Effects of marine reserves on the relative abundance, distribution and ecology of tropical reef elasmobranchs: Caribbean reef sharks and southern stingrays

A Dissertation Presented

by

Mark Bond

to

The Graduate School

in Partial Fulfillment of the

Requirements

for the Degree of

Doctor of Philosophy

in

Marine and Atmospheric Sciences

Stony Brook University

December 2015

Stony Brook University

The Graduate School

Mark Bond

We, the dissertation committee for the above candidate for the

Doctor of Philosophy degree, hereby recommend

acceptance of this dissertation.

Demian Chapman, Associate Professor and Dissertation Advisor
School of Marine and Atmospheric Sciences
Stony Brook University

Robert Cerrato, Professor and Chair of Defense
School of Marine and Atmospheric Sciences
Stony Brook University

Ellen Pikitch, Associate Professor
School of Marine and Atmospheric Sciences
Stony Brook University

Dr. Michael Heithaus
Dean, School of Environment, Arts and Society
Florida International University

Dr. R. Dean Grubbs
Associate Director of Research
Florida State University Coastal and Marine Laboratory

This dissertation is accepted by the Graduate School

Charles Taber
Dean of the Graduate School

Abstract of the Dissertation

Effects of marine reserves on the relative abundance, distribution and ecology of tropical reef elasmobranchs: Caribbean reef sharks and southern stingrays

by

Mark Bond

Doctor of Philosophy

in

Marine and Atmospheric Science

Stony Brook University

2015

Many shark populations have declined due to overexploitation and it is imperative to determine whether or not protected areas like marine reserves are an appropriate approach for shark conservation. The Caribbean reef shark (*Carcharhinus perezi*) is a large reef-associated predator endemic to the Western Atlantic. Belize, Central America, has implemented marine reserves as part of its national strategy for coral reef ecosystem conservation, including *C.perezi*. The overarching objective of this dissertation is to use Belize's marine reserves network to investigate their effect on *C.perezi* populations and elucidate its ecological role. A global literature review provides evidence of positive reserve effects in sharks and rays but only from spatial comparisons of reserves and fished sites. Annual catch per unit effort and total length of *C.perezi* was analyzed from 2000-2013 at Glover's Reef Marine Reserve (GRMR). This population exhibited a stable trend and broad, stable size distribution of individuals, suggesting the reserve is effective at reducing fishing pressure. Automated acoustic telemetry and baited remote underwater video (BRUV) surveys demonstrated that *C.perezi* of all life-stages are residential at GRMR and relative abundance is significantly higher inside marine reserves than similar fished reefs. Bulk stable isotope analysis of *C.perezi* white muscle collected at GRMR demonstrated that it feeds at a similar trophic level to other large predators in the system, feeds across a wide variety of habitats and that larger individuals derive more of their carbon from seagrass based food webs. There was a significant effect of reserve, habitat, and their interaction on the presence of stingrays (F. Dasyatidae) on BRUVs at two reserves and two fished reefs. Specifically, stingrays were less common on the fore-reef inside reserves. This pattern supports stingrays increasing their use of deep fore-reef habitat when *C.perezi*, the only common potential predator, is at low abundance. Overall and despite important data gaps, marine reserves appear effective for the conservation of residential shark species like *C.perezi*. This species might not exert top down control of the ecosystem due to functional redundancy and/or omnivory but there is evidence of behaviorally mediated interactions between *C.perezi* and stingrays.

To Gordon and Veronica Bond thank you for the inspiration, love and support that got me here. We started this journey together, growing moss in the garden, I wish you could have been with me at the finish line.

Frontispiece

Table of Contents

List of Figures

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

List of Tables

Chapter 3

Chapter 4

Chapter 5

Acknowledgments

Although this dissertation only has one name on the cover there are so many individuals who can take pride and credit for helping me get here and its completion. The following is a list of everyone who directly or indirectly supported me during the testing but rewarding process of getting a Ph.D., for which I sincerely thank you all.

First and foremost to Demian Chapman for being a great academic advisor, mentor and friend. For always being willing to help and a great sound board for ideas and motivation. I really appreciated all the learning experiences and opportunities you gave me and so many good times from the field, thanks man. To the rest of my dissertation committee Drs. Dean Grubbs, Michael Heithaus, Ellen Pikitch, and Robert Cerrato I am grateful for the patience and willingness to help whenever I needed it. Thank you for sharing your expertise and helping guide the dissertation with constructive criticism. I am sure you will be relieved that I won't be knocking on your door for stats help anymore, Bob! A huge thank you to my co-authors Nigel Hussey and Elizabeth 'Xena stats warrior' Babcock, whose inboxes were always open and constantly flooded my emails when I got stuck, I really appreciated your inputs. Other academic thanks must go to Lis Henderson for constantly allowing me to pester her when I was stuck coding in R, 'the Brad's' Peterson and Furman for helping me decide on my habitat survey approach and all for being great friends. Thank you to Emily Tolentino for additional help in R and to all the volunteers who helped review the many BRUV videos, I couldn't have done it without you. For every fisherman at sea it creates five jobs onshore and I think for every graduate student it takes ten administrative staff to help them get through it. With that I must thank Carol Dovi, Kim Knoll, Steve Ortega, Paulette Guerrier, John Graham, Mark Lang, and Christina Fink for letting me know which forms must be signed when and not yelling at me (too much!) when I forgot to return the tax exempt card. SoMAS has been an amazing experience for me and made all the better for the great people I have met and now have the fortune to call friends. Thank you to all of SoMAS for making these years so much fun but in particular Alex Malvezzi, Chris 'Murdog' Murray, Amber Stubler, Christine O'Connell, Hannes Baumann, Zosia Baumann, Carla Furman, Jenny George, Alex Sanchez-Franks, and Kaitlin Childers. A special mention to Natasha Gownaris for testing me to my limits with her intense field work in Kenya, we can look back and laugh now but man was that challenging!

I had the great fortune to conduct my fieldwork in Belize an experience enhanced by the characters I was in field with. Nothing brings you closer then field work and thank you to Jasmine Valentine-Albanese for being a great field companion and jaguar porgy hunter. Captain Norlan Lamb and Ashbert Miranda for so many laughs, adventures and saving me more than once, "Sean Paul….?!". All the past and present staff at Wildlife Conservation Society Belize, Glovers Reef Marine Station (Middle Cay), and Earthwatch.

The Bimini Biological Field Station 'Sharklab' who gave me my first start and ignited the passion and motivation to pursue this as a career. In particular Doc and Marie for the opportunity to work there, Kat Gledhill, Tristan Guttridge, Steve Kessel, and Joey DiBattista for the career support, so many laughs and unforgettable experiences.

To my family, Ma, Dad, Gemma, and Cube for all the support through the years, the encouragement to find a job I loved, and putting up with me. The Welsh boys and members of the ‘Gentleman’s Club’ for so many laughs when I was stressed, and the encouragement. My chommies back in South Africa, nothing but love.

They say you can’t pick your family but you can pick your friends. I am very fortunate to have many friends that I consider family. To Debbie Abercrombie who provided constant support both academically and personally, without whom I would have never survived a single winter in New York. I guess your adopted African child has finally flown the coup! Lucy Howey and (W)Lance Jordan, for the generosity, so many laughs in and out of the field, and having such confidence in me. Stephen and the rest of the Heck family for treating me as one of your own every holiday and the reassurance through the tough times. Kelley and the rest of the Coughlin family for being so welcoming and providing Long Island logistical support. . Kevin Feldheim for the brojitos, great support, and talking me off the statistics ledge!

I’m sorry if I have forgotten anyone please know, I am extremely grateful to everyone who has helped me over the years

Chapter One: Introduction

Introduction

The Subclass Elasmobranchii is the larger of the two subclasses of Chondrichthyes, (cartilaginous fishes) and is comprised of the sharks, skates and rays. There are over 500 species of sharks and more than 600 species of rays and new species are being regularly described (Compagno et al. 2005; Castro 2011). Within the elasmobranchs there is great diversity with species occurring in a variety of ecosystems from the sub-arctic to the tropics and the depths from the epipelagic to the abyssal zone.

Elasmobranchs are currently experiencing pressure from anthropogenic activities, which has led to severe declines of many populations (Myers and Worm 2003; Baum and Myers 2004; Ferretti et al. 2008). Elasmobranchs are characterized by K-selected life history traits such as slow growth rates, low fecundity, and late to reach sexual maturity (Musick 1999). These characteristics make them particularly vulnerable to overexploitation and populations slow to recover. Annual global shark landings are estimated to be100 million individuals (Worm et al. 2013) and one quarter of the 1,041 species of chondrichthyan fishes are assessed as threatened by the International Union for the Conservation of Nature (IUCN). This extinction risk is considerably higher than other vertebrates (Stevens et al. 2000; Dulvy et al. 2014). In particular large-bodied, coastal species are at greatest risk and five out of the seven most threatened families are batoids (Dulvy et al. 2014).

Targeted commercial fisheries, both legal and illegal, exploit elasmobranchs for the meat and to supply the lucrative Asian dried fin market. The high value shark fins, sometimes selling for $800 per kg, are consumed in shark fin soup a delicacy throughout Asia and a dish that is revered as a status symbol. Large quantities of sharks are also landed as bycatch particularly in high seas longline fisheries targeting swordfish or tuna, due to the high value of shark fins it is common to see the fins detached and retained and the rest of the meat discarded when storage is limited (Myers and Worm 2003; Myers and Worm 2005; Worm et al. 2013). Degradation and loss of critical habitat from coastal development and pollution also contribute to elasmobranch population declines (Jennings 2000; Jennings et al. 2008).

Declining sharks and rays populations have been observed through the world's oceans but it are particularly prevalent in tropical coral reef ecosystems (Stevens et al. 2000; Dulvy et al. 2004; Dulvy 2006). Tropical coral reefs are some of the most productive ecosystems in the world and global hotspots for marine biodiversity for all taxa. Many species of sharks and rays are considered reef-associated, hereafter referred to as 'reef sharks', meaning they complete all life-stages over coral reef habitats. Reef sharks are large-bodied, upper trophic level piscivores and commonly thought of as apex predators on coral reefs (Heupel et al. 2014). Apex predators are defined as species that exert top down control over the community structure and have few, if any, natural predators (Heupel et al. 2014). Large-bodied upper trophic level species are generally targeted first by fisheries and have become rare in many coral reef ecosystems (Friedlander and DeMartini 2002; Friedlander et al. 2010). This is a serious environmental concern because we have yet to fully understand the ecological role of reef sharks and the consequences of removing them. It has been suggested that a reduction in reef sharks could initiate a trophic cascade that could contribute to a regime shift from a coral to an algae dominated reef (Bascompte et al. 2005; Estes et al. 2011b). A reduction of reef sharks functioning as apex predators could lead to an increase in mesopredatory piscivores (e.g. F. Serranidae). The subsequent increase in predation on herbivores (e.g. F. Scaridae) could diminish their grazing capacity, thus contributing to regime shifts (Bascompte et al. 2005; Ruppert et al. 2013). This could have broader consequences as many other commercial fisheries are reliant on healthy coral reefs.

Current rates of population decline and negative predictions on the outlook for many elasmobranch species has led to the listing of sharks and rays as a conservation priority by the governments and non-governmental sectors throughout world. Improved fisheries management and monitoring of trade is imperative to arrest population declines and promote recovery (Ward-Paige et al. 2012; Worm et al. 2013). Directed research and funding to support such efforts are accumulating, though exactly what conservation approaches are the most effective are not entirely clear. Marine reserves, which are areas of the ocean where anthropogenic activities are restricted in particular extractive practices like fishing, are being implemented all over the world to restore and conserve marine biodiversity (Halpern 2003; Halpern et al. 2007). Marine reserves can have a positive effect on local biodiversity and, for exploited taxa, can enhance local biomass, body size, and density (Bohnsack 1993; Lester and Halpern 2008; Lester et al. 2009). This has been well documented for slow moving, sedentary species with r-selected life history traits but there is limited data to support them as conservation and management tool for sharks and batoids (Bohnsack 1993; Bonfil 1997; Halpern 2003).

Objectives and Hypotheses

The aim of this dissertation was to evaluate the effectiveness of marine reserves as a conservation tool for shark and ray conservation in a tropical coral reef ecosystem. The primary study species were the Caribbean reef sharks (*Carcharhinus perezi*), a large-bodied reef associated species and possible apex predator, and the southern stingray (*Dasyatis americana*), a mesopredator. A network of existing marine reserves throughout the Belizean section of the Mesoamerican Barrier Reef provided an experimental framework to assess direct and indirect effects of reserve establishment on both study species.

The following hypotheses and objectives were addressed:

Chapter Two: A review aimed to synthesize the current literature to determine i) how often tracking studies of elasmobranchs inside marine reserves find evidence that individuals spend a large fraction of their time in these zones, ii) the evidence for positive reserve effects on elasmobranch populations inside marine reserves iii) how these effects have been measured and what biological parameters have responded positively, and iv) whether positive reserve effects are typically associated with certain species, habitats or marine reserve traits (e.g., reserve size).

Chapter Three: To elucidate the ecological role of Caribbean reef sharks and to test the hypotheses that i) large (subadult and adult) Caribbean reef sharks primarily feed on large carnivores in the ecosystem (e.g., black grouper, Nassau grouper, great barracuda), ii) the population feeds across multiple reef habitats, iii) there are ontogenetic shifts in trophic level and foraging habitat and iv) individuals primarily living in lagoon habitats are isotopically differentiated from individuals living on the fore-reef.

Chapter Four: To investigate population trends through a standardized long-term longline time series 2000-2013, to test i) the null hypothesis that year and habitat had no effect on

Carcharhinus perezi CPUE over this period ii) that year and habitat had no effect on the size of *C. perezi* caught throughout the survey. Additional objectives were iii) to further describe the demography of the *C. perezi* population at Glover Reef Marine reserve and iv) to examine growth rates based on mark-recaptures obtained throughout the study.

Chapter Five: To examine site-fidelity and relative abundance of Caribbean reef sharks in a marine reserve in Belize. Given the hypothesis that Caribbean reef shark populations can benefit from no-take marine reserves and increase in abundance in these areas, we predicted that i) acoustically tagged Caribbean reef sharks at GRMR would exhibit site-fidelity to the reserve and ii) the relative abundance of Caribbean reef sharks would be higher at GRMR (and other reserve reefs) when compared to fished reefs.

Chapter Six: To investigate factors affecting the relative abundance and distribution of stingrays and reef sharks inside and outside reserves primarily using baited remote underwater video (BRUV) surveys. Our hypothesis was that the i) frequency of stingray observations on BRUVs would be significantly lower and distributed differently (shallower) inside marine reserves due to the presence of potential predators (sharks) in deeper habitats in these areas, ii) we hypothesized the opposite pattern for reef sharks based on results of previous studies in these areas. We also tested the hypothesis iii) that stingrays inside marine reserves were more likely to carry wounds potentially caused by sharks, such as bite marks or truncated tails and iv) the duration of time that stingrays spent at the bait-cage of the BRUV was compared between sites and habitats, testing the hypotheses that site and habitat affected time spent attempting to forage as a proxy for perceived risk of encountering a predator.

Chapter Two: Review of the effectiveness of marine protected areas as a tool for shark and batoid conservation

Abstract

Marine reserves, areas of the ocean where anthropogenic activities are restricted in particular extractive practices like fishing, are being implemented all over the world to restore and conserve marine biodiversity. Marine reserves can have a positive effect on local biodiversity and, for exploited taxa, can enhance local biomass, body size, and density. Sharks and batoids are among the most threatened marine vertebrate taxa, yet the role of marine reserves in the conservation and management of these large predators has not been reviewed and assessed. A search of published literature on sharks and batoids occurring within marine reserves (n= 176 studies) revealed an increase in research over the past ten years. Studies were skewed towards certain habitats (e.g., coral reefs) and regions (e.g., Australia), with large research gaps identified in the coral triangle region, a hotspot for biodiversity and shark fishing. Movement studies dominated the literature and across all study types the majority were conducted on a small subset of primarily reef associated shark and batoid species. Studies that exclusively examined for evidence of reserve effects (n=44) on one or more biological parameters for both sharks and batoids demonstrated that 60.5% of studies showed a positive response to reserve establishment compared with only 16% which showed a negative response. Of these 44 studies most were either spatial (n=36) or temporal (n=15) in design with few (n=7) using the Before-After-Control-Impact (BACI) approach. There is a growing body of evidence that marine reserves are an effective tool for the conservation of at least reef associated sharks and batoids in the tropical coral ecosystems. There is a need to conduct studies with a BACI approach in ecosystems outside of tropical coral reefs, and on less-reef associated species to determine whether marine reserves are an effective conservation tool in these habitats.

Introduction

Marine reserves, defined here as areas closed to fishing, which prohibit various other extractive or consumptive activities, and within which anthropogenic interference is minimized to the best extent (Sobel and Dahlgren 2004), are now being widely used as a tool for biodiversity conservation and fisheries management (Roberts 2012). Marine reserves are deemed to be effective when the biomass, density, and/or body size of previously exploited species, and sometimes overall ecosystem biodiversity, increases within their borders (Lester et al. 2009). Changes in these biological indicators are most frequently inferred from spatial comparisons of similar habitats inside and outside reserve boundaries (Halpern 2003; Halpern and Warner 2003; Lester et al. 2009). A more convincing but resource intensive way to assess reserve performance is the Before-After-Control-Impact (BACI) design, which is the monitoring of trends in these indicators starting before reserve establishment and continuing over time, both inside the reserve and at appropriate control sites (Stewart-Oaten 1986; Sobel and Dahlgren 2004). Both types of study have provided evidence of positive reserve effects in marine species and ecosystems, primarily in sedentary species and those with r-selected life history traits (Halpern and Warner 2002,2003). Reserve effects are more difficult to study in species with K-selected life histories given the long time required to document signals of population recovery. They are also difficult to study in mobile species that still experience fishing mortality outside the reserve, which can also delay recovery. Large-bodied, upper trophic level predators often have these traits and are becoming focal species for conservation (Pauly et al. 1998; Ferretti et al. 2010; Dulvy et al. 2014).

Elasmobranchs (sharks and batoids) are a group of primarily live-bearing, K-selected predators that have experienced widespread population declines, primarily due to overexploitation and/or habitat loss (Stevens et al. 2000; Dulvy et al. 2014). It is now generally thought that elasmobranchs are in need of stronger management (Musick et al. 2000; Barker and Schluessel 2005), which has become a priority given that the depletion of some species may have a strong influence on community dynamics due to their role as large predators (sharks) or bioturbators (batoids)(Heithaus et al. 2008a). The life history of many elasmobranchs predicts that overexploited populations would be slow to recover but could benefit from zero or near zero fishing mortality if individuals spent significant time within marine reserves (Pauly et al. 1998; Stevens 2000). The primary scientific literature documenting whether or not marine reserves are an effective tool for preserving or restoring elasmobranch populations has never been reviewed and synthesized, leaving important questions about the role of reserves in emerging global elasmobranch conservation efforts.

Elasmobranchs exhibit certain traits that may weaken the benefits provided by marine reserves. The widespread establishment of marine reserves is a relatively recent phenomenon and significant population recovery in elasmobranchs may be contingent upon marine reserves being successfully enforced for very long periods of time given their low rates of intrinsic population increase (Musick 1999). The low rate of intrinsic population increase also means that elasmobranchs are vulnerable to even light fishing pressure (Pauly et al. 1998; Dulvy et al. 2014). Currently only ~10% of global marine protected areas are true marine reserves, which means that fishing mortality may still be too high even from reduced but non-zero fishing mortality (Wood et al. 2008). Elasmobranchs are typically mobile and many individuals could therefore routinely move outside of reserves, resulting in mortality rates that neutralize population recovery or fail to arrest population decline unless reserves are very large.

In light of the current status of many elasmobranch populations it is imperative to assess the role of marine reserves in global efforts to conserve these animals. This review aims to synthesize the current literature to determine i) how often tracking studies of elasmobranchs inside marine reserves find evidence that individuals spend a large fraction of their time in these areas, ii) the evidence for positive reserve effects on elasmobranch populations inside marine reserves iii) how these effects have been measured and what biological parameters have responded positively, and iv) whether positive reserve effects are typically associated with certain species, habitats or marine reserve traits (e.g., reserve size). I will use the results of this review to highlight critical knowledge gaps and suggest future research priorities.

Methods

I limited this review to studies taking place within “marine reserves”, defined once again as areas closed to fishing, which prohibit various other extractive or consumptive activities, and within which anthropogenic interference is minimized to the best extent (Sobel and Dahlgren 2004). Unless they explicitly took place within a marine reserve zone, I did not include studies within Marine Protected Area (MPA) defined as “any area of intertidal or subtidal terrain together with its overlying water and associated flora, fauna, historical and cultural features, which has been reserved by law or other effective means to protect part or all of the enclosed environment” (Kelleher 1999). The recent prioritization of sharks for fisheries management and conservation has in some areas led to shark specific management policies and protective measures that are reminiscent of those offered by marine reserves (Bonfil 1997). These include de-facto marine reserves for certain species that are prohibited from landings within a particular jurisdiction; “Shark Sanctuaries” which are whole Exclusive Economic Zone (EEZ) closures of shark fishing and trade (Davidson 2012); and “Shark no-take reserves” which are smaller marine management zones in Australia where fishing is allowed but the landing of sharks is not (Walker 1998). I omitted studies taking place in such areas on the grounds that there remains the potential for shark by-catch in these fished areas that make them less than a true marine reserve as I define them. To review the evidence for marine reserve effects on sharks and batoids I queried several search engines using the keywords “shark”, “batoid”, “stingray”, “ray”, and “elasmobranch” coupled with “marine reserve,” “marine protected area,” “MPA,” “no-take,” “reserve,” or “sanctuary.” The studies found were then sorted into: 1) studies that examined the movement and residency of elasmobranchs, which provides evidence of the potential efficacy of marine reserves, and 2) those that explicitly examined potential reserve effect across at least one of four biological parameters (biomass, density, body size and biodiversity) in elasmobranchs.

The shark (but not batoid) movement literature in general has been reviewed recently and some conclusions drawn on the potential for reserve to protect certain species based on particular movement patterns (e.g., residency; (Hueter et al. 2005; Chapman et al. 2015)). I narrowed my focus to movement studies conducted within existing reserves, which explicitly measured how long individuals were spending inside these areas. Marine reserves with established shark or batoid provisioning sites were excluded from this analysis given the potential for altered behavior.

The remaining studies investigated reserve effects on at least one of the four basic biological parameters observed to increase inside marine reserves: biomass, density, body size and species diversity. Each study was classified by the reserve effect that was statistically demonstrated (i.e., “positive”, “negative”, “neutral” and “not tested”) and in which of the four parameters the positive effect was observed. Studies were further categorized as either being comparisons of these parameters inside reserves and control sites (“spatial” study) or changes in these parameters over time with standardized, repeated sampling (“temporal studies, e.g., BACI). Studies were sorted by survey method (e.g., underwater visual census [UVC]; Baited remote underwater videos [BRUV] and Catch per Unit Effort [CPUE] from nets or baited hooks) and for each one the duration of the study, habitat type it was conducted in, species studied and which of the five features presented by Edgar et al. (2014) that determine marine reserve effectiveness (i.e., large size, advanced age, effective enforcement, no-take and isolated) the reserve in the study met.

Results

A thorough literature search that concluded on 25th March 2015, revealed 176 studies conducted within marine reserves examining various aspects of shark and batoid ecology, movement, biodiversity and effects of reserve establishment (Fig 1). Shark focused studies (n = 155) dominated the literature compared to batoid (n = 21).The majority of studies focused on movements (n = 63) for sharks and ecology or reserve effects (n= 8 each) for batoids.

The number of studies conducted within marine reserves were documented from 1993-present but have markedly increased over the past decade (Fig 2). Studies were primarily movement oriented but with those focused on reserve effects and ecology also increasing. Species inventory studies were common throughout the time period (n = 30) and provided a baseline against which to measure reserve effects on biodiversity but few studies (n = 3) consisted of a follow up assessment. The number of inventory studies shows the potential for time-series or temporal studies for reserve effects in future years.

Studies were geographically limited with 42% of all studies conducted in Australia although this encompassed a variety of habitats: Great Barrier Reef Marine Park (GBRMP, n = 34), Ningaloo reef (n = 18), New South Wales (n = 13), and Tasmania (n = 10; Fig 3). Palmyra Atoll, Northern Line Islands and the Belizean barrier reef were additional hotspots for studies (n = 13 each). Subsequently tropical coral reef was the most prevalent habitat with 64% of studies conducted in such ecosystems involving 29 different species of sharks (n = 20) and batoids (n = 9), which included both barrier coral reef (n = 64) and insular coral reef (n = 52, Fig 4).

Shark and batoid movement studies (n=67) within marine reserves comprised 35.5% of the literature and revealed 23 species which have the potential to benefit from reserve protection given known movement patterns, the majority of which were coral reef species (Fig 5). Movement data derived from telemetry studies identifying residency, therefore proportion of time spent within reserve boundaries experiencing reduced fishing mortality, forms the majority of our knowledge on potential reserve effectiveness. Additionally studies conducted within tropical coral reef ecosystems on reef-associated shark species, notably grey, whitetip and blacktip reef sharks (n = 36, 33, 32 respectively), comprise the bulk of our understanding on the biological effects of reserve establishment on sharks and batoids, with data primarily gleaned from underwater visual census (n= 58) and baited remote underwater video surveys (n = 18). Only studies with sharks and batoids identified to a species level were included for analysis by survey method type.

Forty-four studies specifically tested for biological responses to reserve establishment that resulted in the assessment (n= 205) of 75 combined shark and batoid species (Fig 6). The biological parameters tested were biomass (2.2%), density (22.7%), and relative abundance (75%). Most employed a spatial approach (n = 36) with relatively few temporal surveys conducted (n = 15) and few studies that conducted both (n = 7). The median duration of studies conducted was 730 days, and primarily studies were < 2 years in duration (n = 27). Long (> 5 years) time-series data from marine reserves were only found for Great Barrier Reef Marine Park, Cocos Island, Costa Rica, and Poor Knights Islands, New Zealand, (Heupel et al. 2009; Taylor et al. 2011; White et al. 2015). Despite the purpose of these studies being to investigate reserve effects very few employed a Before-After-Control-Impact survey design (n = 8). Of the negative responses (n = 33) batoids accounted for 18 with multiple cases of decreasing relative abundance of non-targeted spotted eagle ray (*Aetobatus narinari*), fiddler (*Trygonorrhina dumerilii*) and round stingrays (*Urobatis halleri*). Whitetip reef sharks (*Triaenodon obesus*) were the only shark species to have declines in relative abundance in multiple studies (n=2) following reserve establishment. Tiger (*Galeocerdo cuvier*) and silvertip (*Carcharhinus albimarginatus*) sharks also had isolated declines. Both batoids and sharks had equal numbers of neutral responses to reserve establishment (n = 24). Out of the studies showing positive reserve effects on batoids (n = 17), the common eagle ray (*Myliobatis aquila*) was the only species where this response was observed in multiple studies (n = 3). Out of the 107 positive responses of sharks to reserve establishment, most were from grey (*Carcharhinus amblyrhyncos*), whitetip, and blacktip reef (*Carcharhinus melanopterus*) sharks (n = 20, 18, 10 respectively) and wobbegongs (*Orectolobus* spp.; n = 8). Of the studies which observed a negative response to reserve protection only 24% fulfilled ≥ 3 or more of the criteria defined for a successful marine reserve (Edgar et al. 2014) and only half of these studies occurred in reserves > 100km^2. Conversely

54% of positive responses to reserve implementation satisfied ≥ 3 Edgar et al. 2014 criteria and 61% of these were in reserves > $100km^2$ (Fig 7).

Discussion

The study of the effectiveness of marine reserves for elasmobranch conservation is relatively recent and has risen concomitant with the depletion of sharks becoming a mainstream global environmental issue. Most studies on elasmobranchs pertinent to assessing marine reserve effectiveness focused on individual movements in relation to reserve boundaries. Movements were quantified primarily with acoustic telemetry and nearly all studies were on tropical coral reef-associated carcharhinid sharks. Behavioral patterns identified in these species included "residency" and "site-fidelity". Species that almost continuously occupy a defined area for a specified duration are considered resident (Chapman et al. 2015). Examples of residency are sharks with fixed home ranges or, that inhabit nursery areas or particular habitats during certain life stages. Residential species spent significant periods of time within marine reserves. Site-fidelity is defined as repeated return to and residency within an area punctuated by long periods of absence that are as long or longer than the residency period (Chapman et al. 2015). Marine reserves are a potential tool for the conservation of species exhibiting site-fidelity during the residency period, but the benefits probably decrease with increasing time spent outside of the reserve depending on where they go in relation to fishing. The benefits of marine reserves for species displaying site-fidelity are likely greatest when they could otherwise be disproportionately targeted by fisheries in these areas. Overall, movement studies on certain species of elasmobranchs reveal that individuals can be residents of marine reserves for significant periods of time, implying that there is potential for positive reserve effects on their populations.

Movement studies of individuals suggest that marine reserves could effectively protect or restore populations of certain elasmobranchs, which is borne out by the fact that a majority of reported studies detected positive marine reserve effects. Nearly all of these studies were spatial comparisons of relative abundance as a proxy for biomass and/or density inside marine reserves and comparable or neighboring fished control sites. Most took place in tropical coral reef habitat and focused on coral reef-associated carcharhinid sharks that exhibit site-fidelity or residency.

Studies were primarily based on the non-invasive survey methods underwater visual census (UVC) and/or baited remote underwater video (BRUV) and were skewed towards barrier reef or insular ecosystems in Australia and Belize. Neutral or negative reserve effects were reported but much less frequently. Although this probably in part reflects a reporting bias these were typically associated with mobile or migratory species (e.g. tiger shark, Espinoza et al. 2014) that would be expected to spend minimal time inside reserves. Neutral or negative reserve effects were also observed for species that were not fished in the study area (e.g. spotted eagle ray, Amargós et al. 2010).

While there is a growing body of spatial evidence of positive marine reserve effects for certain elasmobranchs these studies were typically conducted over periods of days, which can be affected by short term population fluctuations (e.g., due to seasonal migrations or movements associated with reproduction) and provide no information on long term population trends. While there is no question that spatial studies are necessary to infer reserve effectiveness in the absence of the ability to conduct temporal studies it remains a substantial research gap that there are virtually no BACI studies for elasmobranchs inside marine reserves. This gap needs to be addressed because spatial studies may conclude positive reserve effects even if populations inside reserves are still declining but at a slower rate than those within control fished habitats (Friedlander et al. 2012). These results would imply the reserve is effective, while masking a slow decline and perhaps enabling a population collapse before the need for alternative management is recognized. Further support of BACI designed surveys to assess reserve effectiveness is that some reserves may carry inherent bias when designated. The selection of a pristine area as a reserve makes more sense than a degraded area therefore, the reserve was exhibited “positive” effects from t=0 and would bias any monitoring efforts.

I therefore suggest that BACI studies need to be established in newly designated marine reserves or published if already underway in established marine reserves. I also suggest a need for more time series capable of detecting elasmobranch population trends inside marine reserves. Since reserve effectiveness is positively correlated with time since protection (Espinoza et al. 2014b), time series may enable researchers to quantify the rate of elasmobranch population recovery following reserve establishment, which would be important for setting realistic restoration expectations and targets when adopting this approach.

Time-series of elasmobranch abundance within marine reserves are very few but detected biological changes in response to reserve protection for 18 species across 3 studies. The coral reef associated shark species (n = 5) assessed in Heupel et al. 2009 all demonstrated a positive increase in relative abundance. Taylor et al. 2011 also detected an increase in relative abundance of bronze whaler (*Carcharhinus brachyurus*) sharks over a 50 year time period with uneven sampling but short-tailed stingrays showed no change, potentially as they were not commercially targeted prior to reserve establishment. The 20 year time-series of standardized diver observations from Cocos Island, Costa Rica analyzed by White et al. 2015 detected species specific differences in reserve effects with only 3 out of 11 species showing a positive response in relative abundance. Highly mobile tiger sharks were absent from the early years of the survey but commonly observed more recently but *Mobula* spp. and blacktip sharks sighting frequencies increased over time. Reef-associated shark species and those that interact with longlines, decreased in abundance, which was attributed to illegal fishing within the reserve. Conclusions from the very limited number of BACI studies (n = 8) examining reserve effectiveness on shark and batoid populations were nearly all positive with only one example of a negative reserve effect, on blacktip reef sharks over a 3 year period (Purwanto et al. 2012). Studies that detected a neutral biological response across 8 species included non-targeted batoid species (n = 3) but were also typically of short duration 2-3 years, with the exception of the short-tailed stingray (Taylor et al. 2011). Positive biological responses detected from BACI studies were limited exclusively to reef associated shark species (n = 9) and the crossback stingaree (*Urolophus cruciatus*, Edgar and Barrett 2012) however data were collected from studies conducted over biologically relevant time scales of 3-20 years given what we know of these species life-histories. BACI designed surveys should be prioritized as a method for quantifying biological change after reserve establishment. Continued sampling must occur over biologically relevant time-scales in order to accurately assess reserve effectiveness and long-term time-series data from both inside and outside of reserves is essential.

I confirm previous findings that positive reserve effects for elasmobranchs were associated with marine reserves that exhibited three to five of the five criteria that Edgar et al. (2014) identified as those that dictated marine reserve success. This raises the point that future studies of reserve effectiveness for elasmobranch conservation should focus on marine reserves that meet this criterion. The inclusion of ‘paper parks’ that lack any enforcement, reserves too small in area to benefit a species given their known home-range, and newly formed reserves are potentially misleading in the sense that we already know that positive reserve effects are unlikely when reserves have one or more of these features. These reserves are, for all intents and purposes, non-functional and should not be expected to work and including them as “reserve” treatments is therefore problematic. The level of enforcement should also be considered as an important feature because residential reef shark species exhibit higher relative abundance in ‘no-entry’ than ‘no-take’ marine reserves on the Great Barrier Reef (Ruppert et al. 2013; Rizzari et al. 2015).

Because positive reserve effects for elasmobranchs nearly all stem from spatial studies, there remains some uncertainty about the underlying mechanisms of how this occurs. It is usually inferred that reserve effects result from a respite from fishing on the sharks themselves but it possible that the lack of fishing generally also plays a role. Marine reserves are known to contain higher biomass and density of a variety of potential prey items for sharks that fished control sites. The ideal free distribution model (Kennedy and Gray 1993) predicts that the distribution and density of predators (sharks) would match that of their prey. Positive reserve effects on sharks therefore align with this prediction, raising questions as to how much these patterns are driven by fishing pressure on sharks as opposed to fishing pressure on their prey. One way to resolve this issue would be to test for reserve effects on sharks in places where their prey are fished but they are not, such as the Bahamas. Goetze et al. (2013) suggested positive reserve effects on sharks around Fiji were due to fishing on their prey because local fishermen did not target sharks. It is also possible that positive reserve effects on sharks are due to marine reserves often being designated in relatively healthy ecosystems or those with special features (such as complex structure) that are attractive for ecotourism. These areas could have also have disproportionately large elasmobranch populations, thus creating what appears to be a positive reserve effect if only measured using the spatial approach. BACI studies could resolve whether this is the case.

Aside from a general lack of temporal and especially BACI studies there are several clear deficiencies in the current literature that assesses the effectiveness of marine reserves for elasmobranch conservation. It is most obvious that research must expand to encompass a broader range of species and ecosystems. At present the relatively narrow focus of research provides evidence of reserve effectiveness but for a limited number of shark species, mainly five species of coral reef associated species in the Family Carcharhinidae (grey reef, *Carcharhinus amblyrhyncos*, blacktip reef, *C. melanopterus*, silvertip, *C. albimarginatus,* Caribbean reef, *C. perezi* and whitetip reef, *Triaenodon obesus*). They are also geographically restricted and occurred within the tropical coral reefs where these species occur. The paucity of studies examining reserve effectiveness on large, apex predatory, and highly mobile shark species requires attention. Species that exhibit these traits would likely benefit least from reserve protection considering the large proportion of time potentially spent outside of the reserve. Additionally, species exhibiting these characteristics are frequently targeted by commercial fisheries, and have heavily depleted populations. Directed research identifying what reserve characteristics (if any) increase reserve effectiveness for these species must be a priority, and failing that what alternative spatial management solutions should be considered (e.g. time area closures). Another obvious taxonomic bias is the lack of focused studies on the effects of marine reserves on batoids, especially given the recent finding of the International Union for the Conservation of Nature (IUCN) that batoids have more species in threatened categories than do sharks (Dulvy et al. 2014). Very little is known about the movement of batoids, but it is possible that residency and site-fidelity are common enough in some of the most threatened taxa (e.g., stingrays, F. Dasyatidae, sawfishes, F. Pristidae, wedgefishes, F. Rhynchobatidae and guitarfishes, F. Rhinobatidae) that marine reserves would benefit their populations (Dulvy et al. 2014). To date too few studies have focused on testing for reserve effects on these or any other batoid species.

There is a great deal of interest in the ecological role of elasmobranchs and my review indicates that marine reserves provide a potential but untapped experimental setting with which to assess the effects of removing or restoring these animals. While it is difficult to separate the effects of shark removal on its own from the general removal of large predatory teleosts (e.g., Serranids, Carangids, Sphyraenids) BACI studies that simultaneously monitor the recovery of predators and the response of their prey and the community could possibly disentangle them because sharks will generally recover more slowly than large predatory teleosts (Pauly et al. 1998; Stevens 2000). I suggest that spatial studies that simultaneously survey sharks, batoids, multiple functional groups of teleosts and benthic community structure could be used to generate hypotheses about the ecological role of sharks that could then be tested within select sites using a

BACI approach. Improved understanding of the pathways and magnitude that behaviorally mediated direct/indirect interactions can propagate through marine ecosystems will allow us to predict the ecosystem-wide implications of predator restoration using marine reserves and other approaches. I also suggest that studies need to assess sharks species on a species specific basis. This is because most resident reef-associated sharks (e.g. blacktip, whitetip, and grey reef) known to respond to marine reserves are likely to function as “mesopredators” (i.e., trophic level 3 or 4) with larger, more migratory species (e.g, tiger, bull and silvertips) the systems’ true “apex predators” (i.e., trophic level >4; Heupel et al. 2014). These reef-associated mesopredators are to date the species demonstrated to benefit from reserve protection. Disproportionate restoration of these species relative to apex predators as a consequence of reserve establishment could result in widespread and unexpected community restructuring. I also suggest that the same could be true of batoids as mesopredators and bioturbators in many ecosystems, which means changes in their populations due to marine reserves could also influence community structure.

Conclusions

Marine reserves are gaining traction worldwide as conservation tool and my review suggests that there is evidence that this approach benefits certain elasmobranchs, specifically exploited species that exhibit residency or site-fidelity. Nearly all of the evidence stems from spatial comparisons of relative abundance inside and outside marine reserves, highlighting an urgent need for temporal studies such as BACI. Without more studies like this it is difficult to assess whether observed positive reserve effects are due to slower rates of population decline inside reserves as opposed to population stability or growth. It is also clear that reserve size, age, isolation, enforcement level and strength of the regulations are key to reserve success and sites lacking many of these features should not be considered a true reserve treatment in these studies. It is also important to test whether positive reserve effects from spatial studies are at least partially attributable to increased prey inside reserves or the possibility that many reserves are positioned in areas that may have naturally larger elasmobranch populations. Lastly, I conclude that it is essential research now broadens from coral reefs and reef associated carcharhinid sharks to a wider variety of habitats and species, including highly threatened batoids and apex predatory sharks, as well as taking advantage of reserves as potential tools for better understanding the ecological role of certain elasmobranchs.

Figures and Tables

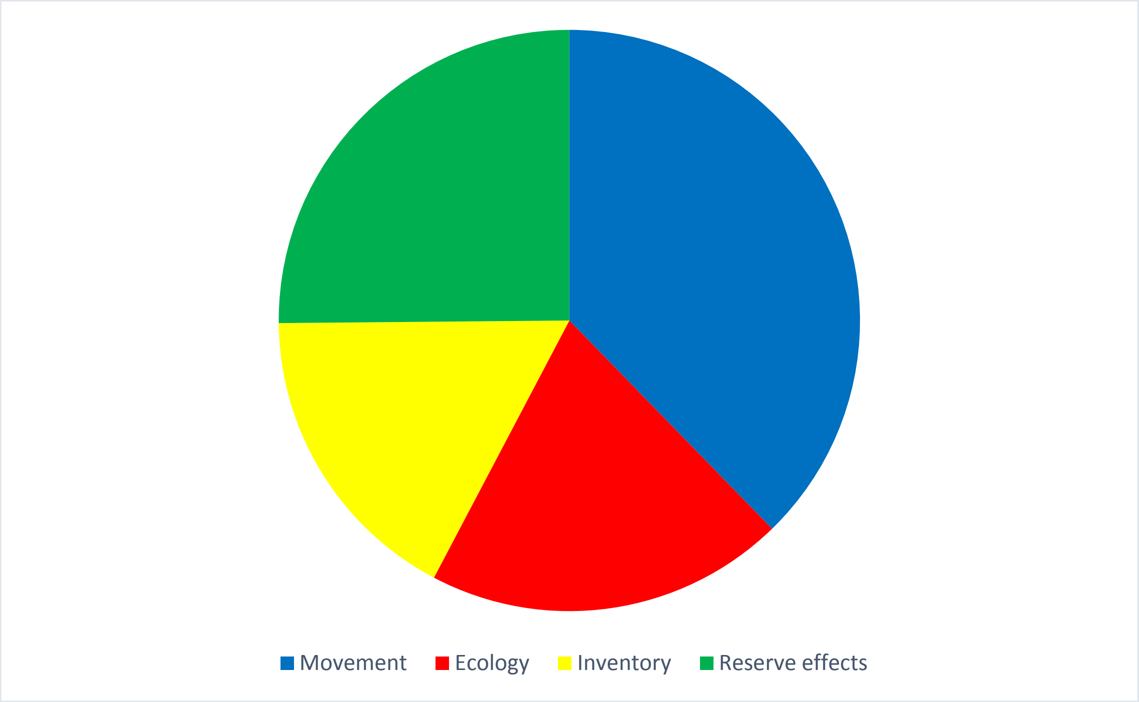


Figure 1. The proportion of studies conducted within marine reserves which have assessed aspects of shark and batoid movement (n = 67), ecology (n = 35), biodiversity (n = 30), and the potential benefits of reserve establishment (n = 44).

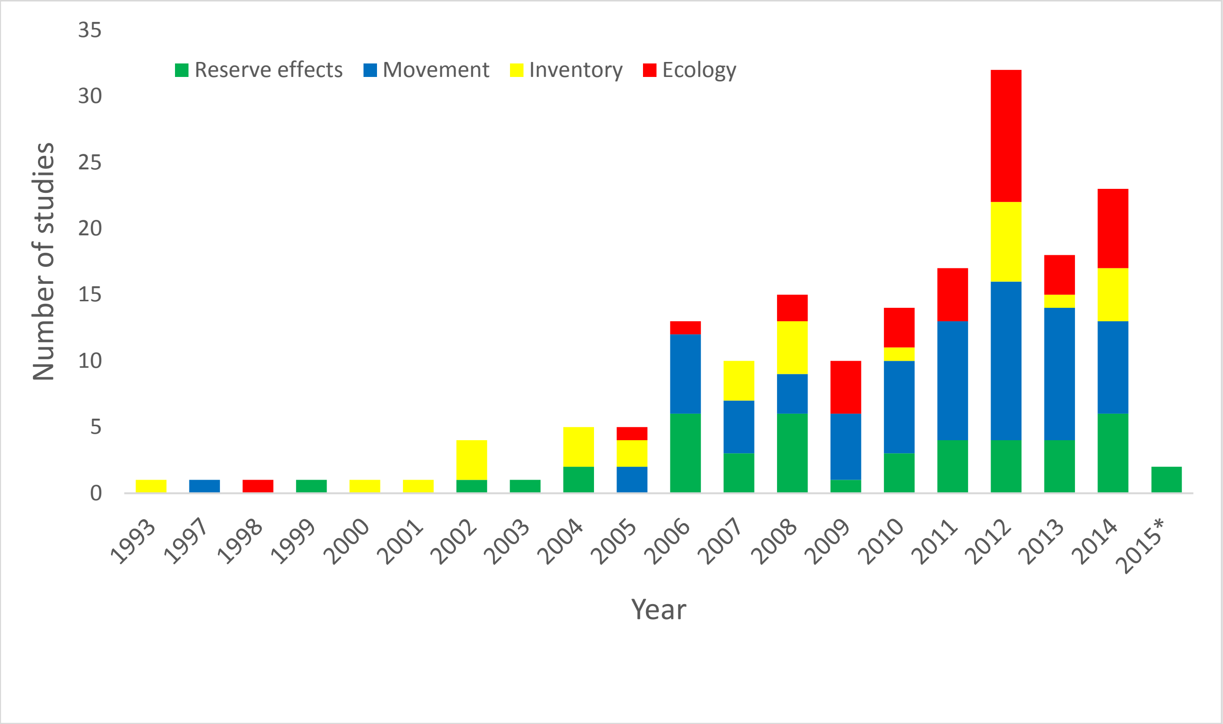


Figure 2. Chronological distribution of studies occurring within marine reserves investigating aspects of shark and batoid movement, ecology, biodiversity and the potential benefits of reserve establishment. The asterisk (*) indicates an incomplete year as the literature search concluded on 25th March 2015.

