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DOCTOR OF PHILOSOPHY

Functional ecology of the southern stingray, *Dasyatis Americana*

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Award date:
2011

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FUNCTIONAL ECOLOGY OF THE
SOUTHERN STINGRAY, *DASYATIS AMERICANA*



PRIFYSGOL
BANGOR
UNIVERSITY

Alexander Tilley

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of
Philosophy in Marine Biology at

Bangor University in 2011

School of Ocean Sciences

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Acknowledgements

The fieldwork phase of this study was made possible by the help and hard work of a number of volunteers. Firstly, the relentless enthusiasm, organisation and sense of humour of my research assistant and close friend Bryan Edwards were appreciated throughout; I couldn't have done it without you. Also, data collection was greatly enhanced by volunteers Marie Smedley, Nick Paige, Barry Shoesmith, Alyson McGahee, Manuel Gonzalez-Rivero, my father Ray Tilley, and many more who generously gave their time and manpower. Additionally, boat captain Faygon Villanueva showed continued passion and enthusiasm for the project even during the midnight shifts, for which I am truly grateful.

Technical aspects of fieldwork were made possible by funding from the Ulysses S. Seal Conservation Award from the Minnesota Zoo, and the Vemco Systems student award. Additionally the flexibility and generosity of the Wildlife Conservation Society allowed me to conduct this research in tandem with my employment as station manager at Glovers Reef, and for enhanced fieldwork immeasurably with provision of boats, equipment and staff time.

To my supervisor John Turner, for having the confidence in me to conduct this study independently at great distance, and his advice and support in times of need. Also thanks to Demian Chapman, Les Kaufman, Bob Michener and Nigel Hussey for help along the way with both methods and manuscript advice.

To my family who have shown tremendous patience through the years of absence, yet have always encouraged and supported my endeavours, I thank you with all my heart.

Finally I would like to thank my wife Juliana for her love and bright ideas, and patient endurance of long discussions, late nights and the cold of Wales with unfaltering support - Te amo para siempre.

Abstract

The global decline of large sharks has fuelled a rapidly expanding field of research into their ecological role within coastal and pelagic systems, yet the ecological importance of smaller mesopredatory elasmobranchs is largely unknown. The goal of this study was to evaluate the functional ecology of the southern stingray in a system of known predator abundance using wildlife surveys, acoustic tracking and stable isotope analysis. Elasmobranch community composition (number of species) and population size were estimated at Glovers Reef Atoll, Belize, and distribution was quantified according to habitat type and prey density. *Dasyatis americana* was the most abundant elasmobranch in lagoon and shallow forereef habitats with an overall atoll population of ~5500 individuals; the lagoon population was dominated by female rays (3:1), and stingray habitat use was influenced by individual size and diel stage. Benthic prey availability correlated with depth and habitat complexity in lagoon margins, yet large female and juvenile rays were abundant in the depauperate shallow sand flat areas during daylight, suggesting the use of this habitat for thermal and safety advantages respectively. Active tracking of rays illustrated heightened crepuscular activity by rays in all life stages, with intermittent activity through day and night. Activity space was larger during daylight and increased with ontogeny, with large (>70 cm) individuals remaining active at night suggesting use of a size refuge. Analysis of movement path structure showed stingray response to two distinct spatial scales, corresponding to topographical features of their landscape. Rays orientate and utilise foraging patches up to a scale of ~100 m, but move randomly at greater spatial scales >3 km, suggesting rays may use the distribution of patch reefs as a network of refuges, connected by pathways of potential foraging areas as seen in some terrestrial animals. Stable isotope analysis revealed stingrays are reliant upon a diverse prey base and forage opportunistically on a number of prey groups, causing little overlap in isotopic niche space with sympatric shark species. Mixing models showed bivalves and invertebrate worms proportionately more important in diet compared to crustaceans and teleost fish. The combined findings of this study indicate that predation risk has a strong influence on stingray behaviour, causing a trade-off of energy for safety in juveniles in terms of movement periodicity and habitat preference. The dietary breadth of stingrays implies their key importance in stabilising benthic communities to trophic perturbations, however population and habitat use changes resulting from a release of predation pressure may significantly impact benthic community structure. This study represents an important step towards a greater understanding of the basic ecology of these organisms, which is crucial to making informed management decisions for species and ecosystem conservation.

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1. General Introduction

1.1 Phylogeny & Taxonomy

Chondrichthyans are the primitive, evolutionarily conserved class of cartilaginous fish comprised of sharks, rays, skates and chimaeras, that have survived in diverse ecosystems for over 400 million years (Fowler et al., 2005). The superorder Batoidae (Batoids) is made up of 631 species in nine sub-orders, and includes the rays, skates, sawfishes and guitarfishes (Fowler et al., 2005). Batoids are extremely widely distributed, ranging from polar latitudes to tropical seas from the shoreline to depths of 3000 metres (McEachran & Aschliman, 2004). The batoids exhibit a dorso-ventrally flattened morphology that arose at least 200 million years after the divergence of sharks and chimaeras (McEachran & Aschliman, 2004). Many chondrichthyans grow slowly, mature at late ages and have few young, so despite their evolutionary success some are now threatened with extinction as a result of human activity and their very conservative life-history traits (McEachran & Aschliman, 2004).

The four major batoid orders are torpediniforms (electric rays), pristiforms (sawfish), rajiforms (skates & guitarfish), and myliobatiforms (stingrays and pelagic rays), having emerged in the late Jurassic to Palaeocene eras in the fossil record (Cappetta, 1987; Cailliet & Goldman, 2004). Despite morphological similarities, myliobatiforms and rajiforms are phylogenetically distinct, having evolved their flattened body shape independently (Hildebrand & Schroeder, 1928; Compagno, 1973). The Whiptail Stingrays, Dasyatidae, are represented by 81 species in 5 genera (Compagno, 1999; White & Sommerville, 2010), exhibiting huge diversity in head morphology, eye position, swimming behaviour, ecology (Bigelow & Schroeder, 1953; McComb & Kajiura, 2008; Aguiar et al., 2009) and size (Compagno & Roberts, 1984; Aguiar, 2003; Le Port et al., 2008). They possess five pairs of gill slits on their ventral side, internal gill arches without filter plates or ridges, and spiracles for drawing water through the gill chamber (Compagno, 1999). Dasyatidae are distributed circumglobally in tropical to warm temperate seas, with 61 species being present in tropical marine systems (Sonnier et al., 1976; Bowman et al., 2000; White & Sommerville, 2010), some occurring exclusively in tropical freshwaters (Berra, 2007), and one exclusively pelagic species *Dasyatis violacea* (Myers & Worm, 2003; Baum & Myers, 2004; Domingo et al., 2005; Baum & Worm, 2009). They are most commonly found in shallow estuarine or coastal regions, and are increasingly recognised as having ecological and commercial importance (Smith & Merriner, 1985; Thrush et al., 1991; Francis, 1998; Kohler & Turner, 2001; Voegeli et al., 2001; Sims, 2010). The 35 currently recognised species of the genus *Dasyatis* are extremely variable in their longevity, with some freshwater rays living as few as 3 years, ranging up to the (male) southern stingray, *Dasyatis americana*, having a T_{\max} value of 28 years

(Cartamil et al., 2003; Cailliet & Goldman, 2004; Vaudo & Lowe, 2006; Collins et al., 2007; Le Port et al., 2008).

