman and Skomal (2009) were used, which provide relative differences in pH and pCO<sub>2</sub> whilst taking into account variation in water temperature over the course of the study.

Bicarbonate (HCO<sub>3</sub><sup>-</sup>) level was calculated via the Henderson-Hasselbalch equation using temperature corrected pCO<sub>2</sub> and pH values (Mandelman and Skomal 2009). Values for pK were derived from equation (3) and the value of αCO<sub>2</sub> was set at 0.414 following investigations into the ventilator responses to hypercapnia in the lesser spotted dogfish (*Scyliorhinus canicula*) (Randall et al. 1976).

$$(3) \quad \text{pK}' \text{ (at } 20^{\circ}\text{C)} = -0.1003(\text{pH}_{\text{TC}}) + 6.67$$

#### 3.2.4 Baseline Blood Chemistry Estimates

The present study took a novel approach to generating baseline estimates of blood chemistry by sampling minimally stressed, free-swimming Caribbean reef sharks underwater at an established shark diving operation on New Providence, The Bahamas. Experienced shark handlers are able to induce a mild form of tonic immobility in free-swimming individuals as they come to feed, allowing an experienced phlebotomist equipped with SCUBA to draw a blood sample underwater. Samples were obtained from three Caribbean reef sharks using identical needles and syringes to those described previously. The time from initial contact by the shark handler to blood draw was estimated to be less than three minutes, and, as such, represents an approximation of a stress-free (control) sample. Underwater blood sampling has seen *a priori* use for both elasmobranchs (Skomal 2006) and teleosts (Pankhurst 1990, Pankhurst 2011). Although blood processing followed identical protocols to the field-based sampling

described above, ~1 mL of blood was discarded prior to processing so as to avoid any potential contamination of the sample by seawater (Pankhurst 1990).

### 3.2.5 Data Analysis

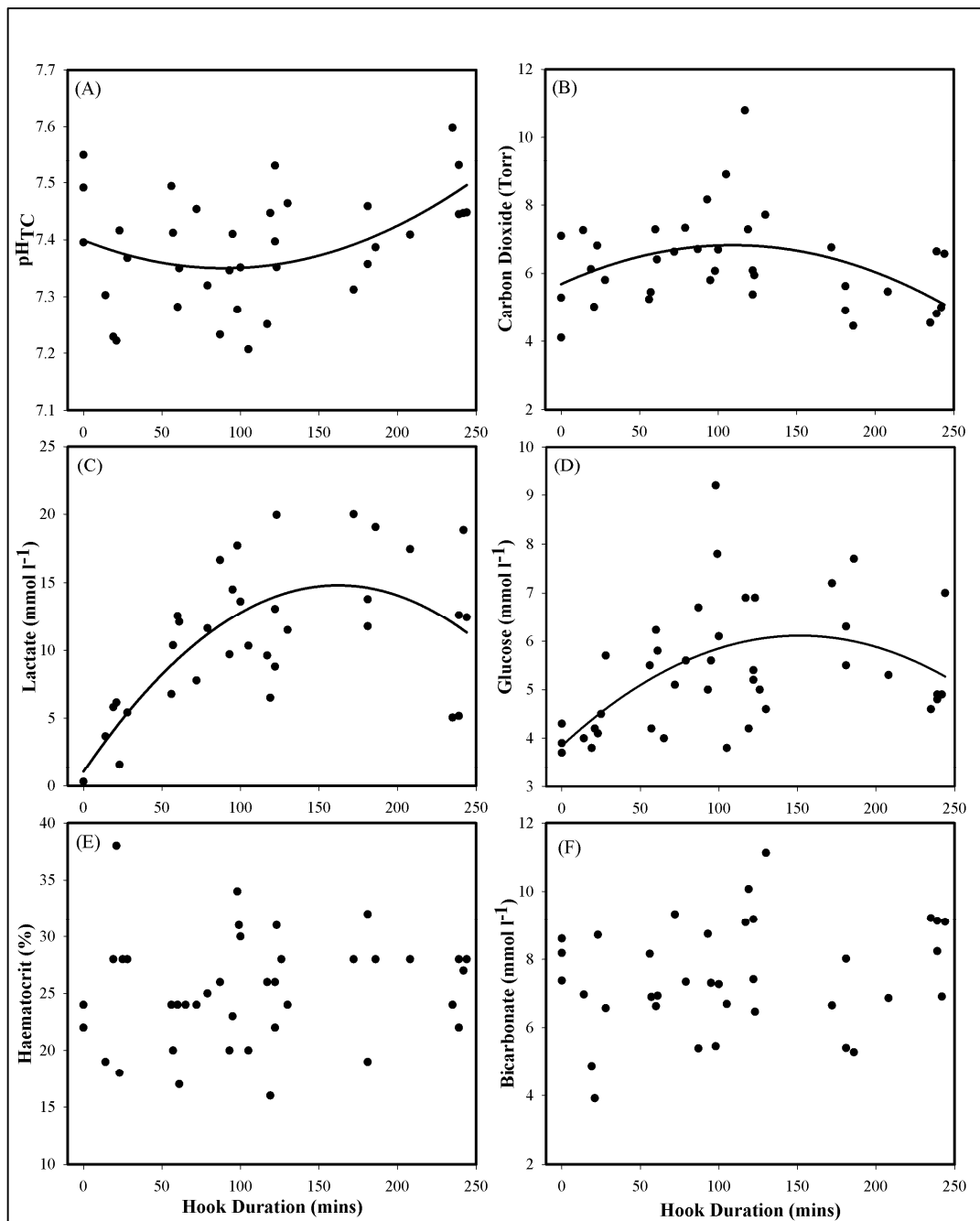
All analyses were performed using JMP 7.0.1 (SAS Institute, Cary, NC, USA) and the level of significance ( $\alpha$ ) for all tests was 0.05. The Box-Cox procedure was used to select the optimum data transformation to improve normality and homogeneity of variances (Box and Cox 1964), as part of a standard data cleaning process (Obourne 2010). Regression analysis was used to estimate relationships between hook duration and blood parameters (Zar 1984). Because it was not assumed that the relationship between variables would be linear, both linear and second order polynomial equations were applied as lines of best fit. In all cases, the best fit line, defined by the highest  $r^2$  value, is reported. Following transformation, only two parameters deviated significantly from a normal distribution: urea ( $p = 0.009$  Shapiro-Wilk W Test) and potassium ( $p = 0.028$  Shapiro-Wilk W Test). For these parameters, hook duration was assigned to 60 min bins and analysed with a non-parametric Kruskal-Wallis tests. Where significant differences were found, post-hoc Mann-Whitney U tests were used to explore the data further (Zar 1984). No Bonferroni adjustments were made to the threshold of significance ( $\alpha$ ) for post-hoc Mann-Whitney U tests (Rothman 1990, Perneger 1998, Nakagowa 2004).

### 3.3 Results

Between June and October 2009, a total of 40 Caribbean reef sharks were sampled: 37 by longline with hook durations ranging from 14 – 244 minutes ( $\bar{x} = 115$  min,  $\pm 11.5$  S.E.), and three underwater-sampled control animals ( $\bar{x}$  Total Length = 165

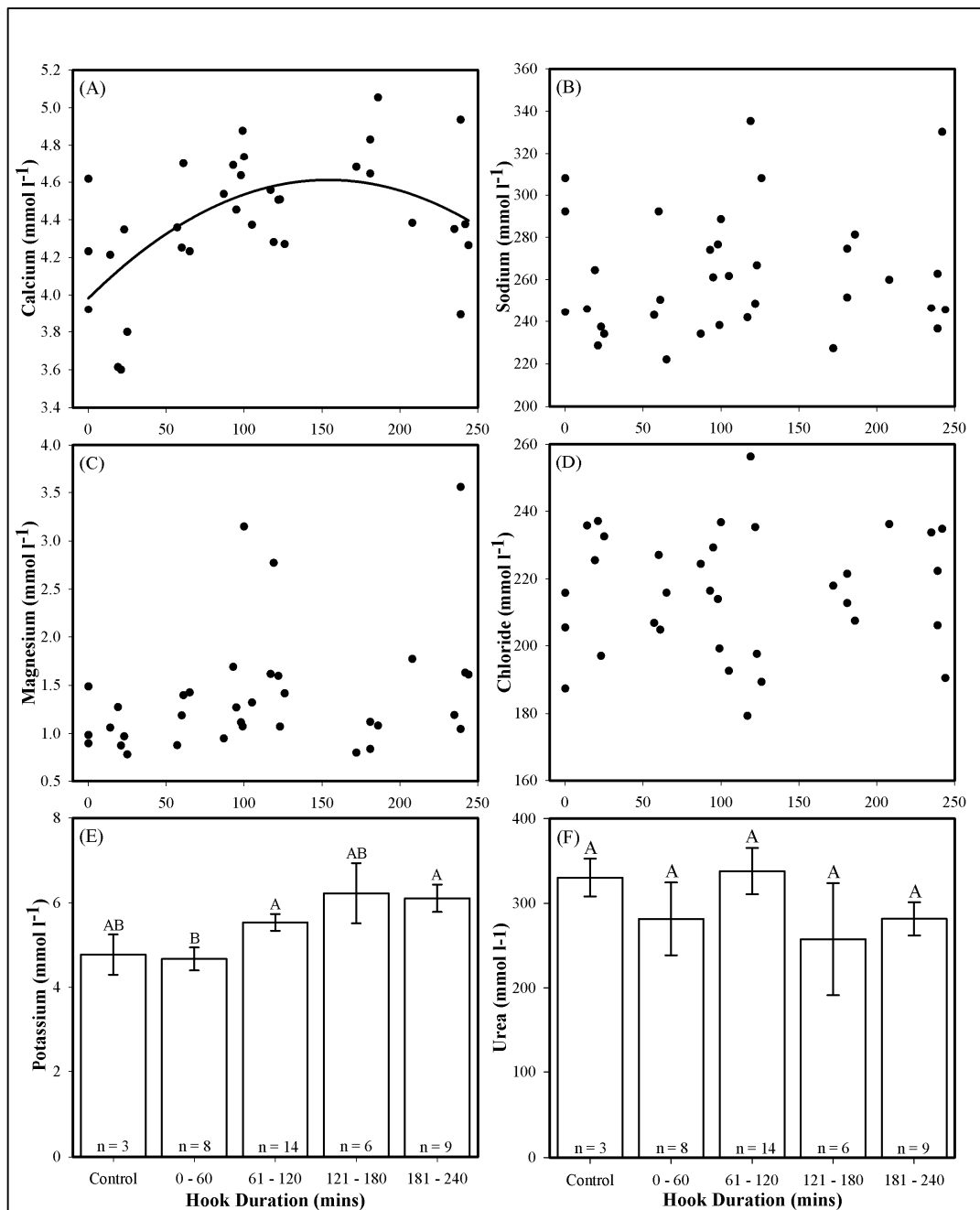
cm,  $\pm 3.7$  S.E.). Sea surface temperatures for both longline-sampled and control animals ranged from 26.3 °C – 29.3 °C ( $\bar{x} = 28.2$  °C  $\pm 0.2$  S.E.).

Significant second-order quadratic relationships were estimated between hooking duration and blood pH<sub>TC</sub> ( $r^2 = 0.221$ ,  $p = 0.016$ ), carbon dioxide ( $r^2 = 0.203$ ,  $p = 0.024$ ), lactate ( $r^2 = 0.622$ ,  $p = <0.001$ ), and glucose ( $r^2 = 0.362$ ,  $p = <0.001$ ) (Figure 10a-d) with disturbances reaching their highest level after 120-180 min of hook duration. There was no significant relationship between hook duration and haematocrit or bicarbonate (Figure 10e-f). A significant relationship was also established between hook duration and plasma calcium ( $r^2 = 0.332$ ,  $p = 0.002$ ) and plasma potassium ( $\chi^2 = 9.969$ ,  $p = 0.041$ ) (Figure 11a, e). Maximum disturbance for both plasma calcium and potassium occurred at approximately 120-180 minutes of hooking. No significant relationships were detected between hook duration and plasma sodium, chloride, magnesium, or urea (Figure 11b,c,d,f).



**Figure 10 - The stress response of the Caribbean reef shark in response to longline capture. Data represent the relationships between hook duration and the physiological response variables (A) pH, (B) carbon dioxide, (C) lactate, (D) glucose, (E) haematocrit and (F) bicarbonate. All parameters were derived from whole blood with field-based portable blood analysers. Curves represent significant second order polynomials and were fitted by  $y = 5.248 (1 - e^{-0.0118x})$ .**





**Figure 11 - The stress response of the Caribbean reef shark in response to longline capture. Data represent the relationships between hook duration and the physiological response variables (A) calcium, (B) sodium, (C) magnesium, (D) chloride, (E) potassium and (F) urea. The curve represents significant second order polynomials and were fitted by  $y = 5.248 (1 - e^{-0.0118x})$ . Potassium and Urea (Panels E and F) deviated significantly from a normal distribution, and as such, hook duration was binned at 60 minute intervals and the data analysed with a non-parametric Kruskal-Wallis test. Data are means, error bars indicate  $\pm 1$  standard error and sample sizes are indicated at the base of the bars.**

### 3.4 Discussion

Results from this study demonstrate a non-linear (parabolic) response of a number of blood chemistry parameters to hook duration for Caribbean reef sharks captured by

mid-water longline. The majority of research published on capture events in sharks suggests that the magnitude of homeostatic disruption initiated by a capture event is proportional to the duration of capture, the type of capture gear, and the metabolic scope of the species in question (Skomal 2007, Mandelman and Skomal 2009). The results of the current study, however, indicate that this is not the case for longline-captured Caribbean reef sharks as sharks subjected to maximal and minimal hook durations exhibited the least disturbed blood chemistry across all parameters with statistically significant trends. This result can potentially be attributed to the characteristics of the longline capture experience, which, when compared to other elasmobranch capture techniques, are relatively benign. Longlines lack the continual active retrieval of rod-and-reel capture, which induces continuous muscular exertion (Skomal 2006) or the forward momentum and physical compaction of a trawl net (Mandelman and Farrington 2007). Moreover, unlike gillnets, longlines do not usually completely entangle the captured animal, which can impede ventilation and consequently gas exchange (Manire et al. 2001, Frick et al. 2010). It is likely that the negative stimuli persisting through the duration of these other capture trigger an acute “fight-or-flight” stress response for the duration of the capture event. In contrast, longline-captured animals are not actively fought following hooking and, in the present study, had relatively unrestricted movement within a 5 m diameter sphere (twice the length of the ganglion). Based on the parabolic response of many blood parameters to hook duration, one hypothesis is that the Caribbean reef shark responds to longline capture with an initial energetic escape response (high anaerobic muscular activity) to the acute stress of hooking, followed by a period of reduced activity associated with a concomitant attenuation of the secondary stress response.

The parabolic response curves associated with the majority of physiological parameters measured in this study are potentially indicative of physiological mediation

of the secondary stress response and the subsequent recovery of physiological homeostasis over longer capture durations. The allostatic overload associated with the initial stress of longline hooking is designed to increase fitness and the capacity for escape in the short term. However, as seen in other aquatic and terrestrial vertebrates, the high metabolic costs of maintaining the stress response can reduce fitness in the long term (Romero 2004, Busch and Hayward 2009, Schreck 2010, Romero and Wikelski 2010). We postulate that Caribbean reef sharks experience a shift in their stress response from acute at the onset of hooking to a sub-acute physiological regime, which promotes physiological recovery and preserves fitness over prolonged capture events. Furthermore, this shift may be mediated by reducing stress hormone production, which facilitates recovery through behavioural (e.g., reduced struggling) and physiological (e.g., reduced heart rates) mediation.

These hypotheses are indirectly supported by somewhat anomalous trends already published within the literature. Firstly, Mandelman and Skomal (2009) found that species-specific differences in at-vessel-mortality were closely linked to species-specific variation in the magnitude of acid-base disruption following longline capture. In a different study, Morgan et al. (2009) found at-vessel-mortality rates to increase proportionally with longline soak time for seven of nine species assessed. In contrast, at-vessel-mortality rates for both bull (*Carcharhinus leucas*) and dusky (*Carcharhinus obscurus*) sharks did not increase linearly with longline soak time but peaked, and then declined over longer hook durations, an anomaly the authors attribute to low sample sizes (Morgan et al. 2009). Given the findings of the present study, these parabolic trends in at-vessel-mortality can be better explained by the ability of some species to shift their stress response from an acute regime at the onset of capture to a sub-acute regime, which facilitates physiological recovery and consequently promotes survivorship. Secondly, support for this shift being mediated hormonally is found in

Hight et al. (2007) who reported that catecholamine concentrations in the blood of viable shortfin mako sharks (*Isurus oxyrinchus*) subjected to up to 3 hours of longline capture were elevated compared with baseline, but lower than those animals subjected to 15-30 minutes of rod-and-reel capture. Although not directly addressed by the authors, a potential explanation of this trend is that viable longline-captured mako sharks were able to reduce the production of catecholamines following hooking and, thereby, mediate the secondary stress responses they induce, which in turn promoted physiological recovery and survivorship. Given the highly species-specific response to capture in sharks (Mandelman and Skomal 2009), and the linear increase in at-vessel mortality with soak time for a number of species (Morgan et al. 2009), the ability to mediate the stress response is not common to all species of sharks.

Blood acid-base properties of Caribbean reef sharks exhibited similar stress responses to other longline-captured carcharhinids (e.g. Cliff and Thurman 1984, Hoffmayer and Parsons 2001, Mandelman and Skomal 2009). However, after lactate anion concentrations increased significantly for animals subjected to 0 – 150 minutes of hook duration, they declined in animals exposed to extended hook durations (>150 min). Due to the lag between intramuscular lactic acid production and the leaching of lactate loads into the blood, the plateau and subsequent decline of lactate concentrations in sharks exposed to longer hook durations suggests that burst exercise might have ceased very early on in the capture event. The increase in pCO<sub>2</sub> is a common stress response in elasmobranchs (e.g. Cliff and Thurman 1984, Hoffmayer and Parsons 2001, Mandelman and Skomal 2009), with hypercapnia a typical product of capture-induced respiratory failure and exhaustive exercise (Skomal and Bernal 2010). Blood pH was significantly depressed following moderate hook durations in this study. This pronounced blood acidosis has been reported in many other elasmobranchs subjected to hook and line (Cliff and Thurman 1984, Hoffmayer and Parsons 2001) and longline

(Mandelman and Skomal 2009) capture. Although this acidosis is thought to be driven by elevated levels of pCO<sub>2</sub> and the metabolic production of H<sup>+</sup> ions in response to capture stress, the specific metabolic source of the latter is at present uncertain (Robergs et al. 2004, Lindinger 2004).

The Caribbean reef shark ranks as a species more resilient to longline capture than several others assessed to date. Mandelman and Skomal (2009) ranked the relative resilience of five species of carcharhinid sharks according to the level of blood acid-base perturbation at the time of longline capture, the characteristics of which are comparable to the current study in terms of ganglion length, hook type, and blood analysis protocols. Individual hooking durations were not obtained by Mandelman and Skomal (2009), but were estimated to range between 3 – 330 minutes compared to the present study which ranged between 14 – 244 minutes. Using the near maximally depressed 10<sup>th</sup> percentile blood pH values reported by Mandelman and Skomal (2009) and corresponding values from the present study, the Caribbean reef shark ranks among the more resilient species assessed to date, second only to the tiger (*Galeocerdo cuvier*) and sandbar (*Carcharhinus plumbeus*) sharks. This resilience is supported by the lack of at-vessel-mortality encountered over the course of the present study and the proportionally lower at-vessel-mortality rates for both tiger and sandbar sharks reported by Mandelman and Skomal (2009).

In addition to mild acid-base disruption, Caribbean reef sharks exhibited elevations in whole blood glucose levels in response to capture. Glucocorticoid stress hormones mobilise hepatic glycogen stores leading to hyperglycemia, which has been identified as a response to stress in a number of elasmobranchs (e.g., Cliff and Thurman 1984, Hoffmayer and Parsons 2001, Skomal 2006, Frick et al. 2010). In addition, it has also been suggested that the stress response in some species of sharks is characterised

by a hypoglycaemic period immediately following the initial hyperglycemia (Manire et al. 2001, Frick et al. 2010). This could account for the parabolic trend in blood glucose identified in the present study. However, given the consistency of the trends across multiple parameters, it is not thought to be the case.

Only two ions ( $\text{Ca}^{2+}$  and  $\text{K}^{+}$ ) showed significant variation with hooking duration in the present study. Elevated levels of plasma potassium (hyperkalemia) have been previously attributed to intracellular acidosis, which causes a net efflux of potassium from the tissue into the blood stream (Cliff and Thurman 1984, Mandelman and Farrington 2007, Moyes et al. 2006, Frick et al. 2010). Hyperkalemia is thought to alter the electrochemical gradients that control the function of excitable tissues such as muscle and, as a result, induce cardiac arrhythmia and muscle fatigue (Martini 1974, Moyes et al. 2006). In mammals, the reported threshold for the onset of cardiac disruption is approximately  $7 \text{ mmol l}^{-1}$ , characterised by bradycardia and reduced cardiac output and muscle tetany (Cliff and Thurman 1984), a threshold which appears to be similar in elasmobranchs (See Martini 1974, Moyes et al. 2006, Frick et al. 2010). In the present study, five Caribbean reef sharks had potassium values in excess of  $7 \text{ mmol l}^{-1}$ , however, the fate of these five animals post-release cannot be ascertained, even though none of the sharks required any form of resuscitation and all swam away strongly upon release. This is in contrast to the muscle tetany observed in moribund gummy sharks (*Mustelus antarcticus*) which was attributed to hyperkalemia by Frick et al. (2010). Elevated plasma calcium is thought to be a natural consequence of acidosis that can impair muscular contraction and neuromuscular nerve transmission (Cliff and Thurman 1984, Moyes et al. 2006). In contrast, elevated levels of calcium might also act as a compensation mechanism to help offset acidosis induced cardiac damage (Wells et al. 1986, Mandelman and Farrington 2006). Although the mean plasma calcium levels in Caribbean reef sharks were higher than those reported in other studies (e.g.,

Moyes et al. 2006, Cliff and Thurman 1984), it is unclear what the physiological implications of this might be.

Although the trends described in this paper are statistically significant, some caution should be employed when interpreting them for two reasons. Firstly, the sample size of 37 experimental animals is relatively small and only six of those sampled were subjected to hook durations in excess of 200 minutes. Secondly, the  $r^2$  values, though comparable to other published field physiology studies (e.g., Skomal 2006, Cooke et al. 2008), are fairly low and there is a great deal of individual variation within the data. The veracity of the parabolic responses identified in the present study can be tested in a number of ways. Firstly, the quantification of variation in the strength of the escape response (e.g., degree of struggling) over the course of a longline capture event will provide further insight as to the level of anaerobic activity and the resulting homeostatic imbalance induced by capture. Secondly, the serial sampling of Caribbean reef sharks over the course of a capture event would account for any individual variability in blood chemistry parameters across a wide array of capture durations, even though it has been suggested that the repeated restraint and sampling of a single animal can itself exacerbate and, thus, potentially mask the effects of the capture event (Frick et al. 2009, Frick et al. 2010). Given the potential hormonal mediation of the stress response over the course of longline capture, an improved understanding of the endocrine stress response in elasmobranchs, which in turn controls behavioural and physiological responses, would be most beneficial (Pankhurst 2011).

In conclusion, the Caribbean reef shark responds to capture stress in a manner similar to other carcharhinids and, according to the magnitude of acid-base disruption, is ranked as one of the more resilient species assessed to date. The non-linear relationship between physiological stress and hook duration is likely unique to longline capture due

to its relatively more benign characteristics when compared to other common capture techniques. It is possible that these characteristics cause a shift from a metabolically costly, escape-driven stress response immediately following hooking, to a sub-acute regime over longer hook durations, which facilitates the recovery of physiological homeostasis. Further investigation of the physiological effects of longline capture in elasmobranchs can help develop protocols to reduce at-vessel-mortality and promote post release survivorship, contributing to the effective management of elasmobranch longline fisheries on a global scale.



## Chapter Four

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### **4. Validating baited remote underwater-video surveys as an alternative to scientific longline surveys for assessing the diversity, distribution and abundance of sharks in the Bahamian Archipelago**

Originally published as: Brooks EJ, Sloman K, Sims D and Danylchuk A. 2011. Validating baited remote underwater-video surveys as an alternative to scientific longline surveys for assessing the diversity, distribution and abundance of sharks in the Bahamian Archipelago. *Endangered Species Research*. 13:231–243.

#### **ABSTRACT**

Baited Remote Underwater Video Surveys (BRUVS) are a novel, non-invasive method of generating relative abundance indices for a number of marine species, including sharks. This technique has become increasingly prevalent in shark ecology literature, but has yet to be formally validated against more traditional shark survey methods. BRUVS and longline surveys were conducted over four seasons from summer 2008 to spring 2009, in three habitat zones in the waters off Cape Eleuthera, The Bahamas. By the end of the project both techniques generated similar values of species richness, however, longline surveys reached these values faster and with less replicates than BRUVS. Overall there was a significant positive correlation of relative abundance between the two survey techniques, however when analysed on a species-by-species basis correlations were not statistically significant for the less abundant species. This suggests a threshold level of abundance, below which relative abundance estimates generated by the different survey techniques do not agree. In addition, this study identified shortcomings in the ability of BRUVS to accurately identify the species, size and sex of individuals captured on video due to the variable behaviour of the sharks in front of the camera, however technological improvements incorporated into

contemporary studies are identified which might improve data quality. In conclusion, BRUVS offer a non-invasive alternative to longline surveys for monitoring broad trends in the relative abundance of sharks, although in the format tested in this study they may lack the resolution required to approach finer scale, intra-species studies.