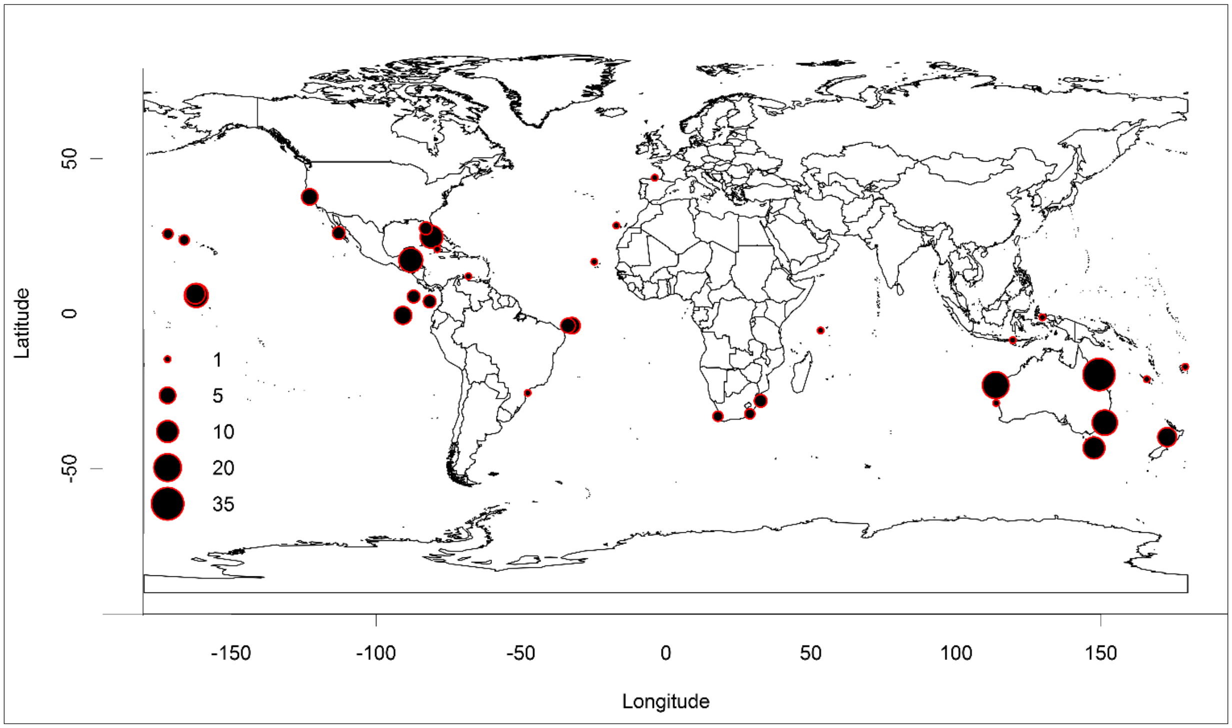


Figure 3. Regional locations of shark and batoid movement, ecology, biodiversity, and reserve effects studies (n = 176).

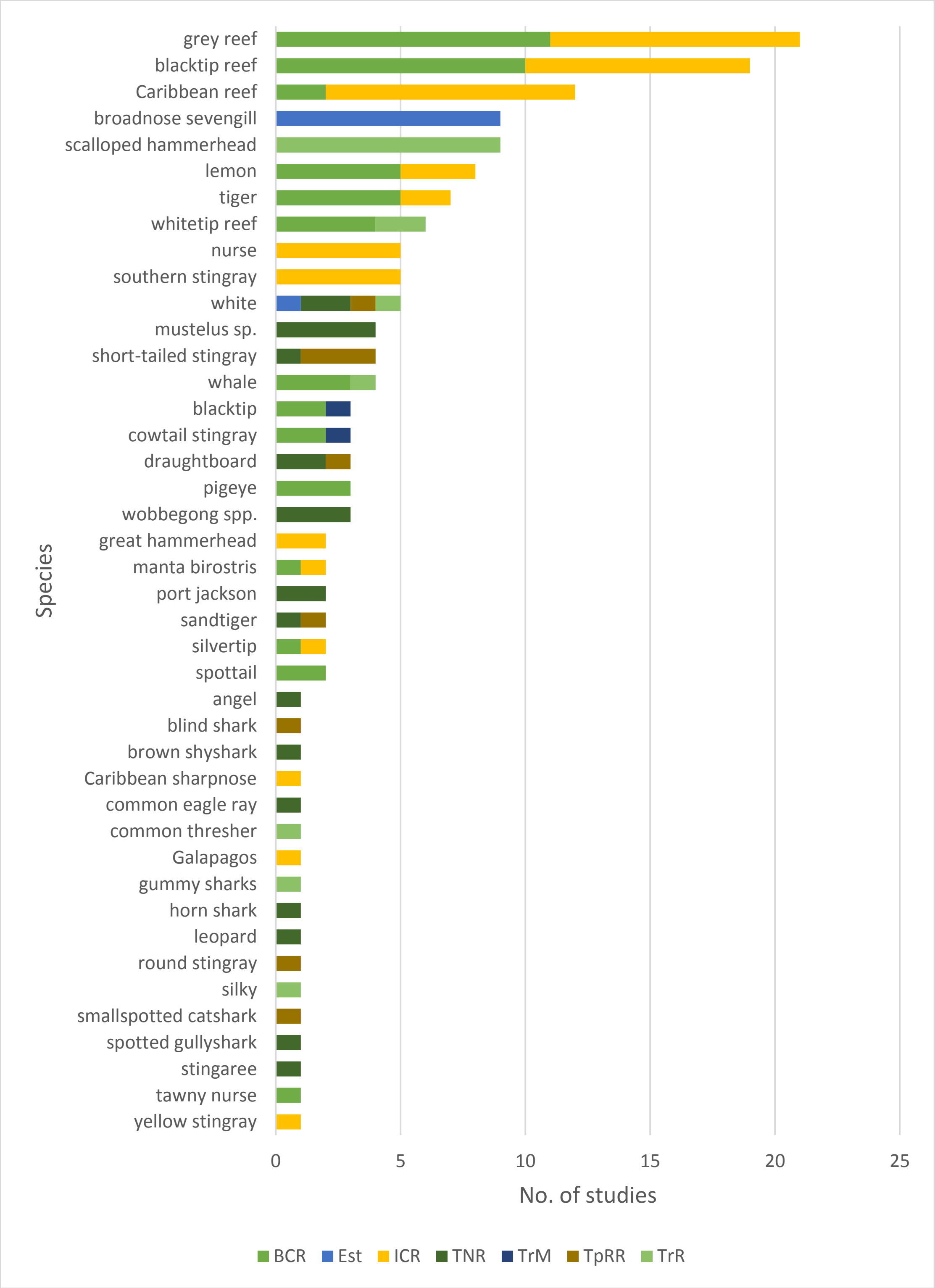


Figure 4. Number of studies conducted within marine reserves across different habitats barrier coral reef (BCR), insular coral reef (ICR), temperate nearshore reef (TNR), temperate rocky reef (TpRr), tropical rocky reef (TrR), tropical mangrove (TrM), and estuary (Est) categorized by study species.

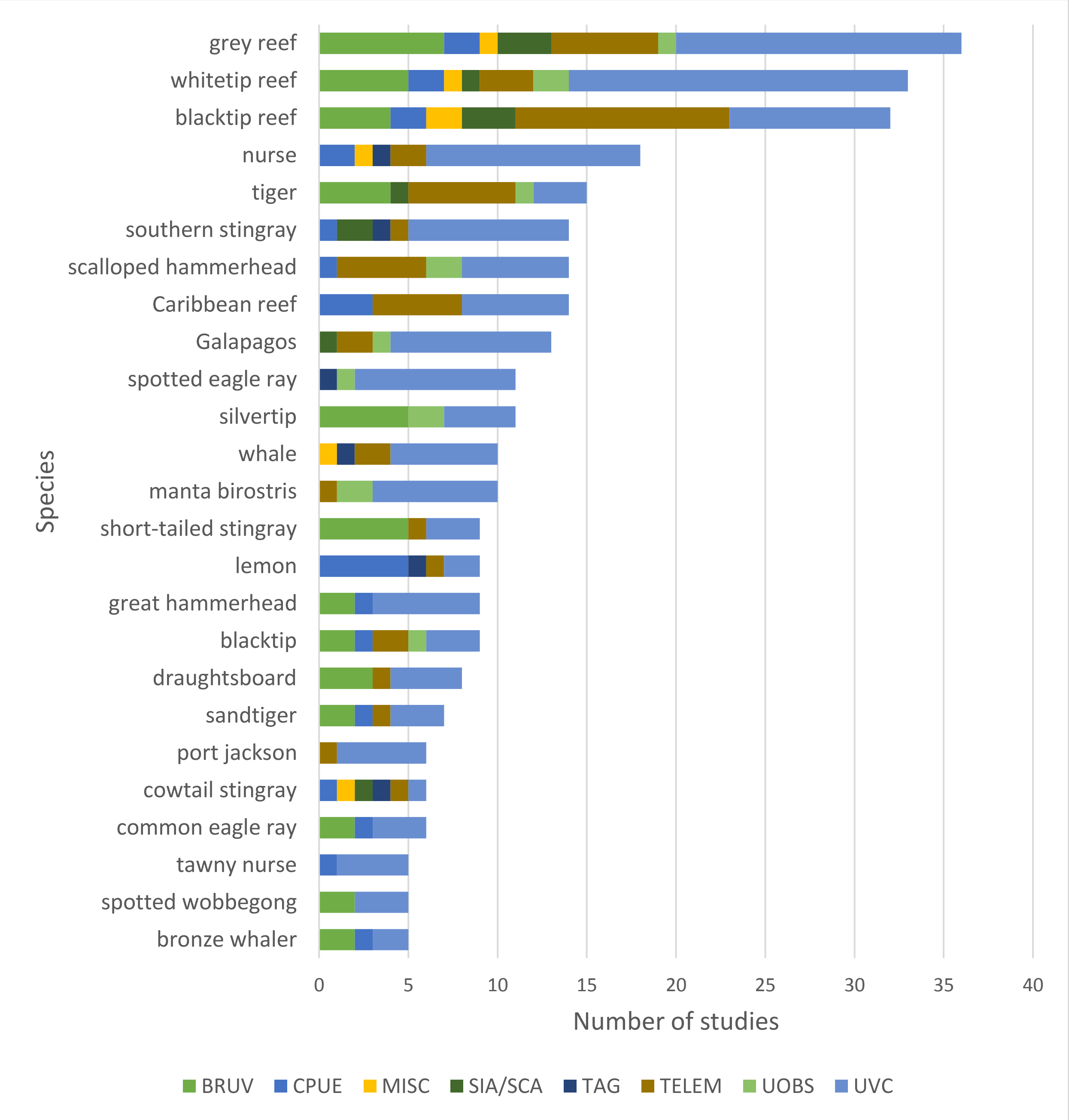


Figure 5. Number of studies conducted on sharks and batoids within marine reserves with different survey methods baited remote underwater video (BRUV), catch per unit effort (CPUE), telemetry (TELEM), external tagging (TAG), stomach contents/ stable isotope analysis (SCA/SIA), underwater observations (UOBS), and underwater visual census (UVC), and miscellaneous (MISC) categorized by species.

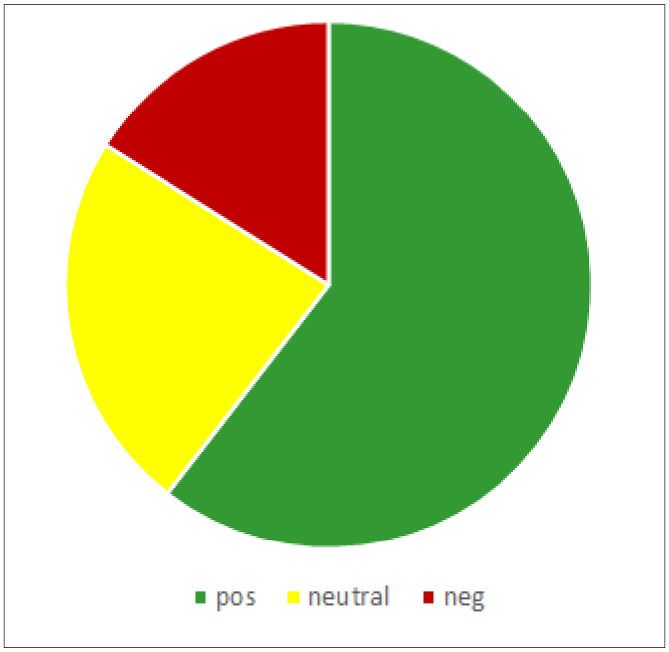


Figure 6. Proportion of species that have exhibited positive (pos), neutral, or negative (neg) changes in relative abundance, density, biomass, and diversity since reserve establishment. Total n=205.

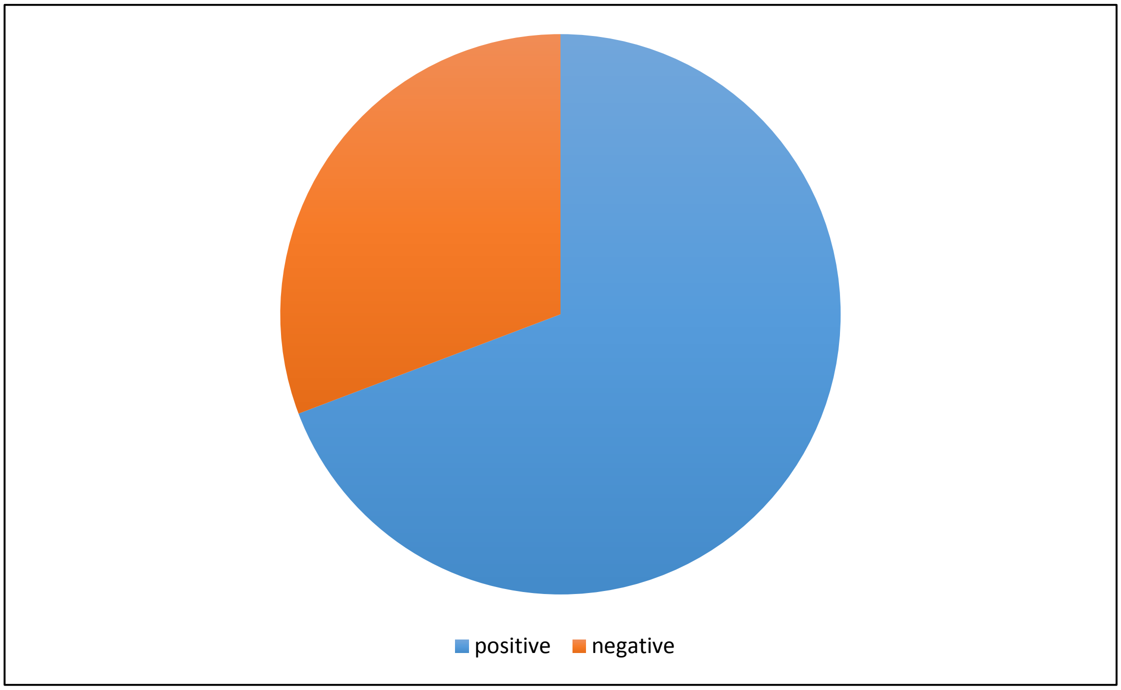


Figure 7. Proportion of positive or negative biological responses to reserve establishment which meet three or more of the criteria for a successful marine reserve defined by Edgar et al. 2014, which are: > 100km^2 in size, enforced, isolated, has a no-take component and > 10 years old. Total n=125.

Chapter Three: Trophic ecology of Caribbean reef sharks (*Carcharhinus perezi*) and large carnivorous teleosts (F. Serranidae; F. Sphyraenidae) assessed using stable isotope analysis.

Abstract

Caribbean reef sharks (*Carcharhinus perezi*) have been assumed to be apex predators in Western Atlantic coral reef ecosystems, potentially exerting top-down control over large-bodied piscivores such as barracuda (F. Sphyraenidae) and grouper (F. Serranidae). Ecosystem models that make this assumption predict that a reduction of Caribbean reef sharks could induce an increase in piscivore populations that in turn reduce herbivore populations (e.g. F. Scaridae) and diminish grazing capacity. This could contribute to a shift from a coral dominated to an algae dominated benthos. Bulk stable isotope analysis of nitrogen and carbon isotopes from white-muscle tissue was conducted on Caribbean reef sharks, and three piscivores that could be their prey: Nassau grouper (*Epinephelus striatus*), black grouper (*Mycteroperca bonaci*) and great barracuda. Southern stingrays (*Dasyatis americana*) were also analyzed as a known mesopredator. All upper trophic level species exhibited a broad range of δ^{13}C, which indicates that they feed across multiple habitats. There was no significant difference between the mean δ^{15}N among upper trophic level species, which suggests that Caribbean reef sharks diet does not primarily consist of these piscivores. There was no significant correlation between δ^{15}N and total length (cm) for Caribbean reef sharks, which indicates there is no ontogenetic increase in trophic level. Caribbean reef shark δ^{13}C was significantly correlated with total length, suggesting an increased contribution of shallow lagoon flats prey with increasing size. These results indicate that Caribbean reef sharks do not fulfill the role of an apex predator in this system, potentially due to omnivory or functional overlap with large-bodied teleost piscivore. This suggests that the widely assumed shark-piscivore-herbivore pathway for a trophic cascade initiated by Caribbean reef shark depletion is not likely to occur.

Introduction

Apex predators can exert top down control in a wide variety of terrestrial and aquatic ecosystems, which is problematic from an ecosystem management perspective because this functional group is usually the first to be depleted by humans and among the slowest to recover

(Pauly et al. 1998; Terborgh et al. 1999; Sergio et al. 2008; McCauley et al. 2010; Estes et al. 2011a; Frisch et al. 2014). Apex predators are typically defined as species that occupy the top trophic level (> TrL 4) and have few, if any, predators themselves during their adult life stage (Stevenson et al. 2007; Creel and Christianson 2008; Wirsing and Ripple 2010; Rizzari et al. 2014). Large sharks are commonly thought of as apex predators in marine ecosystems based on their body size and ability to consume prey much larger than their gape (Cortés 1999; Frisch et al. 2014) (Stevens 2000; Okey et al. 2004; Heithaus et al. 2008b; Estes et al. 2011b). These traits suggest that large sharks may generally occupy a higher trophic level than large carnivorous teleost fish, which are usually constrained to feed prey that are smaller than their gape. Nevertheless many large sharks primarily prey on animals much smaller than their gape or on lower trophic level species, which could result in these species occupying a similar TrL to large carnivorous teleosts. It is important to resolve between these alternatives because local exploitation rates of sharks and large teleosts can be very different due to variation in local seafood consumption and conservation priorities. Disproportionate removal of large sharks is anticipated to have a stronger effect on the ecosystem if they fulfill a unique apex predator role rather than being part of a large predator guild with functional redundancy.

A Caribbean reef ecosystem model run by Bascompte et al. (2005) suggested that there is a strong tri-trophic interaction between sharks (as apex predators), large carnivorous teleosts, such as grouper (as mesopredators in this context), and parrotfish (as primary consumers). The removal of sharks from this model system resulted in increased large piscivores and a reduction in primary consumers. If this interaction reflects reality it would suggest the possibility that the disproportionate reduction in sharks could affect the benthic composition in favor of algae over coral due to a reduction in grazing capacity (Bascompte et al. 2005). Assessing how well this model output captures reality is challenging because there is a dearth of dietary information on large sharks in the Caribbean, particularly the Caribbean reef shark (*Carcharhinus perezi*), the numerically dominant, large, coral reef associated shark in the region (Compagno 1999; Rosa 2006; Ward-Paige et al. 2010; Castro 2011). The diet of this species in the ecosystem model was based on Opitz (1996; 5 stomachs examined all in one location) and since then there have been very few and geographically limited diet studies of this species (Motta et al. 1999; Tavares 2009; Maljković and Côté 2011). These diet studies indicate the species feeds on a wide range of fish species, from tertiary piscivores (F. Sphyraenidae), secondary piscivores (F. Serranidae and F.

Carangidae) to herbivores (F. Scaridae). None of these studies provided data on the size class of prey, so it is possible that large carnivorous teleost species found in reef shark stomachs are primarily consumed while in their juvenile life stages. These studies also did not examine ontogenetic changes in reef shark diet. Resolving the TrL of Caribbean reef sharks relative to the other large carnivores in the system, including the great barracuda (*Sphyraena barracuda*), Nassau grouper (*Epinephelus striatus*) and black grouper (*Mycteroperca bonaci*) is needed to determine if they are a singular apex predator or one member of a large predator guild. It also important to determine at what size Caribbean reef sharks reach their peak TrL, because this parameter has been positively correlated with size and age in some large sharks (Wetherbee et al. 2004b; Estrada et al. 2006).

A second determinant of the ecological role of Caribbean reef sharks is where they feed in terms of habitat and food web. Reef sharks occur in a wide variety of reef habitats from lagoons to the deep reef slope (Pikitch et al. 2005; Chapman et al. 2007; Bond et al. 2012) but it is unclear in which habitats they obtain most of their prey. This makes it challenging to assess which food webs and habitats they might have the greatest effect on. Given that the species is found across multiple reef habitats but individuals are home ranging within a reef system (Chapman et al. 2005; Chapman et al. 2007; Bond et al. 2012) it is also possible that individual diet is influenced by where their home range is in relation to different reef habitats. Individual blacktip reef sharks (*Carcharhinus melanopterus*) primarily fed in one or the other of two adjacent lagoons at Palmyra atoll, highlighting that the location and habitat characteristics of an individual's home range can determine its diet (Papastamatiou et al. 2010). If this is a common pattern in Caribbean reef sharks it suggests that individuals may vary in how they affect the broader ecosystem.

Bulk stable isotope analysis (SIA) can provide a non-lethal alternative to stomach contents studies to help elucidate food web structure (Vander Zanden et al. 1997; Post 2002; Estrada et al. 2003). SIA is based on the principle that ^{15}N and ^{13}C isotopes are transferred from producer to consumer with relatively predictable changes so the isotopic signature of the predator reflects that of its prey (Peterson and Fry 1987; Fry 2006). Carbon (δ^{13}C), the ratio of abundance of ^{13}C to ^{12}C isotopes, experiences minimal changes with each trophic step (Fry et al. 1983). Whereas Nitrogen (δ^{15}N), the ratio of the abundance of ^{15}N to ^{14}N isotopes, becomes enriched ~3.4% per trophic level, though the broad application of this factor across multiple trophic levels and taxa is widely debated (Hussey et al. 2010a; Olin et al. 2013; Hussey et al. 2014). Nitrogen ($\delta^{15}N$) provides an estimate of TrL, and carbon ($\delta^{13}C$) can help trace the primary producer(s) at the base of the food web where the individual obtains its food (Peterson and Fry 1987; Hobson 1999; Rubenstein and Hobson 2004).

Our null hypothesis is that Caribbean reef sharks are one full TrL above the large carnivorous teleosts in the system. We used differences in carbon and nitrogen stable isotopes measured from white muscle tissue to examine the TrL of Caribbean reef sharks relative to large predatory teleosts (great barracuda, black grouper, Nassau grouper) and using an elasmobranch mesopredator (the southern stingray *Daysatis americana*) as a baseline. These data were used to test the hypotheses that (1) large (subadult and adult) Caribbean reef sharks primarily feed on large carnivores in the ecosystem (e.g., black grouper, Nassau grouper, great barracuda), (2) the population feeds across multiple reef habitats, (3) there are ontogenetic shifts in trophic level and foraging habitat and (4) individuals primarily living in lagoon habitats are isotopically differentiated from individuals living on the fore-reef.

Methods

Study Site

Glover's Reef Atoll (16°44'N, 87°48'W) lies approximately 25 km to the east of the Mesoamerican Barrier Reef (MBR) and 45 km east of mainland Belize (Figure 1.1). The atoll is approximately 30 km long and at the maximum 10 km wide (32, 834 ha). The atoll's western reef crest lies submerged with the eastern reef crest being exposed and broken to produce five cuts, which provide connectivity between the ocean reef and lagoon ecosystems independent of tide. The lagoon ecosystem is primarily shallow, sand substrate interspersed with ~ 800 coral patch reefs and sporadic seagrass meadows. The atoll includes six sparsely populated mangrove fringed cayes (Figure. 1.2). The Glover's Reef Marine Reserve (GRMR) was established in 1997 and encompasses the entire atoll. GRMR is comprised of a "no-take zone" on the interior which covers approximately 20% of the atoll, surrounded by a "general use zone" which prohibits gill-nets, fish traps and longlines throughout the entire atoll out to the 180 m depth contour (Gibson 2003). Members of a permanent Government of Belize Department of Fisheries (GBDF) station located on Middle Caye enforce reserve regulations. The relative isolation, time elapsed since protection, large size and permanent enforcement of GRMR has enabled relatively high densities of large commonly exploited predators to persist at this location (Bond et al. 2012; Dahlgren 2014).

Study species

Five species of large carnivorous fish common to Glovers Reef Atoll and considered resident to the atoll were selected for sampling. These species included the Caribbean reef shark, a tertiary piscivore the great barracuda (*Sphyraena barracuda*), two secondary piscivores the Nassau grouper (*Epinephelus striatus*) and black grouper (*Mycteroperca bonaci*), and a known mesopredator the southern stingray (*Dasyatis americana*).

Marker species

Seven additional 'marker species' were selected and sampled at GRMR to define (i) the expected range of δ^{13}C values for distinct habitats/food webs (e.g. lagoon seagrass, lagoon reef and pelagic) since δ^{13}C values of a consumer are representative of the carbon sources/pools of the food web associated with that habitat and (ii) the δ^{15}N values of consumers with defined ecological roles or TrP within the defined habitats, e.g. zooplanktivores that feed at TrP 3. Selected individuals were assumed to be residents of the habitat in which they were collected based on known restricted movement patterns and habitat preferences and have well characterized diets and hence defined ecological roles. The sampled marker species were; for the open ocean facing fore-reef (pelagic derived δ^{13}C); silk snapper (*Lutjanus vivanus*), ocean trigger fish (*Balistes vetula*) and ocean surgeonfish (*Acanthurus bahianus*), for lagoon patch reef δ^{13}C values, lionfish (*Pterois volitans*) and for lagoon seagrass meadow δ^{13}C values, the queen conch (*Strombus gigas*), and white grunt (*Haemulon plumierii*) (Randall 1964,1967; Parker and Mays 1998; Morris et al. 2009).

Field sampling

All sampling was conducted during the summer period from May-August, 2011-2013. Teleosts and queen conch were sampled from the catches of Belizean fisherman, granted traditional fishing rights for GRMR. Fish were caught using handlines, monofilament terminating in a weight and barbed hook, and queen conch were captured by hand. All teleosts were measured for total length (snout to tip of the upper lobe of the caudal fin; TL). Elasmobranchs and some teleosts were collected, sampled, tagged and released alive from scientific longlines deployed as part of an ongoing fisheries independent survey examining elasmobranch abundance [see (Chapman et al. 2005; Pikitch et al. 2005) for description of capture and handling methods]. Moribund individuals (< 6% of captures) were kept for dissection and sampling. For elasmobranchs, TL was recorded. All research, animal handling and associated tissue sampling was conducted under a series of annual Marine Science Research Permits issued from 2011 (#0005-11, #0008-12, and #00014-13 respectively) by the Belize Ministry of Agriculture & Fisheries (GBDF), and in accordance with SUNY Stony Brook's animal care protocol developed under the Institutional Animal Care and Use Committees (IACUC) guidelines.

Stable isotope preparation and analysis

For teleosts and sharks, ~0.5cm³ of white muscle was excised from the hypaxial dorsal musculature below the first dorsal fin using a stainless steel forceps and #11 disposable scalpel. Southern stingrays were sampled for white muscle from a similar position in the dorsal musculature, using the same technique for orientation but in the absence of a dorsal fin. White muscle was selected for stable isotope analysis (SIA) based on its slow turnover rate (~1 year, Sweeting et al. 2007) and therefore its long-term integrated isotopic value of diet and habitat. Tissue samples were placed in labeled 2ml microcentrifuge vials/2ml cryogenic vials on the boat and once on shore were sundried in the absence of a suitable drying oven. Once fully dehydrated, samples were transferred to clean, labeled vials and kept in a freezer until the end of the field season.

In the lab, dried muscle plugs were homogenized using either a pestle and mortar or a Wig-L-Bug (Sigma Aldrich). All samples were lipid extracted following standard procedures (Bligh and Dyer 1959) to correct for variations in lipid content among organisms (DeNiro and Epstein 1977) and the potential effects of urea in elasmobranchs (Hussey et al. 2012). In brief, homogenized samples were lipid extracted by agitation in a 2:1 chloroform-methanol solution and placed in a water bath (30°C) for 24 h. Samples were then removed from the water bath and agitated for 30s, centrifuged for 5 min (15000rpm) and then decanted. A second aliquot of 2:1

chloroform-methanol was added to the samples, followed by another round of agitation and centrifuging. The solvent was decanted and the sample left to dry at room temperature for 48 h to evaporate any remaining solvent.

Between 400-600µg of dried, lipid extracted tissue from each individual was weighed into tin cups and stable isotopes of nitrogen ($\delta^{15}N$ ratio) and carbon ($\delta^{13}C$ ratio) analyzed using a continuous flow isotope ratio mass spectrometer (Delta V Advantage, by Thermo Finnigan (San Jose, California, USA). The delta (δ) values were measured as the deviation from a standard in parts per thousand (‰) using the equation:

$$\delta X = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000$$

where X is ^{15}N or ^{13}C, and R is the ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$, respectively. The standard reference materials were Pee Dee Belemnite carbonate for CO_2 and atmosphere nitrogen for N_2. The analytical precision for $\delta^{15}N$ data was 0.17 and 0.18‰ and for $\delta^{13}C$ data was 0.1 and 0.09‰ for NIST standard 1577c (bovine muscle) and an internal lab fish muscle standard (Tilapia), respectively, across multiple runs and >100 standards analyzed. Analytical accuracy measured throughout this time period, based on the analysis of certified NIST standards (n=77) for $\delta^{15}N$ was 0.09, -0.17, and 0.04‰ (NIST 8573, 8548, and 8549 respectively) and for $\delta^{13}C$ was -0.07 and -0.05‰ (NIST 8542 and 8573 respectively).

Statistical Analysis: Intra/Inter-species variation

A bi-plot of the mean $\delta^{13}C$ and $\delta^{15}N$ values (± SD) for all species was constructed. Analysis of variance (ANOVA) was performed to test for differences in the mean $\delta^{13}C$ and $\delta^{15}N$ values between species and a post-hoc Tukey's multiple comparison test applied where appropriate using the R software (R Core Team 2010). To examine trophic structure and intra-population variation in relative trophic role, the six population metrics developed by Layman et al. (2007) were calculated and compared. Since sample sizes varied by species, the metrics were calculated from the species mean $\delta^{13}C$ and $\delta^{15}N$ values sampled with replacement (n=30) and bootstrapped (n=10000). The two individuals with the highest and lowest $\delta^{15}N$ and $\delta^{13}C$ values for each species produced the $\delta^{15}N$ (NR_b) and $\delta^{13}C$ (CR_b) ranges. Ranges provided a measure of the trophic length and diversity of baseline resources exploited by each species. Mean distance to centroid (CD_b) describes trophic diversity within a population and is the mean Euclidean distance of each individual to the $\delta^{15}N$ - $\delta^{13}C$ centroid for that population. Standard deviation of nearest neighbour distance ($SDNND_b$) provides a measure of trophic evenness, i.e. evenness of species density within isotopic niche space; low values suggest an even distribution. $SDNND_b$ is the SD of Euclidean distances of each individual to its nearest neighbor. The metric total area (TA) of the convex hull was calculated and reported but omitted from further analysis because of inherent bias associated with differing sample sizes (Layman et al. 2007). Instead standard ellipse areas were calculated using Stable Isotope Bayesian Ellipses in R (Parnell and Jackson 2011) see Jackson et al. 2011 for detailed methodology, whereby the standard ellipse area (SEA), provides a measure of the mean core population isotopic niche space, while accounting for variation in sample size (Jackson et al. 2011). Standard ellipses are calculated from the variance and covariance of the original x and y data and contain approximately 40% of the data describing a core niche area (Ricklefs and Nealen 1998). Given that the data is bivariate which leads to a loss in a degree of freedom, a correction of (*n*-2) on the denominator rather than the standard (*n*-1) was used when calculating variances. A sample size corrected version of the standard ellipse area, SEAc, was used to correct for the bias that arises when sample sizes are small (Jackson et al. 2011). The relationship between SEA and SEAc can be described as $\text{SEAc} = -\text{SEAc}\ (n-1)(n-2)^{-1}$. Following this correction SEAc will increase at small sample sizes in order to correct a bias towards underestimation, however it retains the same geometrical shape and asymptotes to 1 as $n \to \infty$. SEAc values were used to estimate the relative trophic similarity, a proxy for dietary similarity, among species by examining the degree of isotopic niche overlap. The OVERLAP command was used to calculate the area of ellipse overlap among species. The percentage of a given SEAc encompassed within another species ellipse was presented. Least squares linear regression analysis was used to assess the effect of predator body size on $\delta^{15}N$ and $\delta^{13}C$ values, to test for ontogenetic shifts in diet or foraging habitat. Based on size at birth and estimated growth rates for young-of-the-year, we estimated that any shark (TL< 90cm) was less than 1 year old (Garla et al. 2006a; Tavares 2009). Individuals <1 year (n=15) were excluded from further analysis to negate potential residual maternal isotopic values given that reef sharks are viviparous and developing embryos are nourished in part from a placental connection in the mid to late stages of gestation (Olin et al. 2011). All metrics were calculated using the R statistical computing package (R Development Core Team 2010).

Calculating relative Trophic Position

Trophic position (TrP) was calculated using a scaled Δ^{15}N framework based on a dietary δ^{15}N value-dependent Δ^{15}N model according to Hussey et al. (2014). The scaled Δ^{15}N framework approach considers that isotope discrimination is not constant but narrows with increasing trophic position (Hussey et al. 2014). The equation to calculate TrP is as follows:

$$TP = \frac{\log(\delta^{15}N_{lim} - \delta^{15}N_{base}) - \log(\delta 15N_{lim} - \delta\ 15N_{TrP})}{k} + TrP_{base}$$

Where $\delta^{15}N_{base}$ is the isotope value for a known baseline consumer in the food web, TrP_{base} is the trophic position of the consumer used to define $\delta^{15}N_{base}$ (e.g. zooplankton, TrP = 2), $\delta^{15}N_{TrP}$ is the δ^{15}N value of the consumer in question, $\delta^{15}N_{lim}$ is the saturating isotope limit as TrP increases and *k* the rate at which $\delta^{15}N_{TrP}$ approaches $\delta^{15}N_{lim}$ per TrL step. Values for $\delta^{15}N_{lim}$ and *k* are estimated from the meta-analysis, for more details see Hussey et al. (2014).

For comparative purposes, TrP was also calculated using an additive Δ^{15}N framework approach (Post 2002) using the following equation:

$$TP_{additive} = TrP_{baseline} + \frac{\delta^{15}N_{fish} - \delta^{15}N_{baseline}}{\Delta^{15}N}$$

Where $\delta^{15}N_{baseline}$ and $TrP_{baseline}$ are the δ^{15}N value and known TrL for a low trophic level or baseline organism in the food web (e.g. zooplankton, TrL = 2), $\delta^{15}N_{fish}$ is the consumer in question, and Δ^{15}N is the tissue specific discrimination factor. For this study, the widely applied Δ^{15}N value of 3.4‰ was used for all species (Post 2002). Both a scaled and additive TrP were calculated for each consumer using multiple TrL 2 baseline primary consumers that define the isotopic baselines of the distinct habitats at GRMR (see methods above) and a mean TrP based from all baseline consumers was calculated. The baseline primary consumers included queen conch (*Strombus gigas*), West Indian top shell (*Cittarium pica*), ocean surgeon (*Acanthurus bahianus*), stoplight parrotfish (*Sparisoma viride*), striped parrotfish (*Scarus iseri*), and redband parrotfish (*Sparisoma aurofrenatum*). This approach was adopted to reduce error associated with variance in individual baseline species isotope values given the focal shark and teleost species are known to occur across all habitats.

Results

Field sampling for stable isotope tissue

A total of 183 muscle tissue samples were collected across our five study species (Table 1.). Belize Department of Fisheries restrictions placed on retaining Nassau grouper meant sample size was very low (n=6) with samples attained from incidental bycatch on research longlines, all being released in good condition. Sex was determined for reef sharks (n=86) and southern stingrays (n=14). Male reef sharks (n=47) had a size range of (74-188 cm, TL) with a mean TL of 124 cm. Female reef sharks (n=39) had a size range of (83-236 cm, TL) with a mean TL of 125 cm. Male stingrays (n=6) had a size range of (35-60 cm, DW), with a mean DW of 44 cm. Female stingrays (n=8) had a size range of (60-90 cm, DW) with a mean DW 74 cm.

Relative trophic levels and isotopic niches of study species

No significant correlation was detected between $\delta^{13}C$ and $\delta^{15}N$ in reef sharks when plotted against each other for either all samples combined ($R^2 = 0.0051$, $F_{1,84} = 0.435$, $p > 0.5$), or, when divided into male ($R^2 = 0.0003$, $F_{1,45} = 0.015$, $p > 0.5$) and females ($R^2 = 0.025$, $F_{1,37} = 0.94$, $p > 0.1$). Across all species sampled CR_b values were larger than NR_b (Table. 2). In particular, the two species thought of as being highly mobile, reef sharks and barracuda, had the broadest CR_b (5.93‰ and 7.36‰ respectively). Despite having the second broadest CR_b value, the reef shark NR_b value of 2.08‰ was only broader than that of Nassau grouper. The CR_b and NR_b for reef sharks were similar to those for black grouper, but both species were substantially smaller than barracuda for both metrics. The low CR_b and NR_b values for Nassau grouper (2.37‰ and 1.14‰, respectively), could have been an artifact of low sample size. Stingrays had broad CR_b and NR_b values (3.96‰ and 2.3‰ respectively), however they had the lowest TrL of all species.

All $\delta^{13}C$ and $\delta^{15}N$ for all species were plotted and SEAc values were generated for each species' ellipse (Fig. 2). The SEAc ellipse generated for southern stingrays had no overlap with any of the other species due to consistently lower $\delta^{15}N$ and was therefore omitted from further ellipse analysis (Fig. 3). The remaining species ranked in descending order based on their SEAc values were: Sba>Zii>Mbo>Est. The ellipse generated for barracuda (5.25 u^2) was almost double that of reef sharks and black grouper both of which were similar, 2.62 u^2 and 2.51 u^2 respectively (Table. 2). Reef sharks overlapped considerably with the remaining upper trophic level piscivores, with their ellipse situated almost right in the center of the other species' (Fig. 3, Table. 2). The lower part (~35%) of the reef shark ellipse overlapped with the tightly packed Nassau grouper. The upper portion of the reef shark ellipse overlapped with black grouper and barracuda (~40% and ~60%, respectively). Reef shark percentage overlap increased with increasing trophic position. Based on the data, reef sharks shared more isotopic niche space with the black grouper and barracuda than Nassau grouper. The two grouper species shared virtually no overlap, the black grouper ellipse positioned above Nassau grouper. Only black grouper and barracuda had portions of their ellipses above the reef shark ellipse (~60% and ~40% respectively), however the black grouper ellipse was almost entirely within that of the barracuda's.

The mean $\delta^{13}C$ and $\delta^{15}N$ and the associated standard deviations for each of the five species were plotted to show the position of each species relative to one another in isotope niche space (Fig. 4). The high proportions of ellipse overlap between reef sharks, black grouper and barracuda was supported by the close proximity of their mean $\delta^{15}N$ values (9.24, 9.7 and 9.84‰ respectively) and associated standard deviations. Only these three species' mean $\delta^{15}N$ values exceeded nine and all three species had broad $\delta^{15}N$ ranges. The mean $\delta^{13}C$ values for reef sharks, black grouper, and barracuda were comparable (-10.48, -11.24 and -10.87‰ respectively) but the black grouper was most depleted. There was considerable intra-species variation in $\delta^{13}C$ values for each species (Table. 1). The plot of mean $\delta^{15}N$ values indicated the mean trophic position of Caribbean reef sharks (9.24 ±0.51‰) was below that of black grouper (9.70 ±0.52‰) and great barracuda (9.84 ±0.75‰) but above that of the Nassau grouper (8.88 ±0.40‰). The plot of mean $\delta^{13}C$ indicated that reef sharks (-10.48 ±1.61‰) have a less pelagic signal than black grouper (-11.24 ±1.5‰) and barracuda (-10.87 ±2.34‰) but a more pelagic signal then Nassau grouper (-10.15 ±0.95‰). Southern stingrays occupied the lowest mean $\delta^{15}N$ (7.14 ±0.65‰) and had the most coastal $\delta^{13}C$ signal (-9.29 ±1.14‰). Between our top guild species: barracuda, reef sharks, Nassau and black grouper, we found significant differences in their mean $\delta^{15}N$ but not mean $\delta^{13}C$ (analysis of variance, F=14.23, p-value <0.001 and F=1.5, p-value >0.1, respectively). A Post-hoc Tukey's multiple comparisons tests showed reef shark mean $\delta^{15}N$ was significantly lower than those of black grouper (p-value <0.01) and barracuda (p-value <0.001) but there was no significant difference between reef sharks and Nassau grouper.

Estimating absolute trophic level

The scaled equation calculated reef sharks to be TrL 3.11 (Table. 3, Fig. 5). This was lower than that of great barracuda (TrL 3.26) and black grouper (TrL 3.22) but above Nassau grouper (TrL 3.01) and southern stingrays (TrL 2.62). The Caut et al. 2009 calculated enrichment factors estimated TrL higher than the scaled model for all species but the positioning of species relative to each other in isotopic niche space did not change. The additive model was applied with the broadly accepted isotopic fractionation of 3.4‰ for teleosts and elasmobranch specific isotopic fractionation values of 2.8‰ for southern stingrays (Tilley et al. 2013), and 2.43‰ for sharks (Hussey et al. 2010b). The calculated trophic position of all species changed as did the order of species in terms of their TrP. Caribbean reef sharks experienced the biggest change increasing in trophic position to TrP 4.17, elevating them above barracuda (TrP 3.73) and black grouper (TrP 3.69) for the first time. Nassau grouper (TrP 3.45) was the lowest of the teleosts but still remained above southern stingrays (TrP 3.13). Southern stingrays were once again the lowest position but for the first time exceeded TrL 3. Overall the application of taxon-specific additive enrichment factors (3.4 for teleosts and 2.43 and 2.8‰ for elasmobranchs) was the only one that put Caribbean reef sharks higher than teleost mesopredators and then by ~ 0.5 of a TrL (Fig. 5).

Intraspecies variation

Great barracuda exhibited the highest overall trophic diversity (2.03), measured as the mean distance to centroid (CD_b). Reef sharks (1.45) followed closely by black grouper (1.32) had the next highest CD_b. Reef sharks and black grouper had comparable variation within their diets when compared together but both species had much less variation than barracuda.

Metrics for intraspecies density and distribution evenness within niche space (NND and SDNND, respectively), found reef sharks were distributed at a similar density to barracuda, roughly double that of black grouper. Reef sharks and black grouper also had comparable SDNND (0.28 and 0.29 respectively), but once again substantially lower than barracuda (0.37). The evenness of the packing of reef sharks within isotopic niche space were relatively high, suggestive of uneven packing, comparable to black grouper. Typically species whose isotopic niche width lies almost completely within that of another species have lower SDNND values, which indicates trophic redundancy. All species' SDNND values were relatively high with the exception of Nassau grouper and southern stingrays with values of 0.01 and 0.17 respectively

(Table. 2). Nassau grouper and stingrays had the two smallest sample sizes, which potentially influenced this metric. However, the samples collected were tightly packed and these were the two species most isolated in niche space, which indicated a degree of evenness in species distribution, therefore trophic redundancy. The high SDNND values for barracuda, black grouper and reef sharks disputed any suggestion of trophic redundancy despite substantial overlap in isotopic niche space.

Linear regressions (TL)

Caribbean reef sharks, black grouper and barracuda were the three species with the largest mean total length, most enriched mean δ^{15}N values, and most depleted mean δ^{13}C values. Total length (cm) was used as a proxy for age and linear regression analyses were performed on the reef shark data to examine for any ontogenetic shifts in diet (δ^{15}N), or foraging habitat (δ^{13}C). Based on size at birth and estimated growth rates for young-of-the-year we estimated that any shark (TL< 90cm) was less than 1 year old. Individuals <1 year (n=15) were excluded from further analysis to negate potential residual maternal isotopic signatures given that reef sharks are viviparous and developing embryos are nourished in part from a placental connection in the mid to late stages of gestation (Olin et al. 2011). There was no significant correlation between reef shark TL and δ^{15}N for the total sample ($R^2 = 0.053$, $F_{1,69} = 3.89$, p-value = 0.053) or for males ($R^2 = 0.093$, $F_{1,37} = 3.79$, p-value = 0.059) or females ($R^2 = 0.017$, $F_{1,30} = 0.54$, p-value = 0.47) analyzed separately (Fig. 6a), There was a significant correlation between reef shark TL and δ^{13}C for the total sample ($R^2 = 0.31$, $F_{1,69} = 30.7$, $p < 0.0001$) and for males ($R^2 = 0.43$, $F_{1,37} = 27.38$, $p < 0.0001$) and females ($R^2 = 0.21$, $F_{1,30} = 7.41$, p-value = 0.01) analyzed separately, as shown in Fig. 6b. Linear regression analyses for total length against δ^{15}N and δ^{13}C values were also performed for stingrays and barracuda as an elasmobranch and non-gape limited comparison for reef sharks respectively. There was no significant correlation when barracuda total length was compared against δ^{13}C ($R^2 = 0.0004$, $F_{1,48} = 0.02$, p-value = 0.89), or, δ^{15}N ($R^2 = 0.02$, $F_{1,48} = 1.18$ p-value = 0.28) values. There was no significant correlation when stingray total length was regressed against their δ^{13}C values ($R^2 = 0.16$, $F_{1,12} = 2.31$, p-value = 0.15), however a significant correlation between total length and δ^{15}N values ($R^2 = 0.3$, $F_{1,12} = 5.15$, p-value = 0.04) was evident.