1.2 Biology, Physiology & Distribution

This study focuses on *Dasyatis americana* (Hildebrand & Schroeder, 1928; Francis, 1998), commonly known as the southern stingray: a large bodied, demersal stingray commonly found throughout the Western Atlantic stretching from New Jersey to Brazil (Bigelow & Schroeder, 1953; CONAPESCA-INP, 2004; Grijalba-Bendeck et al., 2007; Aguiar et al., 2009; Garro et al., 2009). Abundance is high around remote islands such as the Fernando de Noronha archipelago (Aguiar, 2003; Smith et al., 2008); and the Mesoamerican atolls of Mexico and Belize (Pikitch et al., 2005), yet offshore sightings are rare (Sonnier et al., 1976; Bowman et al., 2000; Henningsen, 2000; Chapman et al., 2003), with the species being highly associated with reef fringes and sand flats. Chondrichthyan research has expanded significantly in recent decades in response to increasing evidence of huge global declines e.g. (Stokes & Holland, 1992; Gilliam & Sullivan, 1993; Myers & Worm, 2003; Baum & Myers, 2004; Baum & Worm, 2009), and technological advances in sophistication and size of marine tracking and positioning systems (see section 1.5), which have made it possible to study the movement and habitat use of sharks (Kohler & Turner, 2001; Voegeli et al., 2001; Henningsen & Leaf, 2010; Sims, 2010), and rays (Cartamil et al., 2003; Vaudo & Lowe, 2006; Collins et al., 2007; Le Port et al., 2008; Aguiar et al., 2009; Carvalho et al., 2010). Yet ray (Myliobatiformes) ecology lags far behind that of sharks and skates (Rajiformes), perhaps due to their general absence from commercial fisheries in developed countries. Research has found skate to be highly vulnerable to longline and trawl fisheries (Strong et al., 1990; Francis, 1998), yet the inclusion of stingrays in artisanal fisheries of developing countries and as commercial by-catch is yet to be appropriately investigated. Stingrays have been shown to form a large and important part of targeted commercial and artisanal fisheries in Central and South America (Smith & Merriner, 1985; CONAPESCA-INP, 2004; Grijalba-Bendeck et al., 2007; Garro et al., 2009), and though conservation research remains extremely limited, initial data suggests a low resilience to fishing pressure (Howard et al., 1977; Smith et al., 2008) as might be expected of such long lived species. Research has been undertaken with wild and captive southern stingrays in aspects such as reproduction (Henningsen, 2000; Thrush & Dayton, 2002; Chapman et al., 2003); diet (Stokes & Holland, 1992; Gilliam & Sullivan, 1993; Lohrer et al., 2004); growth (Hines et al., 1997; Meysman et al., 2006; Henningsen & Leaf, 2010); distribution (Thrush, 1999; Aguiar et al., 2009; Carvalho et al., 2010); and behaviour (Strong et al., 1990; Henningsen, 2000; Henningsen & Leaf, 2010), however little has been done in relating this information to overall functional ecology of this species

on a community scale within shallow coral reef systems. This thesis aims to address this issue, and provide information on movement, habitat use, population structure and trophic ecology to gain a better understanding of batoid ecology with relation to community dynamics.

Stingrays have been shown to play an important role as benthic predators, exploiting an ecological niche (Smith & Merriner, 1985; Henningsen, 2000; Cailliet & Goldman, 2004) and considerably modifying the structure of habitat they feed in (Howard et al., 1977; Aguiar, 2003). Marine sediments are geochemical sources and sinks (Hoenig, 1990; Stevens et al., 2000; Thrush & Dayton, 2002), and bioturbation is a key process in seabed systems that enables more oxygenated water to pass deeper into the sediment, and inorganic nutrients to be flushed out, than is possible solely by diffusion between sediment particles (Lohrer et al., 2004; Grubbs et al., 2006). This process is brought about through the suction feeding mechanism of rays, creating pits in the substrate as well as the burrowing of crustaceans, shuffling of gastropods and urchins (e.g. *Strombus* spp., *Tripneustes* spp.) (Hines et al., 1997; Meysman et al., 2006; Stephens et al., 2007). In addition, this disturbance of sediment for infaunal prey is a key biological force in driving the biodiversity of benthic communities by clearing space and changing conditions allowing for the recolonisation and recruitment of prey patches (Connell, 1978; Sheader, 1993; Sih & Christensen, 2001).

However, more information is necessary on the natural history of demersal predators and benthic prey in soft sediment communities, particularly in relation to response to different scales of environmental heterogeneity (Thrush, 1999; Stephens et al., 2007), and the key exogenous drivers of their habitat use.

Dasyatis americana exhibit extreme sexual dimorphism, with females being ~ 53% larger than males (Henningsen and Leaf, 2010), reaching maximum disk widths of ~ 150 cm and ~ 67 cm and weights of 87.7 kg and 17.5 kg respectively (Brown, 1992; Henningsen, 2000; Henningsen & Leaf, 2010). Longevity has been estimated to a mean of 22 years and a T_{max} of ~30 years (Charnov, 1976; Henningsen, 2000; Cailliet & Goldman, 2004). *Dasyatis americana* can exhibit varied colouration on its dorsal surface, from light to dark grey, maroon, olive and brown (Bigelow & Schroeder, 1953; Tilley pers. obs.). *Dasyatis americana* differs from the majority of species in the genus, by exhibiting a caudal membrane only on the ventral surface of the tail, outside of the central longitudinal line of tubercles; and possesses two lines of dorsal tubercles (thorns) on the dorsal disc (Aguiar, 2003; Stephens et al., 2007).

Like most chondrichthyans, *Dasyatis americana* exhibits a K-selected life history strategy. This strategy, exhibited by many elasmobranchs, is commonly defined by long gestation periods, slow growth and maturity, and long life span (Hoenig, 1990; Stevens et al., 2000; Frid et al., 2008).

Dasyatis americana are viviparous (live bearing), aplacental with uterine villi or trophonemata

(Fowler et al., 2005). The exact number of offspring varies directly with maternal size, but mean litter size in a captive environment is 4.2 (Henningson, 2000). Neonates adhere to a 1:1 sex ratio, with size and weight ranging from 200–340 mm DW and 282–1128 g (Henningson, 2000). *Dasyatis americana* has been documented to have a biannual reproductive cycle in captivity (Henningson, 2000) and annual reproduction in the wild (Grubbs et al., 2006) suggesting perhaps that fecundity can be increased under certain conditions, such as a release of predation pressure.

1.3 Foraging and Predation Risk

Optimal Foraging Theory (OFT) is the basis of describing and modelling animal behaviour in search of food (Brown & Kotler, 2004; Stephens et al., 2007). The core principle of OFT is that an organism will forage in a way that maximises energy intake while expending the minimum amount of energy (Ripple et al., 2001; Sih & Christensen, 2001). However, it is important to remember that optimality is a mathematical phenomenon, and somewhat misleading given the coarseness of biological mechanisms such as foraging (Lima & Dill, 1990; Stephens et al., 2007). Foraging models can be split into two main types: 1) Diet models, which analyse decisions by a forager such as to attack, manipulate and eat a prey item, and predict that low energy prey items will be ignored in favour of high energy/larger prey items if they are commonly available (Stephens et al., 2007); and 2) patch models which predict how much time a forager will spend on a patch (concentration of prey) with diminishing returns, before moving on (Schaller, 1972; Brown, 1992; Brown & Kotler, 2004). Charnov's *marginal value theorem* (Charnov, 1976) implies that a predator will select patches of highest prey density, and will only pursue prey until it is more energetically worthwhile to go after alternative prey items, than continue (Brown, 1992). In contrast, Peterson et al. (2001) suggested that hyper abundant schooling rays feed to extinction on site-specific prey species, which could have wider significance in terms of understanding the dynamics of trophic cascades.

Tactical choices made by a forager depend heavily on states such as hunger and fat reserves (*state dependence*), and predation risk or safety (Strong et al., 1990; Cliff, 1995; Chapman, 2002; Stephens et al., 2007). The energy state of a meso-consumer greatly affects its foraging activity (Gudger, 1907; Fowler, 1926; Frid et al., 2008): an animal in a high-energy state craves safety (Dill & Fraser, 1984; Cliff & Dudley, 1991; Brown, 1992), and an animal in a low energy state will take more risks to find food, being unable to afford anti-predator behaviour and vigilance (Frid et al., 2008). The tools available to a forager are time allocation (to a particular activity), habitat selection and vigilance. In this way, fear can be a very powerful ecological force throughout trophic systems (Lowe et al., 1996; Brown & Kotler, 2004), by predation controlling the frequency and intensity of grazing by herbivores (Cortés & Gruber, 1990; Ripple et al., 2001). A decision made by an animal to avoid predation is the

singular most important decision it can make, because survival depends on it, hence fear is the greatest driver of prey behaviour (Lima & Dill, 1990; Dudley et al., 2005).

Although observed predator-prey interactions are relatively rare (Schaller, 1972; Visser, 1999; Brown & Kotler, 2004), it is known that batoids are key prey for large bodied predatory sharks (Fowler, 1926; Compagno, 1984) such as the great hammerhead, *Sphyrna mokarran* (Strong et al., 1990; Cliff, 1995; 1995; Chapman, 2002); smooth hammerhead, *Sphyrna zygaena* (Gudger, 1907; Fowler, 1926; Stevens & Lyle, 1989); bull shark, *Carcharhinus leucas* (Dodrill, 1977; Cliff & Dudley, 1991); tiger shark, *Galeocerdo cuvier* (Lowe et al., 1996); lemon shark *Negaprion brevirostris* (Cortés & Gruber, 1990) and dusky Shark, *Carcharhinus obscurus* (Dudley et al., 2005). Even killer whales, *Orcinus orca* in New Zealand (Visser, 1999) and adult Goliath groupers (*Epinephelus itajara*) in the Caribbean (Randall, 1967) have been found to feed on benthic stingrays. The Sphyrnid (hammerhead) sharks are found worldwide in tropical waters, and are known to favour stingrays and other batoid prey (Compagno, 1984) in South Africa (Cliff, 1995), Australia (Stevens & Lyle, 1989) and the Caribbean (Dodrill, 1977). It has been suggested that the size and orientation of the broad head possessed by sphyrnid sharks, may be a morphological adaptation allowing them to more effectively capture and handle batoid prey (Strong et al., 1990). The rate of consumption (*functional response*) of batoid rays by sharks is largely unknown as a result of logistical and spatial reasons (Heithaus et al., 2002), yet interaction strength between these predators and prey can be estimated from stomach analyses, and studies tend to show a positive correlation between predator size and percentage of non-empty stomachs containing rays (Cliff & Dudley, 1991; Lowe et al., 1996). This is supported by a positive correlation between shark trophic level and body size (Cortés, 1999), as might be expected.