#### **4.1 Introduction**

Many shark populations worldwide are in rapid decline due to chronic overfishing and the slow reproductive life-history characteristics exhibited by most elasmobranchs (Stevens et al. 2000, Baum et al. 2003, Baum and Myers 2004, Myers et al. 2007, Field et al. 2009). Fundamental ecological information pertaining to the diversity, distribution and abundance of sharks is vital for the development of effective management and conservation initiatives (Southwood and Henderson 2000, Garla et al. 2006a). However, for most species, even the most basic ecological information is lacking which has led to 46% of sharks, skates and rays to be listed as ‘Data Deficient’ on the IUCN Red List of Threatened Species (IUCN 2012).

The most common method of deriving basic ecological information from shark populations is through scientific longline surveys. These surveys generate relative abundance estimates which provide comparative estimates of abundance through space and time (Henderson 2003), on both inter and intra-species levels (e.g. Pikitch et al. 2005). Longline relative abundance indices are derived from catch-per-unit-effort (CPUE) calculations, and are usually expressed in sharks caught per hook per hour (sharks hook<sup>-1</sup> hr). Relative abundance estimates generated from scientific longline surveys have been utilised in a number of shark ecology studies including those investigating declines in shark abundance (Simpfendorfer et al. 2002), habitat use and demographic population structure (Pikitch et al. 2005) and variation in seasonal abundance (Wirsing et al. 2006). In addition, the surveys themselves provide a vehicle for deploying numerous acoustic and satellite telemetry devices (Holland et al. 1999, Heithaus et al. 2007).

Despite their utility and widespread application, longline surveys for sharks do have significant drawbacks. Surveying requires the hooking, retrieval, and restraint of

subject animals, which in turn imposes various degrees of physical trauma and physiological stress, the magnitude of which is dependent on the capture method and handling time (Skomal 2006, Skomal 2007). These homeostatic disruptions can potentially impact growth, feeding, and the immune system, and may also impede normal physiological and behavioural function leading to population-level consequences (Cooke et al. 2002, Skomal 2006). If either physiological stress or physical trauma, or a combination of the two, is excessive, then immediate or delayed (post-release) mortality is possible (Skomal 2007). Given the rapid declines in many shark populations, this homeostatic disruption and associated mortality is contrary to conservation-based objectives of many shark research programs.

One of the key benefits of longline surveys is their ability to quantify catch and effort in an easily replicable manner. Nevertheless, these benefits are also inherent to a number of different techniques that do not require the physical capture of sharks. Underwater imaging has been used to study the marine environment and its inhabitants for over 40 years (Bailey et al. 2007). Deep ocean research pioneered the use of this technology and the first baited camera was developed in 1967 in California (Issacs 1969). In more recent years, underwater video surveys have become more prevalent in the literature following video's progress towards being a cheap and accessible medium (Shortis et al. 2009). Currently baited remote underwater videos surveys (BRUVS) have become the standard approach for larger bodied, more cautious reef fish, including sharks (Meekan and Cappo 2004, Meekan et al. 2006, Malcolm et al. 2007).

BRUVS impart a number of major benefits over traditional capture-based survey methods, the most fundamental of which is that they are non-invasive, non-destructive and cause minimal damage to the benthic environment. They detect large, mobile animals that avoid divers and active fishing surveys (Cappo et al. 2004, Cappo et al.

2006), and all animals attracted to the vicinity of the bait are ‘captured’, independent of the effectiveness of the capture process (Armstrong et al. 1992). BRUVS are not size selective like many traditional methods, where hook or mesh size are only effective for a certain size range, and the standardized surveys can be replicated at any depth, in a variety of habitats, and by staff with relatively low levels of training (Cappo et al. 2006). A number of different relative abundance indices have been generated from BRUVS which include time of first arrival (Priede and Merrett 1996), maximum number of animals viewed on the videotape at any one time (Willis et al. 2000, Malcolm et al. 2007), and standard catch-per-unit effort in sharks per hour (Meekan and Cappo 2004, Meekan et al. 2006). Maximum number has become the most common relative abundance index for studying reef fish assemblages where the animals surveyed are numerous and constantly entering and leaving the video (Cappo et al. 2006). For larger, less abundant species such as sharks, where individuals are more easily identified by species, sex and size, catch-per-unit-effort is the more common choice (e.g. Meekan and Cappo 2004, Meekan et al. 2006).

The use of BRUVS to quantify shark abundance and diversity has become more commonplace in recent years for many of the reasons described above. In addition to generating datasets comparable to longline surveys, there are little to no negative impacts on the subject animals. To date, BRUVS have been used successfully to monitor sharks in Australia (Meekan and Cappo 2004, Meekan et al. 2006), Florida (C Simpfendorfer pers. com.), the Cayman Islands (M. Gore pers. com.) and Belize (D Chapman pers. com.). However, despite the increasing prevalence of this technique in the literature there has been no attempt to formally quantify and validate the relative abundance indices it generates against more traditional survey techniques.

Baited video surveys have been validated against both prawn trawls (Cappo et al. 2004), and longline surveys (Ellis and DeMartini 1995). The latter study concluded that baited video surveys can replicate the relative abundance indices generated by longline surveys for the juvenile pink snapper (*Pristipomoides filamentosus*) (Ellis and DeMartini 1995), however the equipment and protocols employed for both baited video and longline surveys, are not comparable to contemporary shark survey techniques. Furthermore, the pink snapper is a relatively abundant teleost which has very different ecology and behavioural characteristics to elasmobranchs.

Given the lack of a rigorous validation of contemporary BRUVS, the objective of this study was to compare and validate the trends in shark diversity and relative abundance generated by BRUVS against traditional scientific longline surveys. In addition, this study compared the quality of the data generated, the degree of ecological impact and the cost effectiveness of the respective survey types.

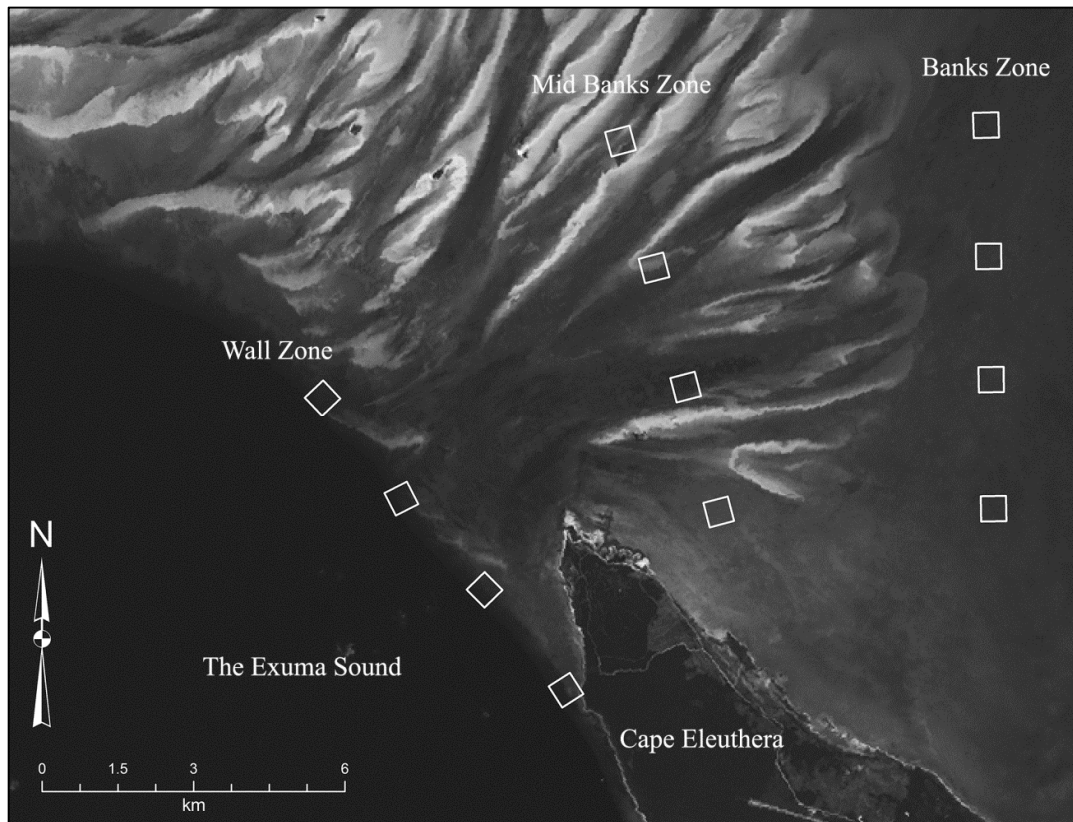
## **4.2 Methods**

This study was conducted between 1st July 2008 and 1st July 2009 in the waters adjacent to Cape Eleuthera, Eleuthera, The Bahamas (24.54° N 76.12° W). All research was carried out under the Cape Eleuthera Institute research permit (MAF/FIS/17 and MAF/FIS/34) issued by the Bahamian Department of Marine Resources in accordance with CEI animal care protocols developed within the guidelines of the Association for the Study of Animal Behaviour and the Animal Behaviour Society (see Rollin and Kessel 1998).

### **4.2.1 Study Area and Sampling Structure.**

The island of Eleuthera is situated on the eastern edge of the Great Bahamas Bank, the largest of the three carbonate platforms which comprise the Bahamian

archipelago (Buchan 2000). The Great Bahamas Bank is divided by two deep-water inlets of the Atlantic Ocean. The north east corner of one of these inlets, the Exuma Sound, is located immediately adjacent to Cape Eleuthera, on the south-eastern tip of Eleuthera (Figure 12). The Exuma Sound ranges in depth from 1500 m – 1800 m and is characterized by steep walls dropping from 20 -30 m to over 1000 m along their margins (Buchan 2000).



**Figure 12 – Baited Remote Underwater Video Surveys (BRUVS) sampling zones in the northeast section of the Exuma Sound.**

The waters off Cape Eleuthera were separated into three zones differentiated by coarse habitat type, water depth, and distance from the pelagic waters of the Exuma Sound (Table 4). Each zone consisted of four, 500 x 500 m (0.25 km<sup>2</sup>) sample sites 2 km apart and orientated approximately north-south along the long axis of the Exuma Sound (Figure 12). These were sampled using both survey techniques across the four seasons (Summer - July/August, Autumn - October/November, Spring - April/May,

Winter - January/February). Standard bait in the form of bonito tuna (*Euthynnus alletteratus*) was used for both survey types. To avoid any potential bias in the BRUVS data due to negative associations between the standardized bait and hooking during longline capture, the surveys were separated temporally. The first month of each field season was dedicated to BRUVS and the second to longline surveys.

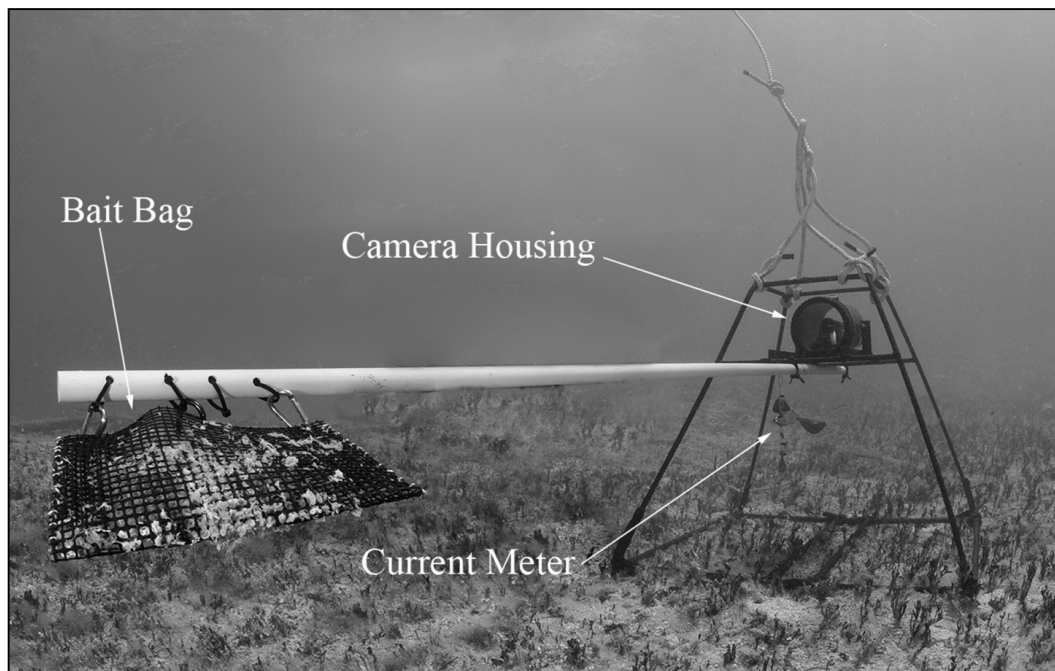
**Table 4 - Key environmental characteristics of the three sampling zones**

<b>Zone</b>		<b>Mean Depth (m)</b>	<b>Habitat Description</b>	<b>Exuma Sound</b>
Wall Zone	WZ	15.3	Coral reef, sand flats and seagrass.	0 Km
Mid-Banks Zone	MBZ	4.1	Shallow sand banks and channels.	5 Km
Banks Zone	BZ	4.3	Seagrass, sand flats and patch reef.	12 m

#### 4.2.2 Baited Remote Underwater Video Surveys.

Baited video units were based on the Australian Institute of Marine Science design described by Meekan et al. (2006). A mini-DV camcorder (Sony Handycam DCR-HC26) with a wide-angle adapter was housed in a PVC and acrylic housing (Aquatix International, St Louis, Missouri, USA), and mounted on a welded steel frame (Figure 13). A bait arm constructed of 25 mm diameter PVC pipe held a 15 cm x 30 cm bait bag 2.5 m away from the front of the camera housing. The bait bag was constructed of ridged extruded plastic with a mesh size of 5 mm holding 300 g of ground bonito tuna. A mechanical current meter (General Oceanics Model 2030 Mechanical Flowmeter, Miami, Florida, USA) was suspended under the camera housing.





**Figure 13 - Baited Remote Underwater Video Survey (BRUVS) unit deployed on the seabed.**

Units were lowered to the sea bed and the cameras recorded continuously for a 90 min period. Tape recordings were analyzed by at least two trained observers. Deployment time, species, sex and tag numbers (if applicable) were noted for any elasmobranchs caught on tape. Catch per unit effort (CPUE) estimates ( $\text{sharks hr}^{-1}$ ) were derived from the deployment time and catch data.

Attempts were made to estimate the length of sharks captured on camera. A 15-cm scale bar was attached perpendicular to the bait arm, 1.5 m in front of the camera housing. A computer program (Screen Callipers, Iconico Software, [www.iconico.com](http://www.iconico.com)), for measuring the length of an object on a computer screen in relation to a known distance, was used to estimate shark lengths from still frames taken from video footage. For these measurements to be made accurately, the entire shark had to be visible in the frame, within  $20^\circ$  of parallel to the bait arm and no more than 1 m behind the scale bar (Harvey et al. 2002b). These requirements were satisfied very rarely during the first two seasons of sampling and attempts were discontinued for the latter two seasons.

#### 4.2.3 Longline Surveys.

Stationary longlines, approximately 500 m in length with ~35 ( $\pm 10$ ) baited gangions were set for 90 min durations. Gangions were 2.5 m in length and spaced ~6 m apart along the mainline with a support buoy attached to 2 m snoods, every six hooks. Each gangion ended in a 16/0, non-offset circle hook baited with a 100 g chunk of bonito tuna.

Due to the shallower water depths in the banks and mid-banks zones, the longline baits were on, or within 1 m of the seabed. In the wall zone, the baits were deliberately kept off the bottom due to the rigidity of the coral reef environment and the risk of entanglement for captured animals. In this area the surveys fished from ~4.5 m of depth closest to the support buoys, to within ~5 m of the seabed. All sharks captured were identified to species, sexed, and measured prior to release. Two external tags were deployed on all captured sharks, firstly a "rototag" style livestock tag attached to the upper third of the first dorsal fin (DuFlex, Destron Fearing, South St. Paul, Minnesota), and a stainless steel dart tag inserted in basolateral dorsal musculature (Hallprint, Victoria Harbour, Australia). For sharks hooked in the jaw, the hook was removed by cutting the barb and rotating the hook free. For sharks hooked in the throat or gut, attempts were made to remove as much of the hook and steel leader as possible prior to release. Fishing effort in hook hours was calculated by multiplying the soak time by the number of hooks set. CPUE (sharks  $\text{hr}^{-1}$ ) was calculated from the hours of fishing effort and the catch.

#### 4.2.4 Survey Replication

To provide the dissimilar survey types equal opportunity to yield statistically significant results the required sample size ( $n$ ) for each technique was determined using a standard method (Harnett 1982). Data generated from a pilot study of sixty replicates

conducted from July 2007 to June 2008 were used to generate minimum replicate estimates for all species encountered. The largest number of replicates indicated for a single species for a single survey was used as the minimum sample size for each survey type. Based on these calculations, the minimum number of replicates for BRUVS and longline surveys per sample site were set at five and three, respectively. The sampling strategy was designed to identify significant differences between sample sites within each zone. As such, the required number of replicates was conducted in each of the four sample sites comprising a single zone, totalling 20 BRUVS and 12 longline surveys per zone per season.

#### 4.2.5 Quantitative Comparisons and Statistical Analysis

All statistical analysis was conducted using JMP 7.0.1 (SAS Institute Inc, Cary, North Carolina, USA). Five metrics were employed to compare the results produced by longline and BRUVS techniques.

##### 4.2.5.1 *Species Richness and Diversity*

The cumulative number of individual species described over the course of the study, relative to project duration and the number of replicates, was graphically compared between survey types. Species richness ( $S$ ), or the total number of species identified by each technique, was quantified. However, given that  $S$  is a non-linear function of sampling effort, absolute values of  $S$  are rarely reached (Southwood and Henderson 2000). An estimate of absolute species richness ( $S_{max}$ ) was calculated from the observed species richness ( $S_{obs}$ ) using the Chao estimator, whereas  $a$  is the number of species represented by single capture or sighting and  $b$  is the number of species represented by two captures or sightings (Colwell and Coddington 1994):

$$S_{max} = S_{obs} + (a^2/b2) \tag{1}$$

#### 4.2.5.2 *Correlation of Mean Catch-Per-Unit-Effort Values*

This metric relies on the assumption that two equally objective survey techniques will generate relative abundance indices that are directly proportional to each other, even if the actual values generated are dissimilar. Mean CPUE for both BRUVS and longline surveys was calculated for each species, in each zone, for each season of sampling, resulting in twelve pairs of mean CPUE per species. Spearman rank correlation ( $\rho$ ) was used to compare resulting pairs of mean abundance indices, for all species grouped, and on a species by species basis.

#### 4.2.5.3 *Comparisons of Spatial and Temporal Trends in Relative Abundance*

For three species of shark where CPUE calculated by BRUVS and longline was found to correlate, the effect of zone and season on shark abundance was determined using data obtained from both survey methods. A Shapiro-Wilks test was used to analyze the distribution of both BRUVS and longline CPUE data and in both cases was found to deviate significantly from a normal distribution, largely due to the abundance of zero values within the data set. Therefore, Kruskal-Wallis tests were used to identify significant differences between habitat types and zones, while for species where significant differences were found post-hoc Mann-Whitney U tests were used to further explore the data. No Bonferroni corrections were applied given the radical lowering of statistical power and the increased likelihood of Type II errors associated with this type of correction (Perneger 1998, Nakagowa 2004, Rice 1989).

#### 4.2.5.4 *Data Quality*

Longline surveys provide high quality demographic and diversity data, including accurate identification of sex, species and size, all in addition to standard relative abundance estimates. During a BRUVS pilot study it was noticed that the quality of data generated varied dependent on the behaviour of the shark in front of the camera. In

some cases, the shark made a single distant pass making it difficult to identify size, sex, and occasionally species. Throughout the course of this study, the number of gaps in the BRUVS data set were recorded and compared to the longline data set.

#### 4.2.5.5 *Survey Impact*

The at-vessel mortality imposed by longline surveys was recorded throughout the project on a species basis. In addition, hooking location, and hook retention was quantified. Negative interactions with the BRUVS equipment (e.g. bumps entanglement, etc) were also quantified however BRUVS were assumed to have zero negative impact on the subject animals.

#### 4.2.5.6 *Cost Effectiveness*

Given the tight budgets often associated with marine research projects, the financial costs of the two survey techniques were tracked over the course of the study and compared on a replicate, seasonal, and project basis. All costs reported are location specific (e.g. local boat rental and daily wages), and are examined on a comparative basis to illustrate the relative expense of each survey type.

### 4.3 **Results**

A total of 279 BRUVS and 153 longline surveys were conducted between 9th July 2008 and 9th June 2009, resulting in the capture of 112 and 372 sharks, respectively (Table 5).

#### 4.3.1 Species Richness and Diversity

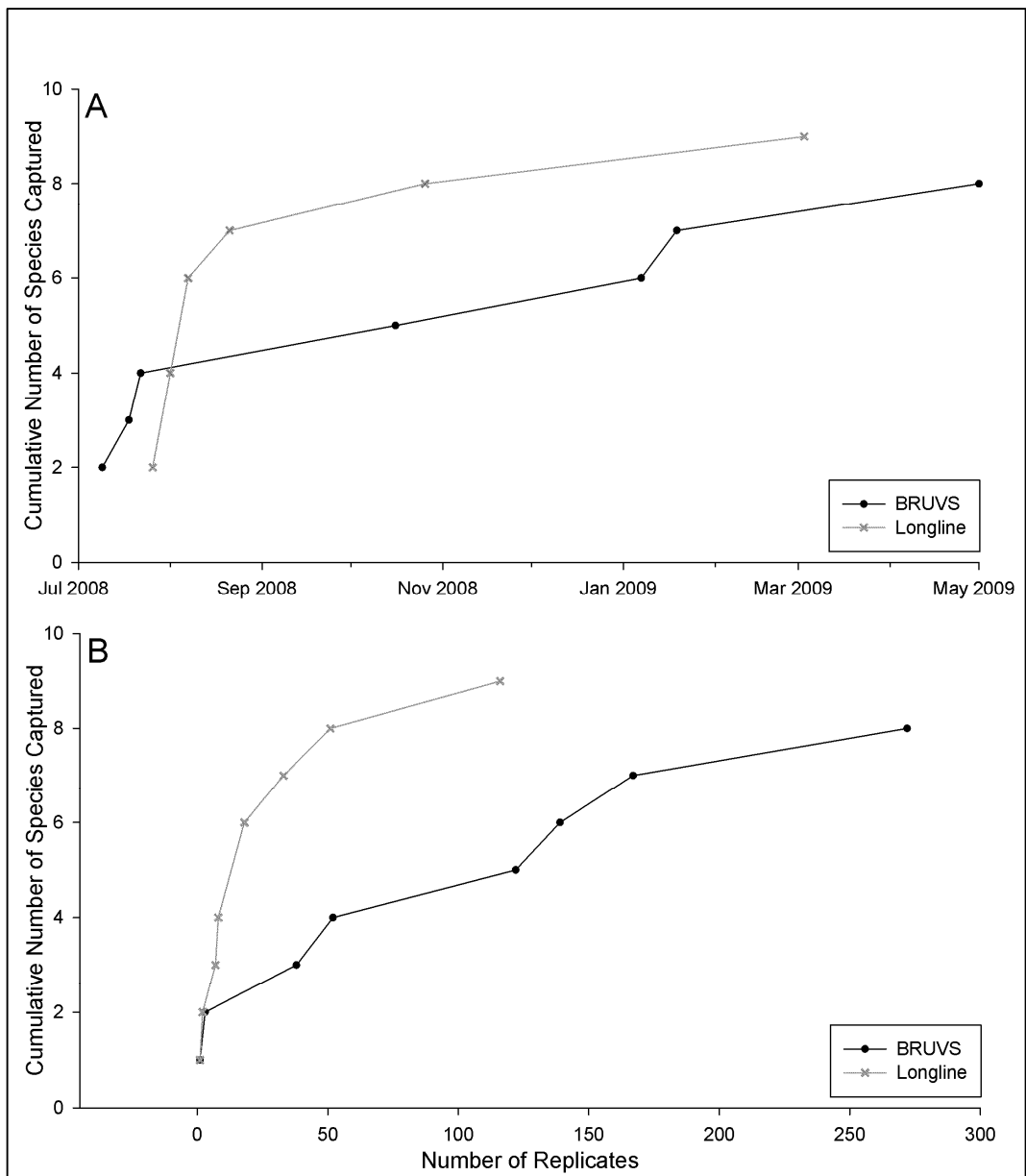
Caribbean reef (*Carcharhinus perezi*) and nurse sharks (*Ginglymostoma cirratum*) were the dominant species during both survey types, the combined catches of which comprised 83.9 % and 73.9 % of BRUVS and longlines, respectively. Consideration of

the proportional catch rates between techniques (Table 5) suggests that on a species by species basis, longlines catch between zero and eight times the number of individuals compared to BRUVS. The one exception to this is the blacknose shark (*Carcharhinus acronotus*) which was caught 37 times more often during longline surveys when compared with BRUVS.