Individual variation in foraging habitat

Caribbean reef sharks were divided into habitat groups (lagoon or fore-reef) based on capture location and the SEAc ellipses of these two groups were plotted and examined for differences (Fig. 3 and ESM 1). ‘Lagoon’ sharks (n=42) were significantly more enriched in $\delta^{15}N$ (Welch’s t-test, p=0.0018) and significantly less depleted in $\delta^{13}C$ (Welch’s t-test, p=0.01) than the ‘fore-reef’ sharks (n=44). The SEAc for reef sharks re-plotted by habitat did differ, fore-reef (2.65 u^2) and lagoon (2.1 u^2), with the combined reef shark SEAc value (2.62 u^2) being closer to that of the fore-reef sampled individuals.

Discussion

Stable isotope analysis enables us to reject the hypothesis that reef sharks, even at large sizes, are a full trophic level higher than the large teleost carnivores at GRMR (black grouper, Nassau grouper and great barracuda). The estimated TrL of these species depends on the diet tissue discrimination factor and integration model used. Two of three models put the TrL of reef sharks at approximately the same level as the groupers and great barracuda, albeit with wide individual variation. An additive model assuming a shark-specific DTDF for the reef shark and generic fish DTDF for the grouper and great barracuda places the reef shark at TrL 4.17, which is ~ 0.5 of a TrL above the carnivorous teleosts. Unfortunately there are no species or even family specific DTDF available for grouper and barracuda, so it remains unclear if using the meta-analytically mean for these species is appropriate. There is evidence that higher TrL species have lower DTDF than species feeding at lower trophic levels, so it is possible that the difference between the reef sharks and large teleost DTDF is in reality smaller than we assumed. Overall, our analyses indicate that reef sharks have a similar or ~ 0.5 level higher TrL than co-occurring teleosts carnivores. This suggests that these species should probably be considered a top predator guild in Caribbean coral reef ecosystems, with the possibility that there is some intraguild predation on large carnivorous teleosts by reef sharks. Resolution of the amount of intraguild predation is a future research priority given that this parameter will determine the interaction strength between large sharks and large carnivorous teleosts.

One interesting observation was that the scaled model of Hussey et al. (2014) produced consistently lower estimates of TrL than the additive models. The scaled model-derived TrL for the sharks, grouper and barracuda indicated that all of these species were ~ TrL 3, which would mean the majority of all of their diets would have to be primary consumers. Although they certainly prey on these species (e.g., scarids) it is implausible that they only rarely consume other piscivores, carnivores or zooplanktivores. Moreover, the southern stingray, a benthic mesopredator, had an estimated TrL of 2.62, which is unrealistically low given a known diet of benthic and infaunal invertebrates and fish (Tilley et al. 2013). The highly competitive and interconnected nature of Caribbean coral reef food-webs or an unsampled primary production baseline may explain the scaled models lower than expected TrP estimates.

All of the large carnivorous fish had higher CR_b than NR_b, suggesting their prey is derived from a broad range of habitats and food webs. Coastal carbon sources (e.g. seagrass, coral and algae) are typically heavily enriched (~ -11.5 ± 3.2‰), with $\delta^{13}C$ compared to pelagic (e.g. phytoplankton, ~ -16‰) sourced carbon (Fry et al. 1983; Peterson and Fry 1987; France 1995; Hemminga and Mateo 1996). The $\delta^{13}C$ of our marker species met our expectations, with the ocean fore reef species being less enriched than the lionfish residing on lagoon patch reefs and all of them being less enriched than the species known to feed over seagrass. Southern stingrays were exclusively sampled inside the lagoon over a seagrass substrate and their mean $\delta^{13}C$ was similarly enriched to the queen conch and white grunt, which is to be expected from species feeding on seagrass and seagrass detritus based food webs. Reef sharks, black grouper and great barracuda had a very wide range of $\delta^{13}C$, with the long term integrated diet of some individuals overlapping with ocean facing fore reef marker species, others overlapping with lagoon seagrass marker species and with many intermediate individuals. The great barracuda had a significantly wider breadth of $\delta^{13}C$ than black grouper and reef sharks. All 3 species had large SEAc and CD_b (great barracuda being the highest), which were all driven by a wide range of $\delta^{13}C$. Overall the analyses indicate that these 3 species feed across a wide range of habitats and/or feed on species feeding in a wide variety of habitats. The Nassau grouper had a narrower and more enriched $\delta^{13}C$ similar to seagrass marker species, which could mean they are more specialized than the other predators. The sample of this species was much smaller than the other predators, however, so this needs to be verified with more research effort.

There was no correlation between TrL and TL of reef sharks, indicating that there is not an ontogenetic shift towards higher TrL as body size increases. An increase in high TrL prey with ontogeny occurs in several sharks, for example, as white sharks grow larger they switch from fish to piscivorous marine mammals as their body size, gape and dentition allow them to subdue larger, higher TrL prey (Wetherbee et al. 2004a; Estrada et al. 2006). Although large reef sharks obviously have greater ability to subdue larger prey than small reef sharks we suggest that this does not necessarily mean they ultimately prey on higher TrL species. There are a number of potential large bodied prey species for large reef sharks that feed at a relatively low TrL, including scarids, pomacentrids and southern stingrays. Conversely, there are many small prey species that are potentially consumed by small reef sharks that have a relatively high TrL, such as small piscivorous grouper, lionfish and snapper. The diet of small reef sharks might have a similar species or functional group composition to larger reef sharks but be composed of smaller bodied individuals from these species or functional groups. Analysis of stable isotopes of nitrogen cannot resolve this.

There was a correlation between δ^{13}C and TL of reef sharks, indicating that at least some of the larger individuals obtain their prey from different habitats/food webs than smaller individuals. The observed change is consistent with them deriving more of their carbon from lagoon habitats. Large reef sharks are frequently captured in the lagoon (Pikitch et al. 2005) and have been observed by us during daylight hours chasing southern stingrays around shallow (< 6 m depth patch reefs). It is therefore possible that animals feeding in relatively shallow lagoon habitats (such as southern stingrays) become more important prey for reef sharks as they get larger. Ontogenetic shifts in foraging habitat have been observed in several sharks (Wetherbee et al. 1990; Lowe et al. 1996; Wetherbee et al. 1997; Wetherbee et al. 2004a) but are usually associated with large-scale ontogenetic migrations between regions or habitats. This study shows that there can also be ontogenetic diet shifts in residential sharks like reef sharks (Bond et al. 2012) even without a substantial change in where they live.

There is growing evidence that predator populations can prey on a wide range of species through individuals having generalized diets or by being composed of individuals with different specialized diets (Munroe et al. 2014). The broad range of δ^{13} C and associated metrics of diet breadth of reef sharks at GRMR suggest the latter, given that we examined long term integrated diet by analyzing white muscle. We used capture location as a proxy for whether an individual’s home range primarily occurred in the fore-reef or lagoon, which is reasonable given results from previous acoustic monitoring studies that showed the primary factor that determined whether an acoustically tagged reef sharks was detected on a particular receiver over a 12-18 month period was the proximity of that receiver to its original capture location (Bond et al. 2012; also see supplementary material for new analysis of these data for habitat use). Using this proxy, we found that lagoon captured reef sharks were significantly more enriched for δ^{13}C than fore-reef captured reef sharks, which is consistent with them deriving more of their diet from this habitat. These findings meet expectations of the hypothesis that the location and distribution of habitat within an individual reef sharks home range influences its diet. It suggests that individual reef sharks may vary in terms of diet and ecological role and the ecological effects of reef shark removal or restoration would partially depend on which individuals (or from what locations or habitat on the reef system) are affected.

Bulk stable isotope analysis of Caribbean reef sharks and large predatory teleosts at GRMR provide new insights into the ecology of this and similar reef systems. The results are inconsistent with the hypothesis that large reef sharks are a full TrL above other large predators and instead occupy a similar or at most only slightly higher TrL than these species. There was considerable amount of overlap between the reef shark, black grouper and great barracuda in isotopic niche space, indicating an isotopically similar diet. This does not mean that they all prey on the same species or have the same effect on prey (their hunting strategies are all very different, for example, and therefore might prompt very different behaviorally mediated indirect interactions) but it does suggest that ecosystem models should treat them more as a collective top predator guild rather than separate apex predator (sharks) and mesopredator (grouper, barracuda) guilds. Reef shark TrL did not change with sex or ontogeny but there was evidence that large individuals derive more of their carbon from different habitats than small individuals, most likely shallow lagoon/seagrass habitats. This could stem from increased foraging on southern stingrays or isotopically similar prey. There was also evidence that the location and habitat characteristics of an individual reef shark's home range influences its diet, indicating that the wide breadth of δ^{13}C observed in the population is driven by individual specialization in terms of where they feed. Overall, these findings indicate that the field should move away from considering reef sharks like *C. perezi* to be singular reef apex predators that exert uniform top down pressure on the ecosystem. We should instead think of them as part of a top predator guild that feeds across a wide variety of reef habitats and food webs, with ontogenetic and individual variation in where they feed leading to more complex and contextual interactions between reef sharks and the rest of the coral reef ecosystem.

Figures and Tables

Table. 1. The breakdown of each species' maximum recorded size, sample size (n), range of total lengths (cm), mean TL (cm), and combined mean $\delta^{15}N$ (±SD), range of $\delta^{15}N$ values and $\delta^{13}C$ (±SD) values and range of $\delta^{13}C$ values, broken down by sex for elasmobranchs. Caribbean reef shark (*Carcharhinus perezi* – Zii), great barracuda (*Sphyraena barracuda* – Sba), black grouper (*Mycteroperca bonaci* – Mbo), Nassau grouper (*Epinephelus striatus* – Est) and southern stingray (*Dasyatis americana* – Dam).

Species	**Species Max. Size (cm)**	**Sample size (n)**	**TL range (cm)**	**Mean TL (cm)**	**Mean $\delta^{15}N$ (±SD) ‰**	**$\delta^{15}N$ Range ‰**	**Mean $\delta^{13}C$ (±SD) ‰**	**$\delta^{13}C$ Range ‰**
C. perezi all	295	86	74-236	124.9 (±41)	9.24 (±0.5)	8.12 - 10.57	-10.48 (±1.6)	-13.94: - 6.85
C.perezi ♀	-	47	83-236	125 (±42)	9.28 (±0.5)	8.22 - 10.17	-10.79 (±1.6)	-13.94: -7.18
C.perezi ♂	-	39	74-188	124 (±40.4)	9.28 (±0.6)	8.12 - 10.57	-10.27 (±1.6)	-13.48: -6.85
S. barracuda	200	50	60-119	91.7 (±17.3)	9.84 (±0.8)	8.79 - 12.55	-10.87 (±2.3)	-15.80: -7.94
M. bonaci	140	27	45-98	71.2 (±14.3)	9.70 (±0.5)	8.10 - 10.51	-11.24 (±1.5)	-13.84: -7.69
E. striatus	120	6	38-50	45.8 (±4.6)	8.88 (±0.4)	8.16 - 9.3	-10.15 (±0.9)	-11.19: -8.82
D. americana all	150	14	35-90	61.1 (±17.7)	7.14 (±0.7)	5.31 – 7.76	-9.29 (±1.1)	-10.99: -6.88
D.americana ♀	-	8	60-90	74 (±9.5)	6.69 (±0.3)	6.88 – 7.76	-9.00 (±0.9)	-10.99: -8.03
D.americana ♂	-	6	35-60	44 (±9.2)	7.5 (±0.8)	6.57 – 7.57	-9.50 (±1.4)	-10.93: -6.88

Table. 2. The results of the 6 Layman metrics used to evaluate each species and their roles within the community and the percentage overlap between each predator species ellipse. In the analysis SEAc values were substituted for total area of the convex hull (TA) as the sixth metric, because of its reduced inherent bias towards small sample sizes. The area of overlap between the SEAc ellipses for each species as a percentage of their total ellipse area. There was no overlap between the SEAc ellipse for southern stingrays and any of the other species therefore it was omitted from the table.

								SEAc ellipse percentage overlap				
Species	**NR**	**CR**	**CD**	**NND**	**SDNND**	**TA**	**SEAc**		**Est**	**Mbo**	**Sba**	**Zii**
Zii	2.08	5.93	1.45	0.28	0.28	7.94	2.62	**Zii**	35.4	40.5	59.7	100
Est	1.14	2.37	0.85	0	0.01	1.35	1.39	**Est**	100	1.6	19.6	66.5
Mbo	2.15	5.47	1.32	0.15	0.29	6.46	2.51	**Mbo**	0.9	100	98.3	42.2
Sba	3.13	7.36	2.04	0.26	0.37	13.76	5.25	**Sba**	5.2	47	100	29.7
Dam	2.3	3.97	1.05	0.05	0.17	3.4	1.83					

Table. 3. The estimated relative trophic position (TrP) for 5 carnivorous fish species at GRMR calculated from the data with both the scaled (Scd) and additive (Add) frameworks applied. The mean TrP and standard deviations (TrP ± SD) of the mean trophic position for the species but using multiple baseline species for each equation is adjacent. Finally the reported value and standard deviation from Fishbase for comparison (Froese and Pauly 2012). Stingrays were limited to the lagoon therefore only lagoon specific baseline species were used.

Baseline species	**Zii Scd**	**Zii Add**	**Sba Scd**	**Sba Add**	**Mbo Scd**	**Mbo Add**	**Est Scd**	**Est Add**	**Dam Scd**	**Dam Add**
Zoo-Forereef	3.14	3.57	3.29	3.79	3.26	3.75	3.05	3.51		
Zoo-Lagoon	3.07	3.45	3.22	3.67	3.18	3.63	2.98	3.39	2.58	2.87
Zoo	3.10	3.51	3.26	3.73	3.22	3.69	3.02	3.45		
Queen conch	3.25	3.75	3.40	3.98	3.37	3.94	3.16	3.70	2.76	3.19
Cittarium pica	3.30	3.84	3.45	4.07	3.42	4.03	3.21	3.79	2.81	3.27
Ocean surgeon	2.90	3.20	3.06	3.41	3.02	3.37	2.82	3.13		
Stoplight parrotfish	2.93	3.24	3.09	3.45	3.05	3.41	2.84	3.17		
Striped parrotfish	3.00	3.34	3.15	3.56	3.11	3.52	2.91	3.27		
Redband parrotfish	2.90	3.19	3.05	3.40	3.01	3.36	2.81	3.11		
Mean TrP (± SD)	3.07 (±0.13)	3.45 (±0.22)	3.22 (±0.21)	3.66 (±0.25)	3.18 (±0.13)	3.62 (±0.15)	2.98 (±0.1)	3.37 (±0.12)	2.72 (±0.14)	3.11 (±0.29)
Mean δ15N‰/ Fishbase	9.24	4.5 (±0.8)	9.84	4.5 (±0.8)	9.7	4.3 (±0.5)	8.88	4.1 (±0.7)	7.14	3.5 (±0.6)

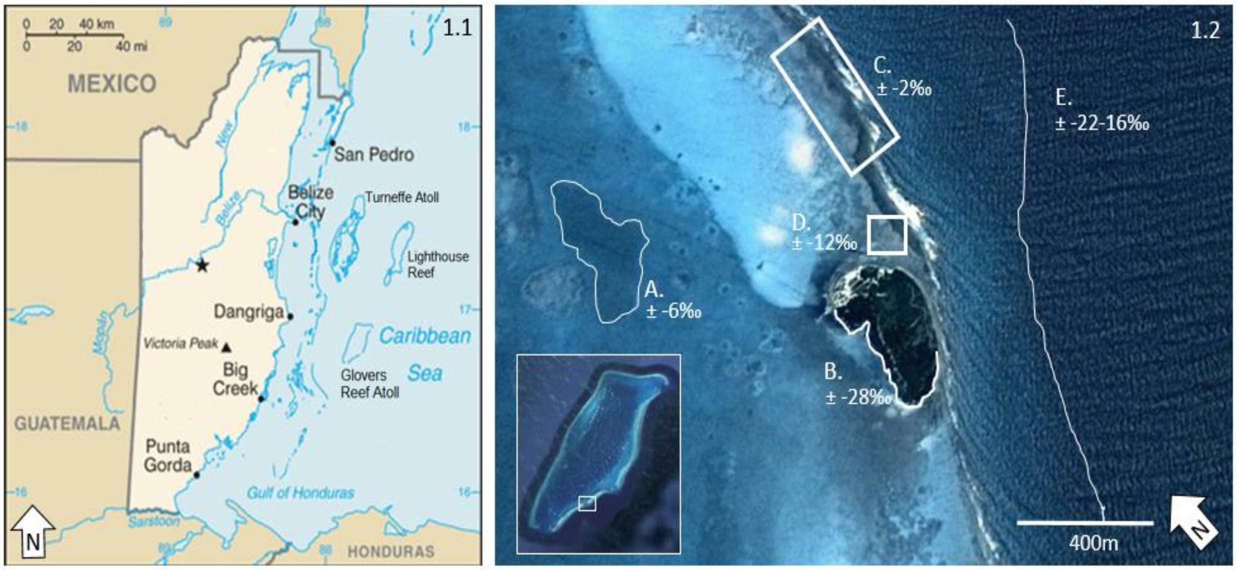


Figure. 1: Location of our study site, Glovers Reef Atoll with respect to mainland Belize, CA (1.1). Diagram showing the location and close proximity of isotopically distinct carbon sources (1.2): Seagrass *Thalassia testudinum* (A), Red mangrove *Rhizophora mangle* (B), coral reef production *Symbiodinium* (C), Macroalgae growth on dead coral *Lebophora variegate* (D), pelagic phytoplankton (E).

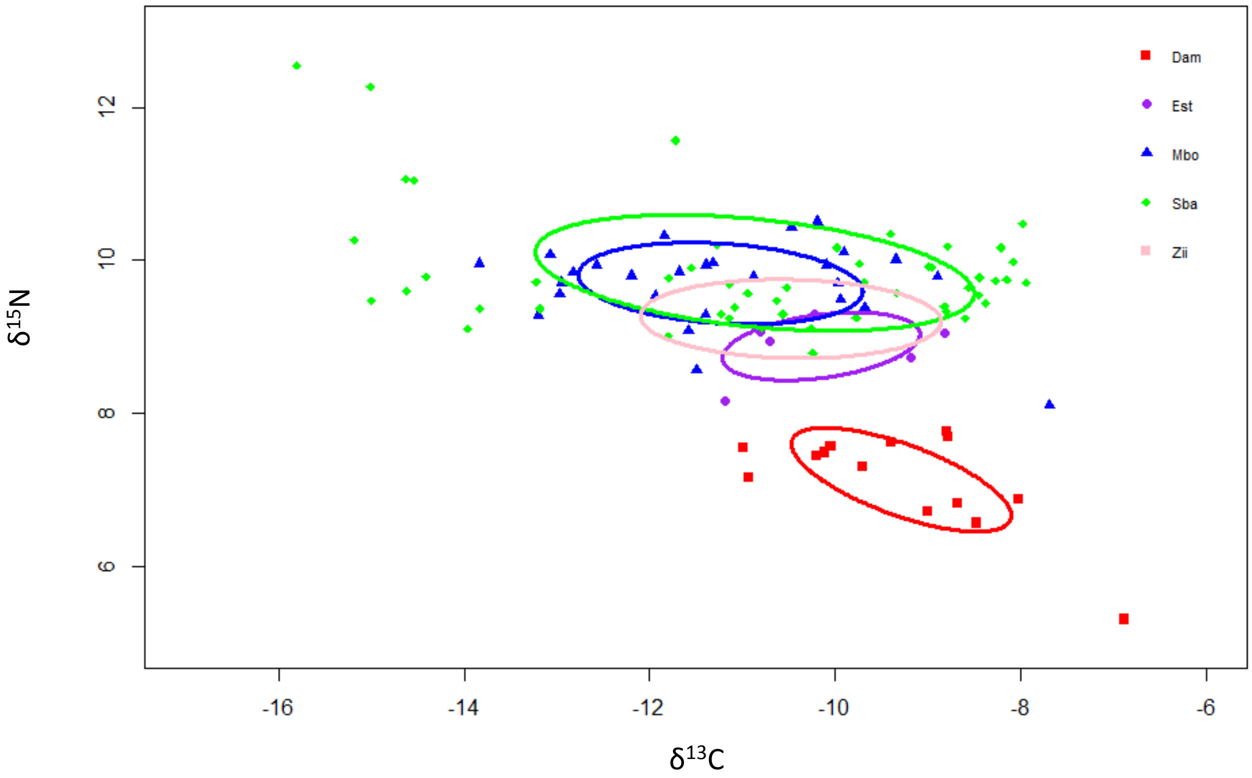


Figure. 2. δ^{15}N and δ^{13}C biplots and SEAc ellipses for all five study species, with Caribbean reef sharks (Zii), southern stingrays (Dam), Nassau grouper (Est), black grouper (Mbo), and great barracuda (Sba).

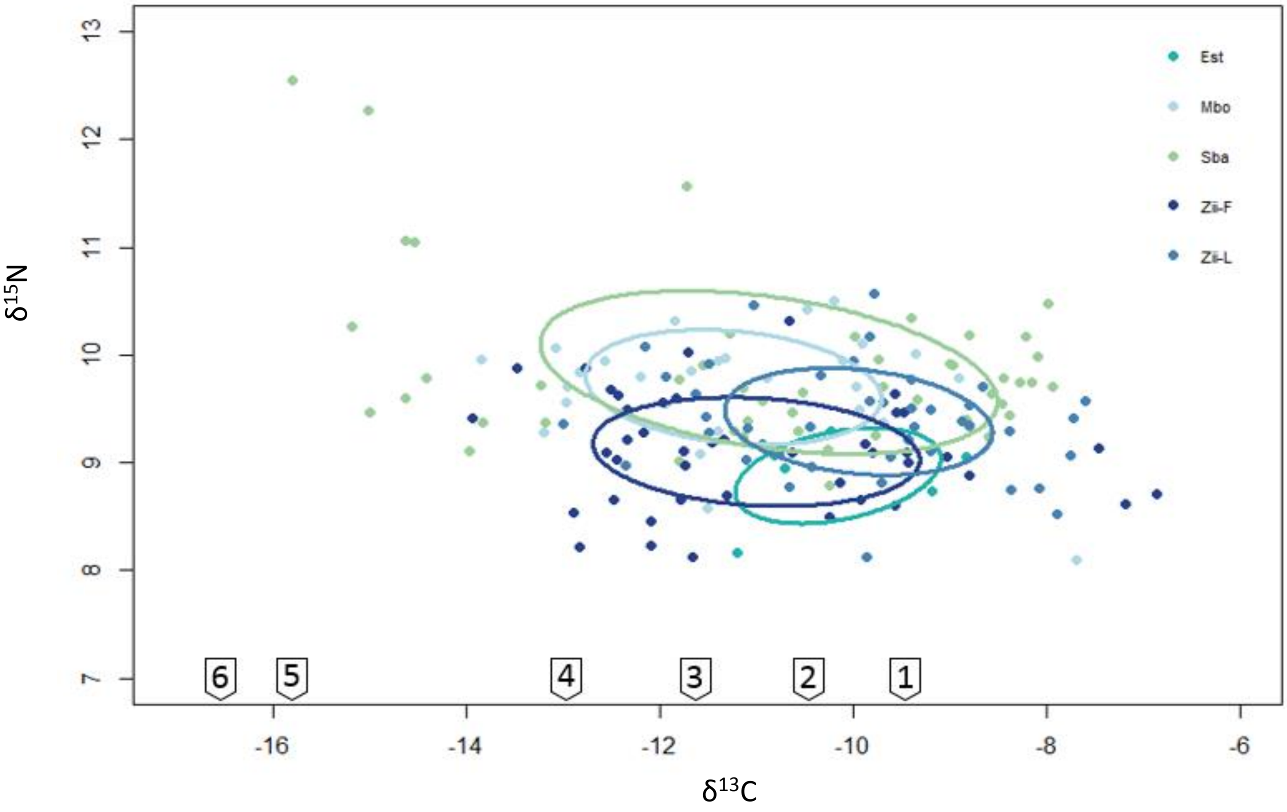


Figure. 3. $\delta^{15}N$ and $\delta^{13}C$ biplots and SEAc ellipses for the four study species, with Caribbean reef sharks divided into forereef (Zii-F) and lagoon (Zii-L) captured individuals. Marker species along the x-axis were used as a point of reference for $\delta^{13}C$ values for specific habitats: *Strombus gigas* (1), *Haemulon plumieri* (2), *Pterois volitans* (3), *Balistes vetula* (4), *Acanthurus bahianus* (5), and *Lutjanus vivianus* (6).

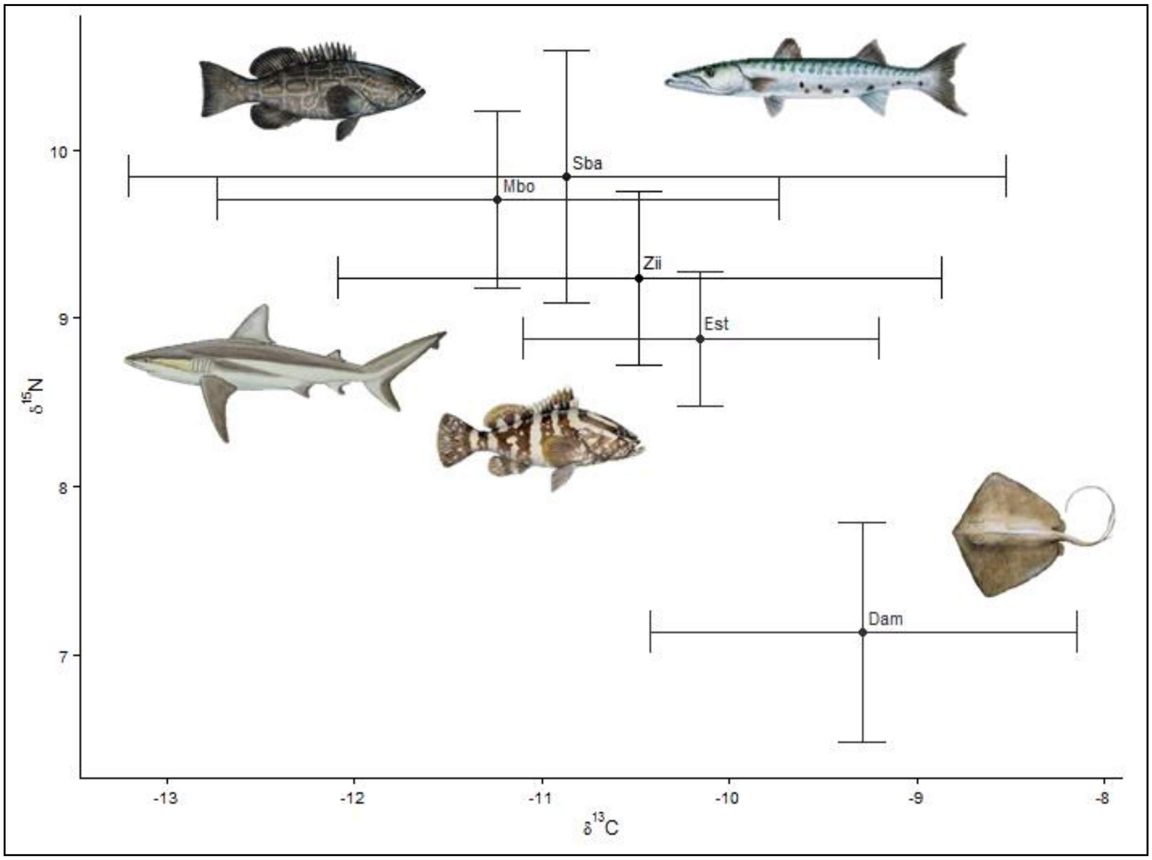


Figure. 4. δ^{13}C and δ^{15}N signatures of our five study species. Data points are group mean, and error bars are standard deviations. Species abbreviations are defined in Table 1.

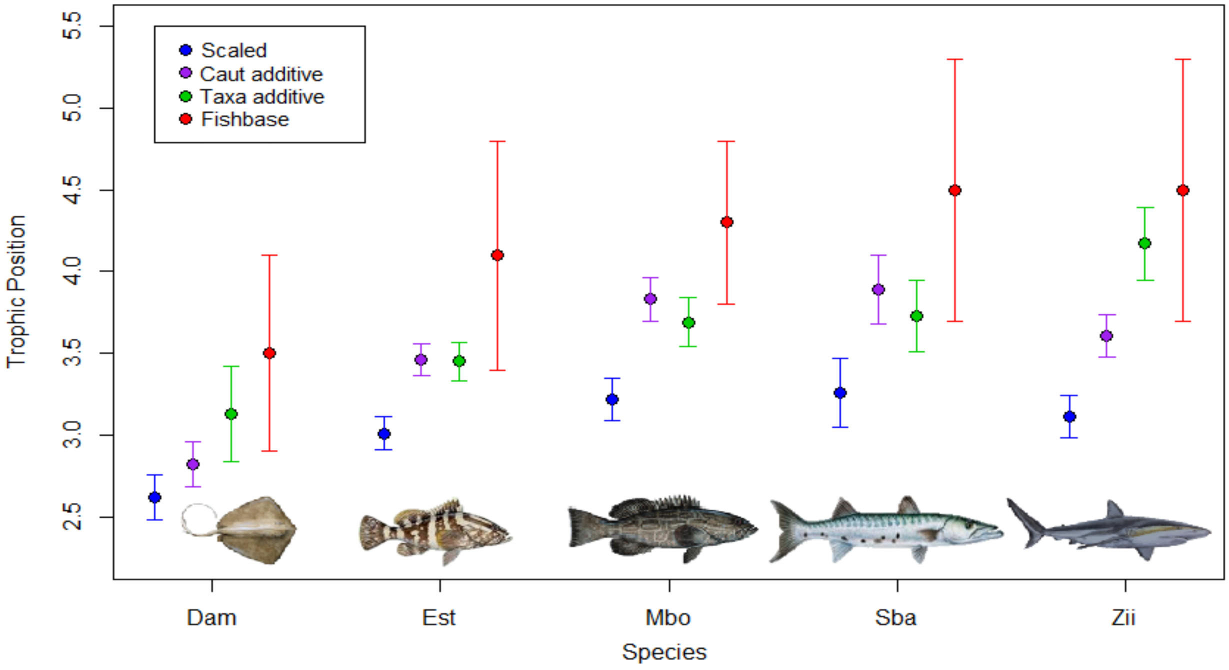


Figure. 5. Results of various trophic position calculations for our study species and the TrP stated by Fishbase as a point of reference (Froese and Pauly 2012). The inter-calculation variation is due to varying trophic enrichment factors (ΔN^{15}) being applied.

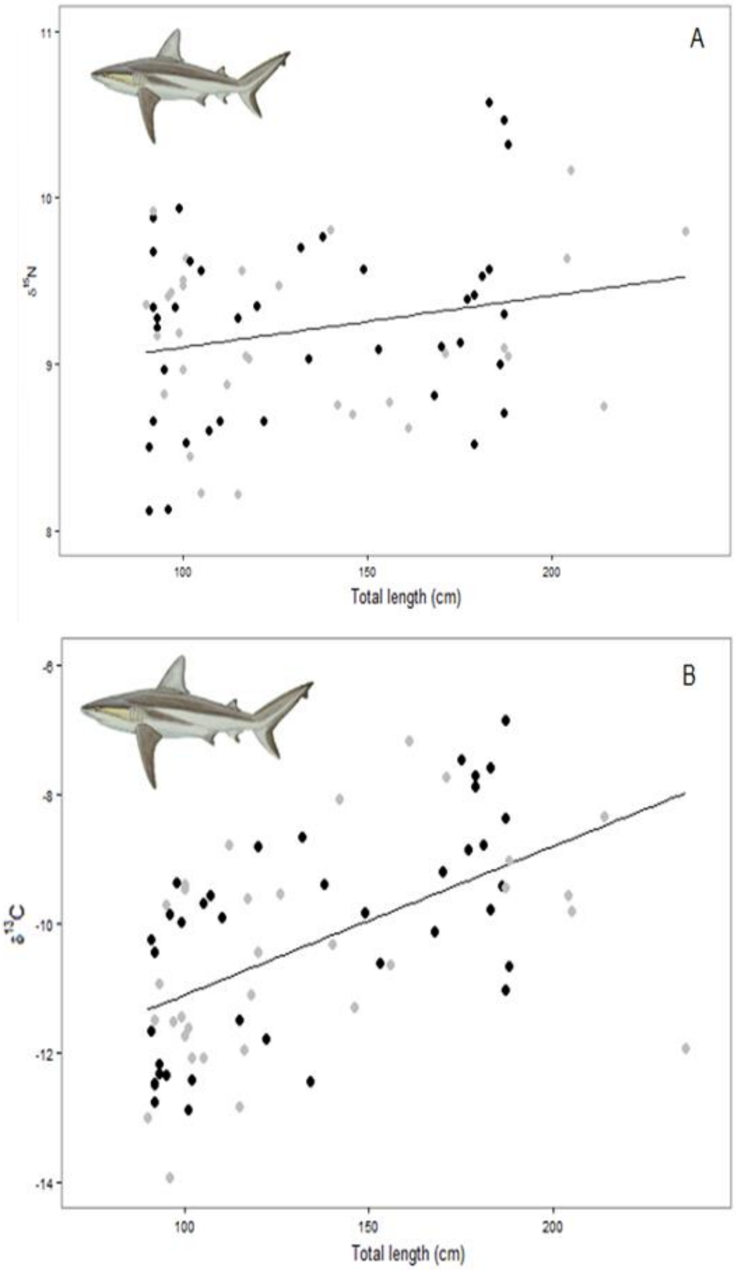


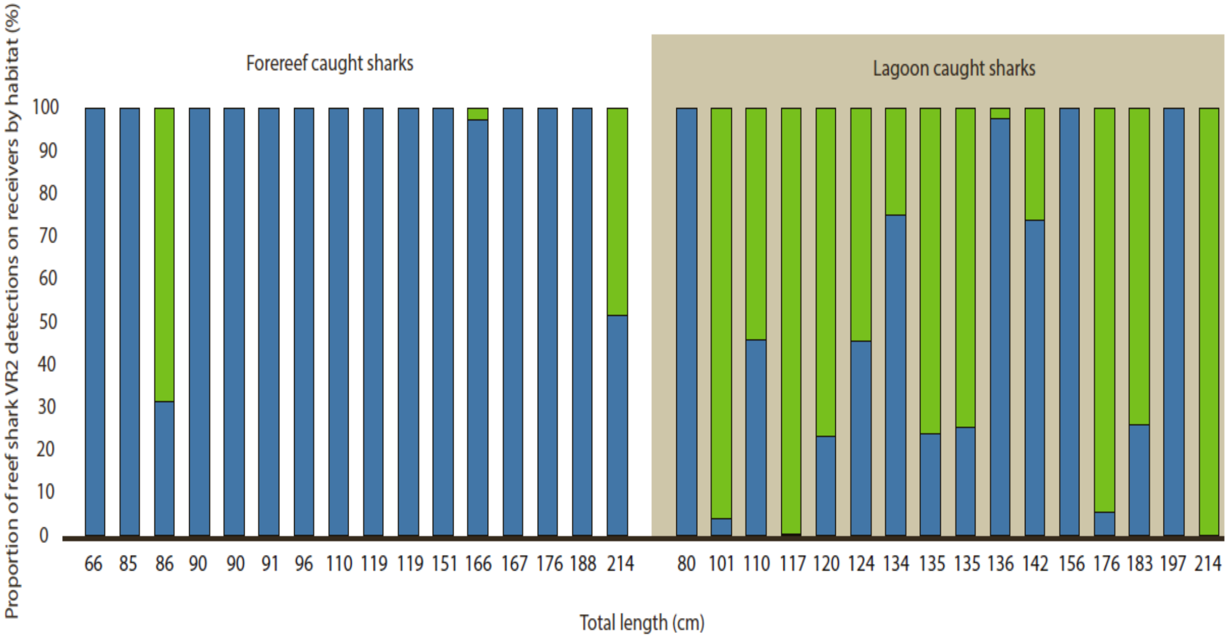
Figure. 6. Caribbean reef shark δ^{15}N and δ^{13}C for individuals ≥1 year old plotted against total length (Figures. A and B, respectively) to examine for ontogenetic shifts in diet or primary carbon source. There was no significant relationship between L_T - δ^{15}N for males (black) or females (grey), but L_T - δ^{13}C was significant for both sexes.

Electronic Supplemental Material

Behavioral support

Interpretation of $\delta^{13}C$ data for Caribbean reef sharks provided insight into the foraging behavior, specifically habitat use, of these highly mobile predators. The close proximity of multiple, unique, habitat specific carbon sources readily accessible to reef sharks provided an opportunity to determine whether individual sharks associate with specific foraging habitats (e.g. fore-reef or lagoon). Previous research at this site used acoustic telemetry to demonstrate that reef sharks are highly site-fidelic to GRMR throughout the year (Bond et al. 2012). Reef sharks exhibited evidence of home ranging behavior [i.e. the use of a fraction of available habitat instead of moving throughout (Burt 1943)], with individuals primarily detected on a subset of receivers (Bond et al. 2012). If an individual spends the majority of its time in a specific habitat, given its need for resources they logically forage within it too and will assimilate any isotopic signatures of the environment. Telemetry data from Bond et al. 2012 was used as a proxy for the reef sharks sampled here for SIA, in order to make inferences on their behavior, see Bond et al. 2012 for details of methodology. It was further analyzed to determine if there are habitat specific (i.e. fore-reef or lagoon) individuals based on movement data, and compared with the $\delta^{13}C$ isotope values from current individuals to see if a similar trend exists. Every shark caught from both telemetry and isotope studies was assigned to a habitat (e.g. fore-reef or lagoon) based on it capture location.

For the telemetry data, each sharks detections were assigned as being ‘lagoon’ or ‘fore-reef’ based on the habitat type where the receiver in question was located. The sum of detections per habitat was divided by the total number of detections for that individual to give a proportion of detections in each habitat. A student’s T-test was used to determine if ‘lagoon’ sharks spent significantly more time in the lagoon and the same for fore-reef sharks, using proportion of detections as a proxy for time. For the SIA data the mean $\delta^{13}C$ and $\delta^{15}N$ isotope values for sharks from each habitat type were calculated and compared.



ESM 1: Caribbean reef sharks tracked for 12-18 months by an array of acoustic receivers and published in Bond et al. (2012) were subject to additional analysis. For each individual a proxy of time spent for forereef and lagoon habitat was calculated by using the proportion of acoustic detections from receivers in each habitat. Estimated proportion of time spent in each habitat, fore-reef (blue) or lagoon (green) compared with the habitat of original capture. Each bar represents an individual. Total length (TL; cm) of individual is shown on the x-axis. With a few exceptions, individuals were primarily detected in the habitat type in which they were tagged.

Chapter Four: Temporal trends in the relative abundance of *Carcharhinus perezi* in a Caribbean marine reserve assessed using standard longlines.

Abstract

Marine reserves, areas of the ocean closed to fishing and other extractive activities, are being implemented as a conservation tool for sharks and batoid populations in response to severe global declines. However limited evidence exists to support marine reserves as an effective conservation approach and the existing data are primarily derived from spatial studies that compare relative abundance inside and outside of reserves. Low growth rates typify shark and ray populations, which means population trends can only be elucidated over long time periods. We analyzed the catch per unit effort (CPUE) from a standardized longline time-series conducted within Glover's Reef Marine Reserve (GRMR) in Belize from 2000-13 to assess the population trend of Caribbean reef sharks (*Carcharhinus perezi*), a resident species. Generalized linear models (GLM) detected no significant influence of the factor 'year' on CPUE however 'habitat' was significant, with a higher CPUE on the forereef. There was a significant influence of the factors 'year' and the 'year × habitat' on the mean total length of *C. perezi* that was evidence of inter-annual variation but not a decrease in mean TL with time. All life stages of *C. perezi* were captured throughout all habitats. The CPUE and total length GLMs suggest that the population of *C. perezi* inside Glovers Reef Marine Reserve is stable despite inter-annual variation in the size of individuals caught and may therefore have benefited from the reduced fishing mortality offered by the marine reserve.

Introduction

Marine reserves, defined here as marine areas closed to fishing, which prohibit various other extractive or consumptive activities, and within which anthropogenic interference is minimized to the best extent (Sobel and Dahlgren 2004), are being widely applied as a tool to protect marine biodiversity and overexploited species (Halpern and Warner 2002,2003; Sobel and Dahlgren 2004). Sharks are frequently important upper level predators in marine ecosystems that also have an economic value as a tourism and fishery resource (Heithaus et al. 2008b; Gallagher and Hammerschlag 2011; Eriksson and Clarke 2015). Many shark populations have declined due to overexploitation and habitat degradation, prompting substantial recent interest in shark conservation (Stevens et al. 2000; Worm et al. 2013; Dulvy et al. 2014). Considering the widespread implementation of marine reserves there is growing interest in their potential to protect or restore overexploited shark populations (Bonfil 1997). Bond et al. (2015, Chapter 1) reviewed the contemporary literature on the effects of marine reserves on sharks and found that studies designed to test for reserve effects, manifested as changes in density, biomass, body-size, relative abundance or species diversity, typically found that such effects were positive. Nearly all of these studies, however, compared shark relative abundance inside and outside marine reserves as opposed to measuring trends inside reserves over time. This is an important research gap because spatial differences inside and outside reserves may be driven by differences in rate of population decline as opposed to population stability or growth within the reserve (Lester et al. 2009). If reserves are simply slowing shark population declines relative to fished areas then they are only a temporary measure that needs to be coupled with better fisheries management or other conservation measures to successfully maintain or restore shark populations (Robbins et al. 2007).

Longlining is a fishing method that is commonly used to catch sharks on a commercial scale (Stevens 2000). Longlines consist of a main line that is typically anchored at both ends to the seafloor that is buoyed by floats. The mainline then has smaller lines stemming from it that terminate in baited hooks called gangions. Longlines have recently been used as a tool to survey shark populations by using catch per unit effort (CPUE) as a metric of relative abundance, with CPUE typically measured as number of sharks caught 100 hooks $^{-1}$ hour^{-1} (Pikitch et al. 2005; Tavares 2009; Brooks et al. 2013). Longline surveys of sharks have been completed in many parts of the world and in some cases have been carried out in a standard way over long periods of time to examine shark population trends (Simpfendorfer et al. 2002; Ingram et al. 2005; Grubbs et al. 2007). Shark population trends can also be inferred from changes in the demographic composition of the catch over time, which can be extracted from longline catch data by measuring the body size or age of captured individuals (Simpfendorfer 2000; Romine et al. 2009). Overexploited populations tend to become dominated by younger (smaller) individuals as a combined result of a reduced probability of survival to older ages (larger sizes) and, in sharks at least, reduced intraspecific predation by larger conspecifics (Stevens 2000).

The Caribbean reef shark (*Carcharhinus perezi*) is a large-bodied predator from the Family Carcharhinidae that can reach 295 cm total length and is endemic to and widely distributed throughout the subtropical and tropical western Atlantic from Bermuda to southern Brazil (Compagno et al. 2005). It is the only true coral reef-associated shark in the Atlantic completing all life-stages over coral reef associated habitats including deep reef (> 350 m deep), forereef (10-30 m), and adjacent lagoons (Chapman et al. 2005; Garla et al. 2006a; Chapman et al. 2007). They prey on a wide variety of species based on examination of a few gut contents (F. Carangidae, Sphyraenidae, Myliobatidae, Scaridae and Lutjanidae) (Rosa 2006; Tavares 2009). Although there is little empirical age and growth data for this species it is presumed they exhibit life-history characteristics typical of K-selected elasmobranchs including slow growth rates, late maturity and low fecundity (1-4 pups per gestation, Compagno et al. 2005). These traits likely make this species vulnerable to overexploitation (Pauly et al. 1998; Musick 1999). Throughout its range Caribbean reef sharks are targeted by commercial or artisanal fisheries and are the primary species observed on shark-feed dives that support a burgeoning regional eco-tourism industry (Pikitch et al. 2005; Gallagher and Hammerschlag 2011; Maljković and Côté 2011). Currently listed as 'Near Threatened' by the International Union for the Conservation of Nature (IUCN) there is potential for *C. perezi* to be relisted as 'Vulnerable' as more data become available (Rosa 2006). It is thought that this species is residential in tropical insular habitats (Garla et al. 2006a; Bond et al. 2012, Chapter 3 this volume) and it has been suggested that they could benefit from marine reserves in such habitats.