The *Ideal Free Distribution* (IFD) represents a theoretical situation where predators (foragers) select habitat patches according to prey density, and in the absence of competition and predation risk, their distribution should be relative to their food availability (Fretwell & Lucas, 1970). This is a useful tool in assessing the cost of predation to foragers, and consequently to assess the predation risk applied to a foraging animal. If predation risk is present this means higher abundances of foragers will be found in safer habitats (Heithaus & Dill, 2002; Ripple & Beschta, 2007; Hammerschlag et al., 2010). IFD is a habitat selection model that can be used as a null hypothesis in studies assessing predation risk and other factors of habitat selection during foraging (Hammerschlag et al., 2010), whereby if relative densities of foragers in safer habitats are higher than they would be according to IFD, they are trading off food (energy) for safety (Fretwell & Lucas, 1970; Lima & Dill, 1990), and predation risk is a likely driving factor in their movement, habitat selection and foraging intensity.

1.4 Ecological Function & Trophic Structure

Food chain dynamics have for many decades been considered the central theory of ecology (Fretwell, 1987; Steneck & Sala, 2005). Hairston et al. (1960) argued that the fact that the world is green suggests that herbivores are not resource limited (from the bottom up), but instead predator limited from the top down, and it is now widely accepted in ecological theory that despite their relatively low biomass, large predators exert considerable and key impacts on community structure (Paine, 1980; Steneck & Sala, 2005). Carnivores impose top down controls on communities by limiting herbivores, which in turn releases primary producers from intense grazing (Estes et al., 1998; Steneck & Sala, 2005; Myers et al., 2007).

Trophic cascades, described by Paine (1980), is a term that has spread throughout trophic ecology in recent decades, yet still eludes precise definition (Polis et al., 2000), due to its broad usage and a lack of distinction between species level and community level cascades (Schmitz et al., 2004). Species level cascades occur within a subset of the community or certain branches of a food web, such that changes in predator numbers affect the success of a subset (one or a few) of the plant species (Polis et al., 2000; Schmitz et al., 2004). Species level cascades do not usually explain habitat wide changes in biomass distribution between plants and animals (Polis et al., 2000). Evidence from a variety of empirical studies suggests that trophic cascades are widespread, yet their occurrence is regulated by many factors (Pace et al., 1999).

The *mesopredator release hypothesis* states that the elimination or reduction of large carnivores results in increases of mesopredators with concomitant declines in their prey species (Estes et al., 1998; Terborgh, 2001). The ability for marine mesopredators to deplete prey populations is well documented (Orth, 1975; VanBlaricom, 1982; Peterson et al., 2001), but linking the decline in apex predators to a rise in mesopredator population is far more contentious, with many critics arguing that the relatively fast population dynamics are not befitting of elasmobranchs' life history strategies (Heithaus et al., 2010). The role of fear in driving behaviour in marine systems is well documented (Heithaus et al., 2002; Heithaus & Dill, 2006; Wirsing et al., 2007a; 2007b; Heithaus et al., 2009), and recent theoretical models utilising state dependence (energy levels, fat stores, starvation) have suggested that the release of mesopredators from predator intimidation may have greater ecological influences than previously thought, as diet and habitat switching caused by predator release may add increased weight to previously weak interactions (Frid et al., 2008). This supports findings of earlier work that stated a need to quantify per capita interaction strength (defined as the effect of an individual predator on the population of its prey), in order to predict the impacts of the reduction or removal of trophic levels (Bascompte et al., 2005; Steneck & Sala, 2005).

1.5 Conservation and Management

Basic information on species composition and abundance in shallow reef environments is often lacking (VanBlaricom, 1982; Heithaus, 2004). Elasmobranch fishes exhibit conservative life history strategies making them vulnerable to extrinsic threats (Stevens et al., 2000; Fowler et al., 2005) such as the potential impacts of climate change (Chin & Kyne, 2007), habitat destruction (Fowler et al., 2005; Lotze, 2006); pollution (Cortés & Parsons, 1996; Gelsleichter et al., 2005) and fisheries (Stevens et al., 2000; Jackson et al., 2001). Such studies demonstrate the need for baseline assessments of faunal composition and predator species ecology in areas that are (or likely to be) subjected to anthropogenic pressures (Jackson et al., 2001).

Research into elasmobranchs lags far behind the conservation issues, and understanding the processes underlying carnivore roles in structuring their communities has become critically important for ecologists concerned about carnivores, their prey, and their conservation (Gehrt & Prange, 2006). Removal of consumers may impact on ecosystem structure and food webs by way of mesopredator release (Rogers & Caro, 1998; Dulvy et al., 2004; Gehrt & Prange, 2006), especially in diverse systems such as coral reefs (McClanahan et al., 2002; Dulvy et al., 2004; Steneck & Sala, 2005). Tropical marine ecosystems typically exhibit huge trophic complexity and biodiversity, which is thought to increase stability (Caldeira et al., 2005) and resilience to trophic perturbations (Steiner et al., 2006; O'Gorman et al., 2010a). However, the indirect effects predators have in driving prey behaviour is increasingly being shown to be extremely influential (Heithaus et al., 2002; Heithaus & Dill, 2006; Wirsing et al., 2007a; Heithaus et al., 2009), and may cause diet shifts in mesopredators leading to trophic cascades (Frid et al., 2008). Benthic elasmobranchs play a major role in structuring intertidal and sub-tidal ecosystems through bioturbation (Thrush et al., 1991; Lohrer et al., 2004) yet the strength of their interactions with known prey types, such as commercially important species of lobster (Smith & Herrnkind, 1992) and conch (Gilliam & Sullivan, 1993), are still unknown and unpredictable.

Very little work has been done linking climate change to batoids, yet some species of Dasyatidae in the Great Barrier Reef have a low to moderate vulnerability to climate change due to habitat specificity and/or immobility (Chin & Kyne, 2007). Current evidence indicates that some sharks, rays and reef-associated species, although not necessarily geographically restricted, are particularly vulnerable to anthropogenic impacts (Vaudo & Lowe, 2006) and now occur in very low numbers (Monte-Luna et al., 2007). Exploitation of elasmobranch populations is increasing on a global scale, and despite some arguing that shark catch-per-unit-effort figures are increasing (Matsunaga & Nakano, 1999), most report massive declines (Holts et al., 1998; Erickson & Berkeley, 2008), with

some species of sharks showing population declines of up to 99%, due in the most part to fishing pressure (Baum et al., 2003; Myers et al., 2007).

Rays have not traditionally made up a large percentage of catches in the largest targeted elasmobranch fisheries (Bizzarro et al., 2007), however in recent years the percentage catch of rays, and their importance to fisheries, have been increasing (Smith et al., 2008). Stingrays are a common by-catch species in long-line fisheries (Piovano et al., 2010), and becoming increasingly targeted commercially in some undeveloped regions (Francis, 1998), such as Colombia (Grijalba-Bendeck et al., 2007), Costa Rica (Garro et al., 2009) and Mexico (CONAPESCA-INP, 2004; Smith et al., 2007), and have been seen to have low resilience to fishing pressure (Smith et al., 2008). Anecdotal evidence suggests serious declines in local populations of sharks in Belize (Chapman, Stony Brook University and Graham & Wesby, WCS pers. comm.), yet little is known of stingray fishing. Additionally, the connectivity of elasmobranch populations in the Caribbean region is unknown for most species, so estimates of regional health remain speculative. Human population growth of >400% in Belize over the past 60 years (UNPP, 2010) and increased pressure on natural resources will compound the problem in forthcoming years, hence research here is required to understand the ecology of locally occurring sharks and rays, and identify key habitats and interactions with prey species.

Glovers Reef Atoll is one of four coral atolls in the Mesoamerican barrier reef system, and its remote location combined with its protection as a marine reserve since 1993, make it extremely suitable for ecological study of a species in a relatively pristine, undisturbed environment. In addition, Glovers Reef is home to a large healthy population of wild southern stingrays *Dasyatis americana* and has the longest running shark longline survey in the Caribbean, conducted by E. Pikitch and D. Chapman (Stony Brook University), illustrating stable shark populations (Chapman, pers. comm.).