**Table 5 - Species counts and proportional catch ratios for all species encountered using BRUVS and longline surveys. Species are ranked in order of abundance during longline surveys.**

Common Name		# Longline Captures	# BRUVS Captures	BRUVS : Longline
Nurse	<i>Ginglymostoma cirratum</i>	160	68	1 : 2.35
Caribbean Reef	<i>Carcharhinus perezi</i>	115	26	1 : 4.42
Blacknose	<i>Carcharhinus acronotus</i>	37	1	1 : 37.00
Tiger	<i>Galeocerdo cuvier</i>	31	4	1 : 7.75
Lemon	<i>Negaprion brevirostris</i>	11	12	1 : 0.91
Caribbean Sharpnose	<i>Rhizoprionodon porosus</i>	9	2	1 : 4.50
Blacktip	<i>Carcharhinus limbatus</i>	5	1	1 : 5.00
Greater Hammerhead	<i>Sphyrna mokarran</i>	3	4	1 : 0.75
Bull	<i>Carcharhinus leucas</i>	1	0	n/a

A total of nine different species of sharks were captured ( $S_{obs}$ ) during the longline surveys, compared to eight species identified using BRUVS. There was discrepancy between the efficiency of the two techniques in identifying the species richness of sharks in the area, with longline surveys acquiring the maximum number of species in a shorter amount of time and fewer replicates (Figure 14). Estimates of true species richness, as generated using the Chao estimator, estimated absolute species richness ( $S_{max}$ ) to be 10 for BRUVS and 9.5 for longline surveys.



**Figure 14 - Cumulative species detected by BRUVS and longlining over the course of the study (A), and by the number of replicates (B). Each point represents the accumulation of a new species. Plots end when the total species count is reached illustrating the difference in time and replicate number required by each method to reach the total observed species richness.**

#### 4.3.2 Catch-Per-Unit-Effort

There was a significant positive correlation ( $\rho = 0.80$ ,  $p = <0.001$ ) between relative abundance indices generated by BRUVS and longline surveys for all species combined (Table 6). In addition, significant positive correlations of mean CPUE were found for the Caribbean reef, nurse and lemon sharks. Only a single bull shark was

caught on a longline survey and, as such, no quantitative comparisons could be made between the two techniques. Where there was a significant correlation between CPUE calculated from longline and BRUVS data, seasonal and/or spatial differences in relative abundance were investigated in more detail.

**Table 6 - Correlation of mean CPUE between BRUVS and longline surveys by species, ranked in order of mean BRUVS CPUE. Statistically significant correlations are given in bold type.**

Species	Mean CPUE (sharks hr <sup>-1</sup> )		Spearman Rank Correlation Coefficient (ρ)	Probability (p)
	BRUVS	Longline		
Nurse	<b>0.2148</b>	<b>0.0116</b>	<b>0.739</b>	<b>0.006</b>
Caribbean Reef	<b>0.0812</b>	<b>0.0081</b>	<b>0.774</b>	<b>0.003</b>
Lemon	<b>0.0351</b>	<b>0.0007</b>	<b>0.577</b>	<b>0.049</b>
Tiger	0.0129	0.0023	-0.147	0.648
Greater Hammerhead	0.0116	0.0002	0.527	0.078
Caribbean Sharpnose	0.0060	0.0006	0.175	0.587
Blacknose	0.0028	0.0024	-0.195	0.054
Blacktip	0.0027	0.0004	-0.132	0.683
Bull	0.0000	0.0001	n/a	n/a
<b>All Species</b>	<b>0.1480</b>	<b>0.0099</b>	<b>0.804</b>	<b>&lt;0.001</b>

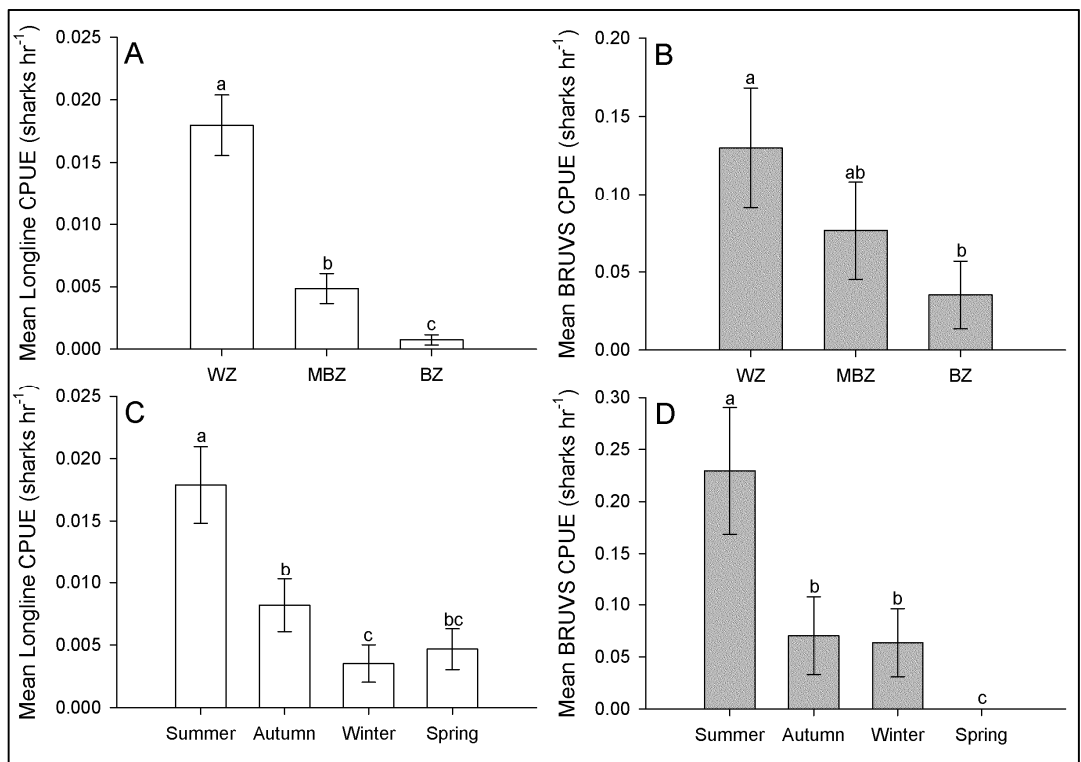
The Caribbean reef shark (Figure 15) was more abundant in the wall zone and decreased in abundance through the mid-banks and banks zones. This trend was replicated graphically by both surveys; however longline surveys indicated significant differences in abundance between all zones, while BRUVS only indicated a significant difference between the wall and bank zones. Caribbean reef sharks were also found to be significantly more abundant in the summer months; a trend replicated both graphically and statistically by the two survey techniques.

The nurse shark was found to be significantly more abundant in the mid-banks zone compared with both the banks and wall zones, a trend replicated both graphically and statistically by both survey techniques (Figure 16). Both techniques showed the nurse shark to be present within the study site throughout the year; however their abundance declined in winter months. This pattern was replicated by both survey

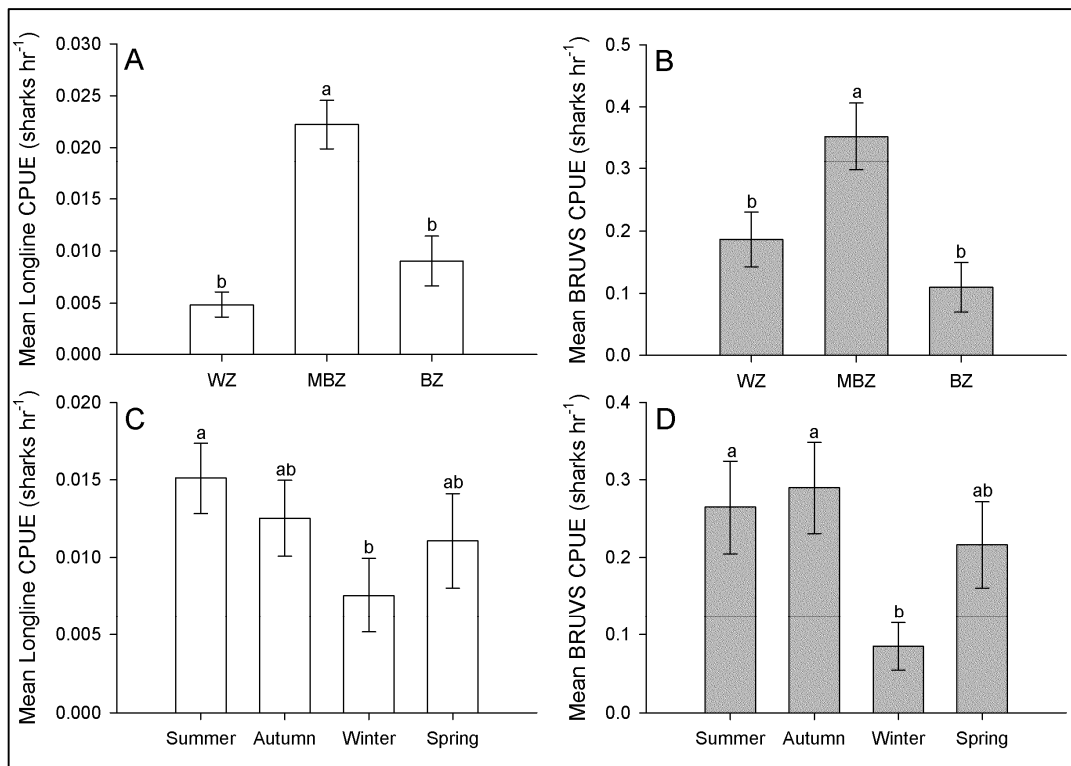


methods; however, there was some variability in the statistical significance of the winter declines offered by the two techniques.

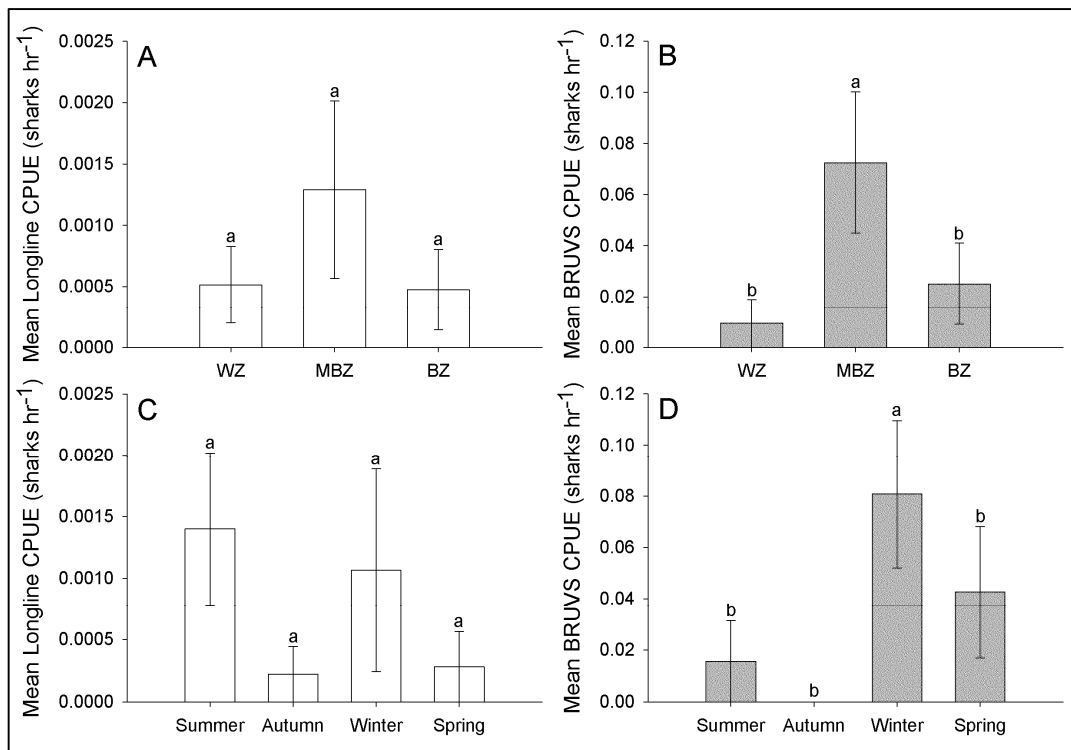
BRUVS indicated that the lemon sharks were significantly more abundant in the winter compared with all other seasons (Figure 17), however this was not replicated by longline data that showed no significant differences in seasonal abundance. Lemon sharks were more abundant in the mid-banks zone, but this was only significant in the BRUVS dataset.



**Figure 15 - Relative abundance of the Caribbean reef shark (*Carcharhinus perezii*) ( $\pm 1$  SE) determined by BRUVS and longline surveys in relation to habitat zone (A and B) and season (C and D). Dissimilar letters above bars signify statistical significance differences.**



**Figure 16 - Relative abundance of the nurse shark (*Ginglymostoma cirratum*) ( $\pm 1$  SE) determined by BRUVS and longline surveys in relation to habitat zone (A and B) and season (C and D). Dissimilar letters above bars signify statistically significant differences.**



**Figure 17 - Relative abundance of the lemon shark (*Negaprion brevirostris*) ( $\pm 1$  SE) determined by BRUVS and longline surveys in relation habitat zone (A and B) and season (C and D). Dissimilar letters above bars signify statistically significant differences.**

#### 4.3.3 Data Quality

Longline surveys provided an assumed 100 % species identification rate. Due to the variable nature of video footage obtained during baited video surveys, only 88 % of 118 sharks sighted were accurately identified to species, including 14 occasions where identification was assigned but was determined to be questionable by the observers. Instances of successful identification of size and sex were quantified as a percentage of the total number of sharks captured for both survey types. Longline surveys were able to determine sex in 97.8% of all sharks captured, whereas sex was only obtained from 39.8 % of animals captured during baited video surveys. Accurate size measurements were obtained for 94.4 % of sharks captured on longlines, however no accurate size measurements could be determined from baited video surveys.

#### 4.3.4 Survey Impact

Longline surveys incurred an at-vessel mortality rate of 5.0 % (n = 19) across all species over four seasons of sampling for a total of 372 sharks captured. At-vessel mortality was observed in three of the nine species including 5.8 % (n = 8) of 121 Caribbean reef sharks, 21.1 % (n = 8) of 38 blacknose sharks, and 44.4 % (n = 4) of 9 Caribbean sharpnose sharks. Of 339 sharks for which hooking location data existed, circle hooks used ensured that 90.0% (n = 305) were hooked in the jaw or the corner of the jaw. A further 8.8 % (n = 30) were hooked in the throat or the gut and 1.2 % (n = 5) were hooked elsewhere on the body, usually in one of the pectoral fins. In some cases it was not possible to remove the entire hook from the jaw of the shark, either due to a difficult hook placement, or the need for a fast release. Of 337 sharks for which hook removal data was obtained, 18.9 % (n = 63) retained some part of the hook, ranging from less than 30 % of the hook for difficult jaw placements, to the entire hook and a portion of the steel leader for gut or throat hooked animals. In addition, when retrieving

lines 31 gangions were encountered which had been bitten off, suggesting there were additional sharks that retained hooks and a substantial portion of the gangion.

In contrast, baited videos surveys were assumed to have zero impact on the surveyed sharks. Physical interactions with the units were limited to minor bumps and scrapes. On occasions (< 20), units were retrieved without bait bags or bait arms, usually taken by nurse sharks; however one Caribbean reef was observed to remove the bait bag.

#### 4.3.5 Cost Effectiveness

The initial construction costs of four BRUVS units was approximately five times that of a single longline, and repairs and consumables costs for BRUVS over the course of the project were approximately twice that of longlines (Table 7). Despite the increased construction and maintenance costs, BRUVS showed considerable savings over longline surveys in the long term, costing approximately one third less over the course of the project. This was largely due to the fact that BRUVS surveys required less boat time (35 vs. 54 days) and less personnel (3 vs. 5 people) than longline surveys. The largest difference in costs was with relation to bait. BRUVS used approximately 84 kg of bait over the course of the project compared with 1,224 kg of bait used by the longline surveys.

**Table 7 - Relative cost breakdown of the two survey techniques. All figures in US dollars.**

	<b>BRUVS</b>	<b>Longline</b>
Initial Construction	\$ 5,500.00	\$ 1,200.00
Consumables/Repairs	\$ 1,200.00	\$ 500.00
Personnel	\$ 6,300.00	\$ 15,300.00
Boat Time	\$ 6,975.00	\$ 10,200.00
Bait	\$ 280.00	\$ 4,150.00
<b>Total Project Costs</b>	<b>\$ 20,255.00</b>	<b>\$ 31,350.00</b>
<b>Cost per Season</b>	<b>\$ 5,063.75</b>	<b>\$ 7,837.50</b>
<b>Cost per Replicate</b>	<b>\$ 72.60</b>	<b>\$ 204.90</b>

#### 4.4 Discussion

This study suggests that BRUVS generates similar relative abundance and species diversity estimates to scientific longline surveys. Longline surveys provided an actual species richness value within 0.5 species of the theoretical maxima (Longline  $S_{obs} = 9$ ,  $S_{max} = 9.5$ ), whereas there was a two species difference described by BRUVS (BRUVS  $S_{obs} = 8$ ,  $S_{max} = 10$ ). By the end of the study the respective survey types generated similar values of species richness ( $S_{obs}$ ), and similar values of estimated maximum species richness ( $S_{max}$ ), although BRUVS required five times the number of replicates and three times the amount of time to reach similar values of species richness.

The highly significant correlation between the CPUE estimates generated by both survey types suggest that overall, BRUVS produces similar relative abundance estimates to longline surveys. This is in agreement with previously published research which concluded that baited video surveys can replicate the relative abundance indices generated by longline surveys for juvenile pink snapper in Hawaii (Ellis and DeMartini 1995). However, this multi-species correlation masks the potential influence of individual species abundance on the comparability of the two techniques. On a species-by-species basis, significant correlations occurred for only three of the eight species encountered on both survey types which represent the three most commonly encountered species on the BRUVS surveys (Table 6).

The lack of correlation between the survey techniques for the lower abundance species suggests a threshold level of CPUE below which data from BRUVS no longer matches those obtained by longline surveys. This idea is further supported by a more detailed analysis of the graphical and statistical agreement between the two techniques. The two most abundant species, the Caribbean reef and nurse sharks, had very clear graphical trends (Figures 15 and 16), and the statistical significance of these trends was

well replicated by the two techniques. The less abundant lemon shark (Figure 17) had clear spatial trends described by both techniques yet they varied in the degree of statistical significance between techniques. In addition, there were conflicting patterns in seasonal abundance described by the respective survey techniques and the statistics which supported them were contradictory. For this study, it appears that the threshold of statistical agreement between the two techniques is approximately that of the mean CPUE of the lemon shark ( $0.0351 \text{ sharks hr}^{-1}$ ) obtained by BRUVS. Relative abundance values calculated from BRUVS for species with a mean CPUE less than the lemon shark should be treated cautiously. Absolute values of relative abundance will vary between studies according to the number of replicates and the variety of habitats in which these replicates were derived. However if these variables are standardised then direct comparisons to the figures generated over the course of this study could be made.

The overarching issue with BRUVS is one of data quality, the most fundamental component of which is accurate species identification. The carcharhinid family, of which seven of the nine species encountered in this study are members, are difficult to identify due to similar body types, colouration and markings (Grace 2001). These identifications are difficult even when the shark is secured adjacent to a boat following longline capture and fine-scale morphological differences can be identified (e.g. presence or absence of an inter-dorsal ridge). The problem is exacerbated during BRUVS surveys due to the variable behaviour of sharks in front of the camera, leading to variable video footage. Indeed, only 88% of sharks captured on BRUVS surveys were accurately identified to species because on many occasions the animal did not approach the camera.

Frequent misidentification of the blacknose shark during the analysis of the BRUVS video sequences could account for the anomaly in the proportional catch rates

between techniques. Longlines captured between 0-8 times the number of individuals when compared with BRUVS for all species other than the blacknose which was caught 37 times as often (Table 5). The blacknose shark is one of the more difficult species to identify as they are morphologically very similar to the Caribbean reef and Caribbean sharpnose sharks, and are present in an overlapping range of sizes. It is likely that blacknose sharks, and potentially Caribbean sharpnose sharks, were often misidentified as small Caribbean reef shark during BRUVS surveys. It is interesting to note that spatial relative abundance trends in Caribbean reef sharks were less statistically defined in BRUVS than in longlines, a potential artefact of erroneously classing a blacknose as a Caribbean reef shark. Longline data suggest that the blacknose is more evenly distributed throughout the three habitat zones (data not shown) than the Caribbean reef shark which would account for the less statistically defined spatial trends described by BRUVS. Longline data also suggest that blacknose sharks are more abundant in the summer which coincides with the increase of Caribbean reef shark abundance and accounts for the lack of dilution in the seasonal trends.

In addition to the issues with species identification during BRUVS surveys, sex could only be established in less than 40% of the sharks captured. Size could not be estimated using the BRUVS in the configuration used in this study, however this problem would be easily rectified by incorporating the stereo-video techniques described in contemporary baited-video literature (e.g. Harvey et al. 2002b, Harvey et al. 2003). Conversely, longline surveys yielded species, sex and size on nearly all occasions, allowing CPUE estimates to be generated for demographics within a species, above and beyond overall species abundance. Given the sexual and ontogenetic segregation prevalent in a number of shark species (Mucientes et al. 2009), there is a need for survey techniques to provide relative abundance data at an intra-species resolution, dependent on the scale of the question being approached. This is beyond the

technical capability of the BRUVS units and protocols tested in this study, although it is potentially possible with the incorporation of the aforementioned stereo-video techniques to provide length estimates. It is also possible that the inclusion of contemporary stereo-video techniques might improve the identification of sex, and could potentially aid in species identification by providing quantitative data on key morphological characteristics such as insertion of the first dorsal fin relative to the axial and inner margin of the pectoral fin, a critical identifier for many carcharhinid species. Furthermore, the mini-DV cameras used in this study are reliant on digital video technology which is several years out of date. The incorporation of more modern high-definition digital video cameras could potentially improve the rates of identification for both species and sex (Harvey et al. 2010).

Efforts were made to ensure that longline surveys incurred the least amount of ecological impact possible for a capture-based survey method. At-vessel mortality rates of 5% for longline surveys were less than those experienced during similar surveys in Belize which ranged from 6-7% (Pikitch et al. 2005, D. Chapman pers. coms.) The circle hooks worked well ensuring the majority (90%) of sharks were hooked in the jaw and relatively few sharks retained any part of the survey hardware. Despite the care taken to minimise the impact of the longline surveys mortality did occur. In addition, the levels of post-release mortality are unknown, although the results of an acoustic telemetry study focusing on Caribbean reef sharks suggest that the majority of captured animals do survive and return to normal behavioural function after approximately six hours (E. Brooks, unpublished data).

Longline surveys are more resource intensive to run than BRUVS, and as such are less cost effective, accruing approximately two thirds the costs of the longline surveys over the course of the project. The major costs for BRUVS are initial equipment costs



and occasionally replacing water damaged cameras, however personnel bait and boat costs are far less than longline surveys. Once BRUVS units have been constructed, long term monitoring with this technique is more cost effective than longline surveys. The addition of more expensive high definition cameras, and more advanced stereo video techniques would add to the initial construction costs of BRUVS but would likely still be more cost effective over the course of a long term project.

In addition to the financial costs of bait, another important consideration is the ecological costs of removing large volumes of wild caught fish from the marine environment. Over the course of the project, longline surveys consumed 92.1% more bait than BRUVS, requiring over 1000 kg of wild caught bonito tuna at an unknown environmental cost. It is conceded that both the environmental and ecological costs of bait can be mitigated to a certain degree by using waste fish (e.g. carcasses from fish docks and marinas), however if any effort is to be made to standardise bait across seasons and locations, then commercially purchased fish is the only viable option.

In conclusion, BRUVS are a viable, less invasive and more cost effective alternative to longline surveys dependent on the specific research question being approached. They are especially suited for long term monitoring of species richness and relative abundance over wide geographical and temporal scales, as they are easily replicated by relatively untrained personnel without specialised equipment. However, the BRUVS tested in this project lacked the resolution required to approach finer scale ecological questions, which would be better approached with longline surveys. Longlines, if structured correctly, provide a higher quality data set with the added benefit of acting as a vehicle for conventional tagging, or deploying and retrieving a suite of biotelemetry devices. Longlines, even those run in the most benign manner possible, will still incur mortality, however the ethical debate required to define an

acceptable level of mortality to better understand and protect a species is outside the scope of this study. An experimental design which incorporates long-term BRUVS sampling and discrete, temporally stratified periods of longline sampling can provide the benefits of both techniques.