Glover's Reef Marine Reserve (GRMR; [16° 44' N, 87° 48' W], Belize, Central America encompasses an isolated coral atoll adjacent to the Mesoamerican Barrier Reef and was previously the subject of a longline study examining the demography of the elasmobranch assemblage including *C. perezi* (Pikitch et al. 2005). This study revealed all life-stages were present across lagoon and forereef habitats from 2000-2004 (Pikitch et al. 2005). Juveniles were common in both habitats but adults were more abundant on the forereef where *C. perezi* was the most abundant shark species (Pikitch et al. 2005). A broader study of the region that compared two marine reserves (GRMR and Caye Caulker) and two fished reefs (Southwater Caye and Turneffe Atoll) using telemetry and baited remote underwater video (BRUV) demonstrated all life-stages were resident throughout the year at GRMR and *C. perezi* were more abundant in this and the other surveyed marine reserve than the fished sites (Bond et al. 2012; Chapter 4). However it is possible the *C. perezi* population within GRMR and the other reserve could be simply be declining at a slower rate than the other reefs and this would not detected with a spatial study of short duration. Nearby Lighthouse Reef atoll has a commercial shark fishery that targets *C. perezi* (R. Carcamo, Belize Department of Fisheries *per comm*; D. Chapman *unpubl. data*). Some individuals tagged at GRMR do make movements to and from to Lighthouse Reef, potentially exposing them to this fishery (Chapman et al. 2005).

There is a clear need for long-term time series of shark populations within marine reserves to assess trends in abundance and body size in order to better assess reserve effectiveness. In this chapter I extend the annual longline survey of Pikitch et al. (2005) from 2001-2013. My objectives were two-fold. First, I tested the null hypothesis that year and habitat had no effect on *Carcharhinus perezi* CPUE over this period. My second hypothesis was that year and habitat had no effect on the size of *C. perezi* caught throughout the survey. Additional objectives were to further describe the demography of the *C. perezi* population at Glover Reef Marine reserve and to examine growth rates based on mark-recaptures obtained throughout the study.

Methods

Site description

Glover’s Reef atoll (16° 44’ N, 87° 48’ W) lies approximately 25 km to the east of the Mesoamerican Barrier Reef and 45 km east of the Belizean mainland (Fig. 1). The Glover’s Reef Marine Reserve (GRMR) comprises an interior no-take ‘conservation zone’ (7226 ha [Gibson et al. 2004]; Fig. 1), surrounded by a regulated ‘general use zone’ (32 834 ha), which encompasses the whole atoll out to the 180 m depth contour and prohibits the use of longlines or gillnets, the two primary shark fishing gears used in Belize [Gibson et al. 2004]; Fig. 1). This gear restriction essentially precludes a commercial shark fishery within GRMR, even though sharks are occasionally landed in hook and line fisheries that are permitted in the general use zone. Reserve regulations are actively enforced by resident members of a permanent Government of Belize Department of Fisheries station located on Middle Caye. To the atoll's north and west water depths range from 300-400 m, however of the eastern (windward) side these drop steeply to > 1000 m. The insular slope edge depth ranges from 15-45 m and the fore-reef is typically < 500 m wide before transitioning to the steep reef wall. The eastern ocean forereef, composed primarily of low-relief spur and groove formations (mainly *Montastrea* spp., *Diploria* spp.), are more developed and wider than the leeward (western) ocean reefs. The atoll itself is approximately 30 km long and a maximum of approximately 10 km wide. The atoll's western reef crest lies submerged (ca. 1.5 to 2 m depth), with the eastern reef crest being exposed and broken to produce five cuts, which allow shark movements between the ocean reef and lagoon ecosystems. The lagoon is basin shaped and is up to 18 m deep in some areas, with approximately 850 patch reefs (10 to 300 m+ wide) scattered throughout the interior, which are composed largely of massive corals (*Montastrea* spp., *Diploria* spp., *Siderasteria* spp.). The atoll also includes six sparsely populated cayes to the east of the lagoon, some are partially fringed with mangroves and surrounded by shallow water (<1.5 m) seagrass flats (*Thalassia testudinum*). Salinity in the lagoon ranges 32-38 ppt throughout the year and water temperature ranges from 27-32°C (summarized from Gibson 2003).

Longline sampling

All sampling was conducted under a series of annual research permits issued by the Belize Department of Fisheries to capture elasmobranchs. Annual expeditions were conducted in summer between May-July 2000-2013 and lasted between 12-46 d. Commercial grade longline gear that consisted of a 5.6 mm tarred, braided, nylon main- line anchored to the substrate at both ends. Baited gangions were placed at 20 m intervals along the mainline, and floats were attached at regular intervals to buoy the line. Each 3.5 m gangion was composed of a 2.5 m section of 2 mm monofilament connected to a 1 m section of 1.6 mm braided stainless steel cable, terminating in a 16/0 Mustad circle hook. Hooks were baited with similar-sized pieces of resident fish species captured locally by handline (primarily from the families Lutjanidae, Haemulidae, Sphyraenidae, and Carangidae). Circle hooks were selected to reduce the incidental catch of large teleosts and to reduce the incidence of gut hooking in sharks. Throughout the study, 2 types of longline sets were made standard and non-standard lines. Standard lines were used to provide quantitative estimates of elasmobranch abundance and to describe their distribution at Glover's Reef. These lines that consisted of 50 hooks were deployed for 3 h at fixed locations each year (Fig 1). For quantitative data analysis, we used catch per unit effort (CPUE, sharks 100 hooks^{-1} h^{-1}) as the statistical sampling unit. Non-standard lines included sets made with varying amounts of hooks (n = 5-30), non-uniform soaks times, and deployed in different locations. We did not use CPUE data from non-standard longline sets in the statistical analysis, but the biological data from the specimens caught were used for delineating the demography of the shark assemblage.

Elasmobranch handling and data collection

All captured elasmobranchs were secured to the side of the 7 m fishing vessel with the gangion tied to the bow and a rope-noose looped around the tail and attached to the stern. This allowed data collection while keeping the shark in the water. The total length (TL) of all sharks was measured on a straight line from the tip of the rostrum to the tip of the upper caudal lobe, and sex was determined by examination of the pelvic region for the presence or absence of claspers, present in males. Small sharks were examined for an umbilical opening, to determine whether they were neonates. Live *C. perezi* were then tagged using individually numbered nylon-tipped dart tags (Hallprint). Each hook was completely removed using a pair of bolt cutters to cut the barb and pliers to rotate the remainder of the hook free before the shark was released. Total handling time typically ranged from 5-8 min. Moribund sharks were retained for dissection, collection of biological samples (DNA and white muscle tissue), stomach content analysis and measurements of embryos in gravid females. Teleost by-catch was released alive when caught within the no-take zone of GRMR, or otherwise retained for bait.

Quantitative analyses

Standard longline set locations were categorized into two classes in order to compare overall abundance, species composition and patterns of species-specific abundance in different macrohabitats around Glover's Reef atoll (Fig. 1). Classifications were based on benthic habitat data for Glover's Reef provided by the Belize Coastal Zone Management Authority and Institute (CZMAI), coupled with depth measurements made along the standard longlines. Longlines set along the reef-slope and fore-reef fringing Glover's Reef over a coral reef substrate in 6-30 m of water were defined as 'forereef', and 'lagoon' lines were those set inside the atoll in 6-18 m of water with mixed seagrass, soft bottom and patch reef substrates. To test whether the factors year, as a categorical variable, or habitat or the year × habitat interaction had a significant effect on *C. perezi* CPUE a Gaussian identity link generalized linear model was performed using the R software (R Core Team 2010). In all cases, CPUE data were log-transformed [log10(CPUE+1)] to normalize the data making the gaussian family an appropriate fit for the GLM. A second GLM was performed to test whether the factors year or habitat had significant effect on *C. perezi* total length (TL). Length data were log transformed to normalize and a Levene's test performed using the R software to examine for heterogeneity of variance. The size distributions of *C. perezi* were examined with length-frequency histograms and sex ratios examined. To characterize growth rates, differences in TL between repeatedly captured individuals tagged and measured throughout the study were divided by the number of days at liberty between captures to provide an estimate of growth (cm yr^{-1}). Given the known slow growth rates of other carcharhinids and the relatively short sampling period each year this was not performed on individuals recaptured within the same sampling expedition.

Results

During 13 annual expeditions between May-July 2000-13 a total of 193 standard longlines were deployed across the fore-reef (n = 71) and lagoon (n = 122) habitats of GRMR. The standardized longline survey was supplemented by non-standard longlines (n = 26) and shark captures using other methods (n = 21). Total shark catch throughout the survey period comprised of 733 individuals from 10 species with *G. cirratum* (54.8%) and *C. perezi* (40.1%) being the two most abundant species in both habitats (Table 1). Combined gear catches were 402 individuals (n = 94 recaptures) for *G. cirratum* and 308 (n = 13 recaptures) for *C. perezi*.

Growth rates (converted to cm yr^{-1}) were estimated for seven-recaptured *C. perezi* by dividing the measured change in TL by the number of days at liberty (Table 2). Growth rates ranged from 1.1-17.3 cm yr^{-1}. Average growth rate for males (n = 5) was 8.3 cm yr^{-1} and for females (n = 2) was 9.8 cm yr^{-1} (Fig. 2). Length-frequency histograms for all *C. perezi* caught throughout the survey irrespective of fishing gear show that all life-stages (neonates to adults) occur at Glover's Reef during summer months in both the lagoon and the forereef (Fig 3). Standard longline forereef-caught *C. perezi* ranged in TL from 66-217 cm for females (n = 64) and 74-197 cm for males (n = 68). Lagoon caught *C. perezi* ranged in TL from 70-234 cm for females (n = 64) and 71-195 cm for males (n = 72). Four juvenile *C. perezi* were observed to have open umbilicus when caught, an indicator of recent parturition (4-6 weeks). These neonates (3 males, 1 female) were caught in the months of May, June and July in both habitats (n = 2 individuals per habitat). The sex ratio for *C. perezi* at Glover's Reef was 140:128 respectively, with no significant deviation from 1:1.

Two hundred and sixty eight *C. perezi* were caught on the standard longlines from 2001-2013 and all statistical analyses were conducted on this subset of individuals. The GLM indicated that total CPUE of *C. perezi* was not significantly influenced by the factor year ($p = 0.74$) or the habitat × year interaction ($p = 0.74$) but the factor habitat was significant ($p = 0.0005$). This was because CPUE was higher on the forereef (1.26 ± 0.15) than the lagoon (0.67 ± 0.07; Table 3, Fig 4). The GLM detected a significant effect of the factors year ($p < 0.05$) and the habitat × year interaction ($p < 0.0001$) on *C. perezi* TL (Fig. 5, Table 4).

Discussion

GRMR is used by a wide range of *Carcharhinus perezi* age classes,, from neonates to adults of both sexes. The low recapture rate of 4.4% compared to other *C. perezi* studies in The Bahamas and Brazil (15.4 and 15.3% respectively) suggests a relatively large population of *C. perezi* at GRMR, especially when considering the duration of the study and the high proportion of resident individuals (Garla et al. 2006b; Bond et al. 2012; Brooks et al. 2013). Smaller *C. perezi* grew at a faster rate than larger individuals, with similar growth rates to those observed in juvenile *C. perezi* in Venezuela (17.4-31.6 cm/y-1, Tavares 2009). Neonates were encountered in both the lagoon and forereef habitats in the spring and summer months but were uncommon, which could mean that parturition typically occurs outside of the months when our survey took place. Catch rates of *C. perezi* were higher on the forereef than the lagoon, which may indicate habitat preference but could also be because the forereef is a relatively narrow (< 500 m) wide strip of habitat that is more effectively sampled by longline than the lagoon, which is a larger area interspersed with patch reefs. The demography and habitat use of *C. perezi* at GRMR is similar to atolls in Venezuela and Brazil, and the Great Bahamas Bank (Garla et al. 2006b; Garla et al. 2006a; Tavares 2009; Brooks et al. 2013). All life-stages of *C. perezi* are found in close proximity to one another at all of these sites, suggesting that this species doesn't have nursery areas that occur in discrete parts of the species geographic range but instead may be microhabitats within reef systems occupied by older life-stages as well (Garla et al. 2006a; Tavares 2009; Brooks et al. 2013). While the sex ratio at GRMR and in Venezuela was 1:1 populations in The Bahamas and Brazil were both female dominated (Garla et al. 2006a; Tavares 2009; Brooks et al. 2013). In the tropical atolls *C. perezi* were present throughout the year but in the Bahamas sex segregated seasonal movements occurred and were hypothesized to a response to seasonal water temperature changes (Brooks et al. 2013). Overall the *C. perezi* population at GRMR exhibits traits that appear typical of this species in insular, low latitude reef systems (residency, occurrence of all life-stages, use of both forereef and lagoons, fast juvenile growth rates, spring/summer parturition).

The factor "year" had no discernable effect on longline CPUE at GRMR. This is consistent with the population being stable or that a population trend (positive or negative) is not detectable with the level of sampling conducted. The results of a baited remote underwater video (BRUV) survey conducted in two marine reserves (one of which was GRMR) and two similar fished reefs demonstrated that *C. perezi* were more common in reserves, with GRMR have the highest sightings per unit effort of any of the sites (Bond et al. 2012). While spatial studies such as this represent the majority of the literature on the effectiveness of marine reserve they are rarely robust enough to determine if populations inside reserves are still declining/increasing just at a slower rate than fished control sites. Long-term temporal studies like the standardized longline survey presented here are the only way to determine the population trend inside reserves. My results are consistent with population stability within GRMR, which is promising because shark fishermen in Belize are increasingly fishing further from the coast and barrier reef to supplement their catch. *C. perezi* are rarely landed by fishermen at GRMR based on surveys of catch (J. Gibson Wildlife Conservation Society *pers comm*), indicating fishing mortality at this atoll is low.

Studies on the size and age structure of a population can provide information on how heavily the population is exploited (Hutchings and Reynolds 2004). If the mean size of individuals within the population decreases with time that can be an indication of population decline (Stevens 2000). Significant decreases in the mean and median total length of pigeye (*Carcharhinus amboinensis*), blacktip (*Carcharhinus limbatus*), and female white (*Carcharodon carcharias*) sharks was reported in a fisheries independent survey in KwaZulu-Natal, South Africa, from 1978-2003. This reflected decreasing catch rates for these species as well (Stevens 2000; Dudley and Simpfendorfer 2006). In contrast there was no evidence of a decline in *C. perezi* TL with time during the survey. While year did have a significant influence on *C. perezi* TL there was no clear trend over time, rather inter-annual fluctuations in the mean TL of captured sharks was most likely a result of relatively small sample sizes each year. Similarly the strength of the 'habitat × year' interaction was most likely due to higher catch rates on the forereef which included larger sharks during certain years in concert with the relatively small sample sizes.

GRMR fulfills all five of the criteria that are associated with effective marine reserves identified by Edgar et al. (2014): it is larger than 100 km ^{2}, isolated, enforced, partially no-take, and has met these criteria for more than 10 years. I would therefore predict that *C. perezi*, as a resident species, would be either stable or increasing within GRMR. The 13 year standard survey of GRMR provides evidence that *C. perezi* population size and size structure at the atoll is not changing or not changing at a rate detectable with the level of survey effort expended. Standard time-series like this one are essential to assess whether or not marine reserve are effectively reducing fishing mortality enough to maintain or restore elasmobranch populations. The results suggest that the spatial management plan employed at GRMR with the central 'no-take' area encompassed within the 'general use' zone where longlines and gillnets are prohibited (Gibson 2003; Gibson et al. 2004) is effectively conserving *C. perezi*. This spatial plan could serve as a template for other insular coral reef ecosystems where this species occurs given apparent similarities between the life-history, movements and habitat use observed between studies at GRMR and other similar systems (Garla et al. 2006b; Garla et al. 2006a; Tavares 2009; Graham et al. 2010; Field et al. 2011; Bond et al. 2012). The IUCN assessment of the Caribbean reef shark (*Carcharhinus perezi*) lists the species as 'Near Threatened' with evidence of a generally decreasing population trend (Rosa 2006), however, this assessment is almost a decade old. Given the high economic value of *C. perezi* to the ecotourism industries of many countries throughout its range this species should be prioritized for conservation (Gallagher and Hammerschlag 2011). The results of this survey and others indicate that marine reserves can be an effective conservation approach for Caribbean reef sharks (*Carcharhinus perezi*).

Figures and Tables

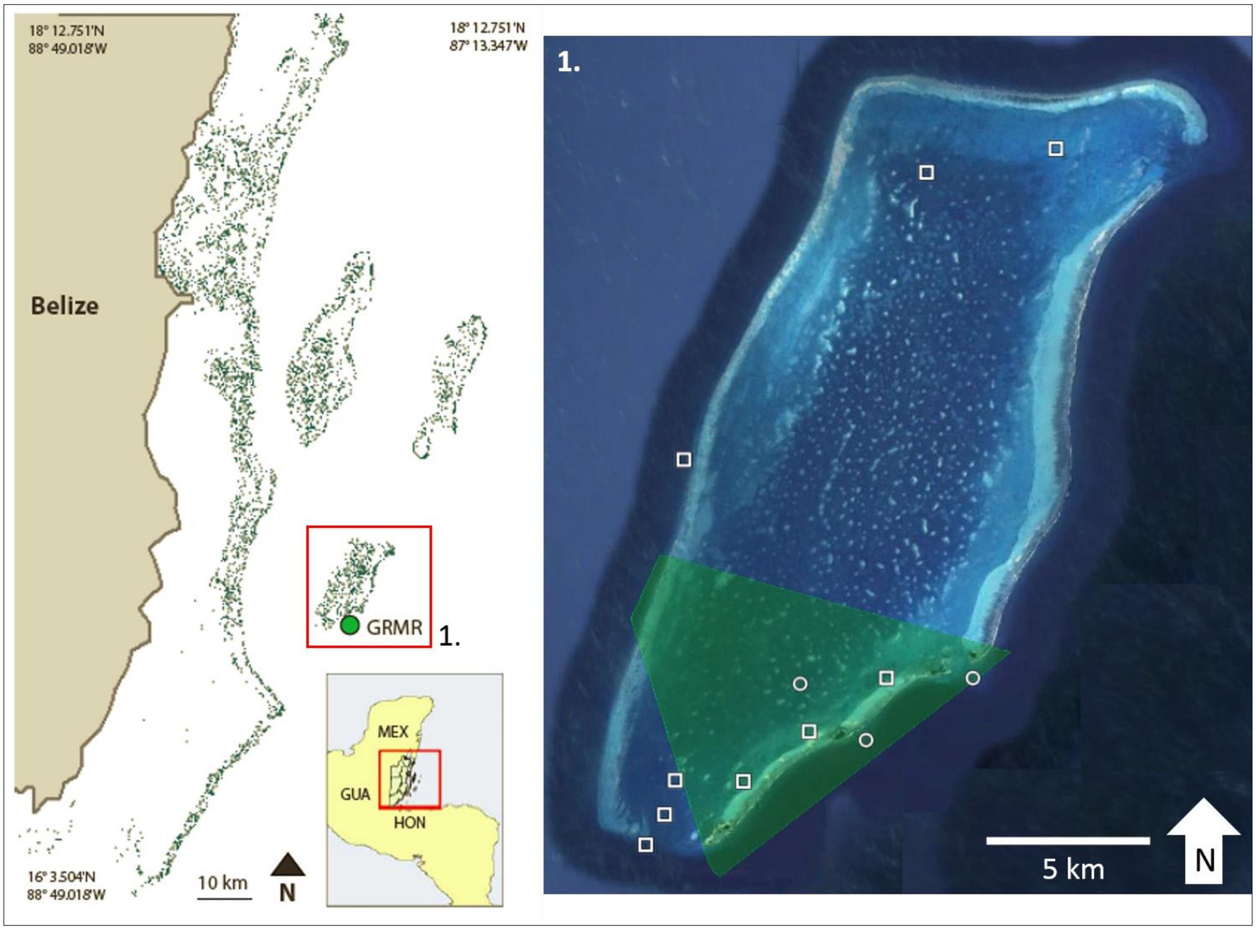


Figure 1. Map of Glover's Reef Marine Reserve. Green polygon shows the "Conservation Zone", which is no-take. Squares depict locations of standard lines set once per expedition, circles depict standard lines set twice per expedition.

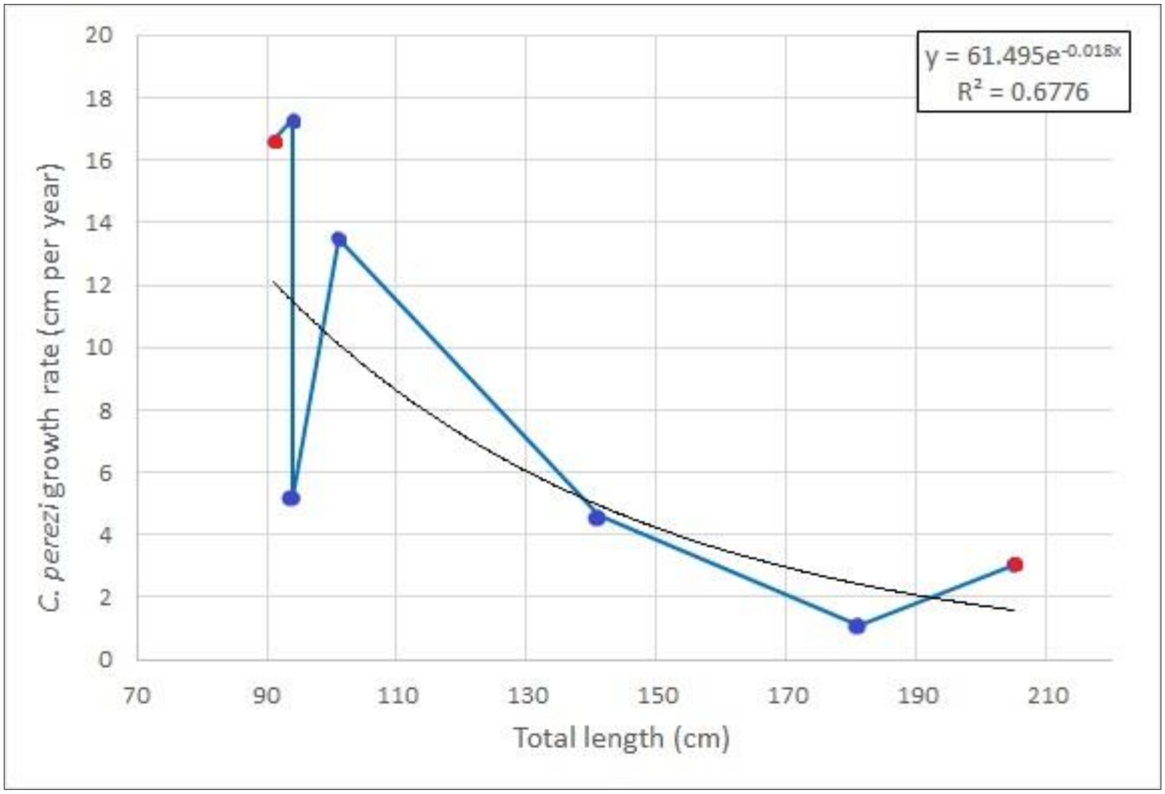


Figure 2. Estimated growth rates (cm/year) by total length (cm) derived from mark-recapture study on *C. perezi* captured at Glover's Reef Marine Reserve from 2000-2013, for males (blue, n=5) and females (red, n=2).

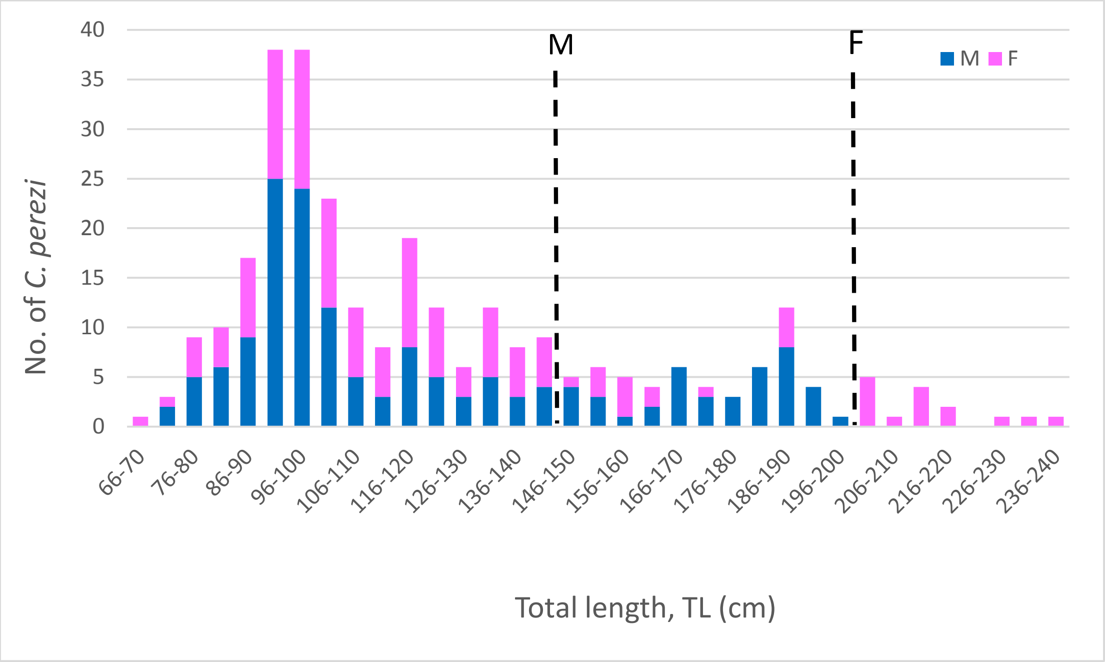


Figure 3. Length-frequency histogram for male (blue) and female (pink) *C. perezi* captured at Glover's Reef Marine Reserve from 2000-2013. Vertical dotted lines show approximate total lengths at maturity (Compagno et al. 2005) for males (M) and females (F).

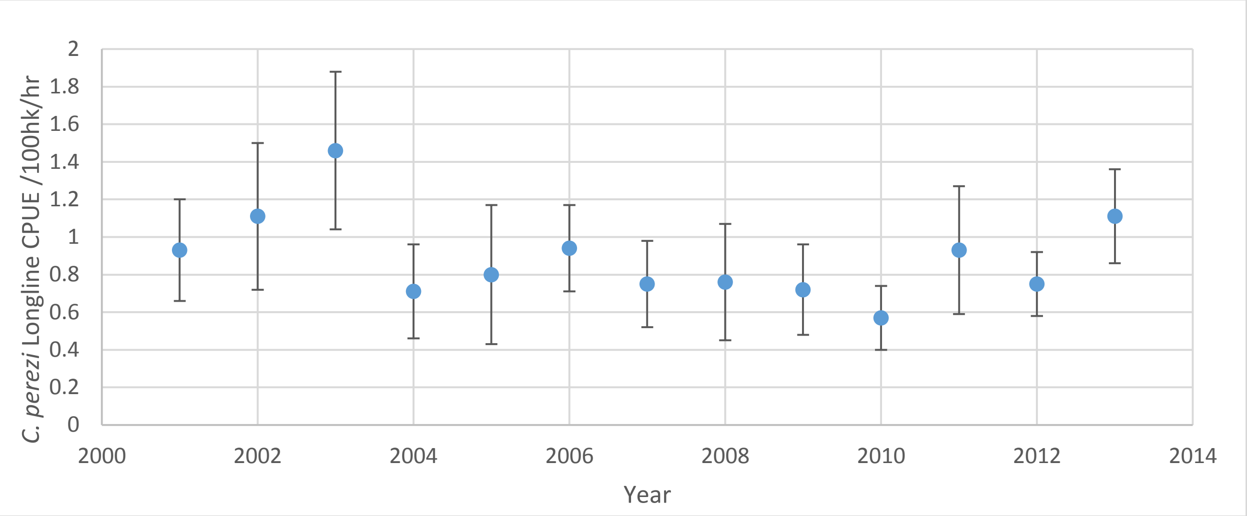


Figure 4. Glover's Reef Marine Reserve *C. perezi* shark longline mean CPUE and standard error time-series 2001-2013.

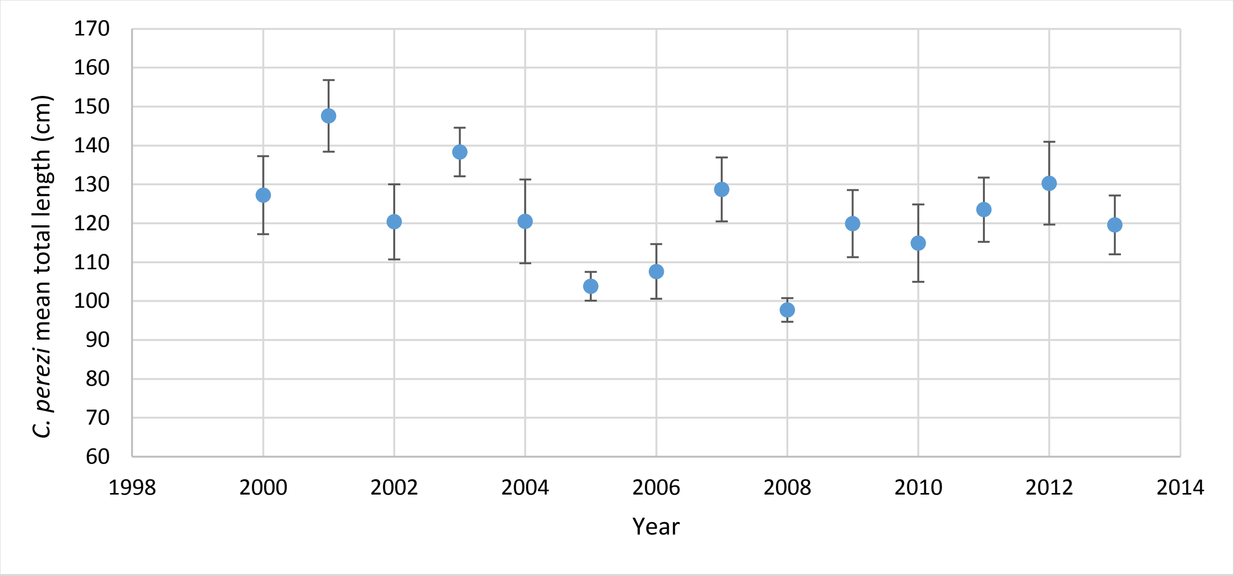


Figure 5. *C. perezi* mean total length and standard error (cm) from standardized longlines Glover's Reef Marine Reserve 2000-2013.

Table 1. Shark species composition at Glover's Reef Marine Reserve derived from standardized longline survey 2000-2013.

Species	**n**
Nurse, *Ginglymostoma cirratum*	402
Caribbean reef, *Carcharhinus perezi*	293
Caribbean sharpnose, *Rhizoprionodon porosus*	17
Lemon, *Negaprion brevirostris*	10
Tiger, *Galeocerdo cuvier*	4
Silky, *Carcharhinus falciformis*	2
Blacktip, *Carcharhinus limbatus*	2
Blacknose, *Carcharhinus acronotus*	1
Galapagos, *Carcharhinus galapagensis*	1
Great hammerhead, *Sphyrna mokarran*	1

Table 2. Growth rate (cm yr^{-1}) of *C. perezi* (n = 7) calculated from differences in total length (TL) from tag-recapture data (cm).

	Sex	Tag date	Recap date	Liberty	Tag TL	Recap TL	TL diff	Growth
	M/F	dd/m/yyyy	dd/m/yyyy	days	(cm)	(cm)	(cm)	(cm yr^{-1})
C. perezi 1	F	10/5/2007	8/6/2011	1491	91	159	68	16.6
C. perezi 2	M	7/5/2006	30/4/2007	359	94	111	17	17.3
C. perezi 3	M	29/5/2013	27/7/2014	425	94	100	6	5.2
C. perezi 4	M	3/5/2006	9/6/2011	1864	101	170	69	13.5
C. perezi 5	M	22/5/2003	12/6/2012	3310	141	183	42	4.6
C. perezi 6	M	12/6/2009	3/5/2010	326	181	182	1	1.1
C. perezi 7	F	22/5/2001	22/5/2005	1460	205	217	12	3.0

Table. 3. The GLM analysis on the influence of the factors year and habitat and their interaction on Caribbean reef shark catch per unit effort from standardized longlines surveys 2001-2013 at Glover’s Reef Marine Reserve, Belize.

	Df	Deviance	Residual Df	Residual Deviance	P(>│Chi│)	Percent of Deviance
NULL			192	8.87		
Year	1	0.005	191	8.86	0.7374	0.06
Habitat	1	0.532	190	8.33	0.0005***	6.0
Year × habitat	1	0.005	189	8.33	0.7411	0.06

Table. 4. The GLM analysis on the influence of the factors year, habitat and their interaction on Caribbean reef shark total length (cm) from standardized longlines surveys 2001-2013 at Glover’s Reef Marine Reserve, Belize.

	Df	Deviance	Residual Df	Residual Deviance	P(>│Chi│)	Percent of Deviance
NULL			263	3.981		
Year	1	0.0600	262	3.921	0.037*	0.24
Habitat	1	0.0051	261	3.916	0.543	0.13
Year × habitat	1	0.3436	260	3.573	<0.0001***	8.64

Chapter Five: Reef sharks exhibit site-fidelity and higher relative abundance in marine reserves on the Mesoamerican Barrier Reef.

A version of this chapter has been published in:

Bond ME, Babcock EA, Pikitch EK, Abercrombie DL, Lamb NF, Chapman DD (2012) Reef sharks exhibit site-fidelity and higher relative abundance in marine reserves on the mesoamerican barrier reef. PloS one 7:e32983

Abstract

Carcharhinid sharks can make up a large fraction of the top predators inhabiting tropical marine ecosystems and have declined in many regions due to intense fishing pressure. There is some support for the hypothesis that carcharhinid species that complete their life-cycle within coral reef ecosystems, hereafter referred to as “reef sharks”, are more abundant inside no-take marine reserves due to a reduction in fishing pressure (i.e., they benefit from marine reserves). Key predictions of this hypothesis are that (a) individual reef sharks exhibit high site-fidelity to these protected areas and (b) their relative abundance will generally be higher in these areas compared to fished reefs. To test this hypothesis for the first time in Caribbean coral reef ecosystems we combined acoustic monitoring and baited remote underwater video (BRUV) surveys to measure reef shark site-fidelity and relative abundance, respectively. We focused on the Caribbean reef shark (*Carcharhinus perezi*), the most common reef shark in the Western Atlantic, at Glover's Reef Marine Reserve (GRMR), Belize. Acoustically tagged sharks (N=34) were detected throughout the year at this location and exhibited strong site-fidelity. Shark presence or absence on 200 BRUVs deployed at GRMR and three other sites (another reserve site and two fished reefs) showed that the factor "marine reserve" had a significant positive effect on reef shark presence. We rejected environmental factors or site-environment interactions as predominant drivers of this pattern. These results are consistent with the hypothesis that marine reserves can benefit reef shark populations and we suggest new hypotheses to determine the underlying mechanism(s) involved: reduced fishing mortality or enhanced prey availability.

Introduction

Many tropical nations are turning to marine reserves to help maintain coral reef biodiversity, ecosystem function, ecotourism and fisheries (Roberts et al. 2001; Sobel and Dahlgren 2004; Lester et al. 2009; Molloy et al. 2009). Marine reserves can clearly enhance exploited coral reef species that have relatively sedentary adult life-stages, in which some individuals live almost exclusively within reserve boundaries (i.e., reef-associated bony fish and invertebrates (Russ and Alcala 2003; Gibson et al. 2004; Sobel and Dahlgren 2004; Newman et al. 2006; Russ et al. 2008)). This enhancement occurs because the reserve provides a respite from fishing mortality that leads to an increase in local abundance and reproductive output (Russ and Alcala 2003; Gibson et al. 2004; Sobel and Dahlgren 2004; Newman et al. 2006; Russ et al. 2008). However, can marine reserves also benefit large, roving reef predators that are potentially mobile throughout their life? This group includes sharks, which make up a significant fraction of the top predators on relatively pristine coral reefs (Friedlander and DeMartini 2002; Stevenson et al. 2007). Sharks are currently experiencing intense fishing pressure worldwide, largely due to the Asian shark fin trade (Clarke et al. 2006), which is worrisome in light of their relatively low reproductive potential (Musick 1999; Au et al. 2008).

There is a modest body of data supporting the hypothesis that marine reserves can benefit certain shark populations (Meekan and Cappo 2004; Garla et al. 2006b; Garla et al. 2006a; Robbins et al. 2006; Heupel et al. 2009; Heupel et al. 2010). Most of the focal species of these prior studies belong to the family Carcharhinidae (requiem or whaler sharks) and complete their life-cycle within coral reef ecosystems. Species with these general characteristics are hereafter referred to as “reef sharks”. No temporal monitoring studies have been conducted to show an increase in reef shark abundance following marine reserve establishment. However, existing studies can be divided into those demonstrating that reef sharks reside inside reserves and those showing differences in reef shark relative abundance between reserves and fished sites. Juvenile Caribbean reef sharks (*Carcharhinus perezi*) in Brazil were more abundant inside than immediately outside a marine reserve at an oceanic archipelago (Garla et al. 2006b). Acoustic monitoring of several individuals revealed year round residency to small home ranges within the reserve, indicating that this protected area reduced the exposure of these individuals to fisheries (Garla et al. 2006a). On Australia’s Great Barrier Reef surveys of reef sharks (mainly grey reef, *C. amblyrhynchos* and whitetip reef, *Triaenodon obesus*) revealed higher relative abundance of sharks inside than outside parts of the reef that are zoned for no entry or no fishing (Meekan and Cappo 2004; Robbins et al. 2006; Heupel et al. 2009). Limited acoustic monitoring, however, suggests that long range movements between reefs and across marine reserve/fishing zones may be common at least among large juveniles and adults in some of these species (Heupel et al. 2010), which raises some questions about how marine reserves are contributing to the observed spatial abundance pattern. It is possible that juvenile site-fidelity is high enough to drive the observations of increased shark abundance in these areas, even though large juveniles and adults are vulnerable to fishing as they move between management zones (Heupel et al. 2010).

Marine reserves are increasingly being used for marine conservation in the Caribbean (Gibson et al. 2004; Newman et al. 2006), yet very little is known about the effectiveness of this strategy in conserving the regional shark fauna. A recent survey of recreational SCUBA divers in the Caribbean found that shark sightings are quite rare, except for some places that have shark conservation regulations or large marine reserves in place (Ward-Paige et al. 2010). Relatively few shark sightings occurred in the Mesoamerican Barrier Reef area of this survey (Ward-Paige et al. 2010) even though this region has a relatively large number of marine reserves. For example, Glover’s Reef atoll is a large, zoned marine reserve that has been protected since 1996 (Gibson et al. 2004). A stable catch-per-unit effort (CPUE) of Caribbean reef sharks was reported on research longlines set in Glover’s Reef Marine Reserve (GRMR) from 2001-2005, which suggests that reserve protection may be maintaining reef sharks in this location (Pikitch et al. 2005). Short term (150 day) acoustic monitoring of 4 individuals (2 adults, 2 juveniles) of this species showed they were residential to GRMR and could benefit from reserve protection over at least this time-scale (Chapman et al. 2005). However, one adult male made a short term (4 day) return movement between GRMR and a nearby fished atoll across 30 km of open water (Chapman et al. 2005), while several other adult individuals moved into deep water off the reef platform outside of the reserve boundary (Chapman et al. 2007). These telemetry studies suggest that movements outside of reserve boundaries might undermine reserve protection for this species, as has been suggested for congeners in the Indo-Pacific (Heupel et al. 2010). More information on shark movements and relative abundance in different management zones is needed to understand the extent to which marine reserves benefit Caribbean reef sharks and reef sharks in general.

Here we combined acoustic monitoring with baited remote underwater video (BRUV) to examine site-fidelity and relative abundance of Caribbean reef sharks in a marine reserve in Belize (GRMR). Given the hypothesis that Caribbean reef shark populations can benefit from no-take marine reserves and increase in abundance in these areas, we predicted that (1) acoustically tagged Caribbean reef sharks at GRMR would exhibit site-fidelity to the reserve and (2) the relative abundance of Caribbean reef sharks would be higher at GRMR (and other reserve reefs) when compared to fished reefs.

Methods

Study species

The Caribbean reef shark (*Carcharhinus perezi*) is a large requiem shark (growing to 295 cm total length) that is endemic to the Western Atlantic from Bermuda to southern Brazil (Compagno et al. 2005; Rosa 2006). It is the only carcharhinid in this region that completes its entire life cycle within coral reef ecosystems and is rarely found away from this type of habitat (Compagno et al. 2005; Rosa 2006). Caribbean reef sharks do not have geographically discrete nursery areas, instead all life-stages occur over the fore-reef, at depths of 10-30 m (Chapman et al. 2005; Compagno et al. 2005; Pikitch et al. 2005; Rosa 2006; Maljković and Côté 2011). Adults also frequently occur over the reef slope at depths of at least 352 m, especially during daylight hours (Chapman et al. 2007). Caribbean reef sharks frequent lagoons associated with coral reefs, but are not typically found in shallow seagrass or mangrove habitats within these lagoons (Pikitch et al. 2005; Rosa 2006). This species feeds on a wide variety of reef fish and is exploited by humans for the seafood trade (Rosa 2006). They are also one of the most common sharks observed by SCUBA divers, either naturally or under baited conditions, and are therefore important for the ecotourism industry in many countries (Rosa 2006; Maljković and Côté 2011). They are considered “Near Threatened” by the International Union for the Conservation of Nature, with a range-wide population trend listed as “Decreasing” (Rosa 2006). The IUCN assessors of the species indicate that it may meet the criteria for the more serious listing of “Vulnerable” as more fisheries and population trend data become available (Rosa 2006).

Primary study site

Glover’s Reef Marine Reserve (GRMR) encompasses Glover’s Reef Atoll (16°44’N, 87°48’W), which lies approximately 25 km to the east of the Mesoamerican Barrier Reef and 45 km east of mainland Belize (Figure 1). The atoll is approximately 30 km long and at the maximum 10 km wide. The atoll’s western reef crest lies submerged with the eastern reef crest being exposed and broken to produce five cuts, which allow shark movements between the ocean reef and lagoon ecosystems. The atoll also includes six sparsely populated cayes. The GRMR was established in 1997 and is comprised of a “no-take zone” on the interior, surrounded by a “general use zone” (32, 834 ha) which prohibits the use of gill-nets and longlines throughout the entire atoll out to the 180 m depth contour (Gibson 2003). This gear restriction essentially precludes a commercial shark fishery within GRMR, even though hook and line fishing is permitted in the general use zone. Reserve regulations are actively enforced by resident members of a permanent Government of Belize Department of Fisheries station located on Middle Caye.

Acoustic monitoring at GRMR

Caribbean reef sharks were collected using longlines and fitted with transmitters under permit from the Belize Department of Fisheries, see (Pikitch et al. 2005) and (Chapman et al. 2005) for description of capture and handling methods. All animal handling procedures were reviewed and approved by the Belize Ministry of Agriculture and Fisheries (Department of Fisheries) under a series of annual research permits issued from 2000 to the present (most recent #00005-11). We hereafter use total length (TL), the length from the tip of the snout to the tip of the tail as the standard measurement. Individually coded transmitters (V9 for small sharks < 110 cm TL, V16 for larger sharks; Vemco Ltd. Nova Scotia, Canada) that had previously been coated in beeswax to alleviate physical irritation and prevent an immunological reaction were implanted into the shark’s coelom. All transmitters emitted acoustic pulse trains with a semi-randomized signal delay for between 180-360 seconds. The individual was positioned upside-down until it entered a state of tonic immobility. The transmitter was then inserted through a ~ 5 cm incision made just anterior to the origin of one of the pelvic fins. Following implantation, the opening was closed with braided-nylon sutures. Upon completion of surgery the shark was rolled back over, the hook was entirely removed and the shark was released. Circle hooks were used to reduce instances of gut-hooking and the lines were checked frequently (every 90-180 minutes, depending on location) to minimize the physiological stress of capture. In May and October 2006 two adult female sharks (3291, 3292) were each fitted with an external V16 coded transmitter. The transmitter was anchored with a plastic umbrella dart in the shark's dorsal musculature just below the dorsal fin (Pflegler Institute for Environmental Research, CA, U.S.A.) and tethered with a 5 cm length of coated stainless steel wire. Externally mounted transmitters were fitted to these individuals instead of performing intracoelomic insertion because of inclement weather and rough sea conditions.

An array of 21 VR-2 receivers (Vemco Ltd., Nova Scotia, Canada) were anchored to the substrate in various locations at GRMR from May 2004 to May 2008 (Figure 1) to monitor shark presence or absence. Fifteen receivers were arranged in a roughly elliptical transect along the edge of the reef slope surrounding the entire atoll, at depths of 15-30 m. Receivers were attached with shackles and heavy duty plastic cable-ties to a length of polyurethane braided rope, anchored to the substrate by cement blocks chained together, and held upright in the water column by a subsurface float. The remaining receivers were positioned inside the atoll using a similar anchoring system at depths of 2.5-19 m. The position of each receiver was obtained using a hand-held Garmin GPS and plotted on an ArcGIS generated map of GRMR. Field testing indicated that the maximum detection range for these receivers was approximately 300 m (V9 transmitters) and 500 m (V16 transmitters) for units on the reef, and 200 m (V9) and 300 m (V16) for those inside the lagoon. Receivers were collected by SCUBA divers each May and October, their data downloaded and the units refurbished and returned to GRMR. Minor variations in the array configuration occurred between monitoring sessions due to occasional receiver malfunction and theft. We estimated total array coverage was ~ 6% of the reef platform. The array was not expected to provide continuous monitoring of shark movements, but rather to detect whether sharks were present at GRMR on any given day.