1.6 Overall Hypotheses and Objectives

The overall aim of this study was to investigate the functional ecology of the southern stingray and assess the influence of predation risk in driving stingray spatial and trophic behaviour at Glovers Reef on different temporal and spatial scales, according to physical and biotic factors.

H₁ The extent to which the spatial (distribution, movement, habitat use) and trophic ecology of southern stingrays are driven by biotic and abiotic factors reflects the influence of predation risk on southern stingray behaviour.

Null hypotheses and associated objectives

H₀₁ Abundance and distribution of *Dasyatis americana* is similar to sympatric elasmobranch species and even across macro- and microhabitats.

Obj. 1. To investigate the large-scale distribution and abundance of elasmobranchs in macrohabitats at Glovers Reef Atoll, and to elucidate fine scale habitat selection by *Dasyatis americana* according to biotic and abiotic factors. [Chapter 2]

H₀₂ Stingray movements and habitat selection exhibit no relationships with biotic or abiotic factors.

Obj. 2. To investigate the spatial ecology of the southern stingray, analysing the physical cues driving movement and habitat use of *Dasyatis americana* throughout diel and tidal cycles and according to environmental factors. [Chapter 3]

H₀₃ Stingray movement paths show no variation in structure or orientation with spatial scale.

Obj. 3. To study the structure of stingray movement paths to elucidate mechanisms of search behaviour, orientation, prey patch size and spatial domains. [Chapter 4]

H₀₄ Southern stingray feeding ecology and diet composition is similar to sympatric elasmobranchs and shows no variation according to individual size.

Obj. 4. To investigate the trophic ecology of the southern stingray *Dasyatis americana* at Glovers Reef compared to sympatric elasmobranchs, elucidating trophic level and diet composition using stable isotope analysis of stingray and prey tissues. [Chapter 5]

2. Abundance, distribution and habitat use of *Dasyatis americana* at Glovers Reef Atoll, Belize

Abstract

The southern stingray is an abundant benthic mesopredator in Caribbean shallow reef systems, yet little is known of its population size or behavioural ecology. Knowledge of habitat use and preference of mesopredators is crucial to understanding their functional role in relation to their predators and prey. This study used a combination of in-water and above water wildlife survey techniques and sediment sampling to investigate southern stingray distribution and habitat preference at Glovers Reef Atoll (Belize), testing the hypothesis that habitat use reflects a trade-off between prey availability and predation risk. Southern stingrays were the most abundant elasmobranch within the atoll with a lagoon density of ~252 individuals per km². Juvenile stingrays utilised shallow, near-crest areas of sand flat habitat and forage rarely, predominantly remaining buried or resting on sand throughout the day. Large females (>80 cm disk width) were seven times more abundant in the lagoon than forereef sand flat, and lagoon margin distribution was biased towards warm shallow water (<2 m) with 61% of rays in waters less than 2.5 m deep. Male and female rays of medium size (>40 <80 cm) showed no depth or habitat preference, however small (<40 cm) and medium rays avoided edge habitats at the sand seagrass interface. Size partitioning of habitat against the gradient of prey availability, and avoidance of edge habitat suggest predation risk trade-offs based on size refugia, as well as a possible thermal preference of large females to enhance reproductive processes and for parturition. The influence of predation risk on habitat preference in *Dasyatis americana* has important implications in understanding the functional role of large predatory sharks, and modelling the effects of their decline at a system scale.

2.1 INTRODUCTION

In the absence of competition and predation risk, top predators have been shown to select habitats relative to prey density & availability (Heithaus et al., 2002) in order to maximise their fitness, (Shepherd & Litvak, 2004) thereby adhering to a theoretical *ideal free distribution* (IFD) (Fretwell & Lucas, 1970). The IFD model assumes animals have perfect knowledge of patch profitability, and that this profitability will decrease with increasing density of competitors (Kennedy & Gray, 1993). Consequently, this is a useful tool in assessing the cost and risk of predation to foragers. If predation risk is influential, then higher abundances of foragers will be found in safer habitats more so than dense prey patches (e.g. Ripple and Beschta, 2007, Heithaus et al., 2002, Hammerschlag et al., 2010)

indicating they are trading off food (energy) for safety (Fretwell & Lucas, 1970; Lima & Dill, 1990), and that predation risk is a driving factor in their movement, habitat selection and foraging intensity. Predatory sharks such as *Sphyrna mokarran*, (Strong et al., 1990; Chapman, 2002), *Sphyrna zygaena* (Gudger, 1907), and *Negaprion brevirostris* (Cortes et al., 1990) are the only known predators of batoids in the Caribbean (Dodrill, 1977; Compagno, 1984) and stomach analyses tend to show a positive correlation between predator size and percentage of non-empty stomachs containing rays (Cliff & Dudley, 1991; Lowe et al., 1996), indicating rays may be a prey species increasingly selected by sharks as foraging efficiency and experience increases. The rate of consumption (functional response) of batoid rays by sharks is largely unknown due to logistical and spatial constraints involved in research of such wide ranging predators, however ‘*Crittercam*’ data from tiger sharks tagged with cameras in Western Australia suggest that predation encounters for sharks are much more common on rays than initially thought (Heithaus et al., 2002). Survival and reproduction are the strongest forces driving the movement and behaviour of any organism, and as such the risk of predation has a crucial significance in the decisions and behavioural trade-offs made by prey species during every day, as well as on an evolutionary time scale, where predation has been seen to be a key process in the development of species traits (e.g. crypsis, colouration) and reproductive strategies (Lima & Dill, 1990). Landscape plays a crucial role in habitat selection as a result of either minimising predator encounter rates, or enhancing escape probability (Heithaus et al., 2009), where prey species may utilise fringe habitats to better facilitate escape paths, or merely select habitats where encounter rates with predators are lower. Temporal variations in habitat selection on various scales are also significant in systems where predator numbers or detection (or number of competitors) may fluctuate with time (e.g. seasonal (Heithaus & Dill, 2006; Vaudo & Heithaus, 2009), or diel cycles (Chapman et al., 2007). Thus, it was hypothesised in this research that stingray distribution and habitat preference at Glovers Reef should reflect a trade-off between prey density and predation risk.

The influential role of benthic elasmobranchs in structuring intertidal and sub-tidal ecosystems through bioturbation is well documented (Thrush et al., 1991; Lohrer et al., 2004), but less is known regarding their direct effects on prey species as a mesopredator, or the effects of prey availability on stingray habitat use. A recent study on bioenergetics in Kaneohe Bay, Hawaii showed that brown stingrays (*Dasyatis lata*) exert considerable direct pressure on crustacean prey populations, and are likely to be the predominant driver of prey population dynamics in the area (Dale, 2011). *Dasyatis americana* is an opportunistic forager, feeding on a wide range of benthic invertebrates and teleosts. Stomach content analyses have shown diet to be dominated by decapod crustaceans (alphaeid, penaeid and callinassid shrimp, palinurid juveniles and brachyuran crabs, Gilliam & Sullivan, 1993)

and sipunculid and polychaete worms, (Randall, 1967). The southern stingray is also a known predator of commercially important Caribbean species such as the spiny lobster *Panulirus argus* (Smith & Herrnkind, 1992) and queen conch *Strombus gigas* (Gilliam & Sullivan, 1993). Habitat selection of *Dasyatis americana* is largely unknown, and has only been estimated at two remote (island) sites in Brazil, where age-specific habitat partitioning is consistent with the ‘bigger-deeper’ pattern of many sharks and rays (Yokota & Lessa, 2006; Grubbs, 2010) where juveniles remain in shallow nearshore areas, expanding to deeper reef areas through ontogeny (Aguilar et al., 2009; Carvalho et al., 2010). Habitat use at Atol das Rocas was observed to shift in relation to tidal state, and overall population had a female bias of >5:1 (Carvalho et al., 2010). There are very few assessments of habitat selection according to prey availability for elasmobranchs, yet most carried out with juveniles in nursery areas show limited or no correlation between prey availability and habitat selection (Morrissey & Gruber, 1993a; Heupel & Hueter, 2002; Vaudo, 2011), suggesting rather that predation risk may be a stronger driver of habitat selection of rays and juvenile sharks. Trophic level shows a general increase with ontogeny in elasmobranchs, as energetic demand increases and larger prey items are consumed (Cortés & Gruber, 1990; Ebert & Bizzarro, 2007; Grubbs, 2010). Given the strong influence stingrays can have over invertebrate prey species (Dale, 2011) greater understanding of their habitat use and foraging ecology will enable more effective predictions of mesopredator population dynamics on ecosystems and associated fisheries. The aim of this chapter was 1) to investigate the large-scale distribution and abundance of elasmobranchs in macrohabitats at Glovers Reef Atoll, and 2) to elucidate fine scale habitat selection by *Dasyatis americana* according to biotic and abiotic factors.