## Chapter Five

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### 5. Seasonal abundance, philopatry and demographic structure of Caribbean reef shark (*Carcharhinus perezii*) assemblages in the northeast Exuma Sound, The Bahamas

#### ABSTRACT

The Caribbean reef shark (*Carcharhinus perezii*), an abundant coral-reef associated apex predator, is one of the most economically and ecologically important, yet least studied species of large shark in the greater Caribbean region. The relative abundance and population structure of *C. perezii* off Cape Eleuthera, The Bahamas, was surveyed by standardised longline surveys from May 2008 – October 2011 which resulted in the capture of 331 sharks. Abundance peaked in the summer and was lowest during the winter. Females were 1.6 times more abundant than males and the assemblage was dominated by immature female sharks (45.5%). The abundance of mature male and female sharks peaked a month apart in June and August respectively. All 331 sharks were tagged and released with 15.4% being recaptured after periods at liberty between 5 – 1159 days ( $\bar{x} = 333.4 \pm 42.7$  S.E.). The mean distance between tagging and recapture was only 1.77 km for recaptures in excess of 6 months, indicating seasonally stratified philopatry in this species. In contrast to *C. perezii* populations studied to date, *C. perezii* inhabiting Bahamian waters have evolved complex habitat use patterns that are both spatiotemporally and demographically segregated; most likely in response to the large and diverse habitat mosaic available on the Bahamas Banks compared to contemporary study sites. This study represents the first step in understanding the spatiotemporal population structure of this important apex predator, and illustrates the potential for studies examining behavioural plasticity in response to environmental variation and anthropogenic impacts.

## 5.1 Introduction

The Caribbean reef shark (*Carcharhinus perezi*) is an abundant, large bodied, reef associated, predator (Compagno 1984), distributed throughout the tropical and subtropical western Atlantic, Gulf of Mexico, and greater Caribbean (Castro 2011, Driggers et al. 2011). As the mainstay of the shark diving industry in a number of countries, it is thought to be one of the most economically important species in the region (Cline 2008, Gallagher and Hammerschlag 2011, Maljković and Cote 2011), in addition to playing a vital ecological role as an apex predator in Caribbean coral reef ecosystems (Optiz 1996, Bascompte et al. 2005, Heithaus et al. 2008, Ferretti et al. 2010).

Despite its economic and ecological importance, very few scientific papers have been published to date dealing specifically with the biology and ecology of *C. perezi* (see Chapman et al. 2005, Pikitch et al. 2005, Garla et al. 2006a, Garla et al. 2006b, Chapman et al. 2007, Tavares 2009, Maljković and Cote 2011), and it remains one of the least studied species of large sharks in the region. The maximum reported size of *C. perezi* is 2.43 m total length ( $L_T$ ), but size at maturity data are scarce (Castro 2011). The synthesis of size maturity estimates from contemporary publications is challenging given the lack of standardised reporting, however, based on previous estimates, a size of maturity of male sharks of 1.50 – 1.70 m and of females 1.80 – 1.90 m is reasonable (Pikitch et al. 2005, Tavares 2009, Castro 2011). Mating in The Bahamas is thought to take place in June and July as ascertained by the presence of mature females with fresh mating scars at local shark feeding sites (C. Zenato, pers. com., Maljković and Cote 2011).

The available research suggests that there are ontogenetic shifts in habitat use with smaller juveniles ( $< 1.10$  m  $L_T$ ) being more common inshore than larger conspecifics that tend to frequent the fore-reef area adjacent to deep water ( $> 400$ m)

(Pikitch et al. 2005). Acoustic telemetry and stomach content analysis suggest that larger reef sharks ( $> 1.10$  cm  $L_T$ ) regularly visit shallow reef areas to feed at night (Chapman et al. 2005, Garla et al. 2006a). Furthermore, large reef sharks are known to make deep dives ( $> 356$  m) into cold water ( $\sim 12.4$  °C) on a regular basis, and increase the proportion of time spent in the upper 40 m of the water column at night (Chapman et al. 2007). Juvenile sharks are thought to exhibit a high degree of site fidelity and there is evidence of increased activity space with ontogeny (Garla et al. 2006b).

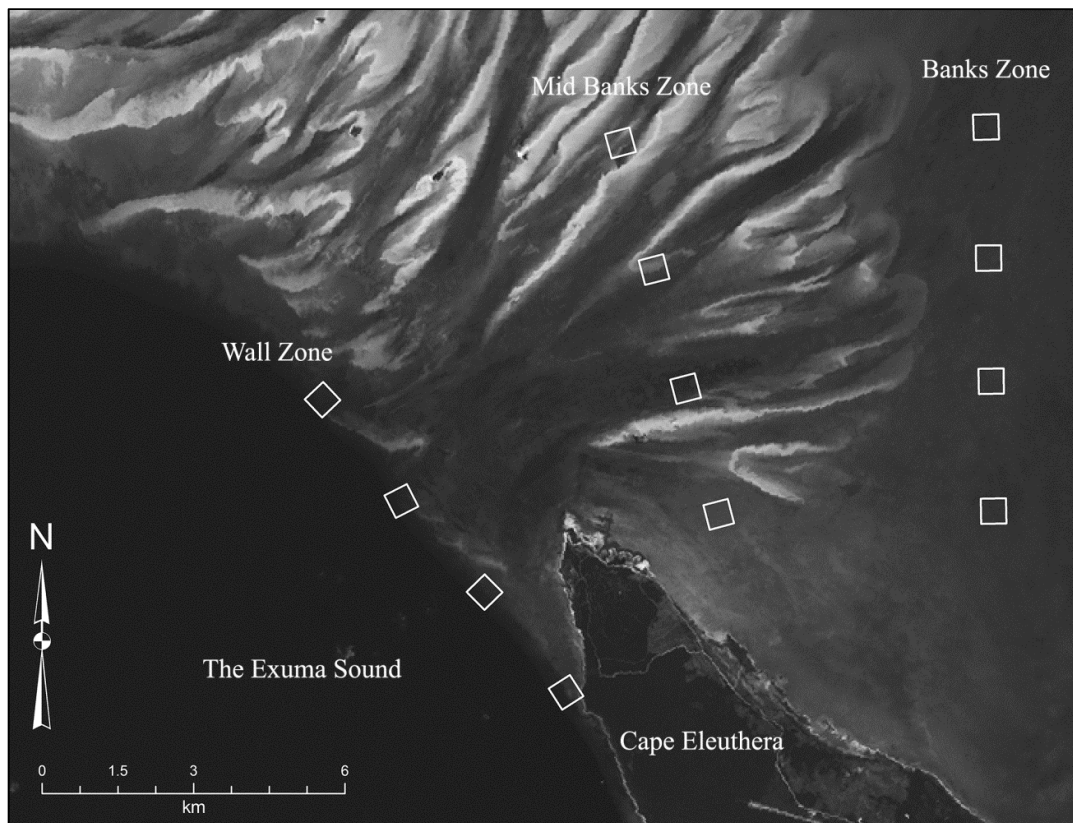
Sharks in the Caribbean, as with populations all over the globe, are in decline (Stallings 2009, Ward-Paige et al. 2010) due to wide-scale fisheries exploitation and habitat degradation (Field et al. 2009, Ward-Paige et al. 2012). Fisheries data specifically pertaining to *C. perezii* are sparse or absent for much of its range, although there are indications that it is fished extensively in much of its range (Amorim et al. 1998, Arocha et al. 2002, Rosa et al. 2006). The IUCN currently lists *C. perezii* as ‘near threatened’, with the caveat that it is likely to meet the criteria of ‘vulnerable’ when additional fisheries data become available (Rosa et al. 2006). In some parts of its range (e.g. USA, The Bahamas), *C. perezii* is protected by fisheries legislation (Morgan et al. 2009), but despite this, there is a paucity of fundamental ecological information that is likely to have hindered the implementation of conservation and management plans throughout its range. Given this lack of information, the purpose of this study was to characterise the seasonal abundance, habitat use, site fidelity and population structure of *C. perezii* in the waters off Cape Eleuthera in the northeast Exuma Sound, The Bahamas.

## **5.2 Methods**

This study was conducted between 8th May 2008 and 11th October 2011 in the waters adjacent to Cape Eleuthera, Eleuthera, The Bahamas ( $24.54^\circ$  N,  $76.12^\circ$  W). All research was carried out under the Cape Eleuthera Institute research permit

(MAF/FIS/17 and MAF/FIS/34) issued by the Bahamian Department of Marine Resources in accordance with CEI animal care protocols developed within the guidelines of the Association for the Study of Animal Behaviour and the Animal Behaviour Society (Rollin and Kessel, 1998).

The island of Eleuthera is situated on the eastern edge of the Great Bahamas Bank, the largest of the three carbonate platforms which comprise the Bahamian archipelago (Buchan 2000). The Great Bahamas Bank is divided by two deep-water inlets of the Atlantic Ocean. The northeast corner of one of these inlets, the Exuma Sound, is located immediately adjacent to Cape Eleuthera, on the south-eastern tip of Eleuthera (Figure 18). The Exuma Sound ranges in depth from 1500 - 1800 m and is characterized by steep walls dropping from 20 -30 m to over 1000 m along their margins (Buchan 2000).



**Figure 18 - Distribution of longline sampling zones in the northeast Exuma Sound, The Bahamas.**

**Table 8 - Key environmental characteristics of the three sampling zones.**

Zone		Mean Depth (m)	Habitat Description	Exuma Sound
Wall Zone	WZ	15.5 ± 0.71 S.E.	Coral reef, sand flats and seagrass.	0 Km
Mid-Banks Zone	MBZ	4.1 ± 0.15 S.E.	Shallow sand banks and channels.	5 Km
Banks Zone	BZ	4.3 ± 0.13 S.E.	Seagrass, sand flats and patch reef.	12 m

### 5.2.1 Longline surveys

Stationary longlines, approximately 500 m in length with ~35 ( $\pm 10$ ) baited gangions, were set for 90 min durations. Gangions were 2.5 m in length and spaced ~6 m apart along the mainline with a support buoy attached to 2 m snoods, every six hooks. Each gangion ended in a 16/0, non-offset circle hook baited with a 100 g chunk of bonito tuna (*Sarda sarda*). Sea surface temperature ( $^{\circ}\text{C}$ ), water depth (m), and location (UTM) were recorded using a boat-mounted chart plotter (Garmin GPS Map 450s, Kansas City, USA) at the centre point of each longline.

All sharks captured were identified to species, sexed, and the pre-caudal ( $L_{PC}$ ), fork ( $L_F$ ) and total length ( $L_T$ ) measured to the nearest cm prior to release. The maturity of male sharks was established by the length and degree of calcification of the claspers, and that of females was estimated by size. Females with a total length in excess of 1.85 m, half way between the most recent estimates of 1.80 m (Tavares 2009) and 1.90 m (Castro 2011), were considered sexually mature. Animals with a visible umbilical scar were considered young-of-the-year. Evidence of mating in the form of bite marks and scars on females and inflamed claspers on males was also noted. Two external tags were affixed to all captured sharks; a "rototag"-style livestock tag attached to the upper third of the first dorsal fin (DuFlex, Destron Fearing, South St. Paul, Minnesota), and a dart tag inserted in basolateral dorsal musculature (Hallprint, Victoria Harbour,

Australia). For sharks hooked in the jaw, the hook was removed by cutting the barb and rotating the hook free. For sharks hooked in the throat or gut, attempts were made to remove as much of the hook and steel leader as possible prior to release.

### 5.2.2 Sampling Structure

An initial sampling period ran from June 2008 –June 2009 and was spatially stratified by three zones differentiated by coarse habitat type, water depth and distance from the deep water of the Exuma Sound (Figure 18, Table 8). Each zone consisted of four 500 × 500 m (0.25 km<sup>2</sup>) sample sites 2 km apart and orientated approximately north–south along the long axis of the Exuma Sound (Figure 18). Sampling in this period was also temporally stratified by season (Summer: June – August; Autumn: September – November; Winter: December – February; Spring: March - May). Part of the data derived from surveys conducted in this period was used in Brooks et al. (2011) for the purpose of baited underwater video survey method validation. The data presented in this study were derived over a much wider time span (3 years), have been subjected to a more detailed statistical analysis, and the conclusions drawn take an ecological focus as opposed to a methodological validation. Based on results from June 2008-June 2009, sampling from June 2009 – November 2011 was restricted to the wall zone and only three of the four seasons (Spring, Summer and Autumn). In 2010 the sampling resolution was higher than previous years, aimed at identifying finer trends in the abundance of specific demographics.

### 5.2.3 Relative Abundance Estimates

Relative abundance indices are a common method of describing the comparative spatial and temporal abundance of terrestrial and aquatic flora and fauna (Southwood and Henderson 2000). The most common expression of relative abundance for longline surveys is Catch-Per-Unit-Effort (CPUE), usually expressed in sharks per hook hour



(sharks hook<sup>-1</sup> hr<sup>-1</sup>) or multiples thereof (Pikitch et al. 2005, Simpfendorfer et al. 2002, Brooks et al. 2011). The traditional expression of longline CPUE is as follows (Equation 1):

$$CPUE = \frac{Catch}{Number\ of\ Hooks \times Soak\ Time} \quad (1)$$

The standard calculation for CPUE relies on the assumption that baits remain on the hook and actively fish for the entire duration of the set, an assumption which has previously been shown to be incorrect (Heithaus 2001). In the present study it was found that the rate of bait loss was significantly higher in the wall zone compared to the other habitat zones (Kruskal-Wallis:  $\chi^2 = 99.4$ ,  $p < 0.001$ ), presumably due to the higher density of scavenging fishes compared to other habitats. To account for these disparate rates of bait loss, the protocols established by Wirsing et al. (2006) for drum line surveys were incorporated into the longline CPUE formula. It was assumed that every hook retrieved without bait, or on which a shark had been captured, had ceased fishing half way through the survey, and fishing effort was adjusted accordingly. The adapted formula used to calculate CPUE in the present study is as follows (Equation 2):

$$CPUE = \frac{Catch}{(Number\ of\ Hooks \times Soak\ Time) - \left( (Number\ of\ Baits\ Lost + Catch) \times \left( \frac{Soak\ Time}{2} \right) \right)} \quad (2)$$

#### 5.2.4 Data Analysis

Catch-per-unit effort data, like most abundance data, are characterised by large numbers of zeroes leading to a heavily skewed distribution (Fletcher et al. 2005, Martin et al. 2005), and as a result, fail the assumptions of the majority of traditional statistical techniques (Zar 1984). Ignoring the characteristics of these zero-inflated CPUE datasets compromises the detection of trends, and alternatively, can lead to the identification of trends that do not exist (Martin et al. 2005).

In the present study, a two-stage hurdle model was used to identify the relationship of reef shark abundance to season and habitat type (Fletcher et al. 2005, Fletcher and Faddy 2007, Bejarano et al. 2010). This technique splits the analysis into two parts using two datasets derived from a single abundance (CPUE) dataset, one binary, indicating the presence or absence of *C. perezi*, and a second continuous dataset of CPUE data which truncates the dataset to exclude sets where reef sharks were not encountered.

The first stage of the analysis modelled presence/absence data using contingency, following which, chi-squared tests were used to test the null hypothesis that the distribution of presence and absence was equal across categories. Where significant differences were indicated, post-hoc, serial chi squared tests were performed to identify category specific differences. If a specific analysis was conducted over multiple years a Cochran-Mantel-Haenzel Test was used instead of Pearson's Chi Squared, as it tests the consistency of trends over a third blocking variable, in this case year. No Bonferroni corrections were applied to the threshold of significance ( $\alpha$ ) for post-hoc tests (Rothman, 1990; Perneger, 1998; Nakagowa, 2004).

Where the presence/absence data identified significant trends, the second stage of the analysis was implemented whereby relative abundance (CPUE) data was analysed using analysis of variance (ANOVA) with post-hoc Tukey analysis (see Fletcher et al. 2005 and Bejarano et al. 2010 for details). Prior to analysis, the distribution of CPUE data were analysed using Shapiro-Wilk W Test and, where necessary, transformed using the Box-Cox procedure (Box and Cox 1964). All analyses were performed using JMP 7.0.1 (SAS Institute, Cary, NC, USA) and the level of significance ( $\alpha$ ) for all tests was 0.05.

### 5.3 Results

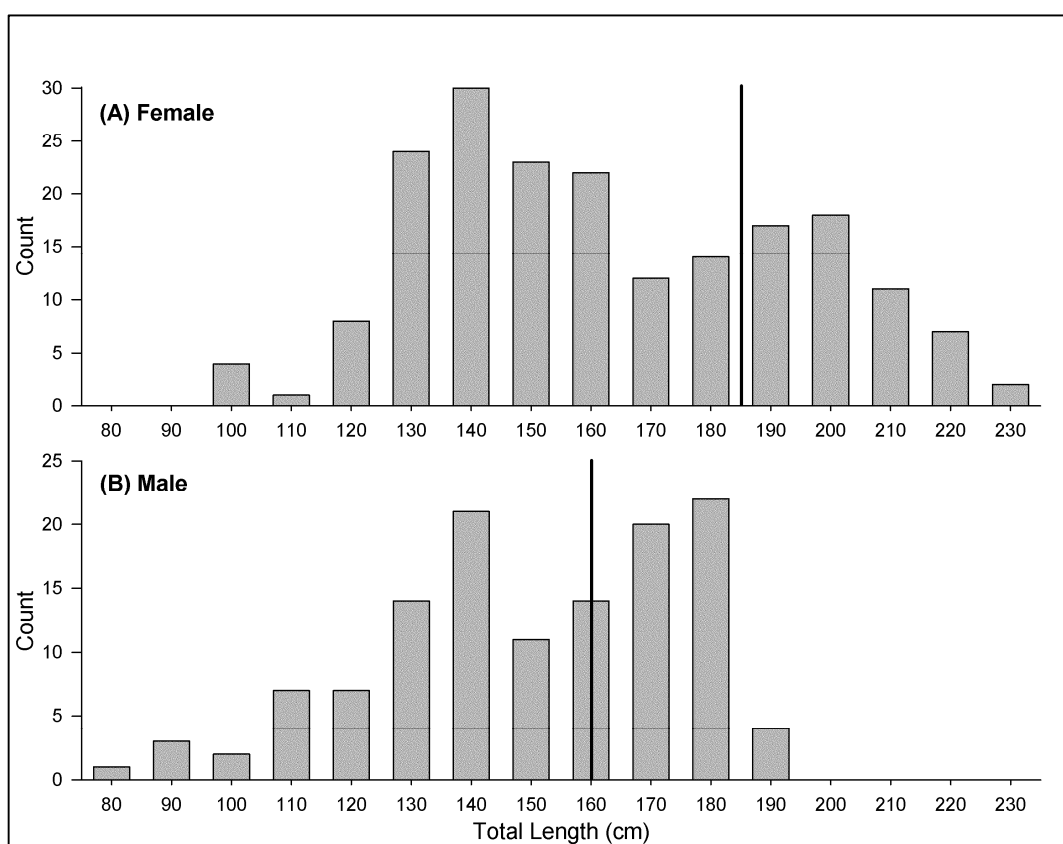
During the study, 377 standardised longline surveys were conducted resulting in the capture of 331 *C. perezii*. At-vessel mortality rates were low at 2.72 % (n = 9), of which approximately half (n = 4) could be attributed to hooking in the gut or throat, and a single death attributed to gastrointestinal blockage from the ingestion of plastic. Subsections of the dataset were selected for specific analyses based on the homogeneity of sampling effort within that period, a key assumption for all relative abundance analyses (Southwood and Henderson 2000).

#### 5.3.1 Demographic Population Structure and Size at Maturity

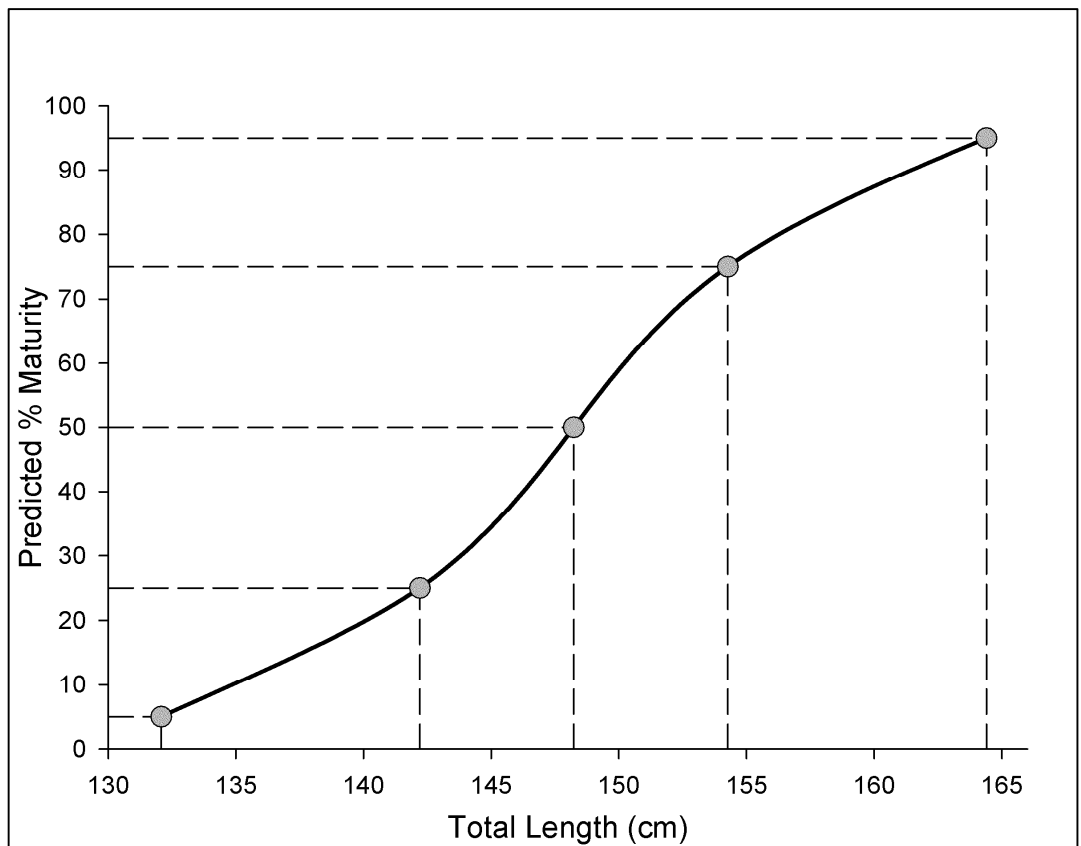
Of the 331 sharks captured during this study, sex was accurately identified in 314 individuals (Table 9). Females were more abundant than males with an observed ratio of 1.6 females for every male captured. Immature sharks were approximately 1.8 times more abundant than mature sharks. The catch was dominated by immature females representing 45.5% of the animals caught. Length frequency distribution suggested a wide range of life stages are present off Cape Eleuthera, with the exception of smaller, young-of-the-year sharks (Figure 19, Table 9). Indeed, only four individuals, all of which were male, were identified as young-of-the-year, ranging in size from 0.75 – 0.89 m  $L_T$ . The smallest mature and the largest immature male animals were 1.37 cm and 1.59 m respectively and the mean size of all mature males was 1.66 m  $L_T \pm 0.15$  S.E. Logistic regression indicated a significant relationship between maturity and total length for male sharks ( $r^2 = 0.69$ ,  $p < 0.001$ ). Based on this logistic function, 50% of the male population are predicted to be mature at 1.48 m  $L_T$  (Figure 20). Fresh mating scars were identified on only three females in the month of June, and well healed mating scars were identified in a further three females in the month of September.

**Table 9 - Population structure of Caribbean reef sharks captured 2008 – 2011 in the northeast Exuma Sound, The Bahamas.**

Demographic	Count	% Catch	Total Length Range (cm)	Total Length Mean (cm)
All	314	100	75 - 222	153.33
Male	122	38.9	75 - 189	145.84
Female	192	61.1	91 - 222	158.22
Mature	112	35.7	n/a	n/a
Immature	202	64.3	n/a	n/a
Immature Females	143	45.5	91 - 184	144.24
Immature Males	60	19.1	75 - 169	129.92
Mature Females	49	15.6	185 - 222	199.88
Mature Males	62	19.7	137 - 189	165.69



**Figure 19 - Length frequency of female (A) and male (B) Caribbean reef sharks (*Carcharhinus perezi*) captured 2008 – 2011 in the northeast Exuma Sound, The Bahamas. Vertical black lines represent the approximate size at maturity based on published articles to date (Pikitch et al. 2005, Tavares 2009, Castro 2011).**



**Figure 20 - Logistic relationship between maturity and total length of male Caribbean reef sharks (*Carcharhinus perezii*). Data points represent the predicted size at which 5%, 25%, 50%, 75% and 95% of the male population is mature.**

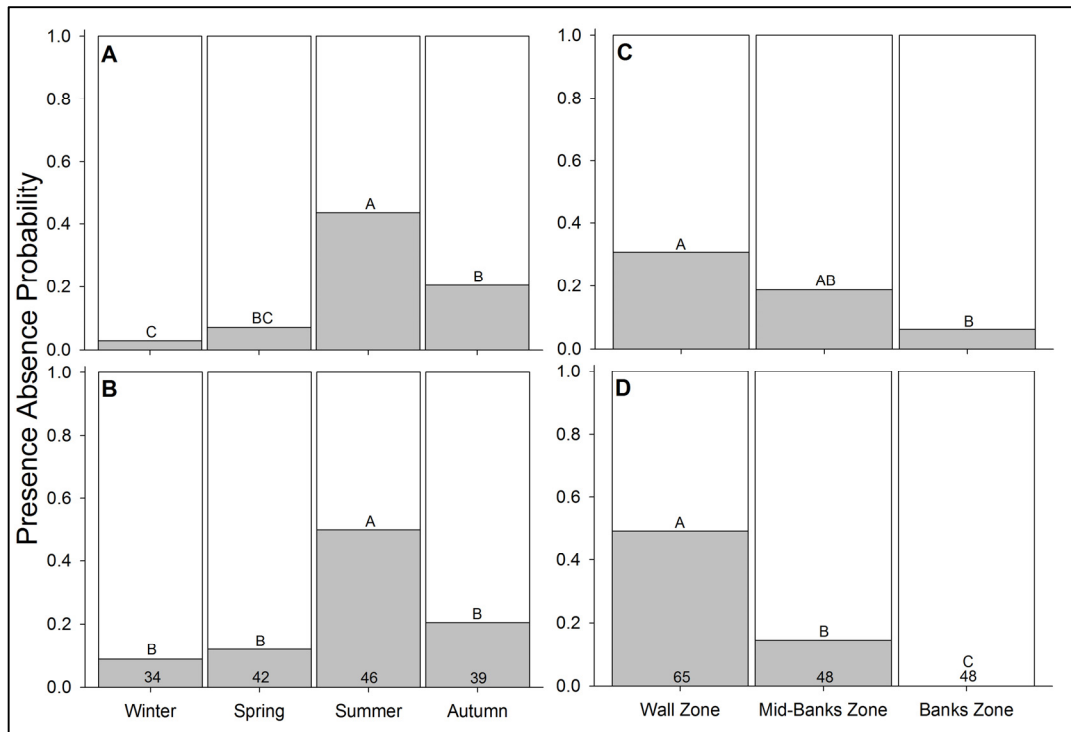
### 5.3.2 Variation in Relative Abundance

#### 5.3.2.1 *Seasonal and Spatial Variation in Abundance*

This analysis incorporated 161 longline surveys conducted June 2008 – June 2009 evenly distributed between the four seasons ( $\bar{x} = 40.25 \pm 2.53$  S.E. per season) and three habitat zones ( $\bar{x} = 53.7, \pm 5.67$  S.E. per zone). There was a significantly higher probability of catching *C. perezii* in the summer compared to all other months, and significantly higher capture probability in the autumn compared to winter (Contingency Analysis –  $\chi^2 = 40.55, p = < 0.001$ ; Figure 21a).