Acoustic monitoring analysis

Detections from all receivers were sorted by transmitter, date and receiver to generate a complete monitoring record for each individual implanted with an acoustic tag. We only used strings of two or more consecutive detections for downstream analysis to avoid using spurious detections that arise from signal collisions or background noise. Caribbean reef shark movements were visualized by plotting presence/absence data, gathered from receivers in the array, over a map of GRMR. Three metrics of shark presence and movement within the array were calculated to test the hypothesis that Caribbean reef sharks exhibit a high degree of site-fidelity to GRMR. The distance between the sharks original capture location and that of each receiver at which it was detected was measured using ArcGIS. This was used to calculate "minimum linear dispersal" (MLD) for each individual, defined as the distance between the two furthest receivers at which it was ever detected. "Monitoring duration" was defined as the number of days elapsed between the date of tagging and the date of the last detection string. Because there is some variation in how long transmitters continue to produce detectable signals after their battery expiry date, a standardized "residency index" (RI) was also calculated for all sharks. RI was defined as the total number of days the shark was detected within the array divided by the number of days it could possibly be detected assuming its transmitter worked only up until the expiry date. Any detections recorded for an individual shark that occurred after the estimated battery life expired were not used to calculate RI. Linear regression was used to test for the effect of increasing shark size (age) on RI and MLD.

If sharks exhibit fine-scale site-fidelity to certain parts of GRMR, then the number of detections on a monitor should decrease with distance from the shark's tagging location. The fraction of days each shark was detected by each monitor was modeled using a delta-lognormal approach (Lo et al. 1992), in which the probability of each reef shark being detected on at least one day during the study was modeled using a logit-link generalized linear mixed model (GLMM) appropriate for binomial (presence/absence) data (Venables and Ripley 2002) and the fraction of days observed if present was modeled as lognormal. Potential explanatory variables were: (1) the log of the distance from the shark's tagging location to the receiver; (2) the habitat type at the receiver (ocean reef [n=15], deep lagoon [n=3], or shallow lagoon [n=3]); (3) the individual receivers as random effects; (4) shark type (adult female, adult male, juvenile female or juvenile male); (5) the individual sharks (n=33, [one shark was never detected]) as a random effect; (6) the number of days the receiver was operational while the shark was tagged (a numerical variable with values 180, 360 and 540, used only for the presence/absence model); and (7) the interactions between ldist and habitat, monitor, shark or shark type. Explanatory variables were included in the model if they were significant, explained more than 2% of the variance, and improved either the Bayesian Information Criterion (BIC) or the Akaike's Information Criterion (AIC) of the model. Analyses were conducted in R, using the MASS and lme4 libraries (Venables and Ripley 2002; Bates et al. 2010; R Core Team 2010). The best model was used to predict whether each shark would be detected at each receiver by rounding the expected probability of detection to zero or one. The expected fraction of days with a detection for each shark × receiver combination was calculated as the probability of any detection from the binomial model multiplied by the expected fraction of days with a detection from the log normal model (Lo et al. 1992).

A logistic (logit-link) generalized linear model (GLM) was used to predict the presence or absence of each shark anywhere in the receiver array by calendar month. The potential explanatory variables were: (1) the month, counted from when the shark was tagged, as a numerical variable and (2) the transmitter type (12 month versus 18 month battery life). The AIC was used to find the best model. Although some sharks were detected after the end of the assumed battery life of their transmitter, only data from within the first 12 months for each individual was included in this analysis so that all sharks could be compared. The two externally tagged individuals (3291, 3292) were omitted from this analysis in order to maintain continuity.

Baited remote underwater video (BRUV)

Caribbean reef shark abundance was surveyed on the fore-reef at four sites (GRMR, [Figure 2] and three other sites, see next section) using baited remote underwater video (BRUV). BRUVs consist of a video camera (Sony Handycam DCR-HC52) inside an underwater housing that is mounted on a metal frame with a small, pre-weighed bait source (1 kg of crushed baitfish) mounted on a pole in the camera's field of view, see (Brooks et al. 2011) for more detail on BRUV design. Data from studies using BRUVs have previously been found to compare well with that obtained from underwater visual census techniques and from baited hook and lines methods for sampling relatively common species (Meekan and Cappo 2004; Watson et al. 2005;

Cappo et al. 2007; Harvey et al. 2007; Heagney et al. 2007; Malcolm et al. 2007; Stobart et al. 2007; Watson et al. 2007; Watson et al. 2009; Brooks et al. 2011). BRUV sampling locations were chosen for each site by using a random number generator to produce latitude and longitude points on the fore-reef of each site from a map constructed using ArcGIS software. BRUVs were then deployed in these randomly selected locations during daylight hours. Upon arrival at a sampling location, the vessel captain would find the closest suitable location for deployment (an area at a depth of 10-25 m and with bottom substrate flat enough to maximize line of sight). The BRUV was deployed from the boat using a rope and in-water personnel to guide it away from live coral and to orient the BRUV facing down current. The BRUV was left for at least 90 minutes, allowing it to film continuously for ~ 85 min after settling to the bottom. No BRUVs were simultaneously deployed within 1 km of another. Units were manually retrieved using the rope, which terminated in a small marker float to facilitate relocation. At both the start and end of each deployment environmental variables were measured including mid water current speed and direction (with a General Oceanics, Mechanical Flowmeter), bottom depth (Lowrance XD85), underwater visibility (secchi disc) and water temperature, salinity, pH and dissolved oxygen (YSI, R85-25). Post deployment, mini-DV cassettes were rendered to digital format and then viewed at normal play speed by one experienced observer (MB). Putative Caribbean reef shark observations were time-logged and then species identity was verified by a second experienced observer (DC). There are no other common carcharhinids likely to be mistaken for this species in the study area (Pikitch et al. 2005). All BRUV deployments were scored as “1” or “0”corresponding to Caribbean reef sharks being “present” or “absent” respectively. Additionally two estimates of the maximum number of Caribbean reef sharks observed per deployment were made: the maximum number Caribbean reef sharks observed in a single frame (Nmax) and the maximum number of individuals observed based on visually definitive differences in body size, sex or markings (Nmax-A).

Additional BRUV survey sites

BRUVs were also deployed at two fished sites and one additional reserve site in order to compare relative abundance between these reefs and GRMR (Figures 2 and 3). Caye Caulker (17°44’N, 88°1’W) lies 1.8 km to the west of the Mesoamerican Barrier Reef and approximately 20 km to the east of the Belizean mainland. It is a sandbar approximately 7.5 km in length and

1.1 km wide, lying over a limestone shelf. The Caye Caulker Marine Reserve (CCMR) was established in 1998 and is co-managed by the Forest and Marine Reserve Association of Caye Caulker (FAMRACC) and the Government of Belize Department of Fisheries. The CCMR is 1,545 hectares in size, extending 1.6 km beyond the barrier reef. A community-based management program works in concert with the Fisheries Department rangers that conduct all day patrols of the reserve. BRUVs were deployed at CCMR in the same way described previously for GRMR, along a ~ 10 km stretch of the fore-reef contained within the marine reserve. Turneffe Atoll ("TU"; 17°21'N, 87°51'W) lies approximately 12 km to the east of the Mesoamerican Barrier Reef and 43 km from mainland Belize. TU is approximately 42 km long and has a maximum width of 14 km. It includes 11 sandy cayes fringed by mangroves arranged around a central lagoon. The majority of the cayes are unpopulated however a few larger cayes, namely Blackbird and Laughing Bird Caye, accommodate dive-based and recreational fishing-based tourism resorts. TU is unique, as it is the only one of Belize's three atolls which is completely open to commercial fishing. BRUVs were deployed at TU in the same way described for the other sites, along ~ 23 km of the fore-reef on the southeast of the atoll. Southwater Caye (16°48'N, 88°04'W; SWC) lies on the Mesoamerican Barrier Reef, approximately 19 km to the east of mainland Belize. SWC is a sand island approximately 610 m long and a maximum of 200 m wide, which accommodates two small tourist resorts and a research station. Given its close proximity to the mainland and more densely populated islands it has been subjected to heavy exploitation from commercial fisherman. In 2010 SWC became a marine reserve and active enforcement of the reserve by Fisheries officers began in early 2011. Because enforcement was initiated after we completed sampling, we consider it a fished site for this study. BRUVs were deployed in the same way described for the other sites, across ~ 28 km of the fore-reef. Caribbean reef sharks are present in all 4 study sites and are exploited at TU and SWC by fishermen, who deploy large monofilament gillnets and longlines to target sharks for their fins and meat (D. Chapman unpubl. data).

BRUV analysis

Analysis of presence and absence data was performed by fitting a logitlink GLM. The R software was used with the MASS4 library (Venables and Ripley 2002; R Core Team 2010).

The GLM was used to examine the effects of reserve versus non-reserve, location nested within reserve or non-reserve, flow velocity and water temperature on reef shark abundance.

Results

Acoustic monitoring at GRMR

A total of 34 Caribbean reef sharks were captured and fitted with transmitters (32 internal, 2 external; Table 1) of which 21 were females and 13 were males. Individuals ranged in length from 66 to 214 cm (mean=134.1 cm, std.dev.= 39 cm). Two (9.5 %) of the females were judged to be sexually mature on the basis of their size, according to published sizes at maturity for this species (Compagno et al. 2005). Six males (47 %) were judged to be sexually mature based on the presence of large, calcified claspers that freely rotated at the base. Sharks were captured throughout GRMR but the most productive fishing and tagging areas were the reef and lagoon area of the southern entrance to the atoll (receivers 1-4, Figure 1; 15 sharks tagged), the fore-reef and reef slope outside Middle Caye (receivers 6 and 25; 8 sharks tagged) and the fore-reef and reef slope outside Middle and Northeast Cayes (“The Elbow”, receivers 10, 21, 22, 23; 7 sharks tagged). The remaining sharks were captured in other parts of the lagoon (Figure 1). Shark capture data (date of capture, sex, size, transmitter type and subsequent monitoring data) are shown in Table 1. There were 14 sharks tagged with smaller V9 tags (estimated 365 day battery life), while 20 sharks were tagged with larger V16 tags (estimated 540 day battery life). We purposefully put the larger tags in larger sharks because of concerns that small sharks might be adversely affected by the V16 transmitters. As a result, the 14 V9 tagged sharks were smaller than the 20 V16 tagged sharks (Table 1).

All but one of the tagged Caribbean reef sharks were detected after release. Single, isolated detections were excluded from the analysis due to the possibility of them being spurious detections. It was highly unusual for more than two individuals to be detected simultaneously on the same receiver. When three individuals were detected simultaneously at a receiver we attempted to verify that detections of the third individual were not an artifact of signal collisions between the other two, which we reasoned would consist of detection strings with an unusually long lag time between detections given the transmission rate of the transmitter. No detection strings met this criterion. Both the total number of days that each individual was detected and the monitoring duration within the array was related to the tag type. For sharks tagged with V9 transmitters (N=14), the number of days detected ranged from 17-361 days (mean=195 days, std. dev.= 109 days; Table 1) and the monitoring duration ranged from 147-468 days (mean=351 days, std. dev.= 84 days; Table 1). For sharks tagged with V16 transmitters and tracked until the battery life expired (N=14; 5 V16-tagged sharks were tracked for ~ 65% of potential tag battery life because they were tagged late in the study), the number of days detected ranged from 5 to 534 days (mean=178 days, std. dev.= 163 days; Table 1) and the monitoring duration ranged from 65-585 days (mean=382.1 days, std. dev.= 171 days; Table 1). Mean Residency Index (RI) among Caribbean reef sharks was 0.43 (i.e., the "average" shark was detected on 43% of the days it had a functional transmitter and there were receivers in the water, std. dev. = 0.3) and ranged from 0.01 to 0.99. (0.53 and 0.36 were the means for V9 and V16 transmitters respectively; Table 1, Figure 4). RI was higher among sharks tagged off on the fore-reef off Middle Caye and the Elbow (mean RI=0.53 and 0.65 respectively) compared to sharks tagged in the southern entrance and lagoon (mean RI=0.33). Most sharks (20, 64%) were detected on at least one day during every month of the year and all but three were detected for six months or more (Figure 5). Individual Minimum Linear Dispersal (MLD) ranged from 1.25-36.4 km (mean 9.4 km, std. dev. 6.3 km for V9; mean 19.3 km, std. dev. 12.6 km for V16, Table 1). Neither RI nor MLD was significantly correlated with shark body size (r^2= 0.02 and 0.04 for RI and MLD respectively).

All Caribbean reef sharks were primarily detected on one or a small number of receivers (Figure 6). The most important factor influencing whether a Caribbean reef shark was ever recorded at a receiver was the log distance between the receiver and the shark's original tagging location; this factor alone explained 23% of the deviance in the presence/absence data (Table 2). There were also significant effects of the number of days sampled and habitat, as well as significant variation between individual sharks. The best mixed effects model according to the AIC (Table 3) allowed the effect of shark length to vary by individual shark. The AIC best fit model correctly predicted the presence or absence of individual reef sharks 83% of the time (Table 4). A model in which days sampled and log distance were the only explanatory variables correctly predicted individual reef shark presence or absence 81% of the time (Table 5). The fraction of days with a detection (given any detection) declined significantly with distance

(Tables 6, 7 and Figure 7). The AIC best fit model included only log distance and habitat, and their interaction. Because the log-distance habitat interaction was not significant we excluded it from further consideration. Log distance explained 36% of the total deviance. Because the expected probability of detection varied by shark type and other factors, there was considerable variability in the expected number of days observed (= expected probability of detection times expected number of days detected divided by the number of days sampled, Figure 7). Nevertheless, for all combinations of the explanatory variables, the fraction of days with detections from individual sharks was expected to be less than 10% for distances from the original tagging site greater than 1 km.

The predicted probability of presence (detection) of sharks by calendar month from the AIC best model, showed that the probability of a shark being detected remains quite high throughout the first 12 months (Figure 8). Although calendar month and tag type (12 month versus 18 month battery life) both had a significant influence over the probability of presence they explained little of the variance (Table 8). The logistic regression predicts that 70-80% of sharks are still within the array, one year after tagging.

Baited remote underwater video at 4 sites

A total of 200 BRUV deployments were made, divided equally across the 4 study sites (total duration of 17,200 minutes). The deployments were made between June 11-19 2009 (BRUV's, n=44) and May 6-12 2010 (n=6) at GRMR, with Caribbean reef sharks observed on 16 BRUVs (32% of deployments at this site), 6 of which recorded 2 individuals. At TU deployments occurred between June 21-26 2009 (n=50), with a shark observed on 6 BRUVs (12% of deployments). Deployments occurred at SWC between July 3-8 2009 (BRUV's n=39) and May 23-29 2010 (n=11), with a shark observed on 2 BRUVs, (4% of deployments). CCMR deployments occurred between 30 June-2 July 2009 (BRUVs, n=21) and 18-20 May 2010 (BRUVs, n=29) with Caribbean reef sharks being recorded on 13 BRUVs (26% of all deployments), with 6 of these recording 2 individuals in frame at once (3 in 2009 and 3 in 2010). Overall, 35 of the 200 BRUV deployments (17.5 % of total number of deployments) recorded at least one Caribbean reef shark. All but 8 of these were in marine reserves, with at least 10 of the marine reserve deployments recording at least 2 different individuals (Figure 9). As a result, whether or not the BRUV was deployed within a marine reserve had a significant impact on reef shark presence in the GLM (Table 9). There was no difference between the two reserve sites or between the two non-reserve sites in reef shark presence/absence.

The two reserve sites (GRMR and CCMR) had smaller total areas when compared to that of the two fished sites (TU and SWC). To ensure that the observed increase in relative abundance of reef sharks at the reserve sites was not biased by a greater proportion of the available habitat being sampled, the number of samples was proportionately reduced by bootstrapping according to total area, to attain an equal sample density per site. The reduced numbers of BRUVs per site of TU (n=50), SWC (n=30), GRMR (n= 25) and CCMR (n=25) were randomly subsampled from the complete data set, using the R software (R CoreTeam 2010).The application of GLM to 2000 bootstrapped subsamples found the marine reserve factor to still be the significant ($p<0.05$) influence on the presence or absence of reef sharks in 84% of the samples. Therefore the difference in size between the reserve and fished sites caused no bias as to the significance of the marine reserve factor and its influence on reef shark relative abundance.

There were also no significant differences in flow, salinity, depth or visibility between locations (ANOVA, $P > 0.05$). Temperature and DO were both significantly higher at SWC than at any of the other three locations (Tukey HSD, $p<0.001$). Because the vast majority of BRUV deployments in the non-reserve sites resulted in zero reef sharks being observed it was not possible to evaluate the impact of environmental variables and reserve versus non-reserve effects in the same model. For the subset of data from marine reserves, there were no significant effects of site, location, water temperature or flow velocity (Table 10). However, there was a significant interaction between flow velocity and location.

Discussion

We tested the hypothesis that Caribbean reef sharks are to benefit from the local respite from fishing occurring within marine reserves by examining two of its key predictions. The first prediction is that Caribbean reef sharks exhibit high site-fidelity to reserve areas. Acoustic monitoring showed that most individuals exhibit a high degree of site-fidelity at GRMR. The mean residency index (RI) indicated that the average shark was detected nearly one out of every two days at GRMR. Notably, RI is a conservative metric considering that receiver array coverage was modest (~6 % of the reef platform). We found that sharks tagged on the fore reef typically had higher RI and were less likely to be lost from the array than lagoon-tagged sharks. These observations most likely reflect differences in receiver coverage and effectiveness between these two reef habitats. Not only were more receivers deployed on the fore-reef, this habitat is so narrow, usually < 500 m, that a line of receivers deployed along the reef slope is likely to regularly detect passing sharks. Reef sharks in the lagoon can swim in most directions and may not necessarily swim close to an isolated receiver despite being close to it. Transmitters are also more likely to be detected in the fore-reef because seafloor relief is low relative to water depth, whereas receivers in the lagoon are partially blocked by emergent patch reefs. Notwithstanding the limitations of acoustic monitoring in the lagoon we found that the probability of detecting tagged sharks by calendar month was high throughout the year following transmitter application. This indicates that sharks were typically year-round residents of GRMR as opposed to being seasonal immigrants. Several large individuals tagged with 18 month (i.e., V16) transmitters were also generally detected right up until or slightly beyond projected transmitter battery life, indicating use of the atoll across successive years. Despite reasonably high RI for many individuals it is important to highlight that these sharks are capable of long range movements over short time periods (days, Chapman et al. 2007). Many individual sharks were sporadically absent from the receiver array, which leaves open the possibility that most sharks occasionally depart GRMR for short periods and may be exposed to fisheries during these movements.

Individual Caribbean reef sharks were mainly detected on a localized subset of receivers within the GRMR array. GLMM analysis indicated that a receiver's distance from the shark's original capture location was an important factor in determining the probability of shark detection. It therefore appears that we typically captured Caribbean reef sharks in an area that they regularly used after release, suggesting that they are a home ranging species (i.e., they regularly use a fraction of the available habitat, rather than moving throughout, Burt 1943). Home ranging behavior has been suggested for several tropical carcharhinid sharks, especially species that live on coral reefs, including Caribbean reef sharks in Brazil (McKibben and Nelson 1986; Garla et al. 2006b; Dale et al. 2010; Heupel et al. 2010; Papastamatiou et al. 2010; Field et al. 2011).

Sharks often exhibit an ontogenetic expansion of home range size (Grubbs 2010) and we would expect a positive correlation between MLD and shark size if this is true for Caribbean reef sharks. Moreover, we would predict a negative correlation between RI and size if large sharks leave GRMR more than small ones. None of the metrics we were able to calculate from monitoring data, however, demonstrated a significant correlation with body size. We suggest that it is still reasonable to hypothesize that large juvenile and adult Caribbean reef sharks have larger home ranges than small juveniles and we recommend that active telemetry tracking should be used to generate activity space metrics (e.g. estimated home range size) that could be more readily compared between individuals than the coarse acoustic monitoring data we collected. This type of information is necessary to make more refined predictions about how different life-stages will respond to different sized marine reserves.

Is the high fidelity of Caribbean reef sharks to GRMR largely driven by the isolation of this reef platform? Large individuals of this species monitored and tracked at GRMR moved across pelagic habitat (Chapman et al. 2005) and dove to depths of at least 352 m (Chapman et al. 2007). These observations suggest that deep, open water separating GRMR from the barrier reef and other atolls is not an insurmountable barrier to dispersal that forces high site-fidelity. We hypothesize that this species may naturally exhibit high site- fidelity, with the degree of fidelity possibly a function of the reef's isolation, climate and carrying capacity. For example, sharks may move between proximate reefs; they may migrate at higher latitude reefs in response to seasonal temperature changes and they may be more likely to emigrate from a reef as competitor density increases or prey availability decreases. Caribbean reef sharks could be acoustically monitored at reefs of different levels of isolation, latitude and prey abundance to further test these hypotheses.

The second prediction of our main hypothesis is that the relative abundance of Caribbean reef sharks is higher in reserves than similar fished reefs. The factor “marine reserve” was the most important predictor of shark presence or absence on BRUVs in the GLM, which is consistent with this prediction. This analysis assumes that random sampling of each site conducted over a few days is representative of relative abundance throughout the year. We suggest that is a reasonable assumption given the high degree of site-fidelity we observed at GRMR using acoustic monitoring and results from other telemetry studies of this species (Garla et al. 2006b; Garla et al. 2006a; Maljković and Côté 2011). Nearly four times as many BRUVs deployed in marine reserves recorded Caribbean reef sharks than on fished reefs. Several of the reserve-deployed BRUVs also recorded 2 individuals, either in frame at once or identified by visually obvious differences in size or markings. Our results are consistent with studies of reef sharks in Brazil and Australia that also show higher relative abundance in reserves (Meekan and Cappo 2004; Garla et al. 2006b; Robbins et al. 2006; Heupel et al. 2010).

The issue of a potential bias arising from the difference in the total area of the reserve sites versus the fished sites was addressed by performing bootstrap sampling techniques. The fished sites had a larger total area to be sampled which could have led to pockets of higher shark abundance being under-sampled due to a lower sample density, when compared to the reserve sites. However, by analyzing reduced sample sizes to correct for the difference in sample density the result was found to be the same, with marine reserve being the only significant predictor of reef shark presence.

The data do not support the competing hypothesis that reef shark relative abundance was primarily driven by environmental variation between sites. Most environmental parameters were not significantly different between the sites and none were consistently different between the reserves and non-reserve sites. All BRUV deployments occurred on ocean-facing fore-reef habitat within 1 km of the reef slope, which is typical habitat for the species (Chapman et al. 2005; Compagno et al. 2005; Pikitch et al. 2005; Tavares 2009). Our data indicates that Caribbean reef shark abundance better tracks the level of local fishing pressure than any of the environmental factors we examined.

Our combined telemetry and survey results support the hypothesis that marine reserves can have a positive effect on the local abundance of reef sharks or at least significantly reduce rates of population decline relative to fished ones. Time series of shark abundance inside marine reserves (e.g., Pikitch et al. 2005, which confirms that GRMR had a stable Caribbean reef shark catch per unit effort from 2001-2005) are needed to determine whether high relative abundance also means that populations are stable or increasing. Given growing support for the hypothesis that reef sharks are more abundant inside marine reserves, it is reasonable to speculate about the potential enhancement mechanisms involved. Potential mechanisms include "direct enhancement", where highly residential reef sharks increase inside marine reserves due to a local respite in fishing pressure on them. A second potential mechanism is "increased prey", in which reserve areas support larger reef shark populations because reserves provide a local respite in fishing pressure for the sharks' prey. Studies that monitor changes in reef shark populations and their prey communities before and after reserve establishment are needed to study the relative importance of these enhancement mechanisms.

In conclusion, our telemetry and BRUV survey results support the hypothesis that Caribbean reef shark populations can benefit from the local respite from fishing pressure provided by marine reserves. Of course, reserve size, placement and compliance will influence whether or not these benefits materialize. We suggest "direct enhancement" and "increased prey" as potential enhancement mechanisms. Our study also underscores that Caribbean reef shark abundance on some fished parts of the Mesoamerican Barrier Reef is relatively low, which is concerning from the perspectives of fisheries sustainability and ecotourism. Although the ecological role of reef sharks is not well studied, it is possible that the local reduction in these upper level predators has significant effects on the coral reef ecosystem (e.g. Bascompte et al. 2005). Our study and others show that marine reserves have an increasingly clear role in the conservation of reef sharks. We suggest that reserves-or larger scale area closures- should be considered as an important tool to preserve the ecological and economic roles of reef sharks in increasingly imperiled Caribbean coral reef ecosystems.

Figures and Tables

Table. 1. Caribbean reef sharks tagged with acoustic transmitters at GRMR. ID = transmitter identity (bolded are V9 tags); T Date = tagging date; TL= total length; Location = Tagging location (see Figure 1); N Days= total number of days with a detection anywhere within the array; DUR= duration between date of tagging and last day detected; MLD=minimum liner dispersal or distance between two furthest receivers with detections; RI=residency index. *= shark externally rather than internally tagged.

ID	T Date	Sex	TL (cm)	Location	N days	DUR (days)	MLD (km)	RI
18	5/3/2007	F	110	MC	259	359	17.92	0.71
19	5/9/2007	F	119	MC	361	356	5.44	0.99
20	5/1/2007	M	96	EL	111	362	5.44	0.30
21	5/2/2007	M	119	EL	320	360	17.92	0.88
22	5/3/2007	F	66	MC	84	229	13.74	0.23
23	5/16/2007	F	91	EL	270	348	5.44	0.74
223	5/3/2007	F	90	MC	229	359	17.92	0.63
234	5/9/2006	F	80	SE	348	355	9.15	0.95
235	5/25/2006	F	90	EL	204	321	1.25	0.56
236	5/12/2006	M	135	SE	134	426	17.92	0.37
237	5/25/2006	F	85	MC	43	468	4.33	0.12
238	5/3/2006	M	101	SE	198	362	8.44	0.54
239	5/6/2006	M	86	EL	161	475	1.25	0.44
240	5/1/2006	F	120	SE	17	147	5.8	0.05
3291*	5/1/2006	F	214	LAG	24	87	5.56	0.04
3292*	10/10/2006	F	214	EL	215	220	11.56	0.39
3346	8/15/2006	F	135	SE	86	274	24.21	0.16
3348	5/5/2004	M	188	MC	386	484	36.46	0.71
3349	5/12/2004	F	134	SE	76	327	36.36	0.14
3372	5/12/2006	F	136	SE	69	358	7.61	0.13
3373	7/2/2005	F	110	SE	170	424	36.46	0.31
3374	5/28/2005	F	142	SE	50	175	3.14	0.09
3376	10/13/2006	F	176	SE	146	303	8.53	0.27
3378	5/11/2007	F	156	NLAG	17	65	26.59	0.05
3379	5/9/2006	M	166	SE	136	466	13.11	0.25
3383	5/31/2005	F	124	SE	143	510	36.46	0.26
3391	5/24/2005	M	167	EL	534	585	20.81	0.98
3340	5/6/2004	M	197	SE	403	534	28.5	0.74
3393	5/6/2004	M	117	SE	458	506	28.5	0.84
4603	12/18/2007	M	176	MC	47	134	4.66	0.31
4604	5/21/2007	F	122	WLAG	5	44	1.25	0.01
4607	5/7/2007	M	151	MC	189	184	23.92	0.52
4608	5/7/2007	M	183	SE	236	359	13.58	0.65

Table. 2. Analysis of deviance for the AIC best model of presence or absence of Caribbean reef sharks by receiver with fixed effects only. “Days” refers to days sampled by each receiver; “ldist” is log(distance).

	Df	Deviance	Resid. Df	Resid. Dev	P(Chi)	Percent deviance
NULL			591	700.62		
days	1	18.06	590	682.55	0.00	0.03
ldist	1	158.43	589	524.13	0.00	0.23
shark type	3	6.13	586	517.99	0.11	0.01
habitat	2	7.05	584	510.94	0.03	0.01

Table. 3. The AIC and BIC values for models with random effects (in bold). "Days" refers to days sampled by each receiver; "ldist" is log(distance).

Model	AIC	BIC	deviance	delta.BIC	delta.AIC
days+ldist+habitat	526.71	548.63	516.71	14.43	17.65
days+ldist+habitat+shark	513.56	539.86	501.56	5.65	4.49
days+ldist+monitor	529.87	547.41	521.87	13.20	20.81
days+ldist+monitor+shark	515.61	537.52	505.61	3.32	6.54
days+ldist+habitat+ldist x shark*	**509.06**	**544.13**	493.06	9.93	0.00
days+ldist+ldist x monitor	**530.77**	**557.07**	518.77	22.86	21.70
days+ldist+ldist x monitor+ldist x shark	**513.96**	**553.42**	495.96	19.21	4.90
days+ldist	529.62	542.77	523.62	8.56	20.55
days+ldist+shark	516.67	534.20	508.67	0.00	7.61
days+ldist+ldist x shark	**519.46**	**545.76**	507.46	11.56	10.40

Table. 4. Observed and predicted presence or absence of Caribbean reef sharks based on the AIC best model (days+ldist+habitat+ldist x shark**)**.

	Observed	
Predicted	absent	present
absent	416	92
present	11	73

Table. 5. Observed and predicted presence or absence of Caribbean reef sharks based on a model including only log-distance and days sampled.

	Observed	
Predicted	absent	present
absent	403	90
present	24	75

Table. 6. AIC best model of log of days with a detection for the fraction of days each shark was observed at each monitor.

	Df	Deviance	Resid. Df	Resid. Dev	F	Pr(>F)	Percent deviance
NULL			164	582.06			
ldist	1	211.33	163	370.73	101.87	0	0.36
habitat	2	28.31	161	342.42	6.82	0.001	0.05
ldist:habitat	2	12.57	159	329.85	3.03	0.051	0.02

Table. 7. AIC best model of log of days with a detection for models with additional random effects.

Model	AIC	BIC	deviance	delta.BIC	delta.AIC
ldist	607.82	617.14	370.73	2.89	11.28
ldist+habitat	598.72	614.25	342.42	0.00	2.17
ldist+habitat+ldist:habitat	596.54	618.29	329.85	4.04	0.00
ldist+habitat+ldist:habitat+**shark**	603.43	628.28	582.54	14.03	6.89
ldist+habitat+ldist:habitat+**monitor**	602.61	627.46	582.73	13.21	6.06
ldist+habitat+ldist:habitat+**monitor**+**shark**	604.61	632.56	582.73	18.32	8.06
ldist+habitat+ldist:habitat+**ldist x monitor**	602.50	627.35	582.48	13.11	5.96
ldist+habitat+ldist:habitat+**ldist x monitor**+**ldist x shark**	604.42	632.37	582.54	18.12	7.87

Table. 8. The AIC best model for the probability of presence, for the duration since tagged (month) and the battery life of the tag.

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)	Deviance
NULL			364	345.03		
sharkmonth	1	14.008	363	331.02	0.000182	0.04
taglife	1	7.226	362	323.79	0.007185	0.02
---						0.06

Table. 9. The GLM analysis on the influence of the conservation boundaries (reserve and non-reserve) and location, on reef shark presence or absence derived from BRUV deployments.

	Df	Deviance	Resid. Df	Resid. Dev	P(>|Chi|)	percent.deviance
NULL			119	108.135		
Reserve	1	9.063	117	98.636	0.002	0.085
Reserve:Location	2	2.7504	115	95.885	0.253	0.023

Table. 10. The GLM analysis on the influence of the environmental parameters (flow velocity and water temperature) on reef shark presence or absence derived from BRUV deployments within the marine reserve sites.

	Df	Deviance	Resid. Df	Resid. Dev	P(>|Chi|)	percent.deviance
NULL			59	69.590		
Location	1	1.375	58	68.215	0.241	0.020
flow	1	2.059	57	66.156	0.151	0.030
start.temp	1	0.149	56	66.007	0.700	0.002
Location:flow	1	7.955	55	58.053	0.005	0.114
Location:start.temp	1	0.664	54	57.389	0.415	0.010
flow:start.temp	1	1.105	53	56.283	0.293	0.016

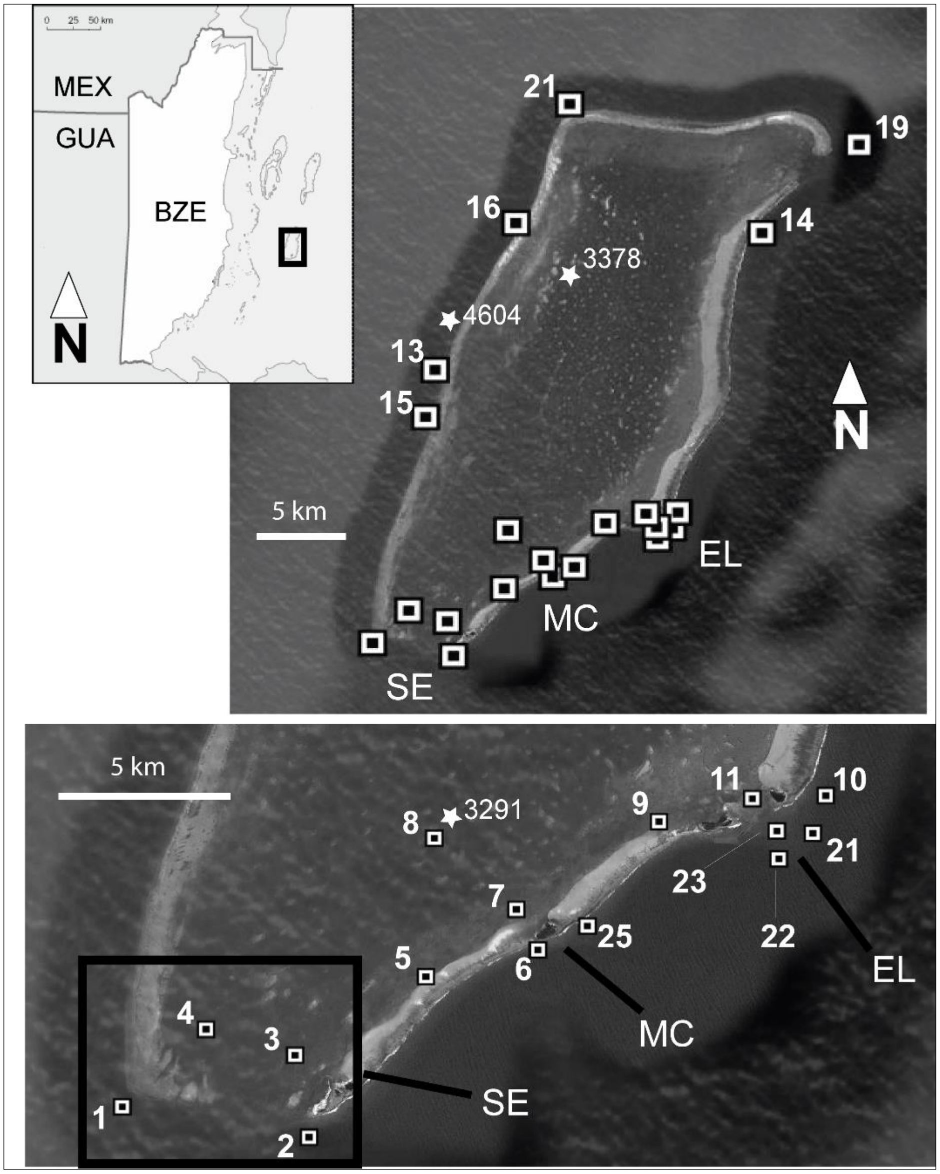


Figure. 1. Top left-Belize (BZE) and surrounding nations (MEX=Mexico, GUA=Guatemala). Box contains Glover’s Reef Marine Reserve (GRMR). Top right: GRMR showing the location of all receivers (black and white squares). The three primary locations where sharks were tagged are labeled “EL”= Elbow, “MC”=Middle Caye and “SE” = Southern Entrance (see Table 1). Tagging locations for other sharks are denoted by their stars and their tag identification number (see Table 1 for capture and biological information). Bottom: Southern part of the atoll showing more detail of receiver locations.

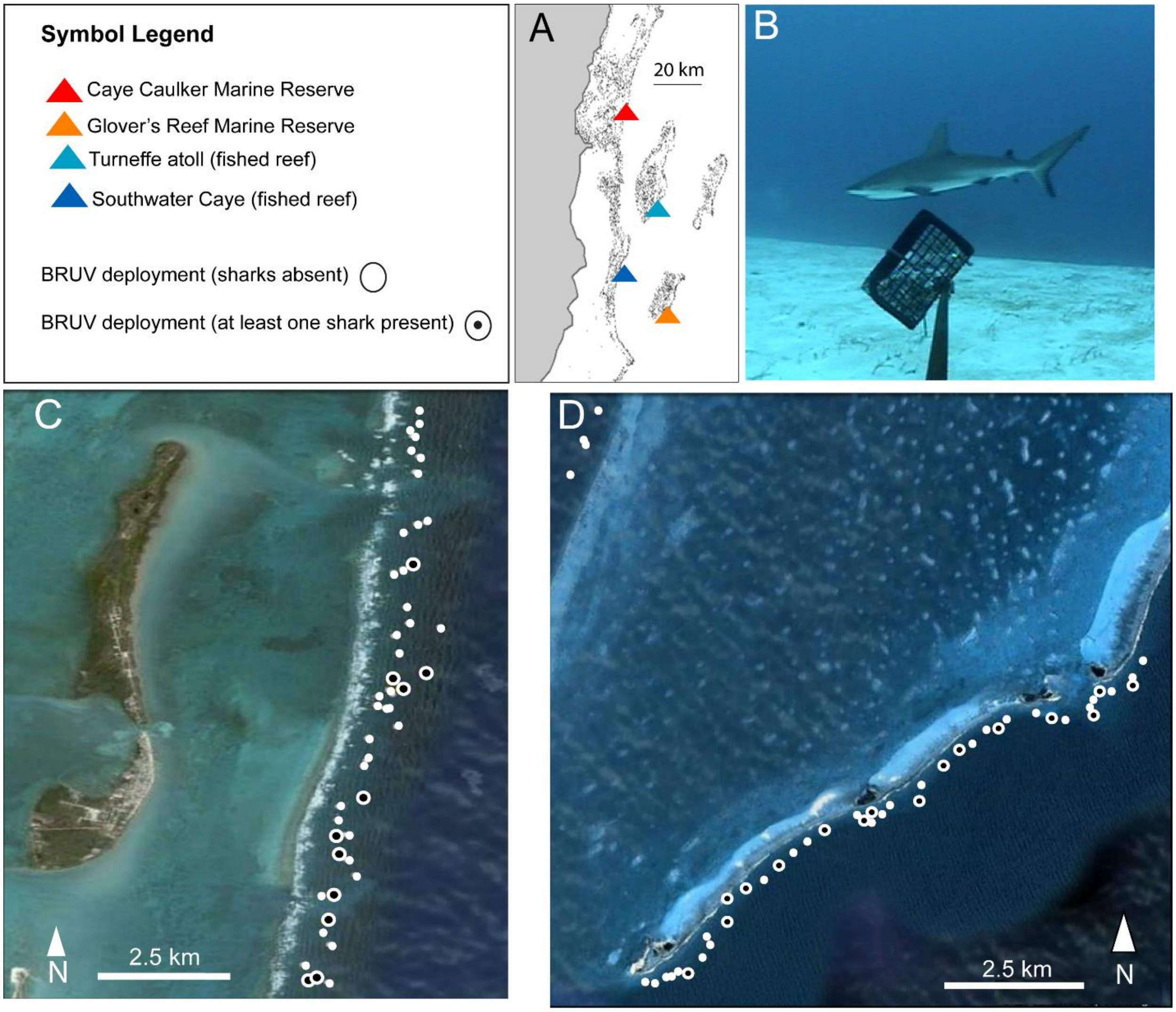


Figure. 2. Deployment locations for Baited Remote Underwater Video surveys. (A) Location of the four study sites along the Belizean coast and Mesoamerican barrier reef: Caye Caulker Marine Reserve (CCMR), Turneffe atoll (TU), Southwater Caye (SWC) and Glover's Reef Marine Reserve (GRMR). (B) Still image captured from a BRUV deployment at GRMR with a Caribbean reef shark in frame. (C) Position of BRUV deployments (see symbol legend) at GRMR. (D). Position of BRUV deployments at CCMR.

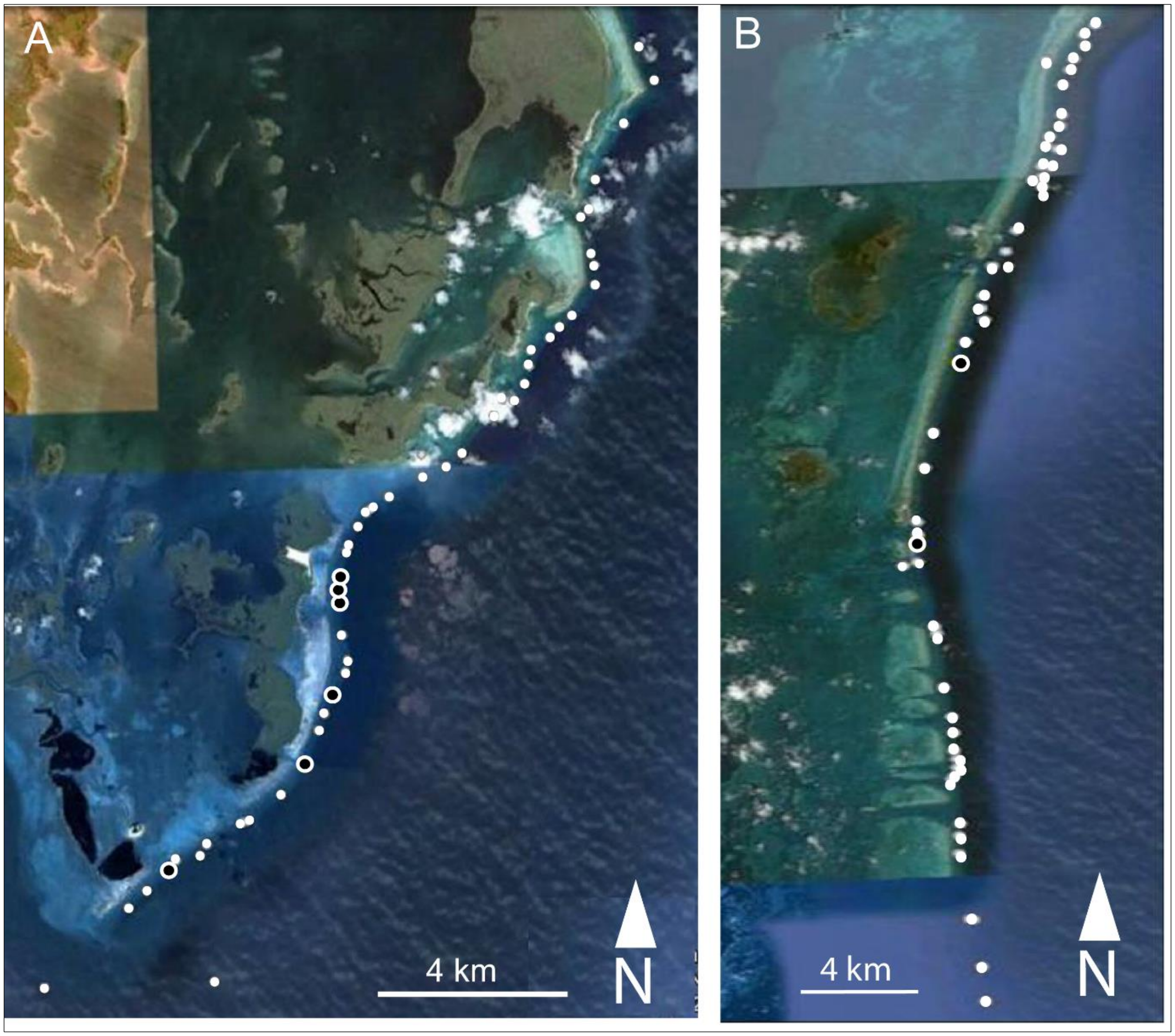


Figure 3: The satellite images show the location of the BRUV deployments (see symbol legend, figure 2) made at the two fished sites: A. Turneffe atoll (TU). B. Southwater Caye (SWC).