Hypotheses & Objectives

H₀ Distribution of elasmobranchs at Glovers Reef does not differ across all macro- and microhabitat types, and across temporal scales. Biotic and abiotic factors have no influence on habitat selection of *Dasyatis americana*.

H_{A1} Stingray abundance in shallow lagoon margin habitat fluctuates relative to season and temperature.

H_{A2} Stingray depth distribution is structured by individual size and water temperature.

H_{A3} Elasmobranch species density and distribution in macrohabitats reflect broad prey choice

H_{A4} Benthic prey species abundance increase with habitat complexity

H_{A5} Stingray habitat selection represents a trade off between prey availability and predation risk.

- Obj. 1.** To investigate seasonal abundance, distribution and population size for elasmobranchs in Glovers Reef shallow lagoon margins using monthly 1 mile long ‘Distance sampling’ transects by boat at 14 sites over a two-year period.
- Obj. 2.** To investigate and compare abundance and depth distribution of *Dasyatis americana* according to size, sex and time of day in shallow lagoon margins and on a forereef sand slope, using random 90-minute swim transects.
- Obj. 3.** To survey forereef abundance of elasmobranchs biannually at Glovers Reef using teams of 6 swimmers at 16 repeated transect sites to elucidate seasonal differences over a two-year period.
- Obj. 4.** To analyse abundance of elasmobranchs in the deep lagoon habitat across diel and seasonal time scales using biannual 30 minute SCUBA transects.
- Obj. 5.** To quantify benthic prey abundance by substrate type and depth in lagoon margins by excavating and sieving sediment from randomly placed 1 m² quadrats at selected sites.
- Obj. 6.** To gather annual water temperature data using in situ data loggers at varying depth in macrohabitats of Glovers Reef over a two-year period.

2.2 METHODS

2.2.1 Study Site

The study was undertaken on Glovers Reef Atoll (W -87. N 16.), the southernmost of four coral atolls in the Mesoamerican Barrier Reef System (MBRS), situated approximately 70 km east of the Belizean coast, and 30 km east of the main barrier reef (Fig. 2.1). Glovers Reef is approximately 27 km long by 10 km wide, with its eastern windward edge sloping from a surface crest to over 1000 m in depth. To the north and west (leeward edge), depth slopes more gradually to \pm 400 m from a submerged crest. The atoll covers approximately 254 km² most of which is made up by the lagoon, surrounded by a reef crest. The total area of marine habitat at Glovers Reef is approximately 253.45 km². Sand flat habitat covers \sim 34.2 km², and forereef slope habitat represents \sim 22.8 km².

The east side is normally exposed to prevailing north-easterly winds and therefore has higher wave energy and water flow (Renken, 2008). The horizontal visibility for the exposed side is approximately 25 m looking along a transect line at approximately 8 metres depth, whereas on the sheltered side horizontal visibility rarely exceeds 10 m (Renken, 2008).

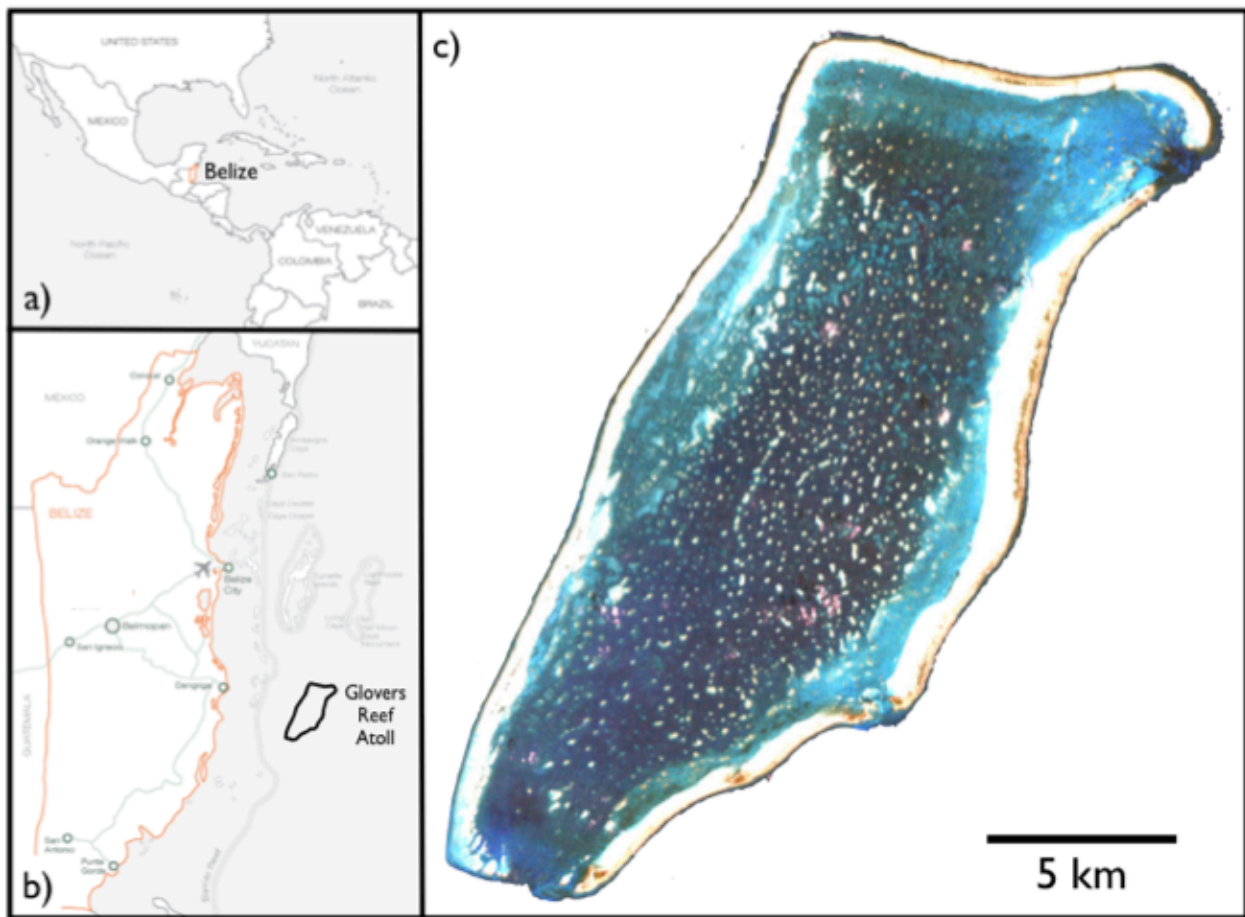


Figure 2.1: Map depicting Belize (outlined in orange) within the Central America region (inset a), the location of Glovers Reef Atoll within Belizean territory (inset b), and a satellite image of Glovers Reef Atoll (c) (Landsat image with inset maps adapted from http://www.belizejungledome.com/dome_location.html).

The fore reef at Glovers Reef drops off between 15–45 m depth and the forereef shelf is less than 500 m wide in most areas. The windward (eastern) forereef, composed largely of low-relief spur and groove formations (of *Montastrea* spp., *Diploria* spp.), is more developed and wider than the leeward (western) forereef. The reef crest on the west side of the atoll is submerged (~1.5–2 m depth), while the eastern crest is exposed, broken by 2 large and 3 minor cuts which connect forereef and lagoon systems. The lagoon is vaguely basin shaped with a maximum depth of ~18 m, dotted with ~860 patch reefs (10 m to >1 km long) throughout the interior, which are composed largely of massive corals (*Montastrea* spp., *Diploria* spp., *Siderasteria* spp.) and macroalgae. Deep lagoon sediments are characterised by silts and muds, sparsely distributed with seagrass. The lagoon margin is defined by a sand apron extending inward from the reef crest to a depth of ~2 m, and a flattened shallow lagoon comprised of seagrass meadows (turtle grass, *Thalassia testudinum* and Manatee grass, *Syringodium filiforme*) and algal fields amongst patch reefs to a depth of ~5 m (Fig. 2.6). There are 5 cayes (reef islands topped with sand) along the south eastern edge of the lagoon (accounting for a total area of 0.6 km²), some of which are at least partially fringed with mangroves

and surrounded on the lagoon side by seagrass and sand flats in shallow water (<2 m depth). *Halodule wrightii* seagrass is common in very shallow, fine sand tidal flats near to the cayes. Average annual rainfall is 175 cm, with the rainy season from June to October, and regular salinities of ~35 ppt occur in the lagoon throughout the year (Renken, 2008). Mean water temperatures range from ~25.5 - 31 °C in the lagoon, and ~26 – 30 °C on the windward forereef (fig. 2.2).