Sharks were more commonly encountered in the wall zone compared to the mid-banks and banks zones, and more commonly encountered in the mid-banks than the

banks zone (Contingency Analysis –  $\chi^2 = 42.16$ ,  $p = < 0.001$ ; Figure 21b). Analysis of the zero-truncated CPUE data indicated that sharks were significantly more abundant in the wall zone compared to both the mid-banks and banks zones (ANOVA –  $F_{2,56} = 14.75$ ,  $p = < 0.001$ ).



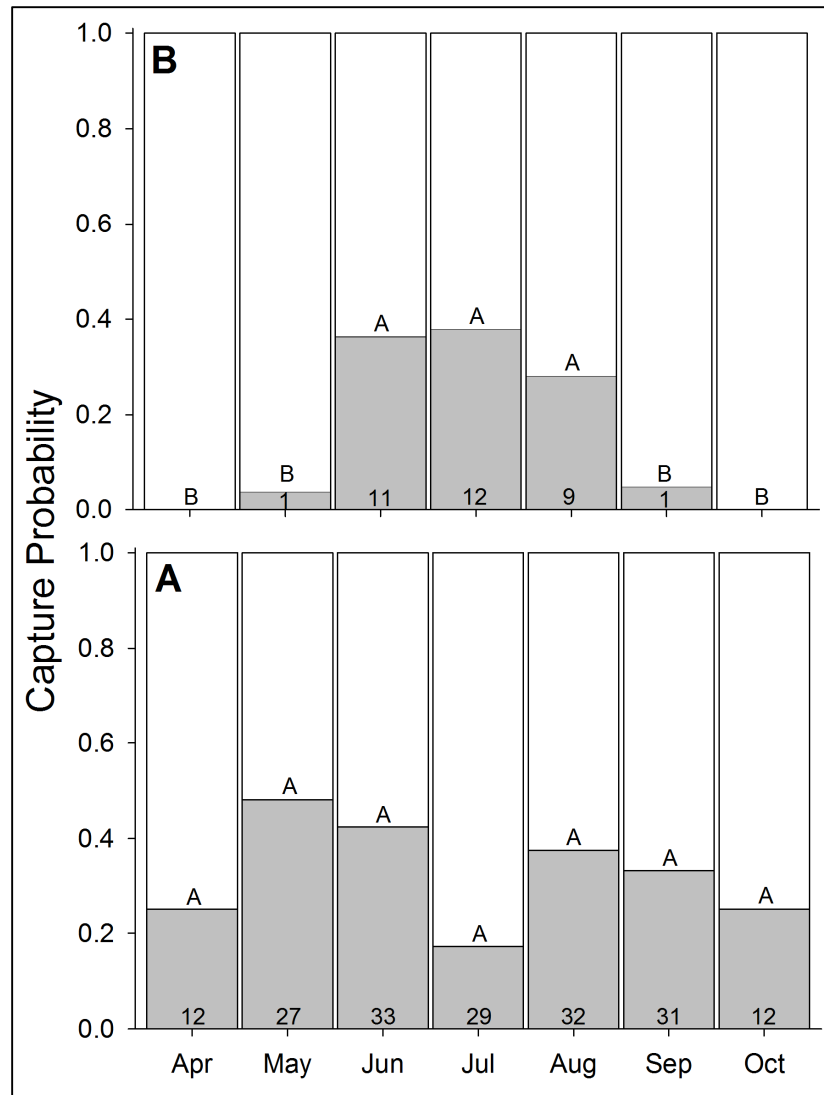
**Figure 21 - Seasonal and spatial variation in relative abundance in mature (A & C), and immature (B & D), Caribbean reef sharks (*Carcharhinus perezii*) in the northeast Exuma Sound, The Bahamas. Significant differences are illustrated by dissimilar letters and sample size is indicated at the column base.**

### 5.3.2.2 Demographic Variation in Temporal Abundance

With a goal of identifying demographic differences in presence/absence and relative abundance, data were subdivided to yield datasets based on maturity and sex. This analysis incorporated 166 longline surveys conducted May – October 2010 and 2011 ( $\bar{x} = 23.7 \pm 3.36$  S.E. per month).

Mature *C. perezii* were significantly more likely to be encountered in June, July and August compared to April, May, September and October (Cochran-Mantel-Haenzel Test –  $\chi^2 = 26.313$ ,  $p = < 0.001$ ; Figure 22). Furthermore, stage two hurdle analysis

indicated that mature *C. perezii* were significantly more abundant in June compared to July (ANOVA –  $F_{4,21} = 3.652$ ,  $p = 0.016$ ). There was no significant variation in the abundance of immature animals over the same period (Cochran-Mantel-Haenzel Test –  $\chi^2 = 8.025$ ,  $p = 0.236$ ).



**Figure 22 - Monthly capture probability of mature (A), and immature (B), Caribbean reef sharks (*Carcharhinus perezii*) in the northeast Exuma Sound, The Bahamas. Significant differences are illustrated by dissimilar letters and sample size is indicated at the column base.**

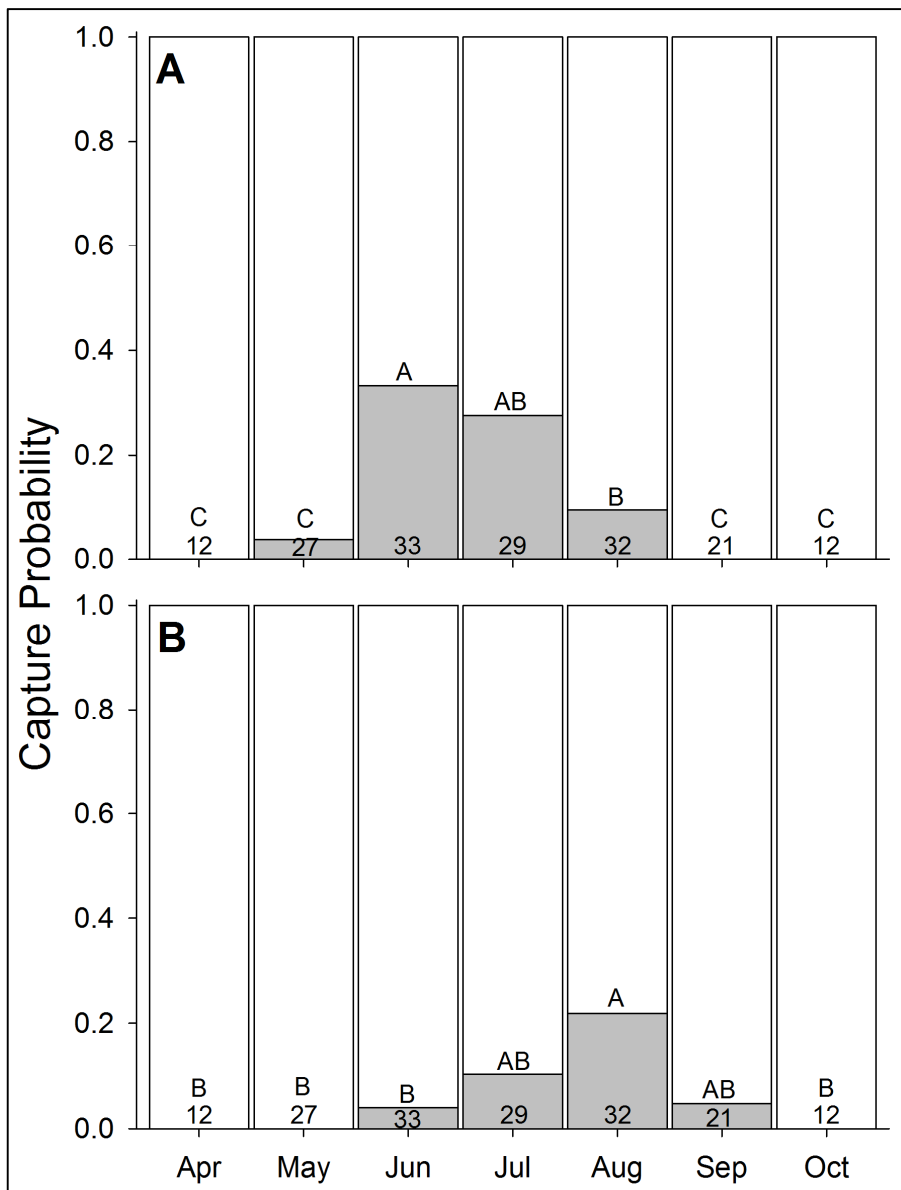
Significant variation in the presence and absence of both mature male (Cochran-Mantel-Haenzel Test –  $\chi^2 = 25.5987$ ,  $p < 0.001$ ; Figure 23a) and mature female sharks were identified (Cochran-Mantel-Haenzel Test –  $\chi^2 = 15.699$ ,  $p = 0.016$ ; Figure 23b),

however, peaks in maximal abundance were a month apart, with maximal mature male abundance in June compared to maximal mature female abundance in August. Stage two analysis of the zero-truncated dataset indicated that mature male *C. perezi* were significantly more abundant in June compared to July (ANOVA –  $F_{3,19} = 5.879$ ,  $p = 0.005$ ), however, no significant trends were identified in mature females ANOVA –  $F_{3,8} = 1.909$ ,  $p = 0.206$ ).

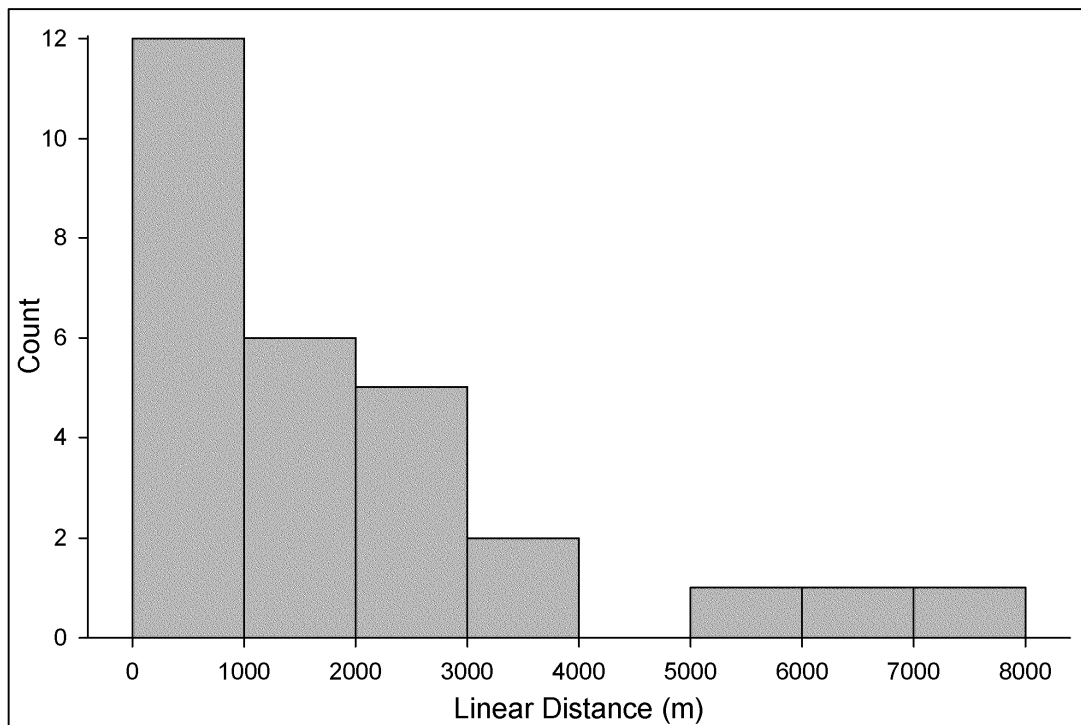
### 5.3.3 Mark Recapture

Of the 331 sharks captured and tagged, a total of 52 (15.4%) were recaptured after periods at liberty between 5 – 1159 days ( $\bar{x} = 333.4 \pm 42.7$  S.E.). There was no significant difference in the sex ratios between captured and recaptured animals (Chi Squared –  $\chi^2 = 0.811$ ,  $p = 0.368$ ). In order to quantify long-term philopatry, the recapture dataset was truncated to include only recaptures in excess of 180 days (6 months) at liberty. For these recapture events ( $n=28$ ), the straight-line distance between the point of capture, and the point of recapture was calculated using Pythagorean Theorem. The mean linear distance between capture and recapture after a minimum of six months at liberty was  $1767 \text{ m} \pm 365.23$  S.E. (Figure 24).





**Figure 23 - Monthly capture probability of mature male (A) and mature female (B) Caribbean reef sharks (*Carcharhinus perezii*) in the northeast Exuma Sound, The Bahamas. Significant differences are illustrated by dissimilar letters and sample size is indicated at the column base.**



**Figure 24 - Linear distance frequency of recaptured Caribbean reef sharks (*Carcharhinus perezii*) in the northeast Exuma Sound, The Bahamas.**

#### **5.4 Discussion**

Identifying spatiotemporal patterns of movement and habitat association, in particular those that incorporate sexual or size based segregation, is critical when developing effective management and conservation strategies for sharks (Dingle 1996, Speed et al. 2012). While the movement patterns of *C. perezii* have been studied in several locations (see Chapman et al. 2005, Garla et al. 2006a, Garla et al. 2006b, Bond et al. 2012), this is the first time spatiotemporal and demographic population structuring has been described. This study identified clear increases in abundance during the summer, in addition to precise, year-to-year philopatry, indicated by the comparatively high recapture rate and short distances between capture and recapture points. In some cases, recaptures occurred after multiple years at liberty suggesting that annual migrations are precise and cyclical. Philopatry, which is often spatiotemporally stratified by sex and ontogeny, is a common behaviour in a large number of marine

species, including sharks (Hueter et al. 2005), and has been previously identified in populations of *C. perezii* in other regions. Recapture rates of juvenile *C. perezii* (< 110 cm) in the Fernando de Noronha Archipelago (15.3%) were almost identical to the present study (15.4%), furthermore, the linear distances between capture and recapture were also comparable (Garla et al. 2006a). Telemetry studies in both the Fernando de Noronha Archipelago and Belize identified distinct philopatry in both juvenile (Garla et al. 2006a) and adult (Chapman et al. 2005, Bond et al. 2012) *C. perezii*, however, no seasonal variation in movements or residency was identified in any of these studies, in direct contrast to the findings of the present study.

Philopatry in species closely related to *C. perezii* found in the greater Caribbean region is common. Natal philopatry, whereby mature females return to their natal area to give birth, has been tentatively identified in lemon sharks (*Negaprion brevirostris*; Feldheim et al. 2004), blacktip sharks (*Carcharhinus limbatus*, Keeney et al. 2005) and bull sharks (*Carcharhinus leucas*; Tillett et al. 2012). Sexually stratified philopatry was identified in nurse sharks (*Ginglymostoma cirratum*) whereby males returned to a mating site annually in contrast to females which followed a biennial cycle (Pratt and Carrier 2001). Philopatry which is seasonally, but not sexually or ontogenetically stratified is exhibited by blacknose sharks (*Carcharhinus acronotus*) which occupy large embayments on the gulf coast of Florida in the summer for mating and feeding (Hueter et al. 2005). The grey reef shark (*Carcharhinus amblyrhynchos*), an indo-pacific species thought to inhabit a similar ecological niche as *C. perezii*, also exhibits philopatric behaviour but is known to undertake large scale movements (> 250 km) on occasions (Heupel et al 2010, Barnett et al. 2012). Given the presence of spatiotemporal, sexual and ontogenetic structure to philopatric behaviour in these closely related species, the structured philopatry identified in the present study is unsurprising; however, the driving forces behind this structure remain unknown.

The movement patterns contributing to the summer increase in abundance are compounded by apparent demographic stratification within the *C. perezii* population in this region. Sexual and size based segregation is considered widespread in shark populations (Springer 1967, Sims 2005, Mucientes et al. 2009, Speed et al. 2012), however, this is the first reported instance of *C. perezii* sex ratios diverging from the 1:1 male to female ratios of populations identified in Belize (Pikitch et al. 2005) and Venezuela (Tavares 2009). Previous studies captured small young-of-the-year sharks ( $< 100$  cm  $L_T$ ) in habitats similar to those sampled in the current study (Garla et al. 2006b) suggesting that habitat use by this life stage is likely different in The Bahamas than other regions. Size based variation in habitat use has been described in this species before, whereby small sharks ( $< 1.10$  m  $L_T$ ) are more commonly found inshore in lagoons, and larger sharks ( $> 1.10$  m  $L_T$ ) are more common on deeper fore-reefs adjacent to deep water (Pikitch et al. 2005). The abundances of these two size classes have been found to mirror each other on a diurnal basis, suggesting that smaller sharks avoid larger conspecifics (Chapman et al. 2007). It is clear that *C. perezii* populations in The Bahamas are also ontogenetically segregated, but over a larger geographic scale than previously described.

Recent studies have focused on the relatively small and isolated oceanic islands of Fernando de Noronha in Brazil, and Glovers Reef in Belize, which are approximately 26 and 400 km<sup>2</sup> in area respectively (Garla et al. 2006a, Garla et al. 2006b, Chapman et al. 2007). In contrast, the present study was conducted on the Great Bahamas Bank which encompasses an area of ~113,000 km<sup>2</sup>, the majority of which is a diverse mosaic of marine habitats interspaced with multiple islands, banks and channels. Animal movements are driven by activities and environmental conditions that promote growth, survivorship and reproductive success (Dingle 1996). *C. perezii* in the Great Bahamas Bank region have access to a larger and more diverse range of habitats which different

population components can use and re-visit on a seasonal basis. We hypothesise that this greater habitat complexity drives the spatiotemporal and demographic population structuring observed in this study. As different habitat types impart advantages and disadvantages to different demographics at different times of the year, it is probable that different life stages have developed more complex habitat use patterns based on their biological requirements in comparison to populations studied to date.

The specific stimuli that have driven the development of these demographically segregated abundance patterns and the precise year-on-year philopatry are not yet clear; however, based on the available data some tentative hypotheses can be proposed. The dominance of immature female sharks, combined with peaks in mature male and female abundance occurring over a month apart, suggest that mating is not the primary activity within the study area. This is further supported by the presence of fresh mating scars on only three mature female sharks all of which were in June which coincides with mating activity observed in other parts of the Bahamas (C. Zenato, pers. com., Maljković and Cote 2011). If mating was the primary activity within the aggregation, a much higher proportion of females bearing mating scars would be expected. Seasonally abundant food sources are known to cause concomitant increases in shark abundance (Dudley and Cliff 2010), although it is suggested that this stimulus would act over an entire population equally, not selecting specific demographics at different times as in the present study. A more likely hypothesis is based on the idea that smaller sharks utilise different habitats to avoid larger conspecifics (Guttridge et al. 2012), either to reduce predation risk, or to decrease foraging competition (Heithaus et al. 2008). The avoidance of larger conspecifics has been identified on a smaller scale in populations of *C. perezi* in Belize (Chapman et al. 2007), and it is possible that the trends observed at Cape Eleuthera are a spatiotemporal expansion of this behaviour in response to the larger and more diverse habitat mosaic of the Great Bahamas Bank. At present, there

are no data with which to test this hypothesis and it is unclear if this sized-based segregation is due to the avoidance of larger conspecifics or the avoidance of larger predators in general.

The present study suggests that *C. perezi* may mature at a smaller size than recent estimates indicate, however, making meaningful comparisons is challenging given the lack of standardised reporting of size-at-maturity parameters in previous studies. Pikitch et al. (2005) states a size-at-maturity of male sharks at Glovers Reef, Belize, of 1.50 – 1.70 m  $L_T$ , which coincides with data from Venezuela (Tavares 2009), but is wider in range than the more recent 1.68 – 1.70 m  $L_T$  estimate of Castro (2011). The most recent female size-at-maturity estimate is 1.90 m  $L_T$  (Castro 2011), whereas the mean length of mature female sharks in Venezuela was 1.83 m  $L_T$ , and the smallest mature female was 1.54 m  $L_T$  (Tavares 2009). In general, this study has yielded the smallest size-at-maturity estimates for male *C. perezi* to date. The mean length of mature males (1.66 m  $L_T$ ) and the smallest mature male (1.37 m  $L_T$ ) in the present study were 0.15 m and 0.12 m smaller respectively compared to populations studied in Venezuela (Tavares 2009). Furthermore, the size at which 50% of the male population are predicted to reach maturity (1.48 m  $L_T$ ) was on the lower bounds of the range given by Pikitch et al. (2005). With respect to females, reports of small (~1.55 m  $L_T$ ) mature female sharks in Venezuela (Tavares 2009) and the presence of several females < 1.80 m  $L_T$  with healed mating scars in the present study, suggests that previous estimates for females might be too large, and there is greater individual variation than previously thought. One caveat is that the aforementioned sized based segregation of local populations could be considered a source of error, however, a theoretical 0.15 m  $L_T$  drop in the size at maturity for females to 1.70 m  $L_T$  still yields a skewed mature to immature ratio of 1:1.7, and immature females would still represent 39.5% of the catch, suggesting that even if the proportions change, the population is still clearly segregated.

In order to facilitate meaningful size-at-maturity comparisons, standardised reporting of size at maturity data is necessary. This should include the smallest mature and largest immature lengths, mean length of mature animals and logistic length predictions at which 50% of the population reach maturity, the latter of which is commonplace in literature pertaining to many species of sharks (e.g. Carlson and Baremore 2003, Norman and Stevens 2007, Papastamatiou et al. 2009). Furthermore, additional research into female size at maturity is needed prior to drawing firm conclusions about sexual segregation and demographically stratified movement patterns. Given the recent development of hormonal assays to establish reproductive status (see Sulikowski et al 2007, Awruch et al. 2008, Heupel and Simpfendorfer 2010), this work can now be conducted via a time series of non-lethal blood samples.

The results of this study suggest that *C. perezii* conforms more closely to the classic life history model of a coastal carcharhinid shark than previous research suggests (Springer 1967). Indeed, demographically segregated populations and seasonally stratified movements are widespread among chondrichthyan species (Speed et al. 2012, Mucientes et al. 2009), and the apparent absence of these behaviours in *C. perezii* was an anomaly. This study suggests that *C. perezii*, given access to a large and diverse range of habitats, will develop seasonal, demographically stratified, movement patterns that create concomitant variation in demographic population structure. However, at present, the driving forces behind these movements and habitat use patterns are unclear. In order to ensure the effective management and conservation of *C. perezii* in the region, further research is needed to elucidate the complex habitat use model it has evolved. Furthermore, the apparent evolution of geographically discrete behavioural patterns within this species indicates the potential for studies investigating behavioural plasticity in response to environmental variation and anthropogenic impacts.