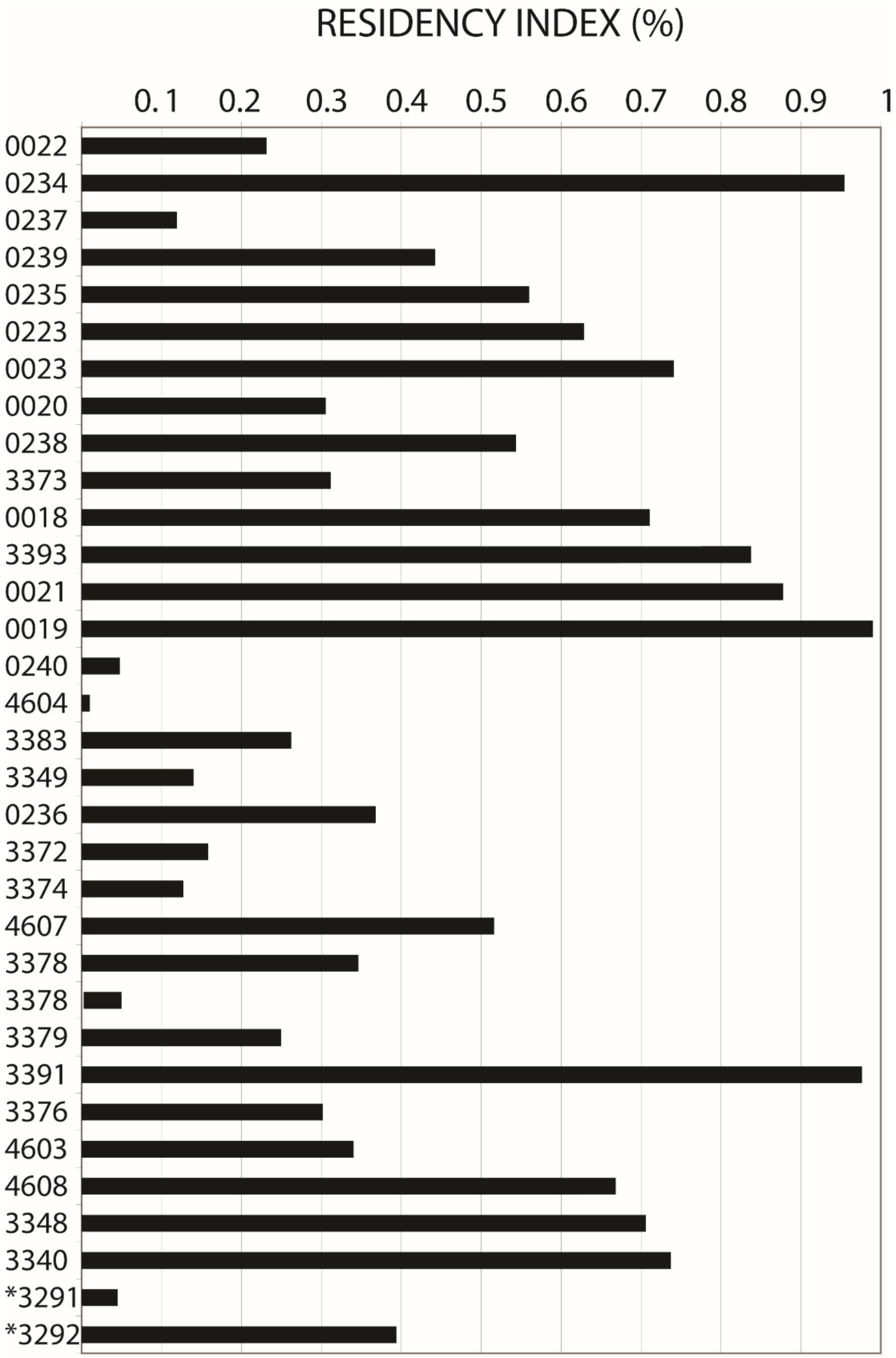


Figure 4: Residency index (RI) of Caribbean reef sharks tagged at GRMR with acoustic transmitters. Individual sharks are denoted by their transmitter code (see Table 1) and are arranged by increasing body size from top to bottom. (*) indicates the shark was fitted with an external transmitter as opposed to having one implanted into its coelom.

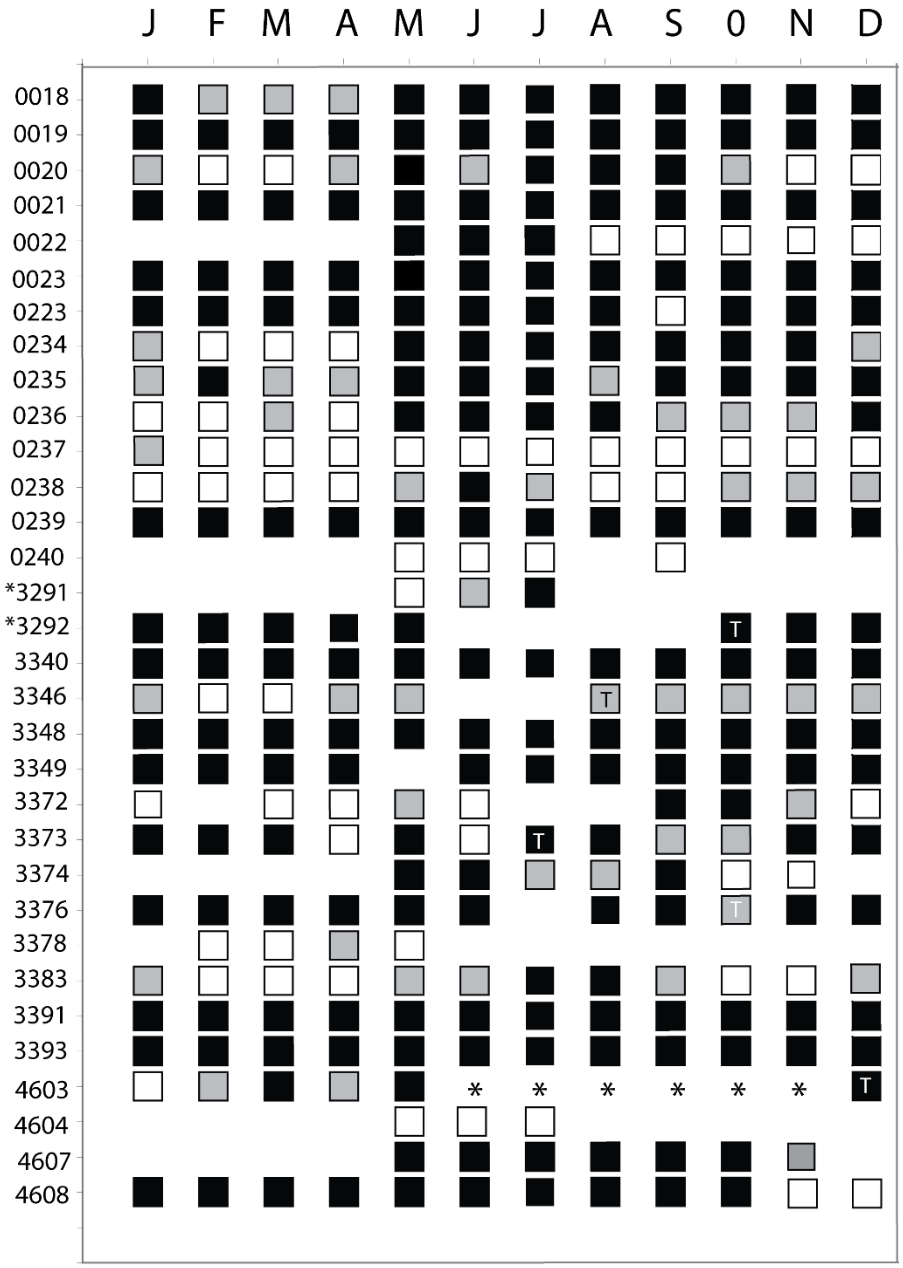


Figure. 5. Monthly occurrence of each of the tagged sharks at GRMR. A square denotes that the individual (transmitter code on y-axis) was detected on at least one day during the given month. The color of the squares indicates the number of days that month that the individual was detected with the scale of white (1-7 days), grey (8-14 days) and black (>14 days). The values are total number of days per month not consecutive days. "T" denotes the tagging time of sharks not tagged in the month of May or June and "*" refers to a break in receiver coverage due to refurbishment or damage.

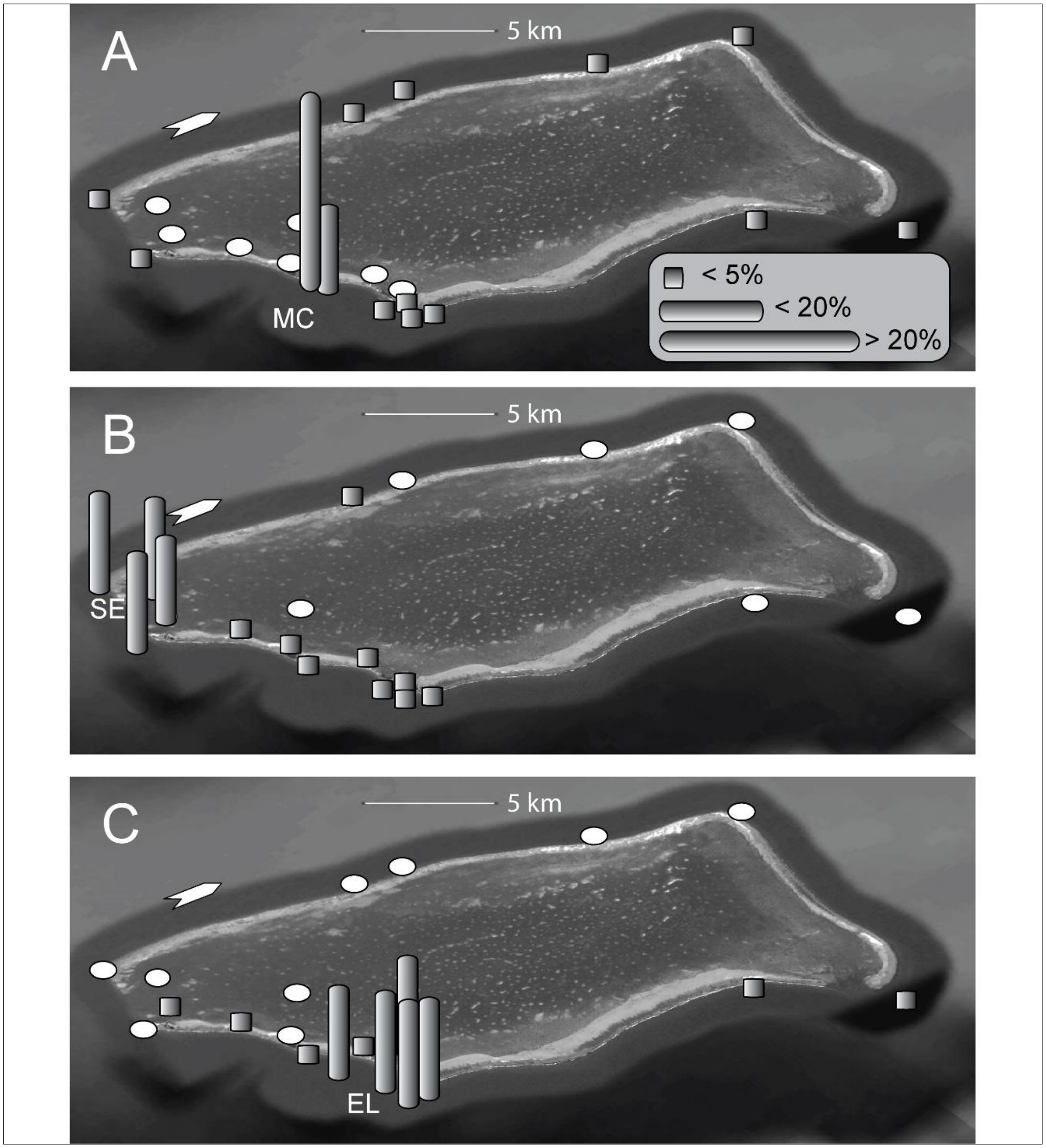


Figure 6: Examples of fidelity of Caribbean reef sharks to sites monitored by receivers. The number of days that all sharks tagged in the specified location were detected anywhere in the array were pooled and then apportioned to receivers. A-Pooled detection days of sharks tagged at Middle Cay (MC); B-Pooled detection days from sharks tagged at Southern Entrance (SE) and C- Pooled detection days of sharks tagged at the Elbow (EL).The height of the bar over each receiver designates the percentage of the pooled days with detections that occurred on that receiver. North is indicated by the arrow. Flat circles show receivers with no detections for any sharks tagged in the specific location.

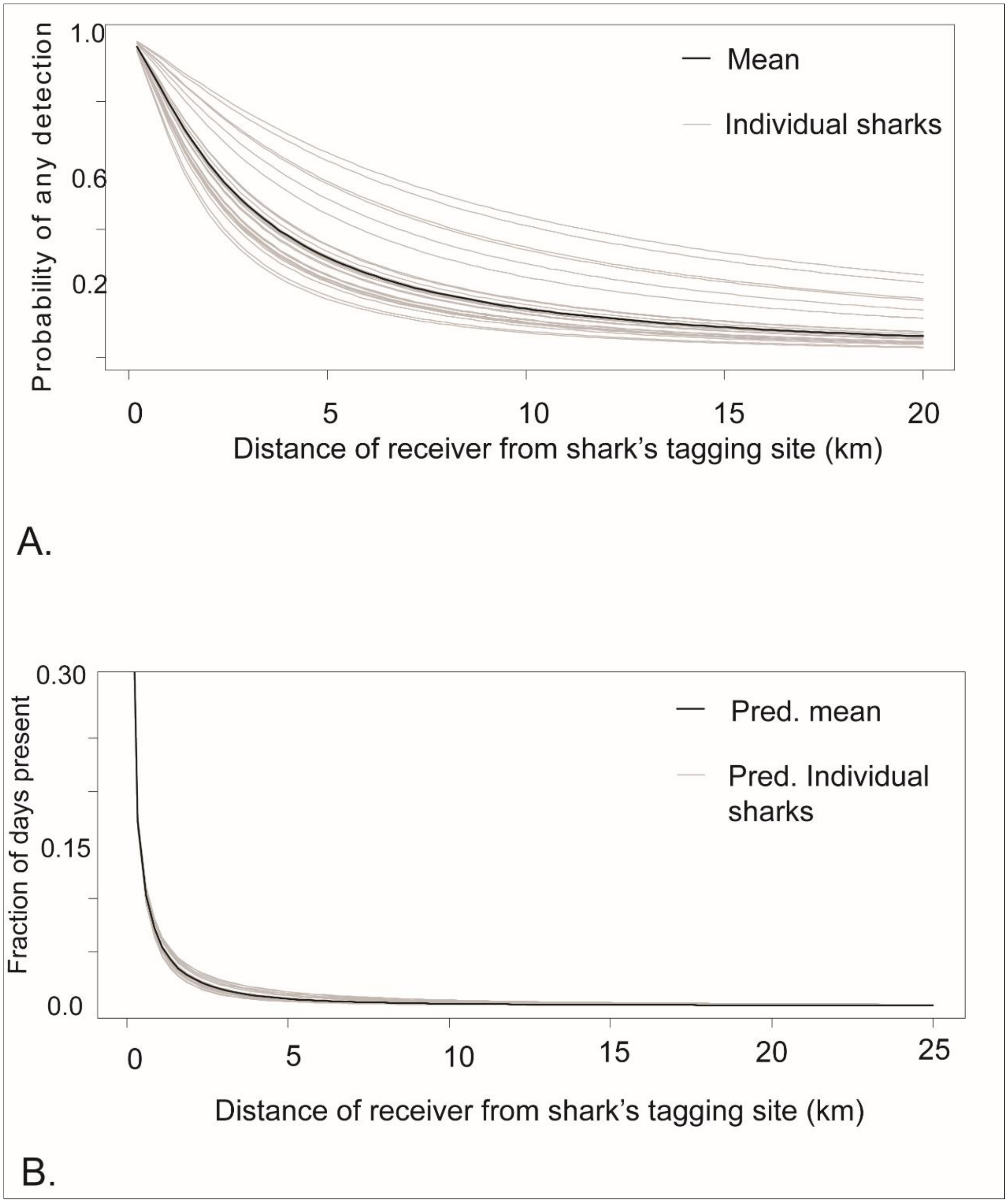


Figure 7: The influence of distance of receiver from sharks tagging site on the probability of detection and number of days detected. **(**a) Probability of detection from the AIC best model of presence/absence, for a receiver operational for one year, in the ocean reef habitat (b) Lognormal predicted fraction of days observed from the two models combined.

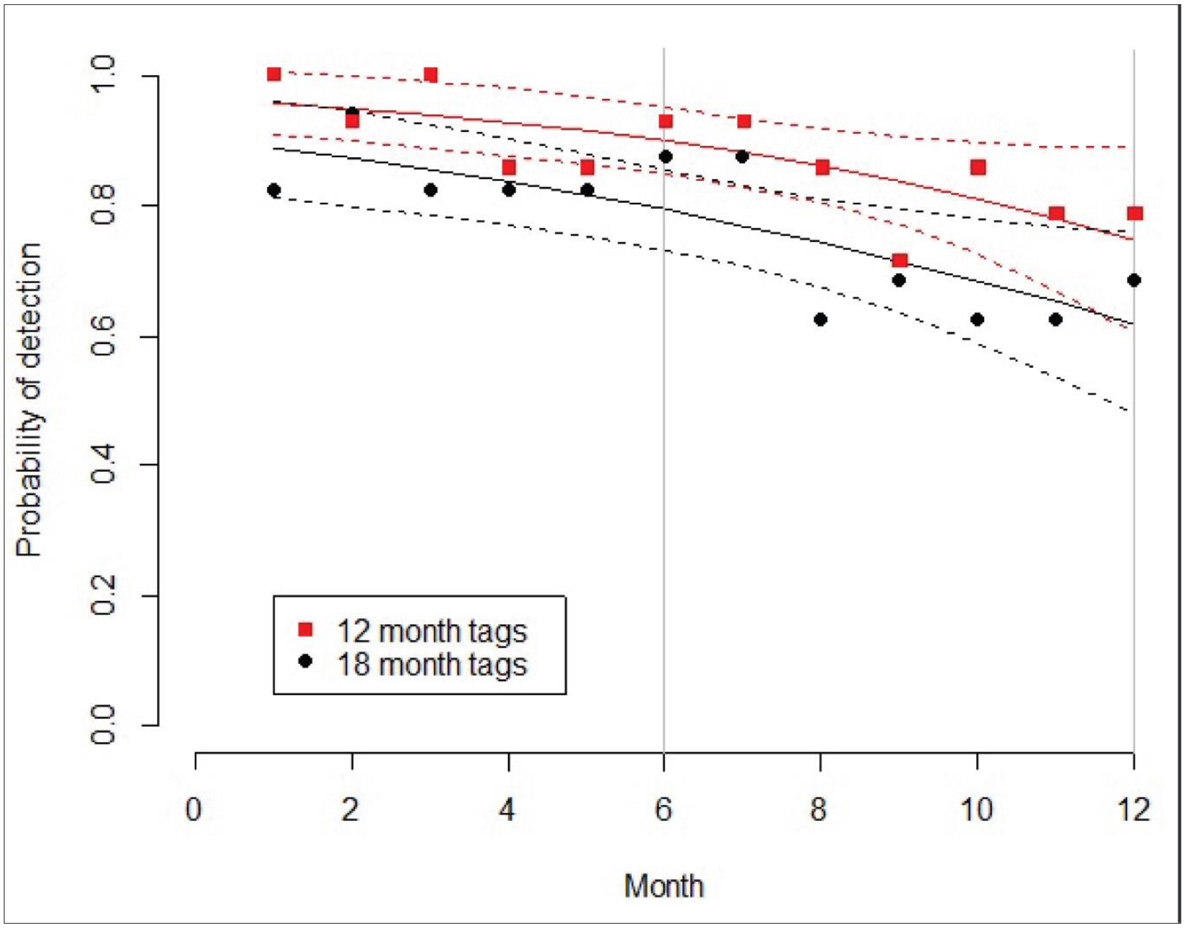


Figure. 8. Predicted probability of presence/absence for the AIC best model (solid lines) plus and minus 2 standard errors (dashed lines). Points are the average values in each month in the data for sharks tagged with V9 (12 month) transmitters and V16 (18 month) transmitters, see symbol legend. The y-axis is the predicted presence or absence of the shark with 0 being absent and 1 being present, the x-axis is the duration since the shark was tagged in months.

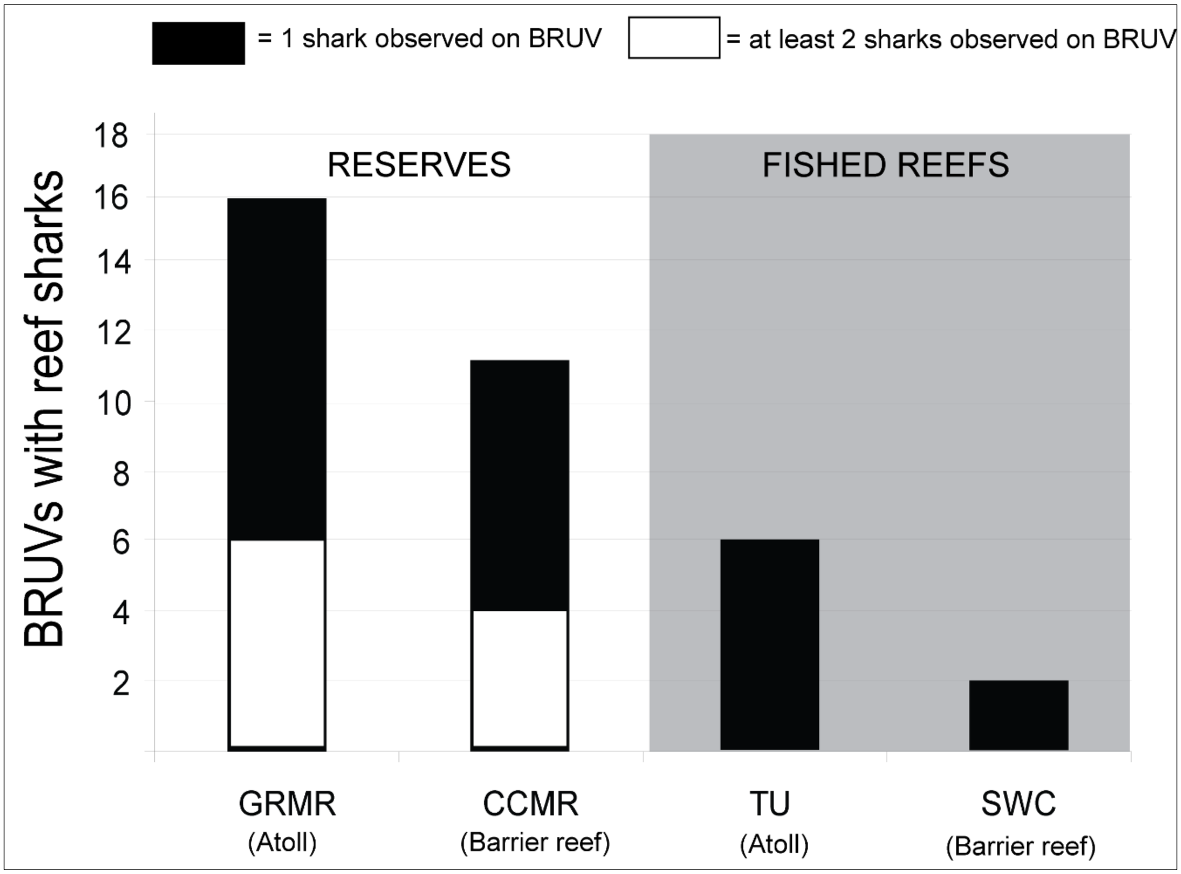


Figure. 9. Number of BRUV deployments out of 50 per site in which one (solid portion of bars) or more (open portion of bars) Caribbean reef sharks were recorded at GRMR (reserve), CCMR (reserve), TU (fished) and SWC (fished).

Chapter Six: Reserves of fear: roving mesopredators avoid dangerous habitats in marine management zones where top predators are common.

Abstract

Marine reserves have been widely applied as a tool for fisheries management and biodiversity conservation. Reserves have demonstrated to have a positive effect on exploited species residing within but their effects on unexploited species are often poorly understood and less predictable. Predator restoration, a common goal of reserve establishment, has been shown to alter the behavior of their prey in terrestrial ecosystems but these behavioral effects are not as well documented in marine reserves, especially on non-surface breathing animals. We investigated factors affecting stingray relative abundance and distribution inside and outside reserves primarily using baited remote underwater video (BRUV) surveys. We predicted the frequency of stingray observations would be significantly lower and distributed differently (shallower) inside marine reserves due to the increased presence, therefore increased risk, of potential predators (sharks) in deeper fore-reef habitats. Generalized Linear Model results indicated that the most significant factors influencing stingray abundance and distribution were the factors: marine reserve, habitat, shark presence, and the associated interactions. Stingrays observed inside marine reserves showed significantly more evidence of predator inflicted damage and stingrays spent significantly longer durations feeding on shallow flats then deeper fore reef. This mesopredator behavioral response to predator restoration highlights the need to consider the indirect consequences of predator restoration prior to reserve establishment, especially in highly interactive in marine ecosystems like coral reefs.

Introduction

Marine reserves frequently have a positive effect on exploited species within their boundaries but their effects on unexploited species are often poorly understood and less predictable (Bohnsack 1993; Roberts et al. 2001; Halpern and Warner 2002; Halpern 2003; Kramer and Heck 2007). Unexploited species may decline within marine reserves if their exploited predators or competitors recover, thus restoring naturally-occurring interspecific interactions (Shears and Babcock 2003). Resulting changes in species density can be transmitted across trophic levels, which can in some cases lead to community rearrangements (Estes et al. 1998; Sala et al. 1998; Ripple et al. 2001; Frank et al. 2005; Mumby et al. 2006; Estes et al. 2011a; Ruppert et al. 2013; Ripple et al. 2014). These density mediated interactions have historically been identified as the primary driver of community restructuring within marine reserves because changes in species density are often relatively easy to measure (Shears and Babcock 2002). The restoration of predators in terrestrial reserves have also been shown to alter the behavior of their prey but these behavioral effects are not as well documented as predators recover in marine reserves (Lima and Dill 1990; Ripple et al. 2001; Dill et al. 2003).

Behaviorally mediated interactions (BMI) between predator and prey can take many forms, such as reduced feeding rates and increased investment in anti-predator behaviors by the prey species (Frid et al. 2007; Heithaus et al. 2007a; Wirsing et al. 2008; Heithaus et al. 2012). One of the most common responses is the redistribution of prey to avoid habitats where there is greater risk of predation, such as habitats where they encounter more predators or those where they are less likely to evade a predator (Heithaus et al. 2008b). BMI can have larger effects than density mediated interactions and can even be important when the actual predation rate (i.e., number of individuals killed over time) is very low. Despite this BMI are often underappreciated because they can be difficult to document and quantify (Madin et al. 2010), especially in the ocean. A clearer understanding of how predator restoration within marine reserves induces changes in prey behavior, together with changes in prey density, is needed.

Stingrays (F. *Dasyatidae*) are common coastal batoids, often subjected to relatively light fishing pressure compared to other large fish (but see Dulvy et al. 2014). The response of batoids to marine reserve establishment has never been the subject of focused investigation even though they are common bioturbators that can potentially influence the structure of benthic communities and effect the chemical composition of surrounding waters (Workgroup 2004; DeWitt 2009; Laverock et al. 2011; O'Shea et al. 2012). Stingrays primarily feed on benthic invertebrates and demersal fishes, which can include commercially important species (Gilliam and Sullivan 1993; Tilley et al. 2013). An increase in these prey species within a marine reserve could enhance local stingray populations. Stingrays have very few natural predators once they exceed a certain size, the majority of which are large-bodied sharks (Strong et al. 1990; Compagno et al. 2005; Papastamatiou et al. 2006). Since certain large-bodied sharks are more abundant within marine reserves (Heupel et al. 2009; Bond et al. 2012; Goetze and Fullwood 2012) it is possible that predation and predation risk for stingrays are elevated within these management zones, potentially reducing their density or changing their behavior.

Belize, Central America, has developed a network of marine reserves that encompass parts of its coast, the Mesoamerican barrier reef and its offshore coral atolls, with the overarching objectives of conserving biodiversity and enhancing neighboring fisheries (Gibson 2003). There are two large species of stingray that occur in Belize, the southern stingray (*Daysatis americana*) and the Caribbean whiptail ray (*Himantura schmardae*), neither of which are commercially targeted (Bigelow and Schroeder 1948). Conversely, Belize has an active commercial shark fishery and the most common large-bodied shark species inhabiting the coral reef that could potentially prey on stingrays is the Caribbean reef shark (*Carcharhinus perezi*, (Michael 2005; Pikitch et al. 2005), hereafter referred to as "reef sharks". In the absence of extensive stomach contents analysis of adult reef sharks, we classify this species as a potential stingray predator on the grounds that other carcharhiniform sharks that are of a similar size and possess similar dentition that have been subject to stomach contents studies (e.g., *Carcharhinus plumbeus, Carcharhinus galapagensis, Negaprion brevirostris*) include batoids in their diet (Papastamatiou et al. 2006; M. Bond *pers obs*). Moreover, batoids have been observed in the few stomach contents of reef sharks that have been examined (Rosa et al. 2010) and we have directly observed and received personal communications of observations of reef sharks chasing or feeding on stingrays in Belize.

Reef sharks have been shown to be more abundant inside certain well established and enforced marine reserves when compared to similar fished reefs in Belize (Bond et al. 2012).

Predation and predation risk for stingrays is therefore predicted to be higher inside marine reserves where reef sharks are more abundant. Belize's marine reserve network therefore provides an opportunity to assess potential predator-mediated effects of marine reserves on stingrays. We investigated factors affecting the relative abundance and distribution of stingrays and reef sharks inside and outside reserves primarily using baited remote underwater video (BRUV) surveys. Our hypothesis was that the frequency of stingray observations on BRUVs would be significantly lower and distributed differently (shallower) inside marine reserves due to the presence of potential predators (sharks) in deeper habitats in these areas (Bond et al. 2012). We hypothesized the opposite pattern for reef sharks based on results of previous studies in these areas (Pikitch et al. 2005, Bond et al. 2012). We also tested the hypothesis that stingrays inside marine reserves were more likely to carry wounds potentially caused by sharks, such as bite marks or truncated tails. The duration of time that stingrays spent at the bait-cage of the BRUV was compared between sites and habitats, testing the hypotheses that site and habitat affected time spent attempting to forage as a proxy for perceived risk of encountering a predator. Lastly, we tested for environmental and habitat differences between sites and the effects of temperature, dissolved oxygen, salinity and flow velocity on the presence of stingrays and reef sharks on BRUVs as alternate hypotheses for any differences in relative abundance or habitat use of these species.

Methods

Study species

The southern stingray (*Dasyatis americana*) is a coastal, benthic dasyatid (growing to 150 cm disc width) endemic to the Western Atlantic and widely distributed from New Jersey (USA) to southern Brazil (Bigelow 1953; McEachran et al. 2002). It has a wide depth range (0-53m) and is associated with a variety of habitats including seagrass, sand flats and coral reefs (Grubbs et al. 2006; Yokota 2006). At Glover's Reef, Belize, southern stingray density is higher in shallow flats habitat compared to deeper fore-reef (Tilley & Strindberg 2012). Its diet has been studied through parts of its range and consists of benthic and infaunal invertebrates and demersal teleosts (Randall 1967; Gilliam and Sullivan 1993; Queiroz 1993). Taken as bycatch in various fisheries and targeted in parts of South America (Buckup 2000; Gadig 2000), where data are available (e.g. USA) the southern stingray is considered 'Least Concern' by the International

Union for the Conservation of Nature (IUCN) (Grubbs et al. 2006). Throughout the rest of its range it is 'Data Deficient' with no known population trend (Grubbs et al. 2006). Increased artisanal fishing pressure (e.g. Brazil, Venezuela and Columbia) and inshore habitat degradation impress the importance of an assessment and continued monitoring for this species (Buckup 2000; Gadig 2000). The other common stingray in Belize is the Caribbean whiptail ray (*Himantura schmardae*), a tropical, benthic species that is poorly known compared to other dasyatids. Caribbean whiptail rays range from Cuba to the Dutch Antilles (Michael 1998, Stehmann *et al*. 1978, Cervigón *et al*. 1992, Charvet-Almeida & de Almeida 2006) and inhabit sandy bottom and coral reef habitats. It can grow to 200 cm disc width and feed on benthic and infaunal invertebrates (Cervigón *et al*. 1992, Michael 1998). Globally considered 'Data Deficient' by the IUCN and requires further assessment (Charvet-Almeida & de Almeida. 2006).

The reef shark (*Carcharhinus perezi*) is a large requiem shark (growing to 295 cm total length) that is endemic to the Western Atlantic from Bermuda to southern Brazil (Compagno, 1984; Rosa et al. 2010). It is the only carcharhinid in the region that completes its entire life cycle within a coral reef ecosystem and is rarely observed away from this habitat (Compagno, 1984; Rosa et al. 2010). Reef sharks do not appear to use geographically discrete nursery areas, instead all life-stages occur over the fore-reef at depths of 10-30 m (Pikitch et al. 2005; Chapman et al. 2005; Compagno, 1984; Rosa et al. 2010; Maljković & Côté, 2011). Adults also frequently occur over the reef slope at depths of at least 352 m (Chapman et al. 2007). Reef sharks frequent lagoons associated with coral reefs, but are not typically found in the shallowest seagrass, sandflat, or mangrove habitats within these lagoons (Pikitch et al. 2005; Rosa et al. 2010). They are exploited by humans for the seafood trade (Rosa et al. 2010) and are the primary species involved in the shark dive tourism industry in the region (Ward-Paige et al. 2010; Maljković and Côté 2011). Considered "Near Threatened" by the IUCN, their range-wide population trend is listed as "Decreasing" (Rosa et al. 2010). IUCN assessors indicate that reef sharks may meet the criteria for the more serious listing of "Vulnerable" as more fisheries and population trend data become available (Rosa et al. 2010). With a diet consisting of a wide variety of reef fishes, reef sharks are typically assumed to fulfill the role of an upper level or apex predator (Motta et al. 1999; Tavares 2009; Maljković and Côté 2011).

Study sites

Our study system consisted of two marine reserves Glover's Reef Marine Reserve and Caye Caulker Marine Reserve and two similar fished reefs South water Caye and Turneffe Atoll (Figure 1.). All four sites included deep fore-reef habitat and shallow, sandy lagoon habitat, hereafter referred to as the 'flats'. These four sites together comprised one fished atoll (TA), one reserve atoll (GRMR), one fished barrier reef site (SC) and one reserve barrier reef site (CCMR). Prior to the study we verified that reef sharks, southern stingrays and Caribbean whiptail rays are present in all study sites using hook-and-line fishing and diver observations. Since Caribbean whiptail rays and southern stingrays are both dasyatids and fulfill a similar ecological role as mesopredators both species were grouped together and will hereafter be referred to as 'stingrays'.

Our two marine reserve study sites meet 3 or more of the 5 features (Edgar et al. (2014)) associated with successful marine reserves around the world (i.e., both are enforced, > 10 years old and no-take; Glover's Reef is also isolated and approaching 100 km^2 in size). Glover's Reef Marine Reserve (GRMR) encompasses Glover's Reef Atoll (16°44'N, 87°48'W), which lies approximately 25 km to the east of the Mesoamerican Barrier Reef (MBR) and 45 km east of mainland Belize. The atoll is approximately 30 km long and at the maximum 10 km wide. The atoll's western reef crest lies submerged with the eastern reef crest being exposed and broken to produce five cuts, which allow shark and stingray movements between the ocean reef and lagoon ecosystems independent of tide. The atoll also includes six sparsely populated cayes. The GRMR was established in 1997 and is comprised of a "no-take zone" on the interior, surrounded by a "general use zone" (32, 834 ha) which prohibits the use of gill-nets and longlines throughout the entire atoll out to the 180 m depth contour (Gibson 2003). Members of a permanent Government of Belize Department of Fisheries (GBDF) station located on Middle Caye provide enforcement, which has led to the recovery of a wide range of exploited fish and invertebrate species (Sobel and Dahlgren 2004; Dahlgren 2014). BRUVs were deployed along ~11 km of the fore-reef and ~10.5 km of the flats within the no-take portion of the marine reserve. Caye Caulker (17°44'N, 88°1'W) lies 1.8 km to the west of the MBR and approximately 20 km to the east of the Belizean mainland. It is a sandbar approximately 7.5 km in length and 1.1 km wide, lying over a limestone shelf. The Caye Caulker Marine Reserve (CCMR) was established in 1998 and is co-managed by the Forest and Marine Reserve Association of Caye Caulker (FAMRACC) and the GBDF. The CCMR is 1,545 hectares in size, extending 1.6 km beyond the barrier reef. A community-based management program works in concert with GBDF rangers that conduct all-day patrols of the reserve. BRUVs were deployed at CCMR along a ~10 km stretch of the fore-reef and ~11 km of the flats within the marine reserve.

Our fished sites were selected because both had active, large-scale shark gillnet fisheries operating during at least part of the study. We confirmed that reef sharks were part of the catch at both sites by surveying landings. Turneffe Atoll ("TA"; 17°21'N, 87°51'W) lies approximately 12 km to the east of the MBR and 43 km from mainland Belize. TA is approximately 42 km long and has a maximum width of 14 km. It includes 11 sandy cayes fringed by mangroves arranged around a central lagoon. The majority of the cayes are unpopulated however a few larger cayes, namely Blackbird and Laughing Bird Caye, accommodate dive-based and recreational fishing-based tourism resorts. TA is unique, as it is the only one of Belize's three atolls that is completely open to commercial fishing, however there are plans to create a multi-use zoned marine reserve at this site. BRUVs were deployed at TA along ~23 km of the fore-reef and ~18 km of the flats on the southeast of the atoll. Southwater Caye ("SC"; 16°48'N, 88°04'W) lies on the MBR, approximately 19 km to the east of mainland Belize. SC is a sand island approximately 610 m long and a maximum of 200 m wide, which accommodates two small tourist resorts and a research station. Given its close proximity to the mainland and more densely populated islands it has been subjected to heavy exploitation from commercial fisherman. In 2010 SC became a marine reserve and enforcement of the reserve by GBDF officers began in early 2011. We considered SC a fished site for this study given that reef sharks would take more than 2 years of protection to recover, and its proximity to populated cayes facilitate poaching within the reserve at night. BRUVs were deployed across ~28 km of the fore-reef and ~18.5 km of the flats.

Baited Remote Underwater Video (BRUV)

Our primary hypotheses were that the relative abundance of stingrays was lower inside marine reserves (GRMR, CCMR) than fished sites (TA, SC) and the converse for reef sharks. We also hypothesized that stingrays would be less common in deep fore-reef habitats at sites where reef sharks are common. Stingray and reef shark relative abundance was surveyed on the fore-reef and the flats at all four sites (GRMR, CCMR, TA and SC) using baited remote underwater video (BRUV©). BRUVs consisted of a video camera (Sony Handycam® DCR-SR68) inside an underwater housing (Aquatix International©) attached to a metal frame with a small, pre-weighed bait source (1 kg of crushed baitfish) mounted on a pole in the camera's field of view (see Brooks et al. 2011, for more detail on BRUV design). Data from studies using BRUVs have previously been found to compare well with that obtained from underwater visual census techniques and from baited hook and lines methods for sampling relatively common species (Meekan & Cappo 2004; Brooks et al. 2011, Watson et al. 2005, Cappo et al. 2007, Harvey et al. 2007, Heagney et al. 2007, Malcom et al. 2007, Stobart et al. 2007, Watson et al. 2007, Watson et al. 2009). BRUV sampling locations were chosen for each site by using the Creates Random Points Tool in ArcToolbox™ produce latitude and longitude points on the fore-reef and flats of each site from a map constructed using ArcGIS software. BRUVs were then deployed in these randomly selected locations during daylight hours. Upon arrival at a sampling location, the vessel captain would find the closest suitable location for deployment (an area at a depth of 10-25 m and with bottom substrate flat enough to maximize line of sight). The BRUV was deployed from the boat using a rope and in-water personnel to guide it away from live coral and to orient the BRUV facing down current. The BRUV was left for at least 90 minutes, allowing it to film continuously for ~85 minutes after settling on the bottom. No BRUVs were simultaneously deployed within 1 km of another. Units were manually retrieved using the rope, which terminated in a small marker float to facilitate relocation. At both the start and end of each deployment environmental variables were measured including mid water flow velocity and direction (with a General Oceanics$^{©}$, Mechanical Flowmeter), bottom depth (Lowrance$^{©}$ XD85), underwater horizontal visibility (secchi disc), water temperature, salinity, pH and dissolved oxygen (YSI$^{©}$, R85-25). Since poor visibility dramatically reduces the likelihood of observing the study species, deployments with visibility <8m were discarded.

Post deployment, the digital video files were copied to an external hard drive and then viewed at normal play speed by one experienced observer (MB). Putative observations were time-logged and species identity was later verified by a second experienced observer (DC). There are no other common carcharhinids or dasyatids likely to be mistaken for these species in the study areas (Pikitch et al. 2005). All BRUV deployments were scored as "1" or "0"corresponding to the study species being "present" or "absent" respectively. Additionally the maximum number observed in a single frame (N*max*) throughout the entire video duration was recorded for reef sharks and stingrays.

BRUV analysis

To test our hypotheses about the effects of marine reserves on stingrays we performed analyses at two different spatial scales. The first (i) used stingray presence/absence data and N*max* and tested our hypothesis at the spatial scale of an individual BRUV. The second (ii) calculated mean reef shark density per habitat per site, and tested its influence on mean stingray presence per habitat across the larger spatial scale of each entire site (multiple km^2) per year. Analysis (i) of presence/absence data was performed by fitting a binomial logit link Generalized Linear Model (GLM). Analysis of N*max* was performed only for BRUVs with stingrays present, using a normal linear model (LM) on log-normally transformed *Nmax* values, because the residuals determined this model had the best fit. The GLMs and LMs were used to examine the effects of reserve (versus non-reserve), location nested within reserve or non-reserve, reef shark presence, habitat (fore-reef or flats), year nested within location, environmental parameters (flow velocity, water temperature, dissolved oxygen, salinity, and conductivity), and the various interactions between these factors on stingray presence and N*max*. The 'stepAIC' function [stepwise model selection using the Akaike's Information Criterion (AIC)] was used to select the best model. Analysis (ii) calculated a mean stingray and reef shark density value for each habitat and site, for a given year, by dividing the number of BRUVs with individuals present by the total number of BRUVs. The reef shark density values were assigned into one of three categories (0.001-0.3; 0.3-0.6; 0.6-0.9) representing low, medium and high reef shark density respectively. A LM with the factors: reserve versus non-reserve, habitat (fore-reef or flats), habitat nested within reserve/non-reserve, reef shark density, the interaction between year and reef shark density, and the interaction between habitat and reef shark density was performed to examine the influence of these factors on mean stingray presence at the larger spatial scale. As with analysis (i) the 'stepAIC' function was used to select the most parsimonious model. To test our hypothesis about the effect of marine reserves on reef sharks we conducted a separate GLM and AIC stepwise model selection, examining how the factors reserve versus non-reserve, location nested within reserve or non-reserve, habitat (fore reef or flat), habitat nested within reserve or non-reserve, year nested within location, and environmental parameters influenced reef shark presence on BRUVs. We did not conduct this for reef shark N*max* data because BRUVs rarely captured multiple sharks in frame at once. All analyses were conducted in R, using the MASS and lme4 libraries (Venables and Ripley 2002; R Core Team 2010).

Stingray appearance and behavior

BRUVs also provided an opportunity to assess the gross body condition and behavior of stingrays, which could provide insights into predation rates and perceived predation risk at our study sites. Stingrays observed on BRUVs in both habitats were assigned to one of four appearance classes: ‘whole’, ‘tail missing’, ‘bite scar’ and ‘too far to assess”. Stingrays classed as ‘whole’ exhibited no external damage. Stingrays that had part or all of their tail missing but otherwise no damage were assigned the ‘tail missing’ class (Figure. 5). If the stingray exhibited a crescent shaped scar/wound or appeared to have a bite-shaped piece of body missing it was assigned ‘bite scar’. Not all stingrays observed came close enough to the camera to be conclusively placed in any of the previous appearances classes and were considered ‘too far to assess’ and excluded from any further analysis. Given the low number of ‘bite scar’ observations, appearance classes ‘bite scar’ and ‘tail missing’ were combined to create ‘ray damage’ class for ease of analysis. Analysis of appearance classes was performed by fitting a binomial logit link GLM. The R software was used with the MASS4 library (Venables & Ripley 2002; R development core team 2010). The GLM was used to examine the influence of reserve versus non-reserve, location nested within reserve or non-reserve and habitat (fore-reef or flats) on ‘ray damage’.

An ‘interaction’ was defined as a behavior when the stingray made contact with the bait cage. It was assumed that contact with the bait cage was an indication that the stingray was interested in the bait and was attempting to acquire food either directly from the cage or on bait particulates that had settled on the substrate below. The time of the first interaction was recorded and was subtracted from the time that the stingray left the vicinity (> 3 body lengths) of the bait cage. Stingrays that interacted with the bait cage were then given a ‘duration’ based on these times that was the total time that the stingray was at the bait cage after its first interaction. A Levene’s test was performed to test for homogeneity of variances. An analysis of variance (ANOVA) was conducted to examine for differences in the mean duration i) between the fore-reef and the flats within a site, ii) between the fore-reefs of the marine reserves and fished reefs. *Post hoc* analyses among means were conducted using TukeyHSD tests.