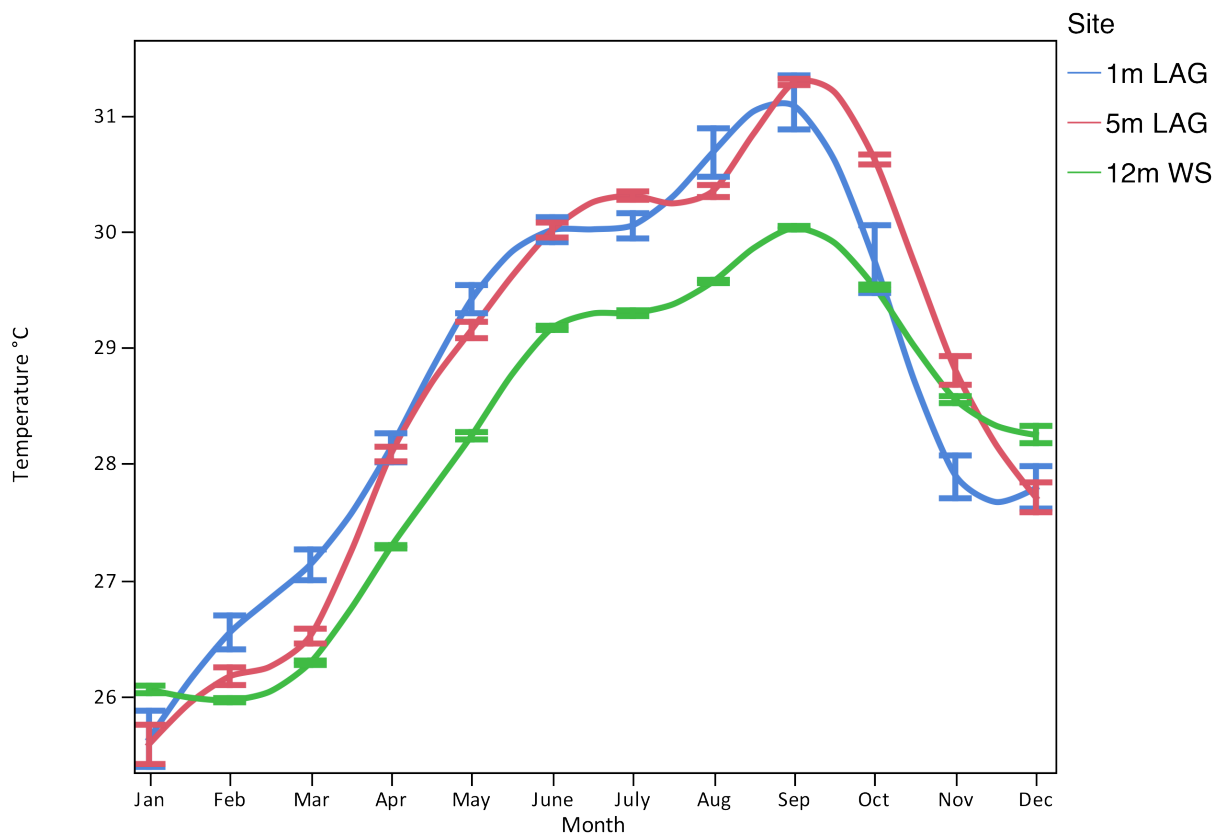


Figure 2.2: Mean monthly temperatures (°C) for lagoon and forereef sampling sites throughout fieldwork period (Jan 2009-Aug 2010). Lagoon temperature was recorded at depths of 1 m (1m LAG - blue) and 5 m (5m LAG - red) using *in situ* Hobo pendant temperature loggers (Onset Corp.). Forereef temperature was recorded at 12 m depth (12m WS - green) near sampling site of White Sands using a Hobo temp pro logger (Onset Corp.).



Figure 2.3: Image of White Sands study site and Long Caye at Glovers Reef Atoll, Belize. The atoll lagoon is at the top of the picture, the forereef drop-off at the bottom. White Sands (600 m²) sits between the island of Long Caye and the reef edge.

Glovers Reef atoll comes under the governance of Belize Fisheries Department (BFD) as the Glovers Reef Marine Reserve (GRMR) established in 1993. The reserve is divided into 3 usage zones: 1) The General Use Zone – the largest proportion of the reserve representing 258 km² of the atoll out to the 180 m depth contour line, is open to fishing regulated by gear type, seasonal species restrictions and special licenses; 2) The conservation zone – a 72 km² no-take area encompassing all 5 cayes on the south-eastern edge of the atoll, and narrowing across the lagoon to the western reef of the atoll; and 3) the wilderness zone – a circular area <1 km² set aside as a pristine habitat, located in the lagoon at the southern end of Middle Caye, where the Wildlife Conservation Society Research Station and the BFD headquarters are located.

2.2.2 Shallow lagoon elasmobranch surveys by boat

Surveys were conducted between June 2009 and July 2010 making use of shallow clear sand flats for identification of elasmobranchs from a skiff (Fig. 2.4). 14 sites were selected in the lagoon margin around the interior edge of the atoll, conducted monthly (fig. 2.6). *Distance* sampling methods were

used to facilitate estimation of population size and species density by calculation of detection probability. Each transect ran for 1.6 kilometres at a constant speed of 2 km/hour, with two observers recording sightings from the bow of a skiff, recording information on species, size category, sex (if distinguishable by presence of claspers) and distance from the midline of the boat. Size of observed rays was categorised into three groups of small (<40 cm), medium (40-80 cm) and large (>80 cm), based upon approximations of neonate and maturity estimates from (Henningsen, 2000). Transects were conducted in conditions of Beaufort scale 2 or less to minimise sampling bias caused by poor visibility. Environmental data for lagoon and fore reef habitats were recorded throughout the year using Hobo pendant and pro temperature and light loggers (Onset Corp.) and a weather station (Oregon Scientific) situated at the Glovers Reef research station on Middle Caye.

Dasyatis americana encounter frequencies from transects were log transformed to achieve normality then frequencies were compared using ANOVA by site, month and time of day (am/pm). Frequencies of other elasmobranch species in lagoon margins were too low to be transformed or effectively analysed. Species frequencies were analysed by sample site and against temperature and tidal cycles using JMP 9 statistical software (SAS Institute Inc.).

Data analysis using *Distance sampling*

Population estimates for stingrays were generated using *Distance* 6.0 release 2 (Thomas et al., 2010). Density of southern stingrays for lagoon habitats was calculated using the equation:

$$D = \frac{nf(0)}{2L}$$

Where D is density of rays, L is the total length of all transects combined, n is the total number of observations and $f(0)$ is the probability density function of observed perpendicular distances evaluated at $x = 0$ (Buckland et al., 2001). Following the protocol outlined in Thomas et al. (2010), data was initially explored using a distribution histogram of observed distances and a quantile-quantile (Q-Q) graph. Distance estimates greater than 20 m were discarded due to deviations from the line in Q-Q plots. Distance estimates were grouped into 7 intervals of distance to rectify rounding of small data values towards zero, and more closely fit a uniform cosine distribution model.

Habitat areas for population estimates were calculated using ArcMap 9.3 (ESRI) using an extant habitat raster map of the atoll created by P. Mumby (MSEL, University of Exeter) and reclassified by C. D'Agrosa (WCS 2005).



Figure 2.4: Clear shallow sand flat waters allowed for sampling of stingray and nurse shark *Ginglymostoma cirratum* (pictured) abundance by boat in the lagoon margins at Glovers reef.

2.2.3 In-water elasmobranch surveys: shallow lagoon and White Sands

Random 90-minute swims (snorkelling) at constant speed were conducted within the lagoon to the north and south of Middle Caye, and at the forereef site of White Sands (WS – Fig. 2.3) between November 2009 and March 2010. Sex and size category (as above) of observed stingrays were recorded, along with habitat, depth and stingray behaviour data. Other elasmobranchs observed during these swims were also recorded. All snorkelling adhered to guidelines in Bangor University snorkelling code of practice.

2.2.4 In-water elasmobranch surveys: forereef

In-water surveys were conducted at 16 forereef sites around the atoll bi-annually in April and November. 1 hour swim transects were run along fore reef habitat <20 m deep, using teams of 6-8 observers spread from ~4 m depth to the reef edge, spaced approximately 10 m apart (Fig. 2.5). Variations in width, depth and visibility of fore reef habitat necessitated adaptation to swimmer number and spacing. Each swimmer counted the total number of each species seen during each 1 hour swim period which were then totalled and recorded at the end of the 1 hour period. To avoid duplicate sightings, swimmers signalled observations to the person on either side of them. Swimmers were external volunteers and staff from the Wildlife Conservation Society and Belize Fisheries Department.