## Chapter Six

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### **6. First characterisation of deep water elasmobranch assemblages in the Exuma Sound, The Bahamas: Diversity, distribution and population structure.**

#### **ABSTRACT**

Deep sea chondrichthyans, like many deep water fishes, are very poorly understood on the most fundamental biological, ecological and taxonomic level. This group of elasmobranchs has amongst the lowest intrinsic rebound potential of any taxa quantified to date, and as such are especially vulnerable to fisheries, both as a targeted resource and as bycatch. Our study represents the first sustained ecological investigation of deep water elasmobranch assemblages in The Bahamas. In addition, we assessed species-specific resilience to capture via both at-vessel and post-release mortality rates. Standardised deep water longline surveys (n = 69) were conducted between September – December 2010 and 2011 between 472.6– 1024.1 m deep. A total of 144 sharks from 10 different species were captured including the Cuban dogfish, *Squalus cubensis*, the bigeye sixgill shark, *Hexanchus nakamurai*, the bluntnose sixgill, *Hexanchus griseus*, the smooth dogfish, *Mustelus canis insularis*, the roughskin dogfish, *Centroscymnus owstoni*, Springer’s sawtail catshark, *Galeus springeri* and the false catshark, *Pseudotriakis microdon*. Preliminary genetic analysis identified three separate species of gulper sharks, *Centrophorus* spp., however, for the present study they were treated as a single species complex. Increased water depth, decreased seabed temperature and increasing distance from the rocky structure of the Exuma Sound wall were inversely correlated with species richness. These variables also had a significant influence on the abundance of many species. Multivariate analysis identified two distinct elasmobranch assemblages, a shallow assemblage (~554.3 m) dominated by *S.*



*cubensis*, and a deeper (~716.8 m) assemblage dominated by *Centrophorus* spp. At-vessel mortality rates increased significantly with depth, and post-release mortality was thought to be high, in part due to high post-release predation rates. This study highlights the importance of utilising strategic geographic locations which provide easy access to deep water, thus facilitating temporally and spatially sustained investigation at lower costs than typically associated with deep sea research. Accelerated acquisition of fundamental ecological and biological insights into deep sea elasmobranchs is urgently required if effective management and conservation initiatives are to be implemented.

## 6.1 Introduction

There is a fundamental lack of basic biological and ecological information pertaining to the majority of deep water species (Haedrich et al. 2001), largely due to the logistical challenges and expense of sustained ecological investigation in this remote ecosystem. The little existing information suggests that deep water fishes have amongst the lowest levels of species productivity ever recorded, making them exceptionally vulnerable to anthropogenic sources of mortality (Koslow et al. 2000). Despite this sensitivity, global population growth, advancement in fisheries technology and the decline of shallow water fisheries have increased both the need and ability to access deep water fish stocks (Morato et al. 2006), causing precipitous declines in some regions (Norse et al. 2012). It has recently been suggested that the economic characteristics of deep sea fisheries are more akin to terrestrial mining operations, and that deep water resources are a finite natural resource that cannot be fished sustainably (Norse et al. 2012).

The deep sea is home to ~50% of all known chondrichthyan species (Kyne and Simpfendorfer 2007). The study of deep water elasmobranchs is very much in its infancy; indeed, many genera need further investigation on the most fundamental genetic and taxonomic level (Last 2007, Naylor et al. 2012). Estimates of species productivity and intrinsic rebound potential only exist for 2.2% of deep ocean chondrichthyans (Kyne and Simpfendorfer 2010), and those that have been assessed have among the lowest values documented for any species of fish to date (Simpfendorfer and Kyne 2009). In areas where deep water sharks have been actively targeted by fisheries, dramatic population declines have been triggered (Anderson and Ahmed 1993, Daley et al. 2002, Graham et al. 2001, Jones et al. 2005, Koslow et al., 2000), and it is likely that bycatch of elasmobranchs in deep water trawl and longline fisheries is having similar negative effects (Graham et al. 2001). Given the slow rate of

scientific advancement in the deep ocean, it has been suggested that many fisheries may become commercially extinct before scientific study can begin (Haedrich et al. 2001).

The effective management and conservation of deep sea shark stocks is dependent on access to pertinent life history, community structure and species resilience data, information which at present is largely absent. Given the recent increase in deep sea fisheries, one of the most pressing areas of research from a management point of view is species-specific resilience to capture. The capture of an animal in commercial fishing gear imposes both physiological and physical insults, which can lead to either immediate (at-vessel), or post-release mortality (Brooks et al. 2012, Skomal and Mandelman 2012). For deep water sharks, the stress of capture is potentially compounded by the additional thermal, barometric and photic stress associated with an ascent from the deep ocean. Consequently, it is likely that deep water sharks have higher rates of both at-vessel and post-release mortality than shallow water species; however, this has yet to be investigated.

Given this acute lack of fundamental data pertaining to deep water elasmobranchs in the greater Caribbean region (McLaughlin and Morrissey 2004), there were two objectives to this study. Firstly, to investigate the diversity, distribution and demographic structure of deep water elasmobranch assemblages in the northeast Exuma Sound, The Bahamas; and secondly, to assess the resilience of the species encountered to longline capture.

## **6.2 Methods**

Research was carried out under the Cape Eleuthera Institute research permit numbers MAF/FIS/17 and MAF/FIS/34 issued by the Bahamian Department of Marine Resources and in accordance with CEI animal care protocols developed within the

guidelines of the Association for the Study of Animal Behaviour and the Animal Behaviour Society (Rollin and Kessel 1998).

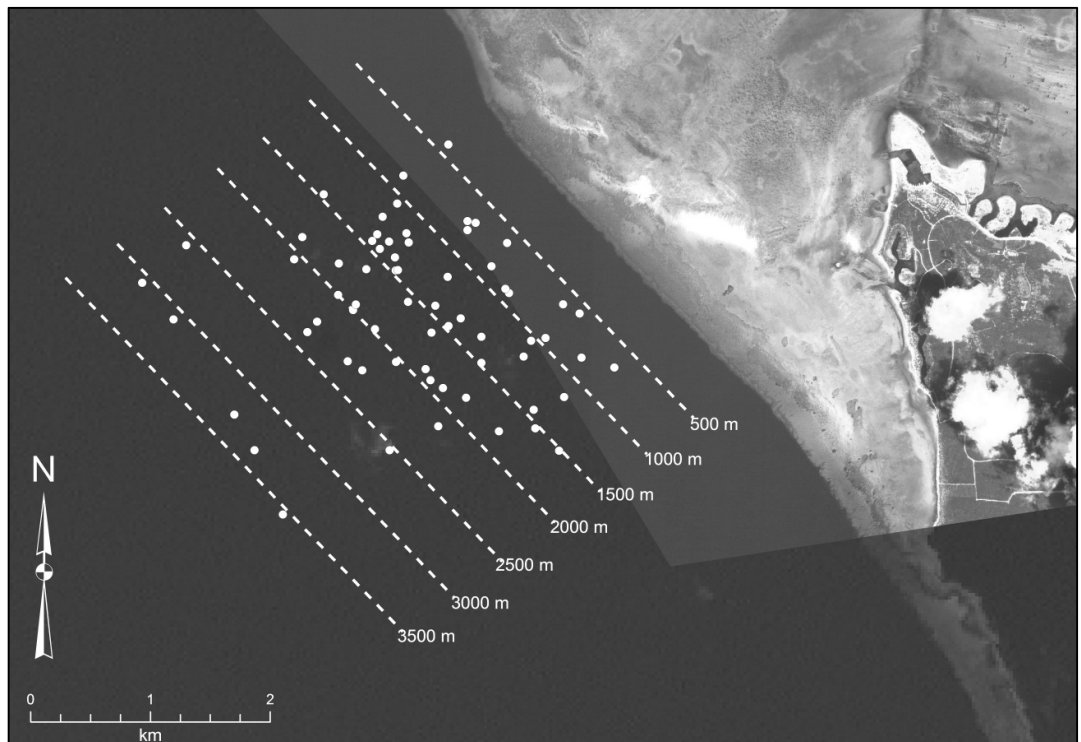
### 6.2.1 Study Area

This study was conducted between September – December 2010 and 2011 in the waters adjacent to Cape Eleuthera, Eleuthera, The Bahamas (24.54° N, 76.12° W). The Exuma Sound is a deep water inlet of the Atlantic Ocean 200 km in length and 50 – 75 km in width, orientated approximately NW/SE on its long axis. The Exuma Sound is surrounded by the shallow waters of the Great Bahama Bank and is characterised by steep walls dropping from ~30 m to over 500 m along its margin, slowly increasing to a maximum depth of 1600 - 2000 m approximately 25 km from the wall (Buchan 2000). The northeast corner of the Exuma Sound is ~2.5 km from Powell Point, on the south eastern tip of the island of Eleuthera, providing access to water depths in excess of 1000 m of water in less than 20 minutes.

### 6.2.2 Vertical Longline Sampling

Standardised demersal longline surveys consisted of a 1000 – 2000 m mainline anchored to the seabed by a single grapnel anchor. Thirty gangions terminating in one of four different sizes of circle hook (5 x 16/0, 5 x 14/0, 10 x 12/0, 10 x 10/0) were spaced approximately 10 m apart originating 5 m from the anchor. An archival temperature and depth recorder (TDR) (Lotek LAT-1400, Newfoundland, Canada), programmed to record temperature and depth every second, was affixed to the mainline 10 m from the last hook. Depth and seabed temperature for each set were taken as the deepest and coldest record from the entire dataset. Soak time was ~4hrs based on deployment and retrieval times, however, more accurate soak times were calculated post-hoc from TDR data, based on time at maximum depth. For each survey, the straight line distance between the survey location and the vertical wall, marking the

edge of the Exuma Sound, was measured using Arc GIS (Version 9.1, ESRI, Redlands, California, USA). Based on these measurements, surveys were assigned to six, 500-m wide geographic zones (Figure 25). Since no surveys were conducted within 500 m of the wall to minimise entanglement and gear loss in the rocky structure, Zone 1 was defined as 500 m – 1000 m from the wall of the Exuma Sound, Zone 2 1000 m – 1500 m, and so on through to Zone 6 which was 3000 m - 3500 m from the drop off (Figure 25).



**Figure 25 - Sampling zones, stratified by distance from the wall of the Exuma Sound, off Cape Eleuthera, The Bahamas. Dotted lines indicate sampling zones and points indicate the geographic location of each longline replicate. Total area sampled over the course of the study was ~10.5 km<sup>2</sup> at depths ranging from 473 - 1024 m.**

All sharks captured were identified to species and pre-caudal ( $L_{PC}$ ), fork ( $L_F$ ) and total ( $L_T$ ) lengths recorded. Sex was established macroscopically. Maturity estimates were based on the degree of calcification and length of the claspers in males, and published size-at-maturity data, when available, in females. In addition, a dart tag was inserted in basolateral dorsal musculature (Hallprint, Victoria Harbour, Australia) and a

small fin clip was collected for genetic analysis. As many deep water sharks are of unresolved taxonomies and species complexes are common, a small genetic sample was taken from the dorsal fin and stored in 95% ethanol prior for further analysis.

### 6.2.3 Estimating Mortality

At-vessel mortality was quantified upon retrieval of the gear and expressed as a percentage of the total catch of a specific species. The quantification of post-release mortality is more challenging given the extreme conditions of the deep water environment. In recent years pop-up archival satellite transmitters (PSAT) have been used to quantify the post-release mortality and behaviour of a number of shallow water species of shark (e.g. Campana et al. 2009; Hoolihan et al. 2011), however, this methodology has yet to be applied to deep water species. In the present study, X-Tags (Microwave Telemetry, Inc., Columbia, MD) 12 cm in length, 43 g weight in air, and rated to a crush depth of 2500 m, were deployed. These tags were pre-programmed to detach after a set period of time (30 days – 6 months), whereupon they float to the surface and transmit archived temperature, depth and sunrise/sunset times through the Argos satellite system.

### 6.2.4 Data Analysis

Abundance data relating to sparsely distributed animals is typically characterised by large numbers of zeros leading to a heavily skewed distribution (Martin et al. 2005), and as a result, fails the assumptions of the majority of traditional parametric statistical techniques. In the present study, Contingency Analysis was used to model species specific presence/absence against capture location (Zones 1 - 6). Following contingency analysis, Pearson's Chi Squared tests were used to test the null hypothesis that the distribution of presence and absence was equal across categories. The effect of water depth and seabed temperature on the presence/absence of each species was

modelled using logistic regression. Elasmobranch species richness, defined as the number of unique species captured on a single longline, was analysed with respect to water depth, seabed temperature and sampling location using non-parametric, Spearman Rank Correlation. Male size at maturity estimates were generated using logistic regression when sufficient numbers of both mature and immature male animals were captured. Inverse prediction based on the logistic function was used to predict the length ( $L_T$ ) at which 50% of the male population reached maturity (Papastamatiou et al. 2009). All the above analyses were performed using JMP 7.0.1 (SAS Institute, Cary, NC, USA) and the level of significance ( $\alpha$ ) for all tests was 0.05.

Spatial variability in the structure of deep elasmobranch assemblages was examined using PRIMER v6.0 (Plymouth Marine Laboratories, Devon, UK). Abundance data were transformed (fourth root) to aid in removal of bias from rare species, and a dummy variable (value = 1) was incorporated to force samples containing no individuals to be 100% similar (Clarke et al. 2006b). Following transformation, a zero-adjusted Bray-Curtis similarity matrix was constructed and a cluster analysis performed. The resulting dendrogram output was accompanied by a SIMPROF test to examine statistical evidence of genuine clusters (Clarke and Gorley 2006). A non-metric multidimensional scaling (MDS) ordination was created from the matrix and the similarity contours in the MDS were derived from the cluster analysis dendrogram.

To test the null hypothesis that no spatial variation exists with the deep water elasmobranch assemblages, the ANOSIM analysis was conducted on the similarity matrix. This analysis, although not a true test of statistical significance, provides a measure of difference in the assemblage structures between factors (Clarke and Gorley 2006), in this case Zones 1 – 6.

The SIMPER analysis was employed to determine, 1) which species drove the difference in assemblage structure identified between pairs of depth zones and 2) the species that provided cohesion within the individual depth zones. This analysis was performed using fourth-root transformed abundance data.

Lastly, to test the effectiveness of longline sampling in describing the species richness of deep water shark assemblages, a species-accumulation plot was constructed, however, given that species richness (S), defined as the number of unique species, is a non-linear function of sampling effort; absolute values of species richness are rarely reached (Southwood and Henderson 2000). Several absolute species richness extrapolators were incorporated in an attempt to predict the diversity of deepwater elasmobranch species that exists in the study area, given an infinite number of samples. Sample order was entered randomly (999 permutations) and five nonparametric extrapolation models used were, in order of conservative to least conservative: Bootstrap, Chao1, Chao2, Jackknife1, and Jackknife2 (Clarke and Gorley 2006).

### **6.3 Results**

A total of 69 deep water longline surveys resulted in the capture of 144 individual sharks from 8 different species (Table 10). Survey depths were between 472.6 m – 1024.1 m (mean = 700.0 m), and seabed temperatures of 5.9 °C – 15.6 °C (mean = 10.4 °C). Surveys were conducted between 462 m and 3588 m from the edge of the Exuma Sound, although sampling density was higher in closer proximity to the wall (Figure 25). Significant, non-linear relationships were established between water depth ( $r^2 = 0.895$ ,  $p < 0.001$ ) and temperature ( $r^2 = 0.906$ ,  $p < 0.001$ ) and distance from the ‘drop-off’ of the Exuma Sound (Figure 26). Furthermore, Spearman Rank Correlation indicated that species richness declined significantly with increasing depth ( $\rho = -0.242$ ,  $p = 0.045$ ), and decreasing temperature ( $\rho = 0.288$ ,  $p = 0.016$ ) (Figure 27).



**Table 10 - Catch composition of deep water elasmobranchs sampled over the course of the study.**

Common Name	Latin Name	n	# Males	# Female	Mean $L_T$ (cm)	Range $L_T$ (cm)	Mean Depth (m)	Mean Temp. (°C)
Cuban Dogfish	<i>Squalus cubensis</i>	55	23	32	61.0	45 - 80	581.81 ( $\pm$ 9.45 S.E.)	12.99 ( $\pm$ 0.25 S.E.)
Gulper Shark	<i>Centrophorus</i> spp.	51	9	38	95.1	52 - 156	731.4 ( $\pm$ 8.12 S.E.)	9.49 ( $\pm$ 0.17 S.E.)
Bigeye Sixgill Shark	<i>Hexanchus nakamurai</i>	14	11	3	148.4	111 - 171	639.54 ( $\pm$ 17.27 S.E.)	11.47 ( $\pm$ 0.39 S.E.)
Bluntnose Sixgill Shark	<i>Hexanchus griseus</i>	8	4	4	287.4	108 - 389	665.39 ( $\pm$ 33.52 S.E.)	11.13 ( $\pm$ 0.86 S.E.)
Dusky Smooth Hound	<i>Mustelus canis insularis</i>	7	1	6	92.1	74 - 113	549.73 ( $\pm$ 20.95 S.E.)	14.13 ( $\pm$ 0.57 S.E.)
Roughskin Dogfish	<i>Centroscymnus owstoni</i>	5	1	3	79.7	72 - 102	888.34 ( $\pm$ 39.58 S.E.)	7.29 ( $\pm$ 0.48 S.E.)
Springers Sawtail Catshark	<i>Galeus springeri</i>	3	1	2	42.2	31 - 53	706.77 ( $\pm$ 64.03 S.E.)	10.40 ( $\pm$ 1.46 S.E.)
False Catshark	<i>Pseudotriakis microdon</i>	1	1	0	227.0	227	790.1	8.7

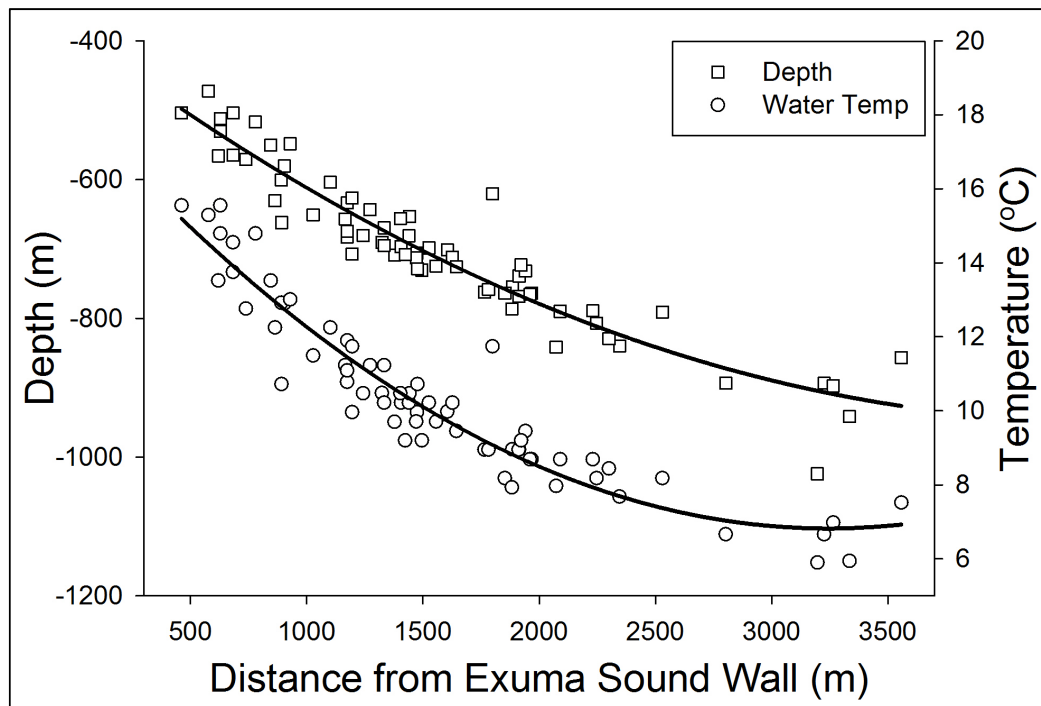


Figure 26 - Scatter plot of significant non-linear relationships between distance from the wall of the Exuma Sound, depth ( $r^2 = 0.895$ ,  $p < 0.001$ ), and temperature ( $r^2 = 0.906$ ,  $p < 0.001$ ).

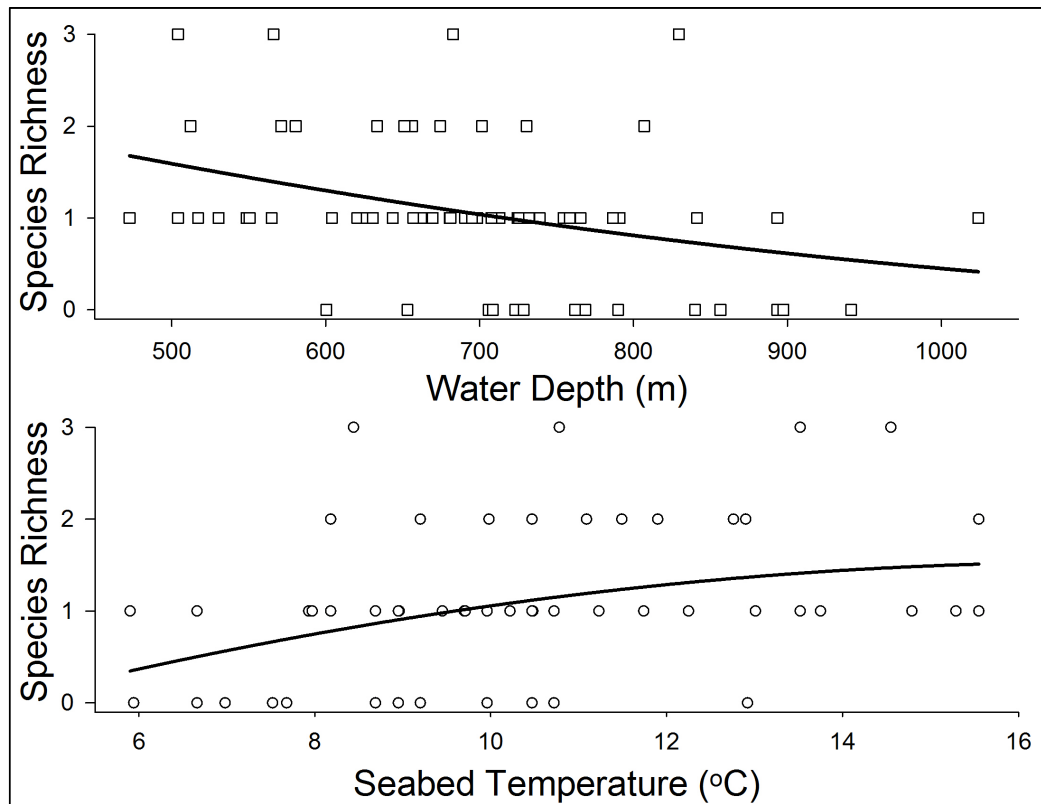


Figure 27 - Species richness was found to decrease significantly with increasing depth ( $\rho = -0.242$ ,  $p = 0.045$ ), and decreasing seabed temperature ( $\rho = 0.288$ ,  $p = 0.016$ ).

### 6.3.1 Distribution and Demographic Structure of Elasmobranchs

The Cuban dogfish (*Squalus cubensis*) was the most numerous species captured over the course of the study (n = 55). It was also one of the shallowest dwelling species, captured at a mean depth of 581.1 m ( $\pm 9.4$  S.E.), a mean seabed temperature of 13.0 °C ( $\pm 0.25$  S.E.), and was significantly higher in abundance less than 1000 m from the drop-off of the Exuma Sound (Contingency Analysis -  $\chi^2 = 23.61$ , p = <0.001; Figure 28a). Depth (Logistic Regression -  $\chi^2 = 36.52$ , p = <0.001) and temperature (Logistic Regression -  $\chi^2 = 33.94$ , p = <0.001) both exhibited a significant effect on the presence/absence of *S. cubensis*. Based on these logistic functions, there was a greater than 50% probability of catching *S. cubensis* at depths < 639.5 m and at a seabed temperature of >11.6 °C. Sex and maturity ratios were skewed with females 1.4 times more abundant than males, and mature sharks 2.6 times more abundant than immature sharks. A significant logistic relationship ( $\chi^2 = 16.36$ , p = <0.001) was established between  $L_T$  and maturity for 13 mature and 9 immature male *S. cubensis*. Based on this logistic function, 50% of the male population are predicted to be mature at 54.4 cm  $L_T$  (Range - 49.1 – 57.9 cm  $L_T$ ). Two female Cuban dogfish that were tagged were recaptured after 30 and 579 days at liberty. The latter, a 72 cm  $L_T$  mature female when tagged, grew 1.8 cm  $L_F$  and 1.01 cm  $L_T$ , yielding growth rates of 1.13 cm yr<sup>-1</sup>  $L_F$  and 1.01 cm yr<sup>-1</sup>  $L_T$ .