Benthic habitat surveys

Stingrays might avoid fore-reef habitat if there is a lack of soft substrate to feed and bury in. Benthic habitat surveys were conducted on the fore-reef at each site to determine if there was a significant difference in the amount and distribution of soft-sediment available. Flats at all of the sites were almost exclusively soft sediment (sand with disjointed seagrass [*Thalassia testudinum*] patches), excluding infrequent patch reefs, and were therefore not sampled for habitat differences. Within the same sampling area used for fore-reef BRUV deployments 100 GPS points were randomly generated using ArcGIS software. Points were navigated to by boat and a weighted line was deployed over the side to mark the location. A weighted PVC square (1.5 m^2) was dropped on the substrate with the weighted line at the approximate center of the square. A photograph of the substrate was taken from 1m above the square, with a digital camera (Nikon Coolpix AW100) with a spirit level affixed to ensure it was parallel to the substrate. The photographs were then cropped to remove the PVC square from view leaving a 1.5 m^2 photograph of substrate.

Benthic habitat survey analysis

“Supervised Image Classification” was conducted with the spatial analyst extension in ArcGIS and two categories ‘hard substrate’ and ‘soft substrate’ were created. Anything that was not sand was categorized as hard substrate. Each photograph was classified and the results were presented as percentage ‘hard substrate’ and ‘soft substrate’ in the attribute table. The soft substrate percentage values, for the fore-reef for each of the four sites, were arcsine square-root transformed prior to analysis of variance (ANOVA) (Jordan et al. 2010). *Post hoc* analyses among means were conducted using TukeyHSD tests when necessary. Additionally, each sample was binned into one of three categories in accordance with the percentage soft substrate it contained: 0-33%; 33-66% and 66-100%. The number of samples within each category was tested between sites using a Pearson’s chi-squared (x^2) test in the R software (R development core team 2010).

Boat-based Transects

We directly measured stingray density on the flats at the two sites we had regular access to (SC and GRMR). Observers (n=6-10) were positioned facing out from the vessel, an 8.5m skiff, and were assigned a fixed field of view within 10 m of the boat to survey. This configuration reduced the likelihood of stingrays being missed by the observer turning their head. The start location and survey direction was randomly generated within the same flats survey area where BRUVs were deployed using ArcGIS software. Each transect was 500m in length, run at a constant boat speed of 4 knots. Each observer independently recorded the number of stingrays they observed throughout the survey and the mean number across all observers for each side of the vessel was calculated. Stingrays commonly fled from the vessel in an outward direction therefore each side of the vessel was independent of the other. The mean total stingrays per transect was calculated by adding the mean from each side of the vessel. On the rare occasion a stingray fled under the boat one was deducted from the mean of the opposite side to ensure it was not counted twice. To maximize visibility surveys were conducted between 12pm-1pm when surface glare is at a minimum. The shallow nature of the lagoon meant visibility was reduced with increased turbidity from wind, therefore no surveys were conducted if the wind exceeded 18 km^{h-1} or cloud cover exceeded 30%. Density data were analyzed using Welch's t-test to examine for differences between sites.

Results

Stingray and reef shark relative abundance

A total of 826 BRUV deployments were made, across the four study sites (total duration of 70,210 minutes). Due to inter-annual variability in field conditions and logistics each site had a different number of BRUV deployments on the fore-reef but an equal amount (n=40) on the flats. Deployments were made between May-July each year and during the years we surveyed the flats at a site BRUVs were simultaneously deployed on the fore-reef (Table. 1). Other than reef sharks, very few other shark species that are potential stingray predators were observed on BRUVs. Great hammerhead *Sphyrna mokarran*, tiger *Galeocerdo cuvier* and lemon *Negaprion brevirostris* sharks were each observed once on fore-reef BRUVs at SC, GRMR and TA respectively. Two small (< 80 cm TL) lemon sharks were observed on flats BRUVs at TA.

At GRMR fore-reef deployments occurred annually, 2009-2013 (BRUV's n=183) with the fore-reef and flats simultaneously surveyed in 2012. Reef sharks and stingrays were observed on 67 and 22 fore-reef BRUVs respectively (36.6% and 12.02% of deployments respectively, Figure. 3). Of the GRMR flats deployments (n=40), reef sharks and stingrays were observed on 0 and 25 BRUVs respectively (63% BRUVs observed stingrays). CCMR fore-reef deployments occurred in 2009 and 2010 with the flats and fore-reef being surveyed in 2013 (fore-reef BRUVs, n=95). On fore-reef deployments reef sharks and stingrays were recorded on 27 and 7 BRUVs respectively (28% and 7% of deployments respectively, Figure. 3). The flats deployments (n=40) observed reef sharks and stingrays on 0 and 23 BRUVs respectively (58% BRUVs observed stingrays). At TA fore-reef deployments occurred in 2009 and 2010 with the flats and fore-reef surveyed in 2013 (fore-reef BRUVs n=150). Reef sharks and stingrays were present on 8 and 53 fore-reef BRUVs (5% and 35% of deployments respectively, Figure. 3). Of the flats deployments (n=40), reef sharks and stingrays were observed on 0 and 19 BRUVs respectively (48% BRUVs observed stingrays). Fore-reef deployments occurred annually at SC, 2009-2013 (fore-reef BRUV's n=238) with the flats and fore-reef surveyed in 2012. Reef sharks and stingrays were observed on 10 and 71 fore-reef BRUVs, respectively (4.2% and 29.8% of deployments respectively, Figure. 3). Stingrays were present on 23 of 40 flats BRUVs (58%), while no reef sharks were observed.

Of the combined 278 BRUV deployments on the fore-reef inside marine reserves (GRMR and CCMR) 94 observed at least one reef shark and 29 observed at least one stingray (33.8% and 10.4% of total combined fore-reef reserve deployments). Only four of the 29 BRUVs that observed stingrays recorded more than two individuals (13.8% of marine reserve BRUVs). On fished reefs, of the combined 388 BRUV fore-reef deployments (TA and SC) 18 observed reef sharks, never more than one, and stingrays were present on 124 (4.6% and 32% of combined fore-reef, fished site deployments respectively). Of the 124 BRUVs with stingrays present 38 (31% of fished reef BRUVs) recorded more than one individual. On the flats when the combined 80 BRUV deployments inside marine reserves (n=40 for GRMR and CCMR) were compared with the fished sites (n=40 for TA and SC), both observed zero reef sharks. The number of stingrays observed on the flats when both reserves and fished sites were combined was 48 and 42 respectively, which was not significantly different (Table. 2). Of the 48 BRUVs which observed stingrays on the marine reserve flats 20 (42% of BRUVs) recorded more than one stingray and only 12 of the 42 BRUVs with stingrays present on the fished sites flats (28% of BRUVs) had more than one individual (Table. 3).

Based on the presence/absence data and at the spatial scale of an individual BRUV the GLM determined the most significant factors influencing whether a stingray was present were habitat (fore-reef or flat), whether it was a marine reserve , the interaction between habitat and marine reserve, and reef shark presence. Together those factors explained 10.74% of the deviance in the presence/absence data (Table. 2). The factor year nested within location was also significant ($P < 0.05$, Figure. 4). The AIC best-fit model included only reef shark presence, year nested within location, and the interaction between reserve and habitat as factors. The LM comprised of the same factors but based only on log(N*max*) instead of stingray presence determined that the interaction between marine reserve and habitat was the only significant factor in determining how many stingrays were observed and accounted for 1.47% of the deviance (Table. 3). The AIC best model only included the factors marine reserve, habitat and the interaction between marine reserve and habitat as the most influential factors on stingray N*max*. This indicated that multiple stingray observations were significantly more common on flats BRUVs compared to fore-reef BRUVs across all four sites. Furthermore the coefficients revealed that there was a marginally significant ($P= 0.057$) greater chance of observing multiple stingrays on the flats inside marine reserves when compared with the flats of the fished sites, suggestive of a greater stingray density there which explained the significance of the interaction A GLM including the same factors used for stingrays determined that marine reserve and habitat were the most significant factors determining reef shark presence on BRUVs and together explained 23.52% of the deviance (Table. 4). The interaction between location and year was also significant (Figure. 4). The AIC best model only included the factors year nested within location and the interaction between marine reserve and habitat. The reef shark presence data was used to generate categories (low, medium, and high) of reef shark density that were included in another GLM together with the previously tested factors determining their influence on mean stingray presence, but at the larger spatial scale of the entire site. This GLM showed that reef shark density and habitat were the only factors that had any significant influence on stingray presence and cumulatively accounted for 79.6% of the deviance (Table. 5). The interaction between marine reserve and habitat was also nearly a significant factor and warrants mentioning. The AIC best model included only reef shark density and the interaction between habitat and marine reserve, as factors influencing stingray presence on BRUVs at the larger spatial scale.

There were no significant differences in water flow velocity, temperature, dissolved oxygen, salinity, conductivity, depth or visibility between locations (ANOVA, $P > 0.05$), across the same habitat. The same environmental parameters were compared between habitats within each site with no significant differences (ANOVA, $P > 0.1$) in any parameter except flow velocity. Across all four sites, flow velocity was significantly higher (TukeyHSD, $P < 0.001$), on the fore-reef than flats. GLM results determined that no abiotic factor had a significant influence (ANOVA, $P > 0.05$) on stingray or reef shark presence (Table. 6).

Benthic habitat

There was no significant difference between the mean percentage of soft substrate on the fore-reef at all sites ($F_3 = 0.229$, $p > 0.1$). There was also no significant difference between the number of samples contained within each percentage sand bin (0-33%, 33-66% and 66-100%) from the fore-reef ($x^2 = 10.13$, d.f. = 6, $p > 0.1$) (Figure.2).

Stingray appearance and behavior

A total of 357 individual stingrays were observed across all habitats and sites throughout the duration of the study (Table. 8). Of these 20 were deemed ‘too far’ to accurately assign to an ‘appearance class’ and were excluded from further analysis. Of the 107 stingrays observed inside marine reserves (GRMR and CCMR) within both habitats 39 individuals (36.5%) showed some type of external damage. Within the combined fished reefs (TA and SC) across both habitats of the 250 stingrays observed 29 individuals (11.6%) showed signs of external damage (Figure. 5). The GLM showed the only factors to influence ‘ray damage’ were marine reserve and habitat (ANOVA, $P < 0.001$ and $P < 0.05$, respectively), with damaged rays being significantly more common in reserves on the flats. Together these factors explained 8.74% of the deviance (Table. 9). The AIC best model included only habitat and marine reserve, as factors influencing stingray damage on BRUVs. The mean duration that stingrays spent at the bait cage was calculated for the fore-reef and flats for each site (Table. 10). Mean duration of time at the bait cage was significantly longer on the flats compared to the fore-reef across all four sites (ANOVA, $P < 0.001$) but not when the fore-reef durations for the fished sites were compared to the marine reserves. A post-hoc TukeyHSD analysis revealed the following significant differences between flats and fore-reefs at each site: GRMR (TukeyHSD, $P<0.05$), CCMR (TukeyHSD, $P<0.05$), TA (TukeyHSD, $P<0.01$) and SC (TukeyHSD, $P<0.001$).

Boat based stingray transects

Boat-based stingray transects were conducted in the flats of GRMR (N=42; 25th May-12th June 2012) and SC (N=40; 24th June-11th July 2012). The mean number of stingrays observed on the boat based transects was significantly higher for the GRMR flats (3.88 s.e. ±0.46 per transect), when compared with the SC flats (1.66 s.e. ±0.26 per transect; Welch's t-test; $P<0.001$).

Discussion

Very little is known about the effects of marine reserves on batoids, even though this group of elasmobranchs includes a large number of species listed in threatened categories by the International Union for the Conservation of Nature (IUCN, Dulvy et al. 2014). Our primary finding was that the factors "marine reserve", "habitat" and their interaction had a significant negative effect on stingray presence on BRUVs on coral reefs in Belize. The habitat effect was driven by stingrays being observed significantly more often on flats than fore-reef BRUVs across all four study sites, which is to be expected because this habitat primarily composed of soft sediments where stingrays can forage and bury themselves for refuge. The marine reserve effect and marine reserve-habitat interaction occurred because there were significantly fewer observations of stingrays on fore-reef BRUVs inside marine reserves compared to fished reefs. None of the measured environmental factors (salinity, temperature, dissolved oxygen, visibility and current velocity) had a significant effect on stingray sightings frequency, nor were there any significant environmental differences measured between like habitats in the four study sites. There were also no significant differences in the relative proportion of soft bottom habitat on the fore-reefs of these four sites, indicating that stingrays were not limited by the availability of this type of substrate on the fore-reef inside the marine reserves. Environmental factors can be important drivers of elasmobranch distribution (Heupel et al. 2003; Ubeda et al. 2009; Udyawer et al. 2013; Heupel and Simpfendorfer 2014; Schlaff et al. 2014) but the absence of significant soft substrate availability or environmental factors in predicting stingray presence and the lack of differences in these factors between our study sites suggests that the negative reserve effect we observed was not driven by spatial variation in habitat composition or environmental parameters.

Reef sharks, the most common upper trophic level shark on coral reefs in Belize, are more commonly observed on BRUVs inside marine reserves than fished reefs (Bond et al. 2012). The present study extended this finding for the same four sites from single sampling period of several days to multiple sampling events made over a five-year period, including five consecutive years at two of these sites. This larger analysis also included flats BRUVs as well as fore-reef BRUVs. The factors “marine reserve” and “habitat” had a significant effect on reef shark sightings frequency on BRUVs. Reef sharks were never observed on the shallow flats even though they occur in adjacent deep lagoon and fore-reef habitats, which is also corroborated by a longline survey at one of these sites (Pikitch et al. 2005). The factor marine reserves had a positive effect on sightings frequency of reef sharks on fore-reef BRUVs, a pattern that was consistent over the five-year study. We attribute this to negligible fishing pressure on the residential sharks living inside these particular well-enforced marine reserves (Chapman et al. 2005, Bond et al. 2012). Although there is a growing body of evidence that carcharhinid reef sharks are more common inside marine reserves around the world (Bond et al. 2012; Goetze and Fullwood 2012 ; Espinoza et al. 2014a), this study is one of very few that provides evidence of population stability within reserves across multiple years. This is an important observation because control-impact spatial comparisons do not directly demonstrate population trends and can occur even if a species is declining in the reserve but at slower rates than at fished sites. Time-series inside and outside reserves, such as those presented in this study, are urgently needed to properly assess the role of marine reserves in reef shark conservation efforts around the world.

We hypothesized that marine reserves could have a negative effect on stingrays when they had a positive effect on their potential predators, the latter of which is evident at our study sites. Our survey results are consistent with this hypothesis. There is no evidence that supports alternative hypotheses explaining the pattern we observed based on site-specific habitat or environmental differences. Predator mediated effects on stingrays in marine reserves could manifest from a direct reduction in population due to increased predator-induced mortality or from behaviorally mediated interactions (BMI) that reduced the frequency of observations on

BRUVs. We did not observe a uniform reduction in stingray sightings on BRUVs in marine reserves, rather the reduction was limited to fore-reef set BRUVs only. If direct predation had reduced the stingray population inside the marine reserves we would expect to see a more uniform reduction in sightings across both habitats. Moreover, boat-based transects actually measured significantly higher stingray densities on the flats inside a reserve than the fished site (Glover's Reef and Southwater Caye respectively). Neither of these findings supports the hypothesis that the stingray populations in the reserves are substantially lower than at fished sites, although we can't entirely rule out a more subtle difference in population size without doing studies of their absolute abundance.

Location- and habitat-specific reef shark density was an important factor in determining stingray presence on BRUVs. We therefore suggest that the negative reserve effect that we observed is driven in part by a BMI, whereby stingrays avoid riskier habitats (forereef) in locations (reserves) where their predators are common. This could also explain why stingray density was significantly higher on the flats of one of the reserves when compared to one of the fished sites. These observations suggest that the presence of sharks on the fore-reef of marine reserves causes stingrays to concentrate in shallower, safer habitats.

We found corroborative evidence that stingrays perceive the flats as being safer than the fore-reef. BRUVs provided a potential feeding opportunity for stingrays that attracted them into the field of view. We measured the time spent at the bait cage attempting to feed and found that stingrays spent significantly longer periods at BRUVs on the flats compared to the fore-reef at all of the study sites. This is consistent with the premise that individuals will spend more time attempting to feed and less time investing in anti-predator behavior, such as vigilance or refuging, in perceived safer habitats. Other large vertebrates, such as dugongs, dolphins and sea turtles at risk of predation by sharks have been previously shown to make similar trade-offs (Heithaus and Dill 2002; Heithaus et al. 2007a; Heithaus et al. 2007b; Wirsing et al. 2007).

Visually assessed body condition of stingrays captured on BRUVs provided evidence that shark predation (and hence risk) is higher inside reserves. Stingrays observed in marine reserves were more likely to be "damaged" in that they were missing their tail or had a bite-shaped scar.

Stingrays have few natural predators in a coral reef environment, especially as they approach adult sizes. With the exception of large sharks all other large predators (e.g., grouper F.Serranidae, snapper F.Lutjanidae, moray eels F.Muraenidae, and barracuda F.Sphyraenidae) are at least partially gape-limited and are unlikely to prey on anything other than very small stingrays. There are sharks besides Caribbean reefs that are likely to be stingray predators (e.g., lemon [*Negaprion brevirostris*], great hammerhead [*Sphyrna mokarran*], and bull [*Carcharhinus leucas*]). These species were rarely observed on BRUVs and given their greater mobility and more heterogeneous habitat requirements than reef sharks they may not be benefiting as much from the small to moderate sized marine reserves in this study (Hammerschlag et al. 2011; Daly et al. 2014; Kessel et al. 2014). Although it is not possible to conclusively attribute observed stingray damage to interactions with reef sharks or sharks in general we would expect these types of injuries to be positively correlated with predator abundance, as they are in this study.

We did not explicitly address the possibility that there were differences in prey availability for stingrays between sites that could influence the patterns we observed. Stingray diets are not that well characterized but are known to be quite broad, including infaunal and epibenthic invertebrates and small teleosts. Tilley et al. (2014) concluded that southern stingrays at GRMR primarily fed on infaunal invertebrates (annelids and bivalves), based on stable isotopes analysis. The wide range of prey possibilities presented an insurmountable logistical challenge for us to be able to conduct meaningful, cross-habitat prey assessments at all of our study sites. We instead relied on using soft bottom as an indicator of foraging habitat availability on the fore-reefs of our sites, finding no significant differences between sites. Even though we were unable to direct assess prey availability and competition as drivers of the patterns we suggest that these processes are unlikely to be important for several reasons. First, the primary species that increase inside Caribbean reef marine reserves are large piscivores and commercially exploited macroinvertbrates such as queen conch (*Strombus gigas*) and spiny lobsters (Sobel and Dahlgren 2004; Dahlgren 2014). None of these species are likely to compete with stingrays for their infaunal prey and increases in juvenile conch and lobster density would actually present an additional prey resource that could reduce competition (Gilliam and Sullivan 1993; Tilley et al. 2013). An increase in large piscivores (e.g., grouper, snapper) that could feed on invertivorous fish might actually reduce potential stingray competitors inside marine reserves. Our observation of higher stingrays densities on the flats of one of the marine reserves than one of the fished sites also argues against competition (intra- or interspecific) driving the patterns we observed. Under this scenario higher stingray densities on the flats would intensify competition and could force more individuals to forage in deeper habitats, potentially including the fore-reef. If this was occurring we would expect to observe more stingrays on fore-reef BRUVs inside the reserves than the fished sites (where flats densities are lower) but we actually observed the reverse pattern. Although more research is needed on stingray diets and the influence of prey on their distribution we conclude that the patterns we observed were more likely to be driven by predator avoidance.

There is considerable interest in the ecological effects of sharks and shark removals on coral reefs and other ecosystems throughout the world. In coral reef ecosystems the overarching focus has been on possible density mediated effects on large piscivores that are transmitted to herbivores and could therefore benefit corals over fleshy algae. Our results highlight that we should also consider BMI between sharks and potential prey in coral reef ecosystems. Human induced changes in shark abundance could trigger a wide range of behavioral changes in potential prey species, including changes in habitat use, time allocation, feeding preys, schooling and spawning. Since reef-associated carcharhinid sharks and some of the larger, transient sharks that also use coral reefs tend to have broad diets it is not clear that predation rates on any one prey species would be sufficient to induce substantial changes in their density and constrain growth. In contrast BMI can affect the majority of individuals even when the predation rate is negligible (Lima and Dill 1990; Heithaus et al. 2008b). Our study also suggests that changes in BMI between sharks and batoids should be considered a possible outcome of shark removals or restoration. Although there are many documented instances of predation on batoids by sharks (Strong et al. 1990; Chapman and Gruber 2002), few studies have found strong ecological interactions between these taxa. The most widely cited evidence of ecological interactions between sharks and batoids is the increase of batoids, specifically cownose rays, *Rhinoptera bonasus*, in certain inshore surveys after large sharks were overfished in the Northwest Atlantic (Myers et al. 2007). This study also showed that cownose ray schools were capable of consuming large quantities of bivalves and might affect negatively the fisheries they support (Myers et al. 2007). Although Myers et al. (2007) favored direct release from predation by sharks as the mechanism behind increased batoid presence in certain survey time series, we suggest

BMI as an alternative hypothesis. We speculate that reduced shark presence in some of the sampling areas may have prompted an influx of batoids and/or reduced the time they spend avoiding predators and increase their foraging rates. We stress that this is simply an alternative hypothesis for their data that would require additional testing, but suggest it warrants consideration in light of our evidence that batoid distribution can be affected by changes in the local abundance of large sharks.

Marine reserves are increasingly being used to conserve biodiversity in the world's oceans. There is evidence that they can enhance the biomass, density and body size of exploited species within their boundaries, which sometimes negatively affects these parameters in other species and can have strong cascading effects through the food web. Our study highlights that marine reserves can also restore naturally occurring BMI, in this case by causing stingrays to reduce their use of riskier fore-reef habitats when large sharks are locally common due to reduced shark fishing. We suggest a need for more studies of BMI that involve species likely to increase inside marine reserves or be disproportionately affected by fishing in order to better predict how we may disrupt or restore these important ecological processes.

Figures and Tables

Table. 1. The number of fore-reef BRUV deployments and total number of sampling days across all four sites, broken down by year. Years where simultaneous fore-reef and flats (n=40) BRUVs deployed denoted by asterisk (*).

Site	2009	2010	2011	2012	2013	Total days	Total BRUVs
GRMR	45	36	30	36*	36	50	183
CCMR	20	34	0	0	41*	19	95
TA	56	54	0	0	40*	24	150
SC	41	54	67	35*	41	40	238

Table. 2. The GLM analysis on the influence of the factors marine reserve, location nested within marine reserve, Caribbean reef shark presence, habitat, habitat nested within marine reserve and year nested within location, on stingray presence. This examines the question at the fine spatial scale of each BRUV and only considers presence/absence data. Factors in bold, represent the variables selected by the AIC model.

	Df	Deviance	Residual Df	Residual Deviance	P(>|Chi|)	Percent of deviance
NULL			825	1006.08		
MPA	1	18.33	824	987.75	1.86 e-05***	1.82
shark presence	1	13.17	823	974.58	0.00028***	1.31
habitat	1	59.38	822	915.2	1.301 e-14***	5.9
MPA:location	2	0.88	820	914.31	0.64105	0.08
MPA:habitat	1	17.11	819	897.21	3.536 e-05***	1.71
location:year	12	26.09	807	871.11	0.01041*	2.59

Table. 3. The LM analysis on the influence of the factors marine reserve, location nested within marine reserve, Caribbean reef shark presence, habitat, habitat nested within marine reserve and year nested within location on stingray *Nmax* This examines the question at the fine spatial scale of each BRUV and considers the influence of these factors on multiple stingrays observed per BRUV from only the stingray present data. This builds on the presence/absence data. Factors in bold, represent the variables selected by the AIC model.

	Df	Deviance	Residual Df	Residual Deviance	P(>|F|)	Percent of deviance
NULL			245	33.13		
MPA	1	0.063	244	33.08	0.49	0.19
shark presence	1	0.027	243	33.05	0.65	0.08
habitat	1	0.357	242	32.69	0.10	1.08
MPA:location	2	0.191	240	32.50	0.49	0.58
MPA:habitat	1	0.486	239	32.08	0.05	1.45
location:year	11	1.891	228	30.19	0.23	5.7

Table. 4. The GLM analysis on the influence of the factors marine reserve, habitat, location nested within marine reserve, habitat nested within marine reserve, and year nested within location on Caribbean reef shark presence. Factors in bold, represent the variables selected by the AIC model.

	Df	Deviance	Residual Df	Residual Deviance	P(>|Chi|)	Percent of deviance
NULL			825	655.65		
MPA	1	90.84	824	564.81	< 2.2 e-16***	13.85
habitat	1	63.40	823	501.42	1.691 e-15***	9.67
MPA:location	2	2.17	821	499.25	0.338687	0.33
MPA:habitat	1	0.00	820	499.25	1.000000	0.00
location:year	12	30.85	808	468.40	0.002078**	4.71

Table. 5. The GLM analysis on the influence of the factors marine reserve, location nested within marine reserve, habitat, year, and reef shark density on the presence of stingrays. Reef shark density broken down into categories to determine if the significant influence observed at a fine scale level of at each BRUV was evident at the larger scale of each entire site using a gradient of shark density (number BRUVs with sharks observed/ total BRUVs, for each year). Stingray presence was averaged per habitat, site and year. Factors in bold, represent the variables selected by the AIC model.

	Df	Deviance	Residual Df	Residual Deviance	P(>|F|)	Percent of deviance
NULL			19	1.53		
shark density category	3	0.96	16	0.57	0.007**	62.7
MPA	1	0.00	15	0.57	0.975	0.00
habitat	1	0.26	14	0.31	0.019*	16.9
shark dens cat:habitat	0	0.00	14	0.31		0.00
MPA:habitat	1	0.13	13	0.17	0.06·	8.5
shark dens cat:year	8	0.06	5	0.11	0.915	3.9

Table. 6. The GLM analysis on the influence of marine reserve, location nested within marine reserve, habitat, and the environmental parameters (flow velocity, water temperature, salinity, conductivity and dissolved oxygen) on stingray presence or absence derived from BRUV deployments.

	Df	Deviance	Residual Df	Residual Deviance	P(>|Chi|)	Percent of deviance
NULL			748	913.89		
MPA	1	13.14	747	900.75	0.0002***	1.44
habitat	1	69.76	746	830.99	$< 2.2e^{-16}$***	7.63
flow velocity	1	0.77	745	830.22	0.38	0.08
water temp	1	0.08	744	830.14	0.78	0.01
conductivity	1	0.21	743	829.93	0.65	0.02
salinity	1	0.28	742	829.66	0.6	0.03
diss. oxygen	1	0.19	741	829.47	0.67	0.02
MPA:location	2	1.99	739	827.47	0.37	0.22

Table. 7. The GLM analysis on the influence of the environmental parameters (flow velocity, water temperature, salinity, conductivity and dissolved oxygen) on Caribbean reef shark presence or absence derived from BRUV deployments.

	Df	Deviance	Residual Df	Residual Deviance	P(>|Chi|)	Percent of deviance
NULL			748	542.12		
MPA	1	71.74	747	470.38	$< 2.2e^{-16}$***	13.23
habitat	1	55.07	746	415.31	$1.16e^{-13}$***	10.16
flow velocity	1	0.46	745	414.85	0.49	0.08
water temp	1	0.14	744	414.71	0.71	0.03
conductivity	1	2.58	743	412.14	0.11	0.48
salinity	1	2.28	742	409.86	0.13	0.42
diss. oxy	1	1.9	741	407.95	0.17	0.35
MPA:location	2	2.23	739	405.72	0.33	0.41

Table. 8. Number of observed stingrays which displayed external damage and which ‘appearance class’ they were assigned to.

	Whole	Tail bite	Bite scar	Too far
GRMR	35	27	2	3
CCMR	30	10	0	0
TA	76	11	0	7
SC	128	18	0	10
Total	269	66	2	20

Table. 9. The GLM analysis on the influence of the factors marine reserve, habitat, location nested within marine reserve, and habitat nested within marine reserve on external stingray damage, derived from BRUV deployments. Factors in bold, represent the variables selected by the AIC model.

	Df	Deviance	Residual Df	Residual Deviance	P(>|Chi|)	Percent of deviance
NULL			316	332.18		
MPA	1	23.41	315	308.77	1.31 e-06***	7.05
habitat	1	5.63	314	303.14	0.01*	1.69
MPA:location	2	0.40	312	302.74	0.81	0.12
MPA:habitat	1	0.59	311	302.15	0.44	0.18

Table. 10. The mean durations (minutes:seconds) that stingrays were observed interacting with the bait cage, broken down by site and habitat type. Stingrays which were observed but failed to interact with the bait cage were omitted from analysis.

	GRMR	CCMR	TA	SC
Fore-reef	01:18	01:37	02:54	06:37
Flats	13:24	12:10	14:38	24:22

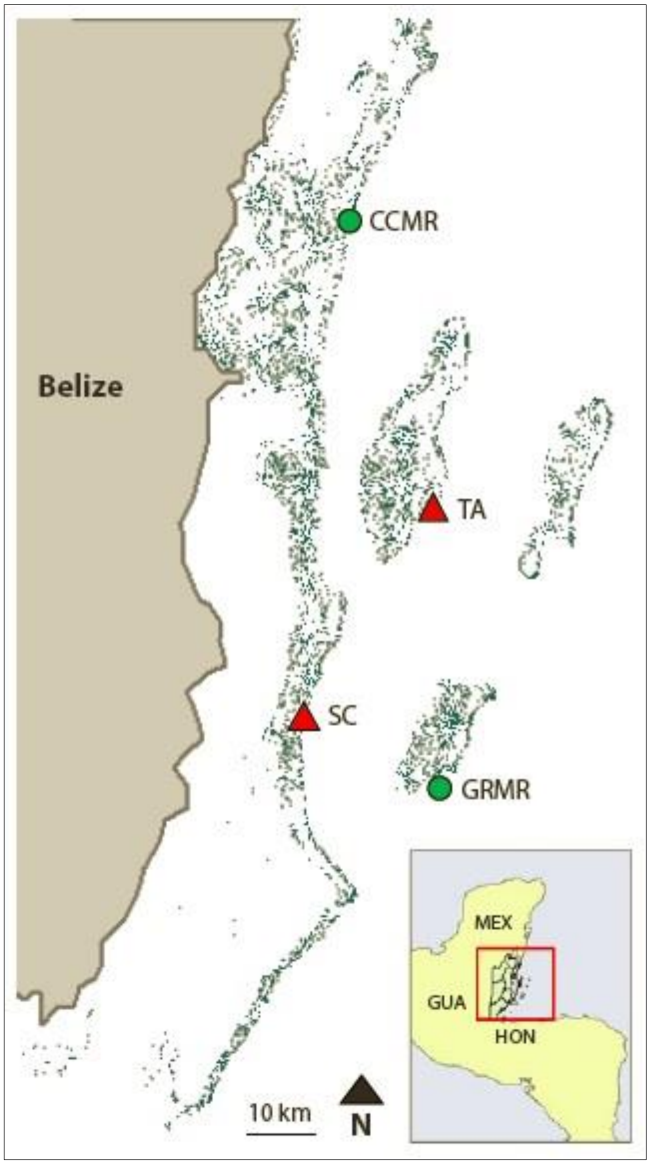


Figure. 1. A map showing the location of our four study sites along the Mesoamerican Barrier Reef of Belize, Central America (BZE). The sites are color coded with marine reserves Glover's Reef (GRMR) and Caye Caulker (CCMR) in green circles and fished reefs Turneffe Atoll (TA) and South water Caye (SC) in red triangles. The inset map shows Belize in relation to its neighboring countries in Central America Mexico (MEX), Guatemala (GUA) and Honduras (HON).

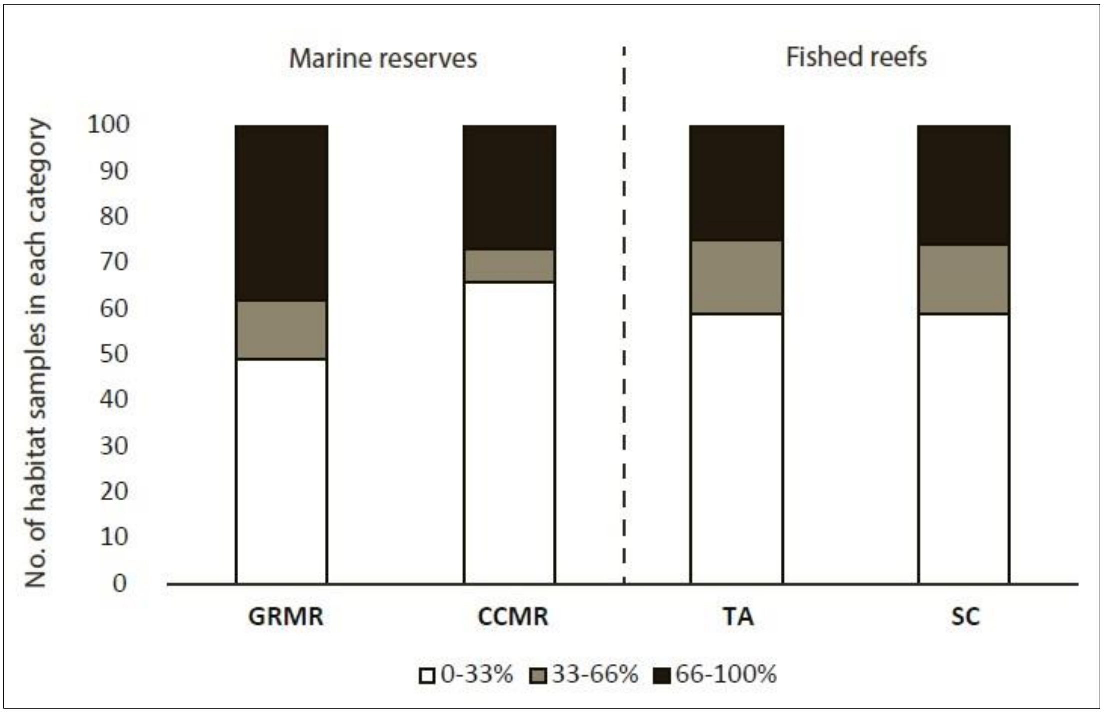


Figure. 2. Stacked bar graph showing the distribution of the amount of soft sediment surveyed on the fore-reef of each site assigned to one of three categories: 0-33% (white fill), 33-66% (grey fill), and 66-100% (black fill).

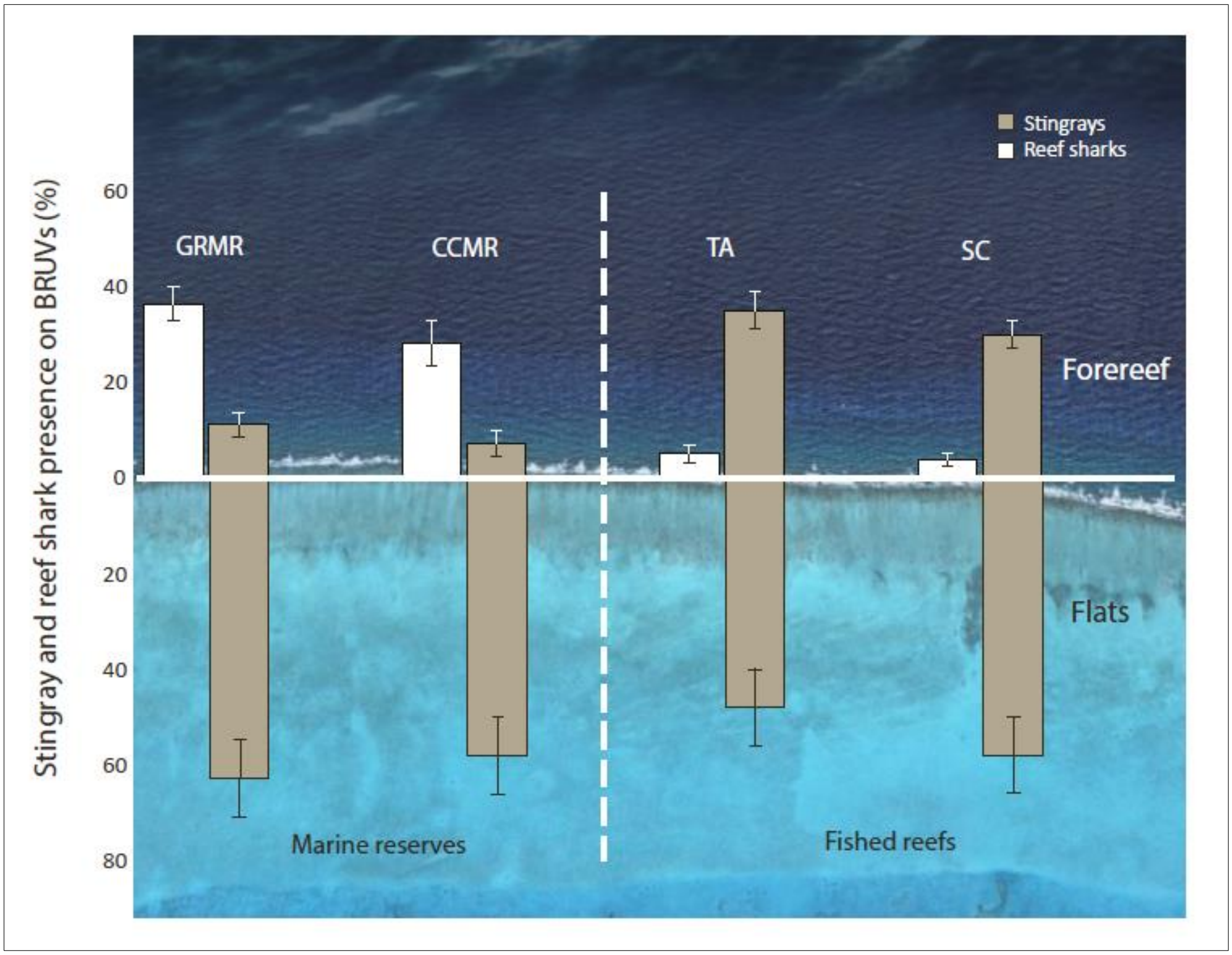


Figure. 3. Total Caribbean reef shark and stingray presence data derived from BRUVs, all years are combined and divided by habitat. All study sites are graphed: Glover Reef Marine Reserve (GRMR), Caye Caulker Marine Reserve (CCMR), Turneffe Atoll (TA) and South water Caye (SC), with stingrays (grey fill) and Caribbean reef sharks (white fill).

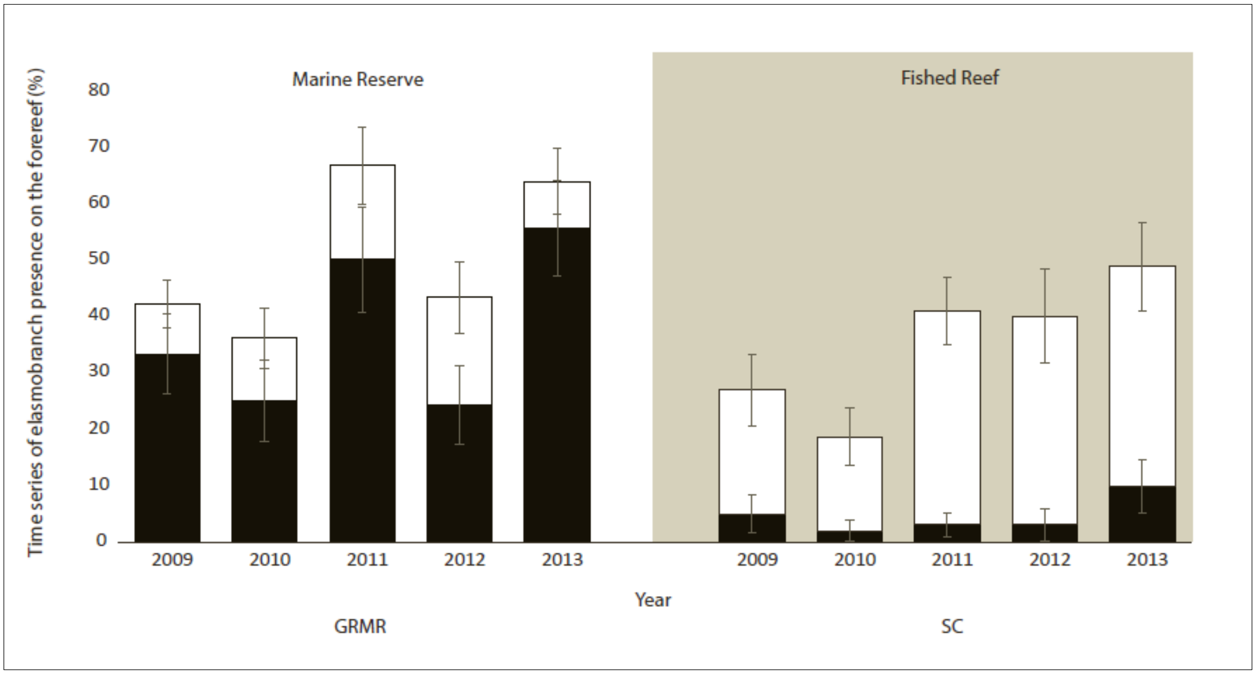


Figure. 4. Time-series of elasmobranch presence data from the fore-reef from one marine reserve (GRMR) and one fished site (SC), Caribbean reef shark (black fill) and stingrays (white fill). Logistics prevented annually surveying all four sites but the figure shows the differential distribution pattern of stingray abundance on the fore-reef is consistent across multiple years despite inter-annual variability.

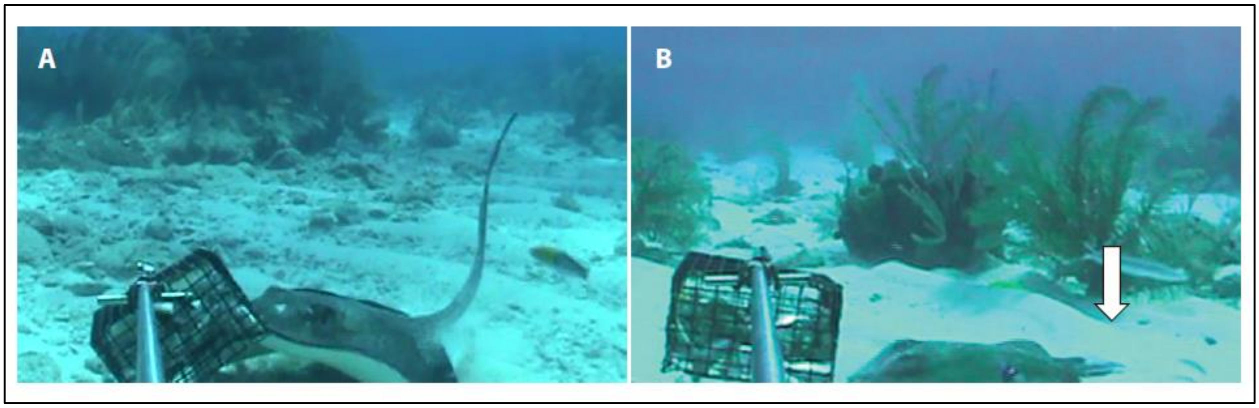


Figure. 5. Still images taken from a forereef BRUV (GRMR) showing the difference between a stingray, in this example *Dasyatis americana*, which was classed as ‘whole’ (panel ‘A’) and one which was assigned to the ‘tail bite’ appearance class (panel ‘B’). The extension from the posterior of the stingray in panel ‘B’ were its claspers.

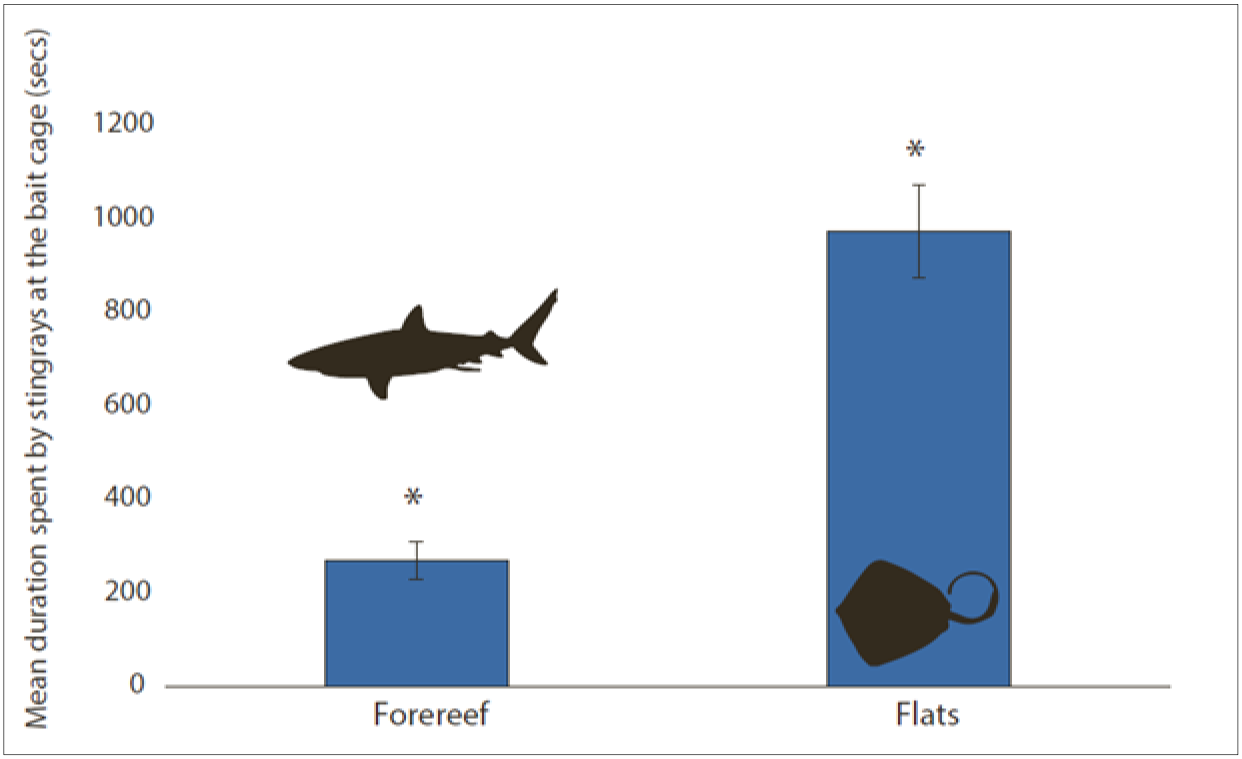


Figure. 6. The mean durations (seconds) that stingrays were observed interacting with the bait cage, broken down by habitat type. Stingrays which were observed but failed to interact with the bait cage were omitted from analysis. The asterisk (*) indicates that there was a significant difference between the means ($p<0.0001$).