Data analysis of in-water sampling

Data resolution from forereef sampling was much lower than lagoon margins due to methodological differences of dealing with multiple observers in deeper water, so population density estimation necessitated much cruder calculation than lagoon margins. Total area surveyed was divided by the total frequency of observations from transects, and multiplied by total available area to calculate atoll forereef population. The mean distance travelled by swimmers in 1 hour transects was used to calculate species densities by dividing the number of each species sighted by the total area of forereef sampled. This value was approximate in order to gauge abundance in comparison with shallow and deep lagoon habitats. This sampling method was not deemed appropriate for estimating deeper dwelling elasmobranchs such as reef sharks, but was designed to estimate the frequency of other elasmobranchs on the shallow forereef (<20 m) in the daytime compared to rays.

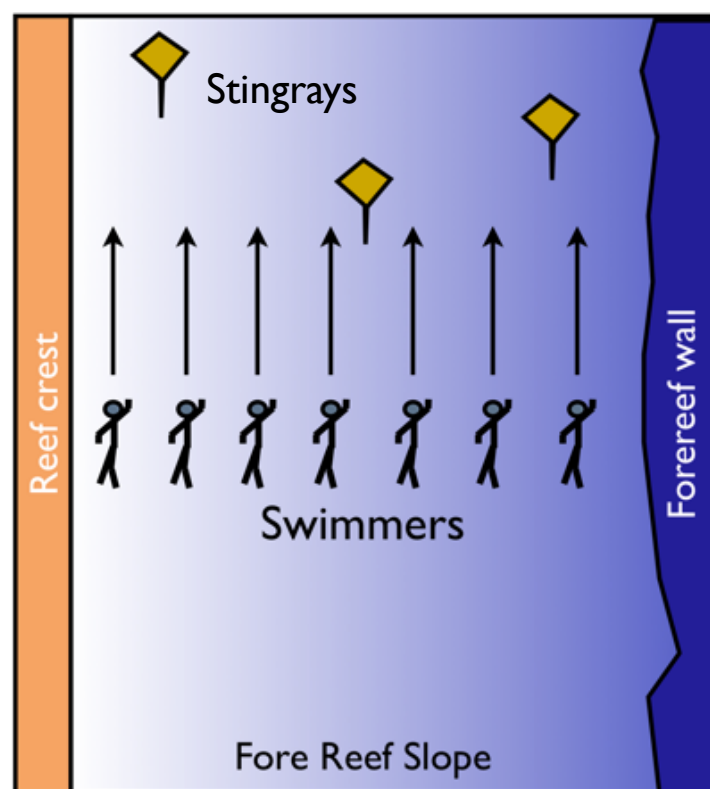


Figure 2.5: Diagram depicting forereef elasmobranch sampling method involving a team of 6-8 swimmers sweeping along the forereef slope parallel to the reef crest, recording individual elasmobranch encounters.

2.2.5 Deep lagoon elasmobranch surveys (SCUBA)

A 30 minute dive survey was initially conducted monthly at two randomly selected sites in the deep lagoon (12-18 m) (fig 2.6), however after initial findings of zero encounters of any elasmobranch species, sampling effort was decreased to bi-annual repetition. Two observers finned at constant speed (~40 kick cycles per minute) in a west to east direction counting elasmobranch species

detected. All diving operations were conducted according to *Diving operations at work* regulations 1992 and Bangor University diving rules, as well as the Wildlife Conservation Society diving policy.

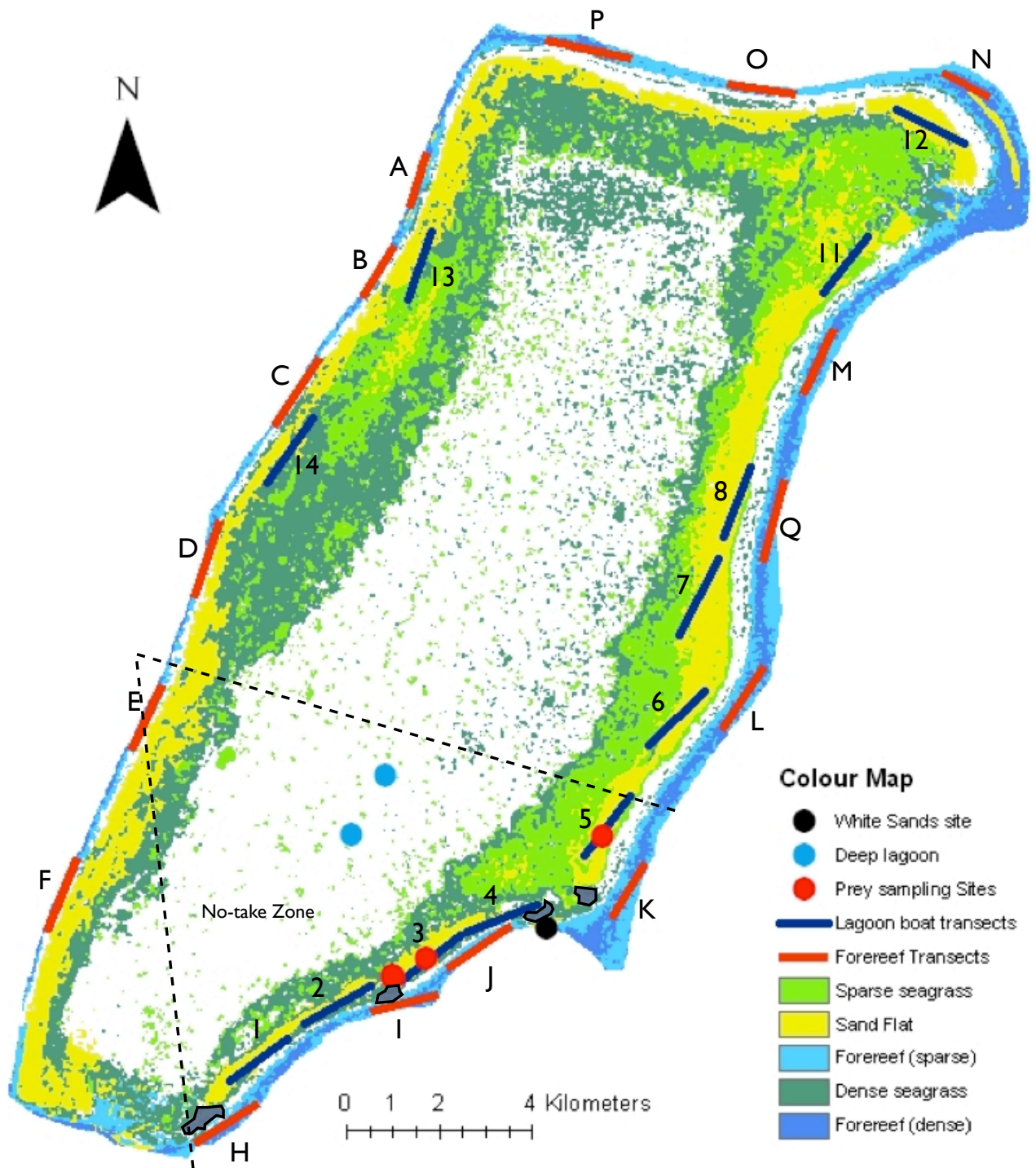


Figure 2.6: Habitat map of Glovers Reef Marine Reserve with sampling sites for boat and swim transects and prey sampling conducted between May 2008-August 2010. Forereef sites are lines labelled from A-Q. Lagoon margin sites are lines labelled 1-14. Benthic prey sampling sites are labelled with red dots, and the forereef sand slope of White Sands is labelled with a black dot. The deep lagoon is white, and sand cayes situated along the south eastern edge are grey. The boundaries of the no-take zone of the marine reserve are marked with a black dotted line.

2.2.6 Sediment sampling methods: benthic prey species abundance

Sediment sampling for benthic prey species was carried out at sites selected to represent a range of depth and substrate types within the lagoon margin, between June and August 2010. Sampled substrate types in the lagoon margin were divided into 5 categories of sand, algae, sparse seagrass (<20% cover), medium seagrass (<50% cover) and dense seagrass (>50% cover). A sample area was chosen for each substrate type (fig 2.6) where three replicate quadrats were excavated. A 1 m² quadrat was laid on the substrate and all the sediment was extracted to a depth of approximately 25 cm to capture all potential prey available to excavating stingray predators. A suction pump was constructed from a 4 m drainage pipe linked to a SCUBA tank of compressed air. The diver released air into the tube which rose to create a suction of sediment and infaunal species up to a sieve in the boat, where it was collected, sorted and identified to the lowest taxonomic level possible in order to assess species richness and diversity according to depth and substrate type. Sampling was not carried out at depths greater than 4 m due to equipment limitations.