The taxonomy of the gulper sharks (Centrophoridae) is globally unresolved and includes multiple circumglobal taxa that are species complexes. Preliminary genetic analysis identified three distinct species of gulper sharks (*Centrophorus* spp.), thought to be the common gulper (*Centrophorus* cf. *granulosus*), the Taiwan gulper (*Centrophorus* cf. *niaukang*) and a previously unidentified species. Morphological and genetic analysis is on-going and as such, for our study, the three species were grouped as single species complex. *Centrophorus* spp. (n = 51), as a group, were the second

most abundant encountered. *Centrophorus* spp. were significantly more abundant 1000 - 2500 m from the drop-off of the Exuma Sound (Contingency Analysis -  $\chi^2 = 15.94$ ,  $p = 0.007$ ; Figure 28b), in a mean water depth of 730.6 m ( $\pm 7.9$  S.E.), and at a mean seabed temperature of 9.5 °C ( $\pm 0.17$  S.E.). As *Centrophorus* spp. were most common at the intermediate depths of our sampling distribution, the presence/absence dataset was bisected at the mean depth (730.6 m) and seabed temperature (9.5 °C) of capture. Separate logistic functions were fitted to the respective datasets which indicated that there was no significant relationship between presence/absence and depth (Logistic Regression -  $\chi^2 = 3.78$ ,  $p = 0.052$ ) or temperature (Logistic Regression -  $\chi^2 = 3.70$ ,  $p = 0.054$ ) for values shallower and warmer than the mean. However, there were significant relationships between depth (Logistic Regression -  $\chi^2 = 10.91$ ,  $p = 0.001$ ) and temperature (Logistic Regression -  $\chi^2 = 5.47$ ,  $p = <0.019$ ) for values deeper and colder than the mean. The significant logistic functions predicted that there was a >50% probability of capturing *Centrophorus* spp. at depths <802.1 m and temperatures warmer than 8.4 °C. Sex ratios were highly skewed as females were 4.2 times more common than males

The bigeye sixgill shark (*Hexanchus nakamurai*) was the third most abundant species ( $n = 14$ ). They were significantly more abundant less than 1500 m from the wall of the Exuma Sound (Contingency Analysis -  $\chi^2 = 7.23$ ,  $p = 0.007$ ; Figure 28c), in at a mean depth of 639.5 m ( $\pm 16.7$  S.E.), and at a mean seabed temperature of 11.5 °C ( $\pm 0.38$  S.E.). Depth had a significant effect on the presence/absence of *H. nakamurai* ( $\chi^2 = 4.94$ ,  $p = 0.026$ ), however, temperature did not. Based on the logistic relationship between presence/absence and depth there was a greater than 50% probability of capturing *H. nakamurai* at depths <426.8 m. Males were 3.7 times more abundant than females. Of the 11 males captured, only one was immature which precluded the mathematical calculation of size-at-maturity, however, the smallest mature male

captured was 131 cm  $L_T$ , and the immature male measured 148 cm  $L_T$ , suggesting the onset of male maturity occurs at approximately 130 – 150 cm  $L_T$ .

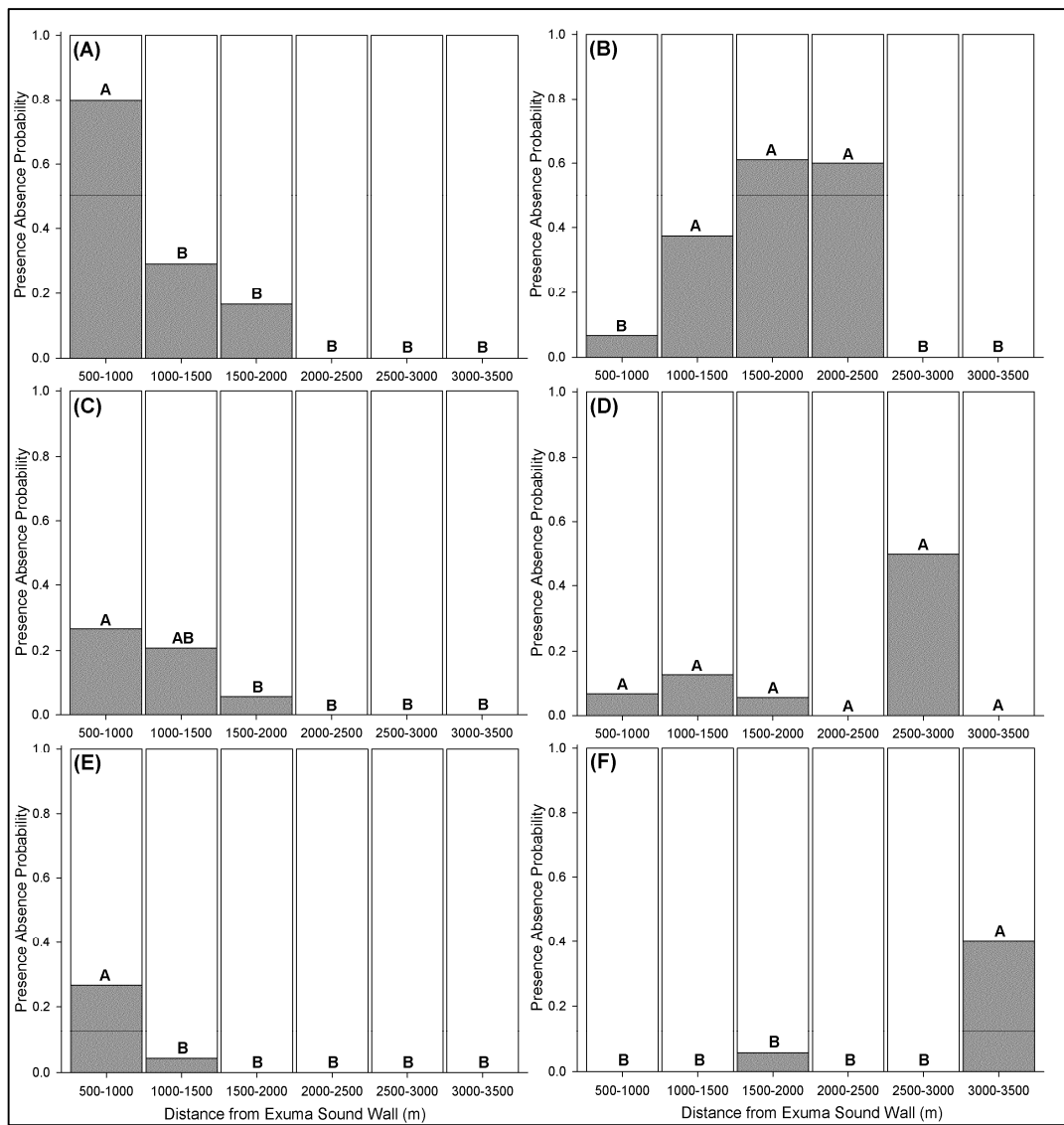
The presence/absence of the bluntnose sixgill shark (*Hexanchus griseus*) (n = 8), exhibited no significant relationship with distance from the Exuma Sound (Figure 28d), or with temperature and depth. The mean capture depth was 665.39 m ( $\pm$  33.52 S.E.), and the mean seabed temperature was 11.13 °C ( $\pm$  0.86 S.E.), however, the range of these values were wide, 565 – 791 m and 8.2 – 13.8 °C. *H. griseus* exhibited an even sex ratio; however, immature animals outnumbered mature animals 3:1.

The insular subspecies of the smooth dogfish (*Mustelus canis insularis*) (n = 7) was most commonly encountered within 1000 m of the edge of the Exuma sound (Contingency Analysis -  $\chi^2 = 11.09$ , p = 0.049; Figure 28e), at a mean depth of 549.73 ( $\pm$  20.95 S.E.) m and at a mean seabed temperature of 14.13 °C ( $\pm$  0.57 S.E.). Depth (Logistic Regression -  $\chi^2 = 10.72$ , p = 0.001) and temperature (Logistic Regression -  $\chi^2 = 11.75$ , p = <0.001) had a significant effect on the presence/absence of *M. canis insularis*. Based on these logistic functions, there was a greater than 50% probability of catching *M. canis insularis* at depths <437.8 m and >15.3 °C. This species also exhibited a skewed sex ratio with only one of the seven sharks captured being male, but had a roughly equal ratio of mature and immature animals. A single immature female (82 cm  $L_T$ ) *M. canis insularis* was recaptured after 62 days at liberty; however, no growth was identified over this short duration.

The roughskin dogfish (*Centroscymnus owstoni*) (n = 5), was the deepest dwelling shark encountered during this project, captured at a mean depth of 888.34 m ( $\pm$  39.58 S.E.) and a mean temperature of 7.3 °C ( $\pm$  0.48 S.E.). This species was almost exclusively captured 3000 - 3500 m away from the wall of the Exuma Sound in the deepest portion of the study site (Contingency Analysis -  $\chi^2 = 17.44$ , p = 0.004; Figure

28f). Depth (Logistic Regression -  $\chi^2 = 12.87$ ,  $p = <0.001$ ) and temperature (Logistic Regression -  $\chi^2 = 11.19$ ,  $p = <0.001$ ) had a significant effect on the presence/absence of *C. owstoni*. Based on these logistic functions, there was a greater than 50% probability of catching *C. owstoni* at depths  $>934.5$  m and seabed temperatures of  $<6.2$  °C. *C. owstoni* also exhibited a female dominated sex ratio of 3:1, although the small sample size precludes any firm conclusions. All specimens except for one female were immature.

The springer's sawtail catshark (*Galeus springeri*) ( $n = 3$ ) was captured at a mean depth of 706.8 m ( $\pm 64.03$  S.E.) and a mean temperature of 10.4 °C ( $\pm 1.46$  S.E.) Location, depth and temperature had no significant effect on the presence/absence of *G. springeri*. All three specimens of *G. springeri* were thought to be mature. A single immature male false catshark (*Pseudotriakis microdon*) was captured in 790.1 m of water at a seabed temperature of 8.7 °C.



**Figure 28 - Presence/absence mosaic plots of (A) *S. cubensis*, (B) *Centrophorus spp.*, (C) *H. nakamurai*, (D) *H. griseus*, (E) *M. canis insularis*, and (F) *C. owstoni* in six 500 m wide zones, spatially stratified by distance from the wall of the Exuma Sound.**

### 6.3.2 Spatial Variation in Elasmobranch Assemblages

Multivariate analysis suggests distinct spatial structure to deep water elasmobranch assemblages. The dendrogram constructed from the cluster analysis (Figure 29), and the corresponding SIMPROF test, reveal distinct groupings of longline survey replicates. The SIMPROF test identified two main ('significant') clusters that separate at ~37 % similarity. One cluster was primarily composed of samples taken from Zones 2 – 4. The second cluster in the dendrogram contains two smaller clusters,





Nakamura). ANOSIM analysis indicated that Zone 1 is characterized by its abundance of *S. cubensis*, and this species is the top contributor to the observed differences in assemblage structure compared to the other zones (Figure 31). ANOSIM analysis also indicated that Zones 2, 3, and 4 were primarily characterized by the abundance of *Centrophorus* spp., which were only recorded in one longline replicate conducted in Zone 1 (Figure 31).

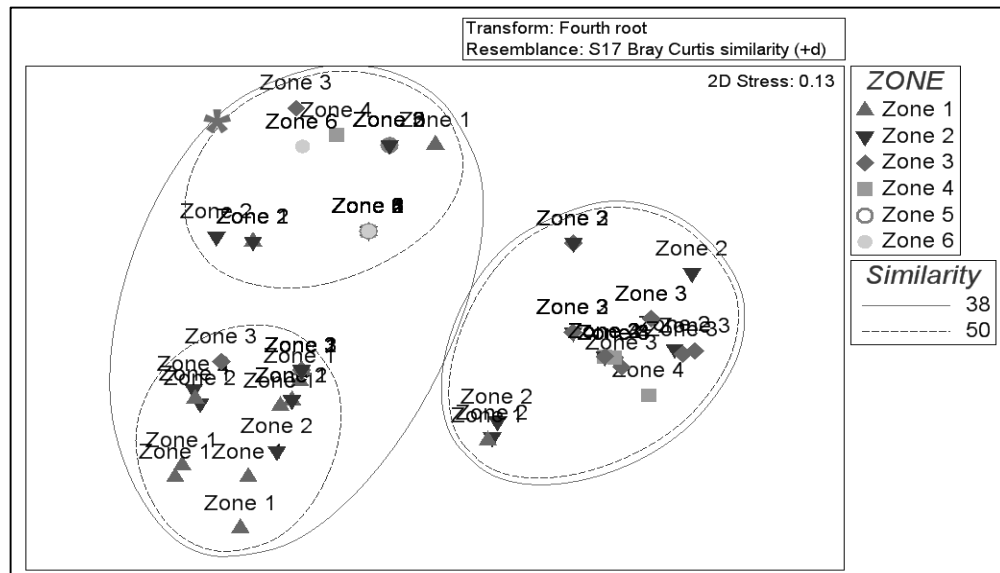


Figure 30 - MDS plot identifying two main clusters that separate at ~37 % similarity, one of which contains two sub clusters which separate at ~50% similarity. The sub-cluster marked with an asterisk contained a mixture of samples with only one species and samples in which zero individuals (n = 13) were recorded.

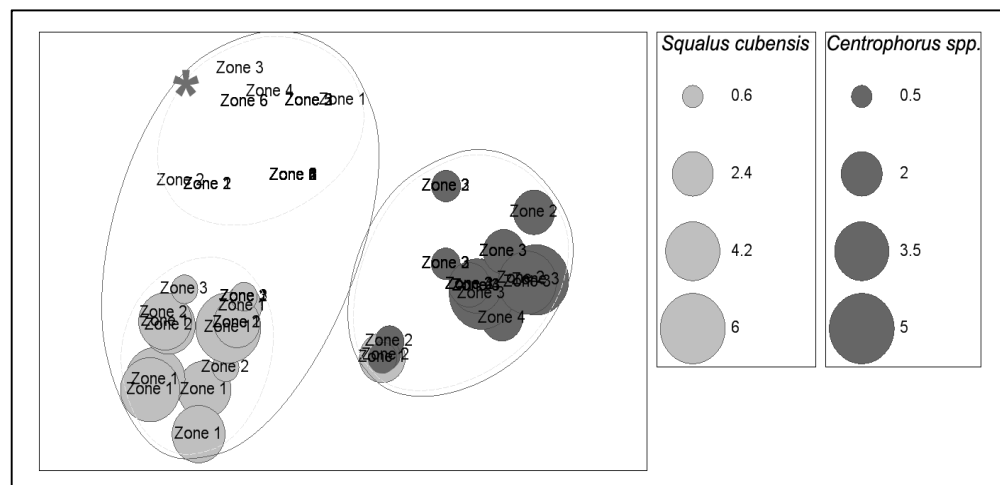
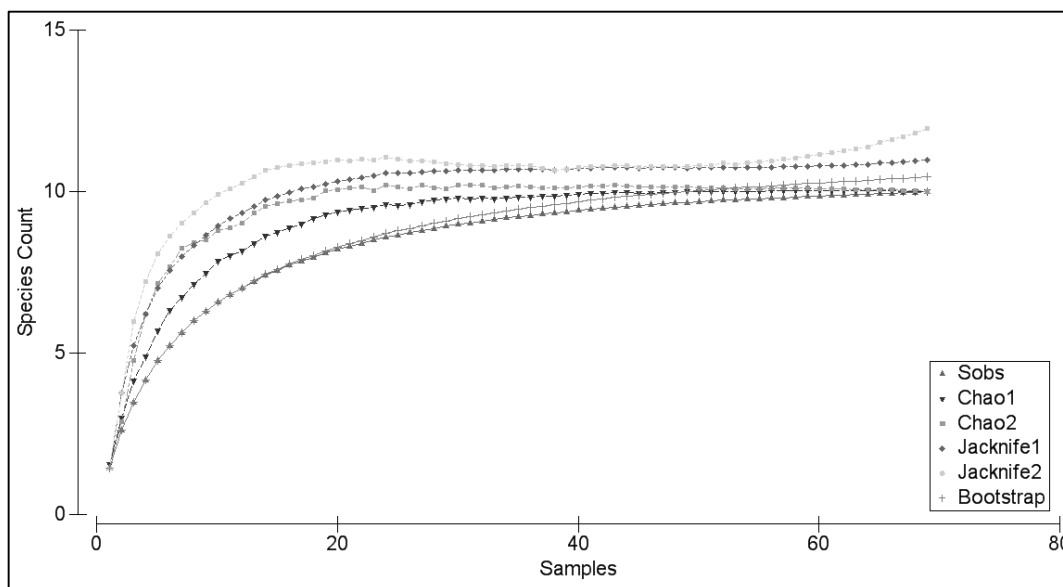


Figure 31 - Abundances of *S. cubensis* and *Centrophorus* spp., the top contributors to structure in the two major clusters. The sub-cluster marked with an asterisk contained a mixture of samples with only one species and samples in which zero individuals (n = 13) were recorded.

### 6.3.3 Species Diversity Estimates

Examination of the species-accumulation plot revealed that observed species richness, based on data from all deepwater longline replicates, was approaching an asymptote (Figure 32). The models used to extrapolate maximum species richness given an infinite number of samples suggest that no more than one additional species would be recorded. This implies that the assemblages described by the sampling efforts to date are representative of the deep water elasmobranch assemblage present within the study area.



**Figure 32 - Species accumulation curves which contain both the observed species richness ( $S_{obs}$ ) and five (Bootstrap, Chao1, Chao2, Jackknife1, and Jackknife2) species richness extrapolators used to predict diversity given an infinite number of samples (Clarke and Gorley, 2006).**

### 6.3.4 Resilience to Longline Capture

Species specific at-vessel mortality rates increased with mean capture depth (Spearman Rank Correlation –  $\rho = 0.77$ ,  $p = 0.04$ ; Table 11). The recapture of two *S. cubensis* and a single *M. canis insularis*, two of the shallowest dwelling species, suggest that both are able to survive the combined insults of capture and ascent. Post-release survivorship was assessed via satellite telemetry in *Centrophorus* spp. ( $n = 11$ ), *H. griseus* ( $n = 3$ ), and *H. nakamurai* ( $n = 2$ ) (Table 12). Of the 16 tags deployed, only two

reported via the Argos system, one deployed on *H. griseus* transmitted 88% of its dataset, and one tag *Centrophorus* spp. transmitted a partial dataset prior to washing ashore. This tag was not recovered. In addition to the partially transmitted *Centrophorus* spp. dataset, two further *Centrophorus* spp. tags were recovered after they had washed ashore allowing the retrieval of the entire dataset. The data from all three tags *Centrophorus* spp. tags indicated that the sharks had been preyed upon almost immediately post release. This conclusion was based on 1) the instantaneous loss of light data in <200 m of water; 2) clear diurnal vertical migrations and surfacing behaviour that is considered uncharacteristic of a deep water shark; and 3) the physical deterioration of the recovered tags by a strong acid, thought to be stomach acid. The data transmitted by the tag deployed on *H. griseus* indicated that this individual survived and returned normal diurnal vertical migrations approximately 60 hours post-release. No tags from *H. nakamurai* either transmitted or were recovered.

**Table 11 - Rank order of species specific at-vessel mortality rates. In general, mortality rates increase with mean capture depth.**

Rank	Species	n	M	% Mortality	Mean Capture Depth (m)
1	<i>C. owstoni</i>	5	4	80.00	888.3
2	<i>G. springeri</i>	3	2	66.67	706.8
3	<i>Centrophorus</i> spp.	51	15	29.41	731.4
4	<i>S. cubensis</i>	55	5	9.09	581.8
5	<i>H. nakamurai</i>	14	1	7.14	639.5
6	<i>H. griseus</i>	8	0	0.00	665.4
7	<i>M. canis insularis</i>	7	0	0.00	549.7
8	<i>P. microdon</i>	1	0	0.00	790.1

**Table 12 - Summary of post release survivorship in *Centrophorus* spp., *H. nakamurai* and *H. griseus* determined by pop-off satellite transmitters.**

Species	Length $L_T$ (cm)	Sex	Pop-Off Duration	Transmission	Fate
<i>Centrophorus</i> spp.	102	F	5 Month	DNR	Unknown
<i>Centrophorus</i> spp.	156	F	30 Day	DNR	Unknown
<i>Centrophorus</i> spp.	152	F	30 Day	Partial	Predated
<i>Centrophorus</i> spp.	80	F	30 Day	Recovered	Predated
<i>Centrophorus</i> spp.	146	F	30 Day	DNR	Unknown
<i>Centrophorus</i> spp.	96	F	30 Day	DNR	Unknown
<i>Centrophorus</i> spp.	85	M	4 Month	DNR	Unknown
<i>Centrophorus</i> spp.	101	F	30 Day	DNR	Unknown
<i>Centrophorus</i> spp.	149	F	30 Day	DNR	Unknown
<i>Centrophorus</i> spp.	100	F	6 Month	DNR	Predated
<i>Centrophorus</i> spp.	94	F	4 Month	Recovered	Unknown
<i>H. griseus</i>	340	M	30 Day	DNR	Survived
<i>H. griseus</i>	389	F	5 Month	DNR	Unknown
<i>H. griseus</i>	306	F	5 Month	DNR	Unknown
<i>H. nakamurai</i>	155	M	4 Month	DNR	Unknown
<i>H. nakamurai</i>	144	M	6 Month	DNR	Unknown

#### 6.4 Discussion

The results of the present study suggest that the northeast Exuma Sound supports a dense and diverse assemblage of deep water elasmobranchs. However, this study represents only a temporal snapshot of the deep water community in the area and seasonal variation in species abundance and assemblage structure is possible. We encountered a large number of sharks ( $n = 144$ ) in a relatively small area ( $\sim 10.5 \text{ km}^2$ ), however, the paucity of information pertaining to typical catch rates in the region makes comparison with other areas virtually impossible. It is unknown if this apparently high density is typical of the Exuma Sound in its entirety, or if the study area represents a 'hot spot' of deep water shark abundance. The latter option is a possibility, given the strong ( $\sim 90 \text{ cm s}^{-1}$ ), highly directional surface current that flows on and off the wall in the area (Rankey and Reeder 2011), potentially concentrating food fall from the shallow water and terrestrial ecosystems in a relatively small area immediately to the north of Cape Eleuthera. The importance of shallow water and terrestrial primary production on the structure of deep water ecosystems, particularly in high surface run off areas, has yet to be investigated. Further investigation by sampling other areas where similar surface currents do not exist, and/or through the use of stable isotope analysis (e.g. Pethybridge et al. 2012, Hussey et al. 2012), is recommended.

The majority of species encountered exhibited skewed sex and maturity ratios. Demographic segregation is considered normal for many populations of shallow water elasmobranchs (Springer 1967, Speed et al. 2012, Mucientes et al. 2009), and has also been reported in some deep water species (Girard and DuBuit 1999). In the present study, clear sexual segregation was identified in *Centrophorus* spp., *H. nakamurai* and *M. canis insularis*. Segregation within *Centrophorus* spp. is unsurprising as female dominated populations of *Centrophorus cf. uyato* in the Cayman Trench (McLaughlin

and Morrissey 2005), and a male dominated population of *Centrophorus squamosus* off the northwest coast of Spain (Banon et al. 2006), have previously been reported suggesting that this is a normal occurrence within this genus. To our knowledge this is the first instance that sexual segregation has been reported in populations of *H. nakamura* and *M. canis insularis*, although conclusions pertaining to the latter are tentative given the small sample size ( $n = 7$ ) and the lack of sexual segregation reported in the shallow water conspecific, *Mustelus canis canis*. Skewed sex ratios were also identified in *S. cubensis*, *C. owstoni* and *G. springeri*; however, given the small sample sizes for *C. owstoni* and *G. springeri*, and the relatively small sexual variation in *S. cubensis*, it is not possible to distinguish true sexual segregation from either sampling bias, or inherent population structure. However, Daly-Engel et al. (2010) reported strong sexual segregation in *Squalus cf. mitsukurii*, a close relative of *S. cubensis*. The segregation of populations by size and sex has important management and conservation considerations, as the imposition of fisheries mortality on a specific demographic can skew sex ratios, disproportionately affecting the reproductive capacity of the entire stock (Mucientes et al. 2009, Field et al. 2009).

Deep water elasmobranchs, with the exception of *H. griseus*, exhibited distinct spatial structure based on depth, seabed temperature and distance from the benthic complexity of the Exuma Sound wall. These factors have previously been reported to control the distribution and abundance of deep sea fishes and the assemblages they create (Clark et al. 2010, McClain et al. 2010), but are also fundamental to the evolutionary development of specific life-history traits within these species (Childress et al. 1980). Based on these traits, it has been concluded that deep sea demersal fishes can be assigned to one of two guilds: those that are typically slow moving, sit and wait predators and scavengers which live at deeper depths, dispersed over the silt plain of the sea floor. This deeper guild is characterised by low energy stores and slow metabolic

rates (Koslow 1996). In contrast, species that inhabit the dynamic, higher current, environment associated with physical structure and large variations in depth (e.g. sea mounts), typically have strong locomotory ability, faster metabolisms and larger energy stores (Koslow 1996). The present study identified two distinct elasmobranch assemblages which largely conform to these two guilds. A “shallow” cluster dominated by *S. cubensis* and centred in Zone 1 (Mean Depth in Zone 1 = 554.3 m ± 13.1 S.E.) closest to the rocky structure of the wall of the Exuma Sound. The second “deep” cluster was dispersed over a wider area in Zones 2 – 4 (Mean Depth in Zones 2 – 4 = 716.8 m ± 8.4 S.E.), further away from the structure of the Exuma Sound and was dominated by *Centrophorus spp.* Little information exists on the life history characteristics of *S. cubensis*, however, it is reported to feed on benthic invertebrates, squid, cuttlefish and teleosts (Cortes 1999) which suggests that it is an active predator, conforming to the active, shallow guild of deep water fishes. Given the uncertainty of the *Centrophorus spp.* identification, and the almost complete lack of life-history information pertaining to the majority of species of this genus, it is impossible to say how well it conforms to the life-history traits to the deeper guild.

In general, survivorship in this study was low, but varied considerably by species. The correlation of at-vessel mortality rates with mean capture depth indicates two possible scenarios. Firstly, the magnitude of thermal, barometric and photic stress, which increases with depth, is a major contributor to at-vessel mortality; or secondly, that deeper dwelling species are inherently more sensitive to capture. In addition to the correlation between depth and mortality, the recapture of a single *M. canis insularis* and two *S. cubensis*, the two shallowest dwelling species, further suggest that these ‘shallow guild’ species are more resilient to the combined insults of capture and ascent from depth. It is thought that survivorship in the deeper dwelling *Centrophorus spp.* was very low based on the almost immediate predation (<200 m from the surface) of all

three individuals fitted with a PSATs where data were recovered, and the high (~30%) at-vessel-mortality rates. However, it is at present unknown if this high post-release predation rate is consistent across individuals without PSATs attached, or across different species. Given that the density of potential predators is likely to be higher closer to the edge of the Exuma Sound where there is an abundance of large coastal sharks such as Caribbean reef (*Carcharhinus perezi*) sharks (Brooks et al. 2011), it is possible that post-release predation rates might increase with proximity to the edge of the Exuma Sound. Survivorship in *H. griseus* was thought to be high based on data derived from the single reporting PSAT tag, which indicated that the individual returned to a normal diurnal dive pattern in about 60 hours. This is supported by the reports of high survivorship in other studies which used longline capture for *H. gresius* (Andrews et al. 2009; D. Grubbs Unpublished Data).