Chapter Seven: Conclusions

Marine reserves are being widely used to manage fisheries and conserve biodiversity (Halpern 2003; Sobel and Dahlgren 2004). Reserves have been shown to positively affect local biodiversity, biomass, body size, and density but primarily for r-selected taxa (Halpern and Warner 2002; Lester and Halpern 2008; Lester et al. 2009). Given the K-selected life-history strategies of sharks and rays and their current global conservation status, my overarching question was to assess how marine reserves affect these species in tropical coral reef ecosystems and what the broader ecological repercussions are likely to be.

A review of the current literature revealed that reef-associated shark and ray species frequently exhibit a positive response to marine reserves. Though data were geographically limited to a handful of countries and further restricted to primarily tropical coral reef ecosystems, it highlighted the conservation potential of reserves. Most data were derived from movement studies and demonstrated that species' with defined home ranges and those that resided within the reserve had the greatest potential to benefit from the reduced fishing mortality. Further research is required to explore conservation strategies for species with larger scale movements partially outside of reserve boundaries. The dearth of Before-After-Control-Impact studies testing for reserve effects among sharks and rays requires attention. Spatial studies which compare differences in a species biological parameters inside and outside of a reserve will not detect if the species inside the reserve is still declining just at a slower rate than populations outside. These results can therefore be misleading and since the majority of evidence of elasmobranch responses to reserve establishment is derived from spatial studies surveys of a B-A-C-I design should be prioritized.

The long term standardized longline time-series from inside a marine reserve, which met all of the criteria outlined for a successful reserve (Edgar et al. 2014), showed the Caribbean reef shark population appeared stable. The robust time-series was as long in duration as one generation for Caribbean reef sharks and was therefore capable of detecting any significant changes in catch per unit effort over time. This was further evidence that reef associated shark species can benefit from reserve protection if known to exhibit residency, which was demonstrated by the results of the acoustic telemetry study (Bond et al. 2012). One single long-distance return migration to a neighboring atoll was detected but otherwise results supported that Caribbean reef sharks of all age classes were year-round residents to Glovers Reef Atoll. The stable population trend inside GRMR could therefore be driven either by the year round reduced fishing mortality provided by the reserve or from increased resources such as prey inside its boundaries (Bond et al. 2012).

Spatial studies are not the most robust method for assessing reserve effectiveness for reasons stated above however in the face of financial or logistical constraints they are still valuable. It was possible to determine that the relative abundance of Caribbean reef sharks was higher inside marine reserves when compared with similar fished reefs throughout the four reef sites surveyed. It is important to note that while video data were scored purely as presence/absence, the most conservative metric, only baited remote underwater video (BRUV) samples deployed inside marine reserve detected multiple sharks simultaneously. Therefore the differences in relative abundance between marine reserves and fished reefs was even greater then reported. There was further support that reduced fishing mortality was the driver behind this pattern because of the infrequent number of other large bodied carcharhinids, not known to exhibit residency and capable of long-distance migrations, detected throughout the study.

Caribbean reef shark relative abundance metrics from the fished reefs suggested that populations are severely depleted. It is possible that the number of individuals were so low that they may not be able to fulfill their proposed ecological role as an apex predator on Caribbean coral reefs. To be able to understand the ecosystem consequences of a reduction in the number of reef sharks it was important to quantitatively determine the ecological role of the Caribbean reef shark. Stable isotope results indicated either functional redundancy and/or omnivory as potential mechanisms describing the Caribbean reef sharks ecological role. If Caribbean reef sharks formed part of a top predator guild with other large-bodied teleost piscivores (e.g. F. Sphyraenidae and F. Serranidae) then the removal or reduction of one species would be buffered by the remaining species weakening the potential for trophic cascades. Similarly if Caribbean reef sharks exhibited an omnivorous diet consuming a mixture of herbivores and upper level piscivores then a reduction in reef shark predation pressure would be distributed among multiple trophic levels dampening the effect. The data suggest the proposed trophic cascade initiated by the reduction of reef sharks would be potentially buffered against by the remaining upper trophic level teleost predators (e.g. F. Sphyraenidae and F. Serranidae) in either scenario (Bascompte et al. 2005; Mumby et al. 2006). It is also important to note that not all individuals within a population will act the same way. If the population is more heavily comprised of omnivores instead of reef sharks which form part of a top predator guild it may respond differently to a reduction in reef shark abundance. Irrespective of whether they exhibit omnivory or functional redundancy it was conclusive that Caribbean reef sharks do not fulfill the role of apex predator. Therefore they should be considered as mesopredators alongside the large bodied teleost piscivores of the system and managed accordingly. Future research efforts should examine whether Caribbean reef sharks resident to fished reefs feed at a different trophic level compared with conspecifics resident to marine reserves.

Through elucidating the ecological role of Caribbean reef sharks we learned what top-down pressure they exert on Caribbean coral reef ecosystems through direct predation and therefore the consequences of their reduction. Similarly top predators can shape ecosystems through behaviorally mediated interactions (Heithaus et al. 2008b). These interactions can be extremely influential in shaping ecosystems even if direct predation levels are low. The reduced use of deeper, risky forereef habitat by southern stingrays inside marine reserves, where the relative abundance of Caribbean reef shark is elevated, is such an example. Unexpected indirect effects of predator restoration/removal can have far reaching consequences both ecologically and economically. The increased use of shallow flats and seagrass beds inside marine reserves could concentrate stingray foraging in these areas instead of distributing it between both the flats and forereef. Seagrass beds are important nursery areas for juvenile lobster (*Panulirus argus*) and queen conch (*Strombus gigas*), which are both commercially important, and some of the species targeted for recovery and motivation for reserve establishment from a fisheries management perspective (Dahlgren 2014). An increase in stingray foraging in seagrass areas because of the perceived risk of predation on the forereef could reduce the number of juveniles therefore limiting recruitment to the adult populations. Increased bioturbation from stingray foraging could damage seagrass rhizomes through excavation and re-suspend buried nutrients thereby altering the water chemistry. It is imperative to consider the indirect effects of reserve establishment and that of predator removal/restoration.

In conclusion, there is a growing body of evidence, including that presented here that marine reserves are an appropriate conservation tool for certain shark and ray species. Reef-associated sharks which exhibit residency are most likely to benefit from the reduced fishing mortality offered by reserve protection. Before-After-Control-Impact should be the priority when establishing surveys to monitor reserve effectiveness. The survey should be of an appropriate duration to be robust enough to detect population trends given the life history traits of elasmobranchs. It is unlikely that migratory species that regularly move large distances will benefit from reserve protection. These species are typically harvested by commercial fisheries therefore it is important that we look for alternatives to marine reserves e.g. time area closures or mega-reserves in our efforts to conserve these imperiled species.

References

2012 IUCN – (2012) Conservation status of north american, central american and caribbean chondrichthyans.www.iucn.org

Amargós FP, Sansón GG, del Castillo AJ, Fernández AZ, Blanco FM, de la Red WA (2010) An experiment of fish spillover from a marine reserve in Cuba. Environmental Biology of Fishes 87:363-372

Au DW, Smith SE, Show C (2008) Shark productivity and reproductive protection, and a comparison with teleosts. Sharks of the Open Ocean: Biology, Fisheries and Conservation:298-308

Barker MJ, Schluessel V (2005) Managing global shark fisheries: suggestions for prioritizing management strategies. Aquatic Conservation: Marine and Freshwater Ecosystems 15:325-347

Bascompte J, Melian CJ, Sala E (2005) Interaction strength combinations and the overfishing of a marine food web. Proc Natl Acad Sci U S A 102:5443-5447

Bates D, Maechler M, Bolker B (2010) lme4: Linear Mixed-Effects Models Using S4 Classes., R Package Version 0.999375-999335

Baum JK, Myers RA (2004) Shifting baselines and the decline of pelagic sharks in the Gulf of Mexico. Ecology Letters 7:135-145

Bigelow H, Schroeder W (1948) 1953. Fishes of the Western North Atlantic. Mem Sears Found Mar Res

Bigelow HBS, W.C. (1953) Sawfishes, guitarfishes, skates, rays, and chimeroids. Fishes of the Western North Atlantic. Mem Sears Mem Found Mar Res 1:1-514

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Canadian journal of biochemistry and physiology 37:911-917

Bohnsack JA (1993) Marine reserves: they enhance fisheries, reduce conflicts, and protect resources. Oceanus 36:63-71

Bond ME, Babcock EA, Pikitch EK, Abercrombie DL, Lamb NF, Chapman DD (2012) Reef sharks exhibit site-fidelity and higher relative abundance in marine reserves on the mesoamerican barrier reef. PloS one 7:e32983

Bonfil R (1997) Marine protected areas as a shark fisheries management tool. Proceedings of the 5th Indo-Pacific fish conference, Noumea, New Caledonia:3-8

Brooks EJ, Sloman KA, Sims DW, Danylchuk AJ (2011) Validating the use of baited remote underwater video surveys for assessing the diversity, distribution and abundance of sharks in the Bahamas. Endangered Species Research 13:231-243

Brooks EJ, Sims DW, Danylchuk AJ, Sloman KA (2013) Seasonal abundance, philopatry and demographic structure of Caribbean reef shark (Carcharhinus perezi) assemblages in the north-east Exuma Sound, The Bahamas. Marine biology 160:2535-2546

Buckup PA, Nunan, G.W., Gomes, U.L., Costa, W.J.E.M. and Gadig, O.B.F (2000) In: Rio de Janeiro 2000. Espécies ameaçadas de extinção no Município do Rio de Janeiro: flora e fauna Rio de Janeiro, Secretaria Municipal de Meio Ambiente pp:52–60.

Burt WH (1943) Territoriality and home range concepts as applied to mammals. Journal of mammalogy 24:346-352

Cappo M, De'ath G, Speare P (2007) Inter-reef vertebrate communities of the Great Barrier Reef Marine Park determined by baited remote underwater video stations. Marine Ecology Progress Series 350:209-221

Castro JI (2011) The Sharks of North America. Oxford University Press, USA

Chapman DD, Gruber SH (2002) A further observation of the prey-handling behavior of the great hammerhead shark, Sphyrna mokarran: Predation upon the spotted eagle ray, Aetobatus narinari. Bulletin of Marine Science 70:947-952

Chapman DD, Pikitch EK, Babcock E, Shivji MS (2005) Marine reserve design and evaluation using automated acoustic telemetry: a case-study involving coral reef-associated sharks in the Mesoamerican Caribbean. Marine Technology Society Journal 39:42-55

Chapman DD, Pikitch EK, Babcock EA, Shivji MS (2007) Deep-diving and diel changes in vertical habitat use by Caribbean reef sharks Carcharhinus perezi. Marine Ecology Progress Series 344:271-275

Chapman DD, Feldheim KA, Papastamatiou YP, Hueter RE (2015) There and Back Again: A Review of Residency and Return Migrations in Sharks, with Implications for Population Structure and Management. Annual review of marine science 7:547-570

Clarke SC, McAllister MK, Milner-Gulland E, Kirkwood G, Michielsens CGJ, Agnew DJ, Pikitch EK, Nakano H, Shivji MS (2006) Global estimates of shark catches using trade records from commercial markets. Ecology Letters 9:1115-1126

Compagno L (1999) Checklist of living elasmobranchs. Sharks, skates, and rays: the biology of elasmobranch fishes., The John Hopkins University Press

Compagno LJV, Dando M, Fowler SL (2005) A Field Guide to the Sharks of the World. HarperCollins UK

Cortés E (1999) Standardized diet compositions and trophic levels of sharks. ICES Journal of Marine Science: Journal du Conseil 56:707-717

Creel S, Christianson D (2008) Relationships between direct predation and risk effects. Trends in Ecology & Evolution 23:194-201

Dahlgren C (2014) Review of the Benefits of No-take Zones: A Report to the Wildlife Conservation Society

Dale JJ, Meyer CG, Clark CE (2010) The ecology of coral reef top predators in the Papahānaumokuākea Marine National Monument. Journal of Marine Biology 2011

Daly R, Smale MJ, Cowley PD, Froneman PW (2014) Residency Patterns and Migration Dynamics of Adult Bull Sharks (*Carcharhinus leucas*) on the East Coast of Southern Africa. PLoS ONE 9:e109357

Davidson LN (2012) Shark sanctuaries: substance or spin? Science 338:1538

DeNiro MJ, Epstein S (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. Science 197:261-263

DeWitt TH (2009) 10.0 The Effects of Bioturbation and Bioirrigation on Seagrasses. Seagrasses and Protective Criteria: A Review and Assessment of Research Status

Dill LM, Heithaus MR, Walters CJ (2003) Behaviorally mediated indirect interactions in marine communities and their conservation implications. Ecology 84:1151-1157

Dudley SFJ, Simpfendorfer CA (2006) Population status of 14 shark species caught in the protective gillnets off KwaZulu–Natal beaches, South Africa, 1978–2003. Marine and Freshwater Research 57:225-240

Dulvy NK (2006) Conservation biology: strict marine protected areas prevent reef shark declines. Current Biology 16:R989-R991

Dulvy NK, Freckleton RP, Polunin NV (2004) Coral reef cascades and the indirect effects of predator removal by exploitation. Ecology letters 7:410-416

Dulvy NK, Fowler SL, Musick JA, Cavanagh RD, Kyne PM, Harrison LR, Carlson JK, Davidson LN, Fordham SV, Francis MP (2014) Extinction risk and conservation of the world's sharks and rays. Elife 3

Edgar GJ, Barrett NS (2012) An assessment of population responses of common inshore fishes and invertebrates following declaration of five Australian marine protected areas. Environmental Conservation 39:271-281

Edgar GJ, Stuart-Smith RD, Willis TJ, Kininmonth S, Baker SC, Banks S, Barrett NS, Becerro MA, Bernard AT, Berkhout J (2014) Global conservation outcomes depend on marine protected areas with five key features. Nature

Eriksson H, Clarke S (2015) Chinese market responses to overexploitation of sharks and sea cucumbers. Biological Conservation 184:163-173

Espinoza M, Heupel MR, Tobin AJ, Simpfendorfer CA (2014a) Residency patterns and movements of grey reef sharks (*Carcharhinus amblyrhynchos*) in semi-isolated coral reef habitats. Marine Biology:1-16

Espinoza M, Cappo M, Heupel MR, Tobin AJ, Simpfendorfer CA (2014b) Quantifying shark distribution patterns and species-habitat associations: implications of Marine Park Zoning. PloS one 9:e106885

Estes JA, Tinker MT, Williams TM, Doak DF (1998) Killer whale predation on sea otters linking oceanic and nearshore ecosystems. Science 282:473-476

Estes JA, Terborgh J, Brashares JS, Power ME, Berger J, Bond WJ, Carpenter SR, Essington TE, Holt RD, Jackson JBC (2011a) Trophic downgrading of planet earth. Science 333:301-306

Estes JA, Terborgh J, Brashares JS, Power ME, Berger J, Bond WJ, Carpenter SR, Essington TE, Holt RD, Jackson JB (2011b) Trophic downgrading of planet Earth. science 333:301-306

Estrada JA, Rice AN, Lutcavage ME, Skomal GB (2003) Predicting trophic position in sharks of the north-west Atlantic Ocean using stable isotope analysis. Journal of the Marine Biological Association of the UK 83:1347-1350

Estrada JA, Aaron NR, Natanson LJ, Skomal GB (2006) Use of Isotopic Analysis of Vertebrae in Reconstructing Ontogenetic Feeding Ecology in White Sharks. Ecology 87:829-834

Ferretti F, Myers RA, Serena F, LOTZE HK (2008) Loss of large predatory sharks from the Mediterranean Sea. Conservation Biology 22:952-964

Ferretti F, Worm B, Britten GL, Heithaus MR, Lotze HK (2010) Patterns and ecosystem consequences of shark declines in the ocean. Ecology Letters 13:1055-1071

Field I, Meekan M, Speed C, White W, Bradshaw CJA (2011) Quantifying movement patterns for shark conservation at remote coral atolls in the Indian Ocean. Coral Reefs 30:61-71

France R (1995) Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. Marine ecology progress series Oldendorf 124:307-312

Frank KT, Petrie B, Choi JS, Leggett WC (2005) Trophic cascades in a formerly cod-dominated ecosystem. Science 308:1621-1623

Frid A, G Baker G, M Dill L (2007) Do shark declines create fear-released systems? Oikos 117:191-201

Friedlander AM, DeMartini EE (2002) Contrasts in density, size, and biomass of reef fishes between the northwestern and the main Hawaiian islands: the effects of fishing down apex predators. Marine Ecology Progress Series 230:253-264

Friedlander AM, Sandin SA, DeMartini EE, Sala E (2010) Spatial patterns of the structure of reef fish assemblages at a pristine atoll in the central Pacific. Marine Ecology Progress Series 410:219-231

Friedlander AM, Zgliczynski BJ, Ballesteros E, Aburto-Oropeza O, Bolaños A, Sala E (2012) The shallow-water fish assemblage of Isla del Coco National Park, Costa Rica: structure and patterns in an isolated, predator-dominated ecosystem. Revista de Biología Tropical 60:321-338

Frisch A, Ireland M, Baker R (2014) Trophic ecology of large predatory reef fishes: energy pathways, trophic level, and implications for fisheries in a changing climate. Marine Biology 161:61-73

Froese R, Pauly D (2012) Fishbase (www database). World wide web electronic publications Available at: http://www fishbase org Accessed June

Fry, B. (2006). Stable isotope ecology. Springer Science & Business Media.

Fry B, Scalan R, Parker P (1983) 13C/12C ratios in marine food webs of the Torres Strait, Queensland. Marine and Freshwater Research 34:707-715

Gadig OBF, Bezerra, M.A., Feitosa, R.D. and Furtado-Neto, M.A. (2000) Ictiofauna marinha do Estado do Ceará, Brasil: I. Elasmobranchii. Arquivos de Ciências do Mar, Fortaleza 33:51-56

Gallagher AJ, Hammerschlag N (2011) Global shark currency: the distribution, frequency, and economic value of shark ecotourism. Current Issues in Tourism 14:797-812

Garla R, Chapman D, Shivji M, Wetherbee B, Amorim A (2006a) Habitat of juvenile Caribbean reef sharks, Carcharhinus perezi, at two oceanic insular marine protected areas in the southwestern Atlantic Ocean: Fernando de Noronha Archipelago and Atol das Rocas, Brazil. Fisheries Research 81:236-241

Garla RC, Chapman DD, Wetherbee BM, Shivji M (2006b) Movement patterns of young Caribbean reef sharks, Carcharhinus perezi, at Fernando de Noronha Archipelago, Brazil: the potential of marine protected areas for conservation of a nursery ground. Marine Biology 149:189-199

Gibson J (2003) Glover's Reef Marine Reserve & World Heritage Site Management Plan. Commissioned by the Coastal Zone Management Authority and Institute (CZMAI) on behalf of the Fisheries Department Belize City, Belize CZMAI, Belize City

Gibson J, McField M, Heyman W, Wells S, Carter J, Sedberry G (2004) Belize's evolving system of marine reserves. Marine Reserves: a Guide to Science, Design and Use The Ocean Conservancy Island Press, Washington, DC:287-315

Gilliam D, Sullivan K (1993) Diet and feeding habits of the southern stingray Dasyatis americana in the central Bahamas. Bulletin of Marine Science 52:1007-1013

Goetze J, Fullwood LAF (2012) Fiji's largest marine reserve benefits reef sharks. Coral Reefs:1-5

Graham NA, Spalding MD, Sheppard CR (2010) Reef shark declines in remote atolls highlight the need for multi-faceted conservation action. Aquatic Conservation: Marine and Freshwater Ecosystems 20:543-548

Grubbs D, Snelson F, Piercy A, Rosa R, Furtado M (2006) Dasyatis americana. 2009 IUCN Red List of Threatened Species

Grubbs RD (2010) Ontogenetic shifts in movements and habitat use. Sharks and their relatives II: Biodiversity, adaptive physiology, and conservation CRC Press, Boca Raton:p319-350

Grubbs RD, Musick JA, Conrath CL, Romine JG (2007) Long-term movements, migration, and temporal delineation of a summer nursery for juvenile sandbar sharks in the Chesapeake Bay region. American Fisheries Society Symposium 50:87

Halpern BS (2003) The impact of marine reserves: do reserves work and does reserve size matter? Ecological applications 13:117-137

Halpern BS, Warner RR (2002) Marine reserves have rapid and lasting effects. Ecology Letters 5:361-366

Halpern BS, Warner RR (2003) Review Paper. Matching marine reserve design to reserve objectives. Proceedings of the Royal Society of London Series B: Biological Sciences 270:1871-1878

Halpern BS, Selkoe KA, Micheli F, Kappel CV (2007) Evaluating and ranking the vulnerability of global marine ecosystems to anthropogenic threats. Conservation Biology 21:1301-1315

Hammerschlag N, Gallagher AJ, Lazarre DM, Slonim C (2011) Range extension of the Endangered great hammerhead shark Sphyrna mokarran in the Northwest Atlantic: preliminary data and significance for conservation. Endangered Species Research 13:111-116

Harvey ES, Cappo M, Butler JJ, Hall N, Kendrick GA (2007) Bait attraction affects the performance of remote underwater video stations in assessment of demersal fish community structure. MARINE ECOLOGY-PROGRESS SERIES- 350:245

Heagney EC, Lynch TP, Babcock RC, Suthers IM (2007) Pelagic fish assemblages assessed using mid-water baited video: standardising fish counts using bait plume size. MARINE ECOLOGY-PROGRESS SERIES- 350:255

Heithaus MR, Dill LM (2002) Food availability and tiger shark predation risk influence bottlenose dolphin habitat use. Ecology 83:480-491

Heithaus MR, Wirsing A, Dill L (2012) The ecological importance of intact top-predator populations: a synthesis of 15 years of research in a seagrass ecosystem. Marine and Freshwater Research 63:1039-1050

Heithaus MR, Wirsing AJ, Frid A, Dill LM (2007a) Behavioral indicators in marine conservation: lessons from a pristine seagrass ecosystem. Israel Journal of Ecology and Evolution 53:355-370

Heithaus MR, Frid A, Wirsing AJ, Worm B (2008a) Predicting ecological consequences of marine top predator declines. Trends Ecol Evol 23:202-210

Heithaus MR, Frid A, Wirsing AJ, Dill LM, Fourqurean JW, Burkholder D, Thomson J, Bejder L (2007b) State-dependent risk-taking by green sea turtles mediates top-down effects of tiger shark intimidation in a marine ecosystem. Journal of Animal Ecology 76:837-844

Hemminga M, Mateo M (1996) Stable carbon isotopes in seagrasses: variability in ratios and use in ecological studies. Marine Ecology Progress Series 140

Heupel M, Simpfendorfer C (2014) Importance of envi-ronmental and biological drivers in the presence and space use of a reef-associated shark. Mar Ecol Prog Ser 496:47-57

Heupel M, Simpfendorfer C, Hueter R (2003) Running before the storm: blacktip sharks respond to falling barometric pressure associated with Tropical Storm Gabrielle. Journal of fish biology 63:1357-1363

Heupel M, Williams A, Welch D, Ballagh A, Mapstone B, Carlos G, Davies C, Simpfendorfer C (2009) Effects of fishing on tropical reef associated shark populations on the Great Barrier Reef. Fisheries Research 95:350-361

Heupel MR, Simpfendorfer CA, Fitzpatrick R (2010) Large–scale movement and reef fidelity of grey reef sharks. PloS one 5:e9650

Heupel MR, Knip DM, Simpfendorfer CA, Dulvy NK (2014) Sizing up the ecological role of sharks as predators. Mar Ecol Prog Ser 495:291-298

Hobson KA (1999) Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120:314-326

Hueter R, Heupel M, Heist E, Keeney D (2005) Evidence of philopatry in sharks and implications for the management of shark fisheries. Journal of Northwest Atlantic Fishery Science 35:239-247

Hussey NE, MacNeil MA, Fisk AT (2010a) The requirement for accurate diet-tissue discrimination factors for interpreting stable isotopes in sharks. Hydrobiologia 654:1

Hussey NE, Brush J, McCarthy ID, Fisk AT (2010b) delta15N and delta13C diet-tissue discrimination factors for large sharks under semi-controlled conditions. Comparative biochemistry and physiology Part A, Molecular & integrative physiology 155:445

Hussey NE, Olin JA, Kinney MJ, McMeans BC, Fisk AT (2012) Lipid extraction effects on stable isotope values (δ 13 C and δ 15 N) of elasmobranch muscle tissue. Journal of Experimental Marine Biology and Ecology 434:7-15

Hussey NE, MacNeil MA, McMeans BC, Olin JA, Dudley SFJ, Cliff G, Wintner SP, Fennessy ST, Fisk AT (2014) Rescaling the trophic structure of marine food webs. Ecology Letters 17:239-250

Hutchings JA, Reynolds JD (2004) Marine fish population collapses: consequences for recovery and extinction risk. BioScience 54:297-309

Ingram W, Henwood T, Grace M, Jones L, Driggers W, Mitchell K (2005) Catch rates, distribution and size composition of large coastal sharks collected during NOAA Fisheries Bottom Longline Surveys from the US Gulf of Mexico and US Atlantic Ocean. National Marine Fisheries Service, Southeast Fisheries Science Center, Pasagoula, MS– SEDAR 11

Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SIBER–Stable Isotope Bayesian Ellipses in R. Journal of Animal Ecology 80:595-602

Jennings S (2000) Patterns and prediction of population recovery in marine reserves. Reviews in Fish Biology and Fisheries 10:209-231

Jennings S, Mélin F, Blanchard JL, Forster RM, Dulvy NK, Wilson RW (2008) Global-scale predictions of community and ecosystem properties from simple ecological theory. Proceedings of the Royal Society B: Biological Sciences 275:1375-1383

Jordan L, Banks K, Fisher L, Walker B, Gilliam D (2010) Elevated sedimentation on coral reefs adjacent to a beach nourishment project. Marine pollution bulletin 60:261-271

Kennedy M, Gray RD (1993) Can Ecological Theory Predict the Distribution of Foraging Animals? A Critical Analysis of Experiments on the Ideal Free Distribution. Oikos 68:158-166

Kessel S, Chapman D, Franks B, Gedamke T, Gruber S, Newman J, White E, Perkins R (2014) Predictable temperature-regulated residency, movement and migration in a large, highly mobile marine predator (Negaprion brevirostris). Marine ecology progress series 514:175-190

Kramer K, Heck KL (2007) Top-down trophic shifts in Florida Keys patch reef marine protected areas. Marine Ecology Progress Series 349:111-123

Laverock B, Gilbert J, Tait K, Osborn AM, Widdicombe S (2011) Bioturbation: impact on the marine nitrogen cycle. Biochemical Society Transactions 39:315

Layman CA, Arrington AD, Montan CG, Post DM (2007) Can Stable Isotope Ratios Provide For Community-Wide Measures of Trophic Structure? Ecology 88:42-48

Lester SE, Halpern BS (2008) Biological responses in marine no-take reserves versus partially protected areas. Marine Ecology Progress Series 367:49-56

Lester SE, Halpern BS, Grorud-Colvert K, Lubchenco J, Ruttenberg BI, Gaines SD, Airamé S, Warner RR (2009) Biological effects within no-take marine reserves: a global synthesis. Marine Ecology Progress Series 384:33-46

Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation: a review and prospectus. Canadian journal of Zoology 68:619-640

Lo NC, Jacobson LD, Squire JL (1992) Indices of relative abundance from fish spotter data based on Delta-Lognornial Models. Canadian Journal of Fisheries and Aquatic Sciences 49:2515-2526

Lowe CG, Wetherbee BM, Crow GL, Tester AL (1996) Ontogenetic dietary shifts and feeding behavior of the tiger shark, Galeocerdo cuvier, in Hawaiian waters. Environmental Biology of Fishes 47:203-211

Madin EMP, Gaines SD, Warner RR (2010) Field evidence for pervasive indirect effects of fishing on prey foraging behavior. Ecology 91:3563-3571

Malcolm HA, Gladstone W, Lindfield S, Wraith J, Lynch TP (2007) Spatial and temporal variation in reef fish assemblages of marine parks in New South Wales, Australia-baited video observations. MARINE ECOLOGY-PROGRESS SERIES- 350:277

Maljković A, Côté IM (2011) Effects of tourism-related provisioning on the trophic signatures and movement patterns of an apex predator, the Caribbean reef shark. Biological Conservation 144:859-865

McCauley DJ, Micheli F, Young HS, Tittensor DP, Brumbaugh DR, Madin EM, Holmes KE, Smith JE, Lotze HK, DeSalles PA (2010) Acute effects of removing large fish from a near-pristine coral reef. Marine biology 157:2739-2750

McEachran J, Carvalho Md, Carpenter K (2002) Batoid fishes. The living marine resources of the Western Central Atlantic, FAO Species identification guide for fishery purposes and American Society of Ichthyologists and Herpetologists Special Publication, Rome 3:2127

McKibben JN, Nelson DR (1986) Patterns of movement and grouping of gray reef sharks, Carcharhinus amblyrhynchos, at Enewetak, Marshall Islands. Bulletin of Marine Science 38:89-110

Meekan M, Cappo M (2004) Non-destructive techniques for rapid assessment of shark abundance in northern Australia. Australian Institute of Marine Science

Michael SW (2005) Reef sharks and rays of the world. ProStar Publications

Molloy PP, McLean IB, Côté IM (2009) Effects of marine reserve age on fish populations: a global meta-analysis. Journal of Applied Ecology 46:743-751

Morris J, Akins J, Barse A, Cerino D, Freshwater D, Green S, Muñoz R, Paris C, Whitfield P (2009) Biology and ecology of the invasive lionfishes, Pterois miles and Pterois volitans. Proceedings of the Gulf and Caribbean Fisheries Institute 29:409-414

Motta F, Moura R, Francini-Filho R, Namora R (1999) Elasmobrânquios dos recifes Manoel Luıs—MA. Resumos do XIII Encontro Brasileiro de Ictiologia 1:267

Mumby PJ, Dahlgren CP, Harborne AR, Kappel CV, Micheli F, Brumbaugh DR, Holmes KE, Mendes JM, Broad K, Sanchirico JN (2006) Fishing, trophic cascades, and the process of grazing on coral reefs. science 311:98-101

Munroe S, Simpfendorfer C, Heupel M (2014) Defining shark ecological specialisation: concepts, context, and examples. Reviews in Fish Biology and Fisheries 24:317-331

Musick J, Burgess G, Cailliet G, Camhi M, Fordham S (2000) Management of sharks and their relatives (Elasmobranchii). Fisheries 25:9-13

Musick JA (1999) Life in the slow lane: ecology and conservation of long-lived marine animals. American Fisheries Society

Myers RA, Worm B (2003) Rapid worldwide depletion of predatory fish communities. Nature 423:280-283

Myers RA, Worm B (2005) Extinction, survival or recovery of large predatory fishes. Philosophical transactions of the Royal Society of London Series B, Biological sciences 360:13-20

Newman MJ, Paredes GA, Sala E, Jackson JB (2006) Structure of Caribbean coral reef communities across a large gradient of fish biomass. Ecology letters 9:1216-1227

O'Shea OR, Thums M, van Keulen M, Meekan M (2012) Bioturbation by stingrays at Ningaloo Reef, Western Australia. Marine and Freshwater Research 63:189-197

Okey TA, Banks S, Born AF, Bustamante RH, Calvopiña M, Edgar GJ, Espinoza E, Fariña JM, Garske LE, Reck GK (2004) A trophic model of a Galápagos subtidal rocky reef for evaluating fisheries and conservation strategies. ecological modelling 172:383-401

Olin JA, Hussey NE, Grgicak-Mannion A, Fritts MW, Wintner SP, Fisk AT (2013) Variable δ15N Diet-Tissue Discrimination Factors among Sharks: Implications for Trophic Position, Diet and Food Web Models. PloS one 8:e77567

Olin JA, Hussey NE, Fritts M, Heupel MR, Simpfendorfer CA, Poulakis GR, Fisk AT (2011) Maternal meddling in neonatal sharks: implications for interpreting stable isotopes in young animals. Rapid Communications in Mass Spectrometry 25:1008-1016

Papastamatiou YP, Wetherbee BM, Lowe CG, Crow GL (2006) Distribution and diet of four species of carcharhinid shark in the Hawaiian Islands: evidence for resource partitioning and competitive exclusion. Marine Ecology Progress Series 320:239-251

Papastamatiou YP, Friedlander AM, Caselle JE, Lowe CG (2010) Long-term movement patterns and trophic ecology of blacktip reef sharks (*Carcharhinus melanopterus*) at Palmyra Atoll. Journal of Experimental Marine Biology and Ecology 386:94-102

Parker R, Mays R (1998) Southeastern US deepwater reef fish assemblages, habitat characteristics, catches, and life history summaries. US Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Scientific Publications Office

Parnell AC, Jackson AL (2011) siar: Stable Isotope Analysis in R. R package version 4.1.3.

Pauly D, Christensen V, Dalsgaard J, Froese R, Torres F (1998) Fishing down marine food webs. Science 279:860-863

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annual review of ecology and systematics:293-320

Pikitch EK, Chapman DD, Babcock EA, Shivji MS (2005) Habitat use and demographic population structure of elasmobranchs at a Caribbean atoll (Glover's Reef, Belize). Marine Ecology Progress Series 302:187-197

Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703-718

Purwanto M, Wilson J, Ardiwijaya R, Mangubhai S (2012) Coral Reef Monitoring in Kofiau and Boo Islands Marine Protected Area, Raja Ampat, West Papua. 2009—2011. The Nature Conservancy, Indo-Pacific Division, Jakarta:3-4

Queiroz EL, Souza-Filho, J.J. and Simões, F.M. (1993) Estudo da alimentação de Dasyatis guttata (Bloch & Schneider, 1801), na área de imfuência de Estação Ecológica Ilha do Medo, BA. Summaries of the VI Meeting of the Work group on Fishes and Research on Sharks and Rays in Brazil, Recife 28

Randall JE (1964) Contributions to the biology of the queen conch, Strombus gigas. Bulletin of Marine Science 14:246-295

Randall JE (1967) Food habits of reef fishes of the West Indies. Institute of Marine Sciences, University of Miami

Ricklefs RE, Nealen P (1998) Lineage-Dependent Rates of Evolutionary Diversification: Analysis of Bivariate Ellipses. Functional Ecology 12:871-885

Ripple WJ, Larsen EJ, Renkin RA, Smith DW (2001) Trophic cascades among wolves, elk and aspen on Yellowstone National Park's northern range. Biological Conservation 102:227-234

Ripple WJ, Beschta RL, Fortin JK, Robbins CT (2014) Trophic cascades from wolves to grizzly bears in Yellowstone. Journal of Animal Ecology 83:223-233

Rizzari JR, Bergseth BJ, Frisch AJ (2015) Impact of conservation areas on trophic interactions between apex predators and herbivores on coral reefs. Conservation Biology 29:418-429

Rizzari JR, Frisch AJ, Hoey AS, McCormick MI (2014) Not worth the risk: apex predators suppress herbivory on coral reefs. Oikos

Robbins WD, Hisano M, Connolly SR, Choat JH (2006) Ongoing collapse of coral-reef shark populations. Current Biology 16:2314-2319

Roberts C (2012) Marine Ecology: Reserves Do Have a Key Role in Fisheries. Current Biology 22:R444-R446

Roberts CM, Bohnsack JA, Gell F, Hawkins JP, Goodridge R (2001) Effects of marine reserves on adjacent fisheries. Science 294:1920-1923

Romine JG, Musick JA, Burgess GH (2009) Demographic analyses of the dusky shark, Carcharhinus obscurus, in the Northwest Atlantic incorporating hooking mortality estimates and revised reproductive parameters. Environmental Biology of Fishes 84:277-289

Rosa RS, Mancini, P., Caldas, J.P. & Graham, R.T (2006) Carcharhinus perezi. The IUCN Red List of Threatened Species. The IUCN Red List of Threatened Species Version 20143

Rubenstein DR, Hobson KA (2004) From birds to butterflies: animal movement patterns and stable isotopes. Trends in Ecology & Evolution 19:256-263

Ruppert JLW, Travers MJ, Smith LL, Fortin MJ, Meekan MG (2013) Caught in the Middle: Combined Impacts of Shark Removal and Coral Loss on the Fish Communities of Coral Reefs. Plos One 8:9

Russ GR, Alcala AC (2003) Marine reserves: Rates and patterns of recovery and decline of predatory fish, 1983-2000. Ecological Applications 13:1553-1565

Russ GR, Cheal AJ, Dolman AM, Emslie MJ, Evans RD, Miller I, Sweatman H, Williamson DH (2008) Rapid increase in fish numbers follows creation of world's largest marine reserve network. Current Biology 18:R514-R515

Sala E, Boudouresque C, Harmelin-Vivien M (1998) Fishing, trophic cascades, and the structure of algal assemblages: evaluation of an old but untested paradigm. Oikos:425-439

Schlaff AM, Heupel MR, Simpfendorfer CA (2014) Influence of environmental factors on shark and ray movement, behaviour and habitat use: a review. Reviews in Fish Biology and Fisheries:1-15

Sergio F, Caro T, Brown D, Clucas B, Hunter J, Ketchum J, McHugh K, Hiraldo F (2008) Top predators as conservation tools: ecological rationale, assumptions, and efficacy. Annual review of ecology, evolution, and systematics 39:1-19

Shears N, Babcock R (2002) Marine reserves demonstrate top-down control of community structure on temperate reefs. Oecologia 132:131-142

Shears NT, Babcock RC (2003) Continuing trophic cascade effects after 25 years of no-take marine reserve protection. Marine ecology Progress series 246:1-16

Simpfendorfer CA (2000) Predicting population recovery rates for endangered western Atlantic sawfishes using demographic analysis. Environmental Biology of Fishes 58:371-377

Simpfendorfer CA, Hueter RE, Bergman U, Connett SM (2002) Results of a fishery-independent survey for pelagic sharks in the western North Atlantic, 1977–1994. Fisheries Research 55:175-192

Sobel J, Dahlgren C (2004) Marine reserves: a guide to science, design, and use. Island Press

Stevens J, Bonfil R, Dulvy N, Walker P (2000) The effects of fishing on sharks, rays, and chimaeras (chondrichthyans), and the implications for marine ecosystems. ICES Journal of Marine Science: Journal du Conseil 57:476-494

Stevenson C, Katz LS, Micheli F, Block B, Heiman KW, Perle C, Weng K, Dunbar R, Witting J (2007) High apex predator biomass on remote Pacific islands. Coral Reefs 26:47-51

Stewart-Oaten A (1986) The before-after/control-impact-pairs design for environmental impact assessment. Marine Review Committee

Stobart B, García-Charton JA, Espejo C, Rochel E, Goñi R, Reñones O, Herrero A, Crec'hriou R, Polti S, Marcos C (2007) A baited underwater video technique to assess shallow-water Mediterranean fish assemblages: Methodological evaluation. Journal of Experimental Marine Biology and Ecology 345:158-174

Strong WR, Snelson FF, Gruber SH (1990) Hammerhead shark predation on stingrays: an observation of prey handling by Sphyrna mokarran. Copeia 1990:836-840

Sweeting CJ, Barry J, Barnes C, Polunin NVC, Jennings S (2007) Effects of body size and environment on diet-tissue δ15N fractionation in fishes. Journal of Experimental Marine Biology and Ecology 340:1-10

Tavares RAT (2009) Fishery biology of the Caribbean reef sharks, Carcharhinus perezi (Poey, 1876), in a Caribbean insular platform: Los Roques Archipelago National Park, Venezuela. Pan-American Journal of Aquatic Sciences 4:500-512

Taylor RB, Morrison MA, Shears NT (2011) Establishing baselines for recovery in a marine reserve (Poor Knights Islands, New Zealand) using local ecological knowledge. Biological Conservation 144:3038-3046

Team RDC (2010) R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. Available: http://www.R-project.org.

Terborgh J, Estes J, Paquet P, Ralls K, Boyd-Heger D, Miller B, Noss R (1999) The role of top carnivores in regulating terrestrial ecosystems. Wild Earth 9:42-56

Tilley A, López-Angarita J, Turner JR (2013) Diet Reconstruction and Resource Partitioning of a Caribbean Marine Mesopredator Using Stable Isotope Bayesian Modelling. PloS one 8:e79560

Ubeda AJ, Simpfendorfer C, Heupel M (2009) Movements of bonnetheads, Sphyrna tiburo, as a response to salinity change in a Florida estuary. Environmental biology of fishes 84:293-303

Udyawer V, Chin A, Knip DM, Simpfendorfer CA, Heupel MR (2013) Variable response of coastal sharks to severe tropical storms: environmental cues and changes in space use. Mar Ecol Prog Ser 480:171-183

Vander Zanden MJ, Cabana G, Rasmussen JB (1997) Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios (δ15N) and literature dietary data. Canadian Journal of Fisheries and Aquatic Sciences 54:1142-1158

Venables W, Ripley B (2002) Modern applied statistics with S Springer-Verlag. New York

Walker TI (1998) Can shark resources be harvested sustainably? A question revisited with a review of shark fisheries. Marine and Freshwater Research 49:553-572

Ward-Paige CA, Mora C, Lotze HK, Pattengill-Semmens C, McClenachan L, Arias-Castro E, Myers RA (2010) Large-scale absence of sharks on reefs in the greater-Caribbean: a footprint of human pressures. PloS one 5:e11968

Ward-Paige C, Keith D, Worm B, Lotze HK (2012) Recovery potential and conservation options for elasmobranchs. Journal of fish biology 80:1844-1869

Watson D, Anderson M, Kendrick G, Nardi K, Harvey E (2009) Effects of protection from fishing on the lengths of targeted and non-targeted fish species at the Houtman Abrolhos Islands, Western Australia

Watson DL, Harvey ES, Anderson MJ, Kendrick GA (2005) A comparison of temperate reef fish assemblages recorded by three underwater stereo-video techniques. Marine Biology 148:415-425

Watson DL, Harvey ES, Kendrick GA, Nardi K, Anderson MJ (2007) Protection from fishing alters the species composition of fish assemblages in a temperate-tropical transition zone. Marine Biology 152:1197-1206

Wetherbee BM, Gruber SH, Cortés E (1990) Diet, feeding habits, digestion, and consumption in sharks, with special reference to the lemon shark, Negaprion brevirostris. NOAA Technical Report NMFS 90:29-47

Wetherbee BM, Crow GL, Lowe CG (1997) Distribution, reproduction and diet of the gray reef shark Carcharhinus amblyrhynchos in Hawaii. Marine Ecology Progress Series 151:181-189

Wetherbee BM, Cortés E, Carrier J, Musick J, Heithaus M (2004b) Food consumption and feeding habits. Biology of Sharks and their Relatives:223-242

White ER, Myers MC, Flemming JM, Baum JK (2015) Shifting elasmobranch community assemblage at Cocos Island—an isolated marine protected area. Conservation Biology

Wirsing AJ, Ripple WJ (2010) A comparison of shark and wolf research reveals similar behavioral responses by prey. Frontiers in Ecology and the Environment 9:335-341

Wirsing AJ, Heithaus MR, Dill LM (2007) Fear factor: do dugongs (Dugong dugon) trade food for safety from tiger sharks (Galeocerdo cuvier)? Oecologia 153:1031-1040

Wirsing AJ, Heithaus MR, Frid A, Dill LM (2008) Seascapes of fear: evaluating sublethal predator effects experienced and generated by marine mammals. Marine Mammal Science 24:1-15

Wood LJ, Fish L, Laughren J, Pauly D (2008) Assessing progress towards global marine protection targets: shortfalls in information and action. Oryx 42:340-351

Workgroup FSSR (2004) Factors influencing seagrass recovery in Feather Sound, Tampa Bay, Florida

Worm B, Davis B, Kettemer L, Ward-Paige CA, Chapman D, Heithaus MR, Kessel ST, Gruber SH (2013) Global catches, exploitation rates, and rebuilding options for sharks. Marine Policy 40:194-204

Yokota LL, Rosângela Paula (2006) A nursery area for sharks and rays in Northeastern Brazil. Environmental Biology of Fishes 75:349-360