2.2.7 Environmental data collection

Water temperature data was recorded at 1 m & 5 m depths using data loggers (Hobo pendant, Onset Computers). Light intensity was measured at 1 m water depth, limited to two-week periods 4 times per year due to marine fouling affecting accuracy of light sensors. Weather parameters of wind speed, air temperature, light intensity and rainfall were recorded on land throughout the field phase using a weather station (Oregon Scientific).

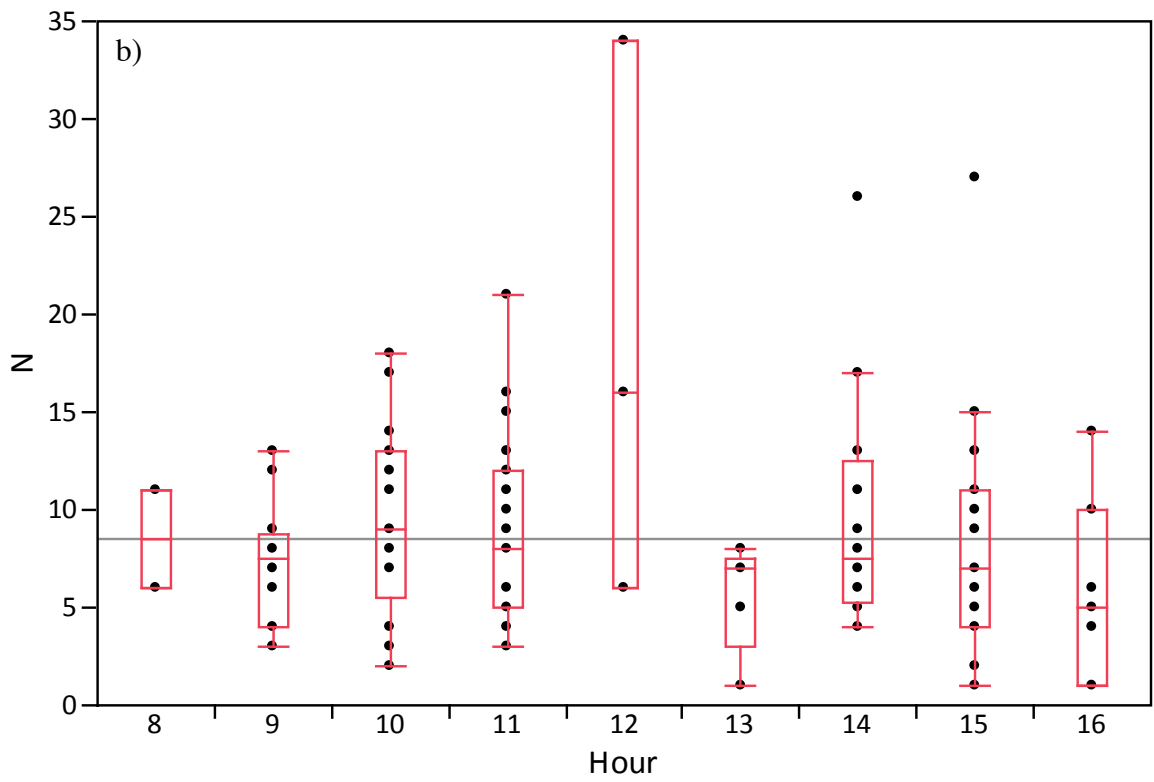
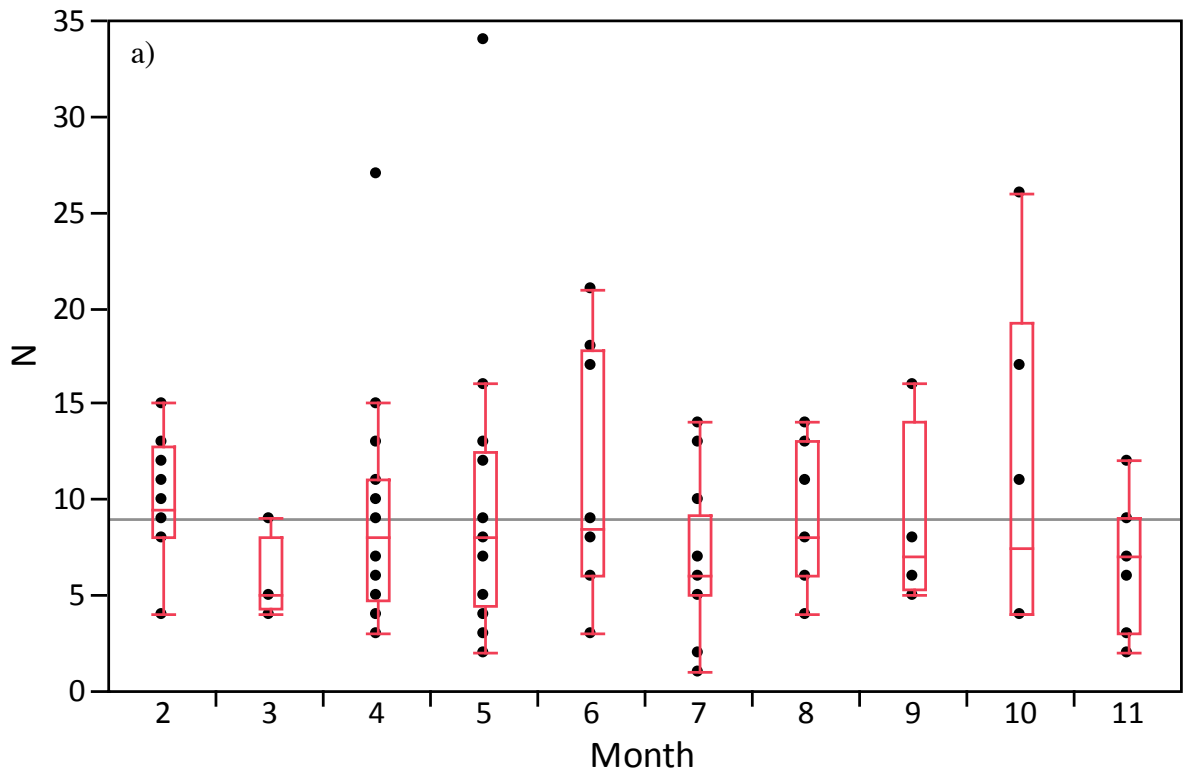
2.3 RESULTS

Nine species of elasmobranch were observed during fieldwork sampling at Glovers Reef: the southern stingray *Dasyatis americana*; spotted eagle ray *Aetobatus narinari*; nurse shark *Ginglymostoma cirratum*; Caribbean reef shark *Carcharhinus perezi*; yellow stingray *Urolophus jamaicensis*; tiger shark *Galeocerdo cuvier*; Caribbean sharpnose *Rhizoprionodon porosus*, lemon shark *Negaprion brevirostris* and the critically endangered Caribbean electric ray *Narcine bancroftii*. Additional species sightings at Glovers Reef communicated (longline sampling, dive encounters, or fishermen captures) during the study period or sighted outside of regular surveys include the whale shark *Rhincodon typus*; Cuban night shark *Carcharhinus signatus*; silky shark *Carcharhinus falciformes*; dusky smooth-hound *Mustelus canis insularis*; Chupare stingray (Caribbean whiptail ray) *Himantura Schmarde*; and great hammerhead shark *Sphyrna mokarran*. Including additional sightings, elasmobranchs recorded at Glovers Reef comprised of 15 species (4 batoids and 11 sharks).

2.3.1 Shallow lagoon transects by boat

95 one mile-long transects were conducted in the shallow lagoon, recording 846 southern stingrays, 14 nurse sharks, 9 eagle rays and unrecorded observations of yellow stingrays and juvenile green and hawksbill marine turtles.

The southern stingray was the most abundant elasmobranch on the sand flats during daylight hours in distance transects (ANOVA $F=75.70$, $p < 0.0001$), with only rare sightings of other species present (*G. cirratum*, *A. narinari* and *U. jamaicensis*). Mean frequency (\pm SD) of rays per transect across all sites and months was 9.06 ± 3.45 . *Dasyatis americana* frequency was unaffected by month (Fig 2.7a) (ANOVA $F=0.88$, $df=94$, $P=0.54$) or time of day (Fig 2.7b) (ANOVA $F=1.73$, $df=94$, $P=0.10$), however significant differences were seen between sampling sites (ANOVA $F=2.84$ $df=94$ **$P=0.0033$**) (Fig. 2.7d) with the highest mean frequencies seen at site 12, near to the main NE channel through the reef. Transects falling outside the conservation zone (sites 6-14) showing significantly higher abundance than those inside (sites 1-5) (ANOVA $F=22.29$, $df=1$, **$P < 0.0001$**). Abundance in sand flat habitat