The energetics of deep water demersal fishes, including elasmobranchs, might account for the divergent mortality rates of shallow and deeper dwelling species. Metabolic rate is known to decrease with increasing depth even when adjustments are made for body mass and temperature (Drazen and Seibel 2007). Furthermore, this relationship is not thought to be related to resource constraint (e.g. oxygen/food), extreme low temperature or extreme high pressure, but rather from the locomotory requirements of visual predators which inhabit the photic zone vs. deeper dwelling benthic or benthopelagic species which are not thought to hunt visually (Seibel and Drazen 2007). In addition, energy stores in the form of lipids and protein are known to decrease with increasing depth (Childress et al. 1980, Koslow 1996). Given that metabolic capacity is a key factor in species susceptibility to longline capture (Brooks et al. 2012, Mandelman and Skomal 2012), it is likely that shallower dwelling species that associate with the wall of the Exuma Sound (*S. cubensis*, *M. canis insularis*, *H. nakamurai*) have higher metabolic capacity, which makes them more resilient to



longline capture. This is in contrast to the potentially lower metabolic capacity of deeper dwelling species (*Centrophorus* spp., *G. springeri*, *C. owstoni*) that are more susceptible. The anomaly to this trend is *H. griseus*, which inhabits a diverse range of depths from ~300 m - >2000 m and is an active apex predator thought to have a high metabolic capacity; furthermore, its large size imparts higher thermal mass, potentially insulating it from the thermal trauma of ascent. In general, mortality following longline capture is high. If individuals survive the combined threats of capture, ascent, descent and post-release predation, it is unknown if they survive long term following the physiological impairment of capture. Based on these low chances of survival, traditional fisheries management techniques (e.g. mandated release of prohibited species), are unlikely to be effective in reducing population mortality in many species of deep water shark.

Our results illustrate the benefits of conducting deep ocean research in areas where bathymetric structure results in narrow continental shelves. The Bahamas is not unique in its proximity to deep water; indeed many nations around the world (e.g. Cayman Islands, The Maldives, and Bermuda) have easy access to the deep ocean. These locations combined with the use of low cost survey techniques (e.g. longline surveys, deep water baited video) can facilitate temporally sustained, high resolution research at much lower costs of when compared to traditional deep ocean science. With the addition of established ocean exploration techniques (e.g. high resolution bathymetric mapping), these areas can facilitate a new deep ocean research paradigm which approaches hypothesis driven questions which require high resolution and temporally diverse sampling, more typically associated with shallow water marine science. Targeted investigation in specific areas can help accelerate species-specific and ecosystem based research to a rate that has not previously been seen, providing

urgently needed data that can help facilitate the effective management and conservation of deep sea sharks.

# Chapter Seven

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## 7. Discussion

Longline fishing is thought to be the predominant commercial capture method for sharks in the world (Gilman et al. 2008). In the majority of longline fisheries all or part of the shark is harvested, however, in some areas large numbers of sharks are released alive, due to low species-specific commercial value (Beerkircher et al. 2002) and/or to comply with fisheries regulations (Morgan et al. 2009). In addition to the commercial application, small scale scientific longline surveys are commonly used to assess the diversity and distribution of a large proportion of shark species investigated to date (e.g. Holland et al. 1999, Simpfendorfer et al. 2002, Pikitch et al. 2005). A capture event, such as those incurred on commercial or scientific longlines imposes various degrees of physical trauma and physiological stress; the magnitude of which is dependent on the capture method and handling time (Skomal 2007). If either physiological stress or physical trauma, or a combination of the two, is excessive, then immediate or delayed (post-release) mortality is possible (Skomal 2006), however, the mortality rates associated with scientific surveys are typically a fraction of the rates associated with commercial capture. Given the precipitous declines in elasmobranch populations on a global scale (Field et al. 2009), an improved understanding of the physiological effects of capture, and the reduced individual fitness and/or mortality it causes, is vital. Firstly, this information will allow informed management choices to be made in commercial fisheries that can help reduce mortality and improve post release viability. Secondly, scientists can make ethical choices about which survey technique to employ, potentially exchanging longline surveys for a lower impact method such as BRUVS. The purpose of this thesis was to assess the physiological impacts of longline capture, in addition to

investigating their scientific application as a survey technique and identifying and validating less invasive alternatives to capture based surveys.

### **7.1 The Physiology of Capture and Restraint in Elasmobranchs**

The response of elasmobranchs to longline capture remains very poorly understood, and the cause of both at-vessel and post-release mortality, in the absence of physical trauma, is not yet known (Skomal and Mandelman 2012). Further research into which physiological system and/or process fails, and how this varies between species, is needed in order to make informed management decisions which can help mitigate the effects of longline capture on the individual, thus promoting survivorship and post-release viability. The results presented in this thesis (Chapter 2, and Chapter 3) have provided insight into the stress response of elasmobranchs subjected to the exhaustive exercise typically associated with a capture event, and the resulting publications (Brooks et al. 2011, Brooks et al. 2012) represent some of the most recent research in this field.

Brooks et al. (2011; Chapter 2) showed that extended duration tonic immobility is not a viable means of generating baseline blood chemistry values as it typically magnifies the stress response in a number of parameters. TI reduced the ventilatory efficiency of the lemon shark (*Negaprion brevirostris*) causing an increase in carbon dioxide in the blood and a concomitant decrease in blood pH in the short term. However, in the long term these values recovered to near baseline values, suggesting that lemon sharks were able to compensate for the reduced efficiency, in part by increasing ventilation rates. There is also evidence to suggest that lemon sharks maintained the primary stress response during TI. Elevated glucose levels throughout the treatments, indicative of sustained circulation of glucocorticoids in the blood (Busch and Hayward 2009), and the lack of recovery in a number of electrolytes, indicative of

sustained vasodilation of the branchial blood vessels (Gonzalez and McDonald 1992, Randall 1982), supports this hypothesis. Perturbation of these parameters is independent of exhaustive exercise and more closely linked to the hormonal cascade of the primary stress response, suggesting that the endocrine/neuroendocrine response was maintained throughout tonic immobility. However, it is uncertain if this is another compensatory mechanism in response to disrupted ventilatory efficiency, or due to the persistent negative stimulus of capture and restraint by a large ‘predator’ in the form of the research assistant, the latter of which is possible given evidence that central neural processing remains intact during TI in some taxa (Gallup et al. 1980). In either instance, lemon sharks were able to recover parameters typically associated with exhaustive anaerobic exercise (i.e. capture) despite sustained negative stimuli, reduced ventilatory efficiency and the maintenance of the stress response. This paper represents the first study of TI in elasmobranchs in over 17 years, and the first time the physiology of TI has ever been investigated.

Brooks et al. (2012; Chapter 3) describes the physiological response of Caribbean reef sharks (*Carcharhinus perezi*) to longline capture, and represents the first time that the effect of longline hooking duration has been accurately quantified for any species of elasmobranch. The results describe a non-linear (parabolic) response of a number of blood chemistry parameters to longline hook duration, in direct contrast to all previous capture physiology studies which described linear relationships between hook duration and the magnitude of physiological perturbation. These results can be attributed to the more benign characteristics of longline capture which lacks the persistent negative stimuli associated with other methods such as the continuous active retrieval of rod-and-reel capture, which induces continuous muscular exertion (Skomal 2006) or the forward momentum and physical compaction of a trawl nets (Mandelman and Farrington 2007). It is hypothesised that Caribbean reef sharks respond to hooking with an initial high

energy escape response which attenuates to a lower energy response over time, facilitating a degree of physiological recovery over longer hook durations. Data pertaining to the behavioural response to longline capture, and how this behaviour impacts physiology, are needed in order to support or refute this hypothesis.

These findings suggest that the recovery of blood chemistry parameters associated with intense anaerobic exercise is possible, even when a persistent negative stimulus is maintained (i.e. capture and restraint in Chapter 2 and longline capture in Chapter 3). However, parameters associated with the hormonal cascade of the primary stress response, which are independent of exhaustive exercise; do not exhibit such clear cut recovery, suggesting the maintenance of the primary response due to persistent negative stimuli. It is hypothesised that this is related to the perceived magnitude of a stressor. For example, the perceived threat during the TI experiments in Chapter 2, which involved continual restraint in an inverted position over long periods of time in close proximity to a 'predator', is likely to elicit a high magnitude, and temporally sustained, stress response. In comparison, an animal captured on a longline which is restrained to a lesser degree without any imminent threat from 'predation' is likely to elicit a lower magnitude stress response. This difference in the magnitude of the stressor is reflected in the results. In the TI experiments, elevated glucose concentrations persisted for the duration of the treatments, whereas evidence for a sustained primary stress response during longline capture was less clear as glucose concentrations recovered, suggesting attenuation in the volume of circulating glucocorticoids.

Attenuation of the stress response given a lower level stressor, and the distinction between secondary stress response parameters that are dependent on anaerobic exercise or dependent on the hormonal cascade of the primary stress response, are important in identifying the causes of mortality. Given that the results of both studies suggest that

elasmobranchs can recover, particularly from those physiological disruptions related to exercise, to near baseline levels despite sustained negative stimuli, indicates that further research is necessary into the direct effects of the primary stress response on the individual. Research in this area is challenging as the primary glucocorticoid in elasmobranchs is not cortisol, but  $1\alpha$ -hydroxycorticosterone (Anderson 2012), and at present there is no commercially available assay (Skomal & Mandelman 2012). Until this assay is available, secondary stress responses, such as hyperglycaemia, can be used to infer the presence of the primary stress response. Electrolyte imbalance could also be used as an indicator for a sustained primary stress response; however, more research is needed to identify the source of excess ions which has been reported to be both internal in origin due to intracellular acidemia (e.g. Cliff & Thurman 1984, Frick et al. 2010), or external due to increased gill perfusion caused by vasodilation of the branchial blood vessels (Gonzalez & McDonald 1992, Randall 1982), the latter of which is caused by the hormonal cascade of the primary stress response.

## **7.2 Generating Ecological Data from Longline Surveys**

Longline surveys are commonly used for generating diversity, distribution and abundance data for elasmobranchs, and used as vehicles for the deployment of both passive tags, and electronic telemetry devices (e.g. Holland et al. 1999). The ability to generate highly accurate size, sex and species data allows longline surveys to generate high resolution demographic data pertaining to a single species (e.g. Caribbean reef sharks, Chapter 5), or to generate holistic assessments of entire apex predator assemblages (e.g. deep water elasmobranchs, Chapter 6). Longlines in various formats can be adapted to surveys sharks in every marine ecosystem, from coral reef and lagoon habitats (e.g. Pikitch et al. 2005), to pelagic species on the high seas (e.g. Simpfendorfer

et al. 2002), and remain one of the most versatile and productive survey techniques available to elasmobranch researchers.

Chapter 5 used data derived from standardised, mid-water longline surveys and an associated passive tagging program to describe the demographic structure, site fidelity and seasonal abundance of Caribbean reef sharks. The results describe the spatiotemporal and demographic structuring of Caribbean reef shark populations, a phenomenon not previously described in this species, and in direct contrast to many contemporary studies (e.g. Bond et al. 2012). Clear increases in abundance during the summer, in addition to precise, year-to-year philopatry were identified, indicated by the comparatively high recapture rate and short distances between capture and recapture points. Furthermore, these seasonal shifts in abundance appear to be demographically stratified, again in contrast to contemporary research published on this species. These contrasting results are thought to be attributed to the greater geographical area and habitat complexity of the Great Bahamas Bank compared to the small isolated oceanic islands where all previous research on Caribbean reef sharks has taken place (e.g. Garla et al. 2006a, Bond et al. 2012). I hypothesise that the diverse habitat mosaic available to the Bahamian sub-population of Caribbean reef sharks drives the spatiotemporal and demographic population structuring observed, as different habitat types impart advantages and disadvantages to different demographics at different times of the year. The apparent evolution of geographically discrete behavioural patterns within this species indicates the potential for future studies investigating behavioural plasticity in response to environmental variation and anthropogenic impacts. This study highlights the benefits of using longline surveys to investigate complex demographic segregation and seasonal habitat usage in elasmobranchs, at minimal cost (mortality) to the target species.



Chapter 6 utilised deep water demersal longlines to assess the diversity, distribution and abundance of deep water elasmobranchs in the Exuma Sound. The deep ocean (> 200 m) is the single largest ecosystem on the planet (Ramirez-Llodra et al. 2011, McClain et al. 2012), and the least explored. This study represents the first temporally sustained investigation into the diversity, distribution and structure of deep water elasmobranch assemblages in the greater Caribbean region. A dense and diverse assemblage of deep water elasmobranchs was identified in the north east Exuma Sound, however, the spatially constrained sampling (~10.5 km<sup>2</sup>) precluded any broad conclusions about the importance of the Exuma Sound as a whole. Skewed sex ratios in the majority of the species captured suggests sexual segregation is as common in deep water elasmobranchs as their shallow water counterparts. Sexual segregation in some deep water genera has been previously described (*Centrophorus*, McLaughlin & Morrissey, 2005); however, this was the first description of segregation in the bigeye six gill (*Hexanchus nakamurai*) and smooth dogfish (*Mustelus canis insularis*). Species-specific abundance was strongly influenced by depth, temperature and distance from the benthic complexity of the Exuma Sound wall. Two distinct elasmobranch assemblages were identified, one shallower at ~550 m and immediately adjacent to the rocky structure of the wall of the Exuma Sound dominated by Cuban dogfish (*Squalus cubensis*), and a second deeper assemblage at ~715 m and separated from the rocky structure of the wall, and dominated by gulper sharks (*Centrophorus* spp.). These discrete assemblages broadly conform to the energetic model of deep water fishes proposed by Koslow (1996), whereby the shallow water assemblage is characterised by strong locomotory ability, faster metabolisms and larger energy stores, and the deeper assemblage by low energy stores and slow metabolic rates (Koslow, 1996). Given that mortality rates in this study increased with depth of capture, and that metabolic capacity is a key factor in species susceptibility to longline capture (Mandelman & Skomal

2012), it is likely that species within the shallower assemblage have higher metabolic capacity, which makes them more resilient to longline capture. This is in contrast to the potentially lower metabolic capacity of species that are more susceptible. In general, the mortality rates generated by this study were high, however, the additional taxonomic and morphometric data that were derived from these specimens offset these additional costs; however, it is uncertain if this balance will remain now these data have been acquired.

Longlines, in various formats, can provide valuable insights into the life histories of single species (Chapter 5), or be used to holistically assess whole assemblages of species (Chapter 6). Furthermore, longlines facilitate mark-recapture studies which give other biological insights into philopatry and growth rates as well as accurate determination of size and sex, allowing the demographic structure of shark populations to be assessed. All capture based surveys carry an inherent risk of mortality, however, the relative cost (mortality) benefit (data) analysis remains an ethical debate.

### **7.3 Ethical Survey Selection for Elasmobranch Research**

Longline surveys carry with them inherent levels of at-vessel mortality. For example, Caribbean reef shark mortality rates published in Chapter 5 were low at 2.7 %, and its resilience to capture, based on the comparison of physiological values derived in Brooks et al. 2011, and previously published data (Mandelman and Skomal 2009), ranks this species behind tiger and sand tigers as one of the most resilient assessed to date. In contrast, mortality rates in deep water sharks (Chapter 6) were much higher, up to 80% in some species, suggesting that they might be far more sensitive to capture, and/or the thermal, barometric and photic stress of ascent is far greater than standard longline surveys. Mortality data from other species also indicated very diverse sensitivity to longline capture ranging from 8.5% in tiger sharks to 91.4% and 93.8% in scalloped

(*Sphyrna lewini*) and greater (*Sphyrna mokarran*) hammerheads respectively (Morgan & Burgess 2007). It is also interesting to note that the blacktip shark (*Carcharhinus limbatus*), a species closely related to the Caribbean reef shark, had mortality rates of 88% (Morgan & Burgess 2007). These variable mortality rates in closely related species highlight the importance of survey design prior to execution. For surveys targeting more sensitive species, shorter soak times, longer gangions lengths which provide more swimming space and fewer hooks are important design considerations, or switching from a longline to drumline surveys, a technique that allows up to 30 m diameter swimming area for the captured animal.

There are alternatives to capture based surveys. Baited Remote Underwater Video Surveys (BRUVS) generate similar diversity and abundance data to longline and drumline surveys, but without physically capturing and restraining the animal. Brooks et al. (2011; Chapter 4) compared diversity indices, catch rates and trends in abundance between BRUVS and longline surveys. The study concluded that BRUVS are especially suited for long-term monitoring of species richness and relative abundance over wide geographical and temporal scales, as they are easily replicated by relatively untrained personnel without specialised equipment. However, BRUVS lack the resolution required to approach finer-scale ecological questions, which would be better approached with longline surveys. Longlines, if structured correctly, provide a higher quality data set which includes accurate size, sex and species data, induce minimal mortality, and have the added benefit of acting as a vehicle for conventional tag and recapture studies.

Given the precipitous declines in many species of sharks in recent years (Field et al. 2009), the ethical choices facing elasmobranch scientists have become more challenging (Heupel & Simpfendorfer 2010, Hammerschlag & Sulikowski 2011). The

impacts of the surveys themselves must be taken into account, especially when dealing with threatened or highly sensitive species. In the case of the deep water surveys, the high mortality rates were considered acceptable as the life history and taxonomic data derived from these mortalities outweighed the environmental costs. However, those data have now been gathered and, given the availability of deep water baited video surveys, longline surveys are no longer the most ethical choice for continued investigation into the deep water in the Exuma Sound. In contrast, the low mortality rates associated with the shallow water longline surveys are outweighed by the wealth of mark-recapture and accurate demographic data generated, allowing far more intricate ecological questions to be approached. Longlines and their alternatives are useful tools in generating much needed data that can support informed management and conservation initiatives, however, given the conservation based objectives of the majority of elasmobranch research, the impact of the survey must be considered alongside the data it will generate.

#### **7.4 Future Research Directions**

The results presented in this thesis suggest numerous intriguing directions for future research. In general, these directions fall into three broad themes which have application beyond elasmobranch conservation science. These themes include research into the behavioural and physiological processes associated with the elasmobranch stress response, and the potential long term costs of stress on the individual; the causes and ecological function of demographic segregation within elasmobranch populations, and the complex cyclical movement patterns which both generate the segregation, and manages to satisfy biological imperatives of reproduction and feeding; and the continued investigation of deep ocean vertebrates, many of which are virtually undescribed by science.

Recent advances in telemetry devices include the use of tri-axial data loggers which record acceleration in three dimensions up to 60 times a second (e.g. Whitney et al. 2010). Similar loggers have previously been attached to longline hooks to record time, temperature and depth profiles of a loggerhead turtle (*Caretta caretta*) incidentally captured on a pelagic longline (Grace et al. 2010). By attaching accelerometer data loggers adjacent to the hook, in a similar manner to the time, temperature and depth recorders described by Grace et al. (2010), a high resolution time series of activity during capture can be generated. In order to validate the assumption that mean tri-axial acceleration is proportional to activity level during capture, small high-definition digital video cameras can be mounted on each gangion to provide an ethogram of behaviours which can be correlated with the accelerometry values. This methodology will be able to quantify the duration and magnitude of the high energy proportion of the escape response, and help support or refute the hypothesis of behaviourally-mediated physiological recovery proposed in Brooks et al. (2012, Chapter 2). Furthermore, this standardised assessment will facilitate both inter and intra species behavioural comparisons. The comparison of behavioural metrics with blood chemistry data across a broad range of species with diverse evolutionary histories will help in understanding the wide variation in mortality exhibited by closely related species, and guide gear and protocol restrictions that can reduce mortality and increase post-release viability.

The variation in the magnitude and complexity of demographic stratification within Caribbean reef shark populations, between those inhabiting small isolated oceanic islands (e.g. Bond et al. 2012), and those inhabiting the large and diverse habitat mosaic of the Great Bahamas Bank (Chapter 5), provides a unique opportunity to quantify the influence of environmental complexity on population structure and movement patterns. Adjacent to the Great Bahamas Banks is a small isolated oceanic island (San Salvador), which shares similar habitat characteristics and fish assemblages

to the Great Bahamas Bank, but is isolated by 50 km of pelagic waters >2000 m deep (Buchan 2000). Caribbean reef sharks have been known to make 30 km journeys across stretches of water up to 400 m deep in Belize, but it is thought to be a very rare occurrence (Chapman et al. 2005), rendering the proposed study populations in the Bahamas independent of each other. San Salvador is similar in size to study areas where previous Caribbean reef shark research concluded that there was no seasonal variation in abundance or cyclical movement patterns (Garla et al. 2006a,b), and a degree of ontogenetic segregation but no sexual segregation of populations (Pikitch et al 2005). Standardised investigation of Caribbean reef sharks at both the Great Bahamas Bank, and San Salvador can test the hypothesis that the complexity of demographic population structure and movements patterns increases with the size and complexity of the available habitat mosaic.

Given the challenges of conducting surveys at depth, the bulk of deep ocean biological research to date has focused on sessile invertebrates and molluscs, as the compounded challenges of surveying mobile vertebrates at depth is at present challenging. The results presented in Chapter 6 represent one of the few temporally sustained, fisheries independent investigations into deep water elasmobranchs ever undertaken, and further research at the most basic taxonomic, biological and ecological levels is needed. Deep water baited video units (landers) have been used to examine the diversity and abundance of deep water marine animals since the late 1960s (Issacs 1969). However, given the high costs of construction and of ship time in order to make deployments, the datasets derived typically contain only a few spatial locations and over relatively discrete portions of the year, precluding the identification of spatiotemporal trends in abundance and assemblage structure. By applying a shallow water sampling paradigm whereby multiple replicates are generated across several spatiotemporal

categories broad ecological insights can be generated in a manner not previously generated in the deep water.

## **7.5 Conclusions**

Despite their infamy, elasmobranchs remain one of the most poorly understood groups of fish in the sea (Kyne et al. 2012) and given widespread declines in shark populations, the need for data which can facilitate meaningful management and conservation plans is greater than ever (Lucifora et al. 2011).

Longlines are the most effective, and consequently the most common, method of shark capture in the world. In commercial form, they are responsible for the vast majority of global shark capture (Gilman et al. 2008), and in scientific configuration, form the backbone of many research programs (e.g. Pikitch et al. 2005). Any capture event carries an inherent level of mortality, yet the physiological and behavioural processes that cause that mortality are still uncertain (Skomal & Mandelman 2012). The data presented in this thesis suggest that both Caribbean reef and lemon sharks are able to recover from the exhaustive exercise associated with various levels of capture and restraint, despite the presence of persistent negative stimuli. Furthermore, in the presence of a persistent stressor, i.e. longline capture, the Caribbean reef shark appears to be able to attenuate the primary stress response, facilitating the recovery of secondary stress response parameters associated with both the exhaustive exercise and the hormonal cascade of the primary stress response. Differentiating between secondary stress parameters that are caused by exhaustive anaerobic exercise, and those caused entirely by the endocrine/neuroendocrine response, is important as it will help guide future research into the cause of capture induced mortality and reduced post-release fitness. Further investigation into the behavioural and physiological responses of

capture should facilitate gear and/or protocol driven legislation which can promote survivorship and post release viability.

Scientific longline surveys yield a plethora of high resolution data pertaining to the biology and ecology of the target species yet, as previously mentioned, carry with them an inherent level of mortality. The results presented in this thesis support the notion that demographic segregation is commonplace in elasmobranch populations, including minimally investigated deep water species. The interesting variation in population structure and movements patterns identified in Bahamian Caribbean reef sharks in contrast to previously studied populations, suggests that the complexity of characteristics might be proportional to the size and complexity of the habitat mosaic which they inhabit. Furthermore, the Caribbean reef shark, and physical structure of The Bahamas, provides an opportunity to test this hypothesis.

The physical structure of The Bahamas also facilitated the first temporally sustained investigation into deep water elasmobranch assemblages in the greater Caribbean region. The results of this study identified a diverse and dense assemblage in the north east Exuma Sound, however, further spatiotemporally stratified sampling is needed prior to drawing firm conclusions about the importance of the sound as whole. Given the high mortality rates associated with deep water longline surveys, deep water baited video surveys would be the more ethically acceptable choice. The close proximity of deep water to land facilitates the application of shallow water sampling paradigms which have the potential to accelerate the acquisition of data from this sensitive and very poorly understood ecosystem.

The research presented by this thesis has furthered elasmobranch conservation science in a number of diverse avenues, and has managed to propose far more new research questions than were answered. Further insights into the areas outlined by these



chapters will require the integration of a number of research techniques, including physiology, telemetry, and traditional capture and non-capture based survey techniques. Given the tenuous conservation status of many shark species, the acquisition of applied, conservation, and management focused data is, and should remain, the primary research focus of shark scientists around the world.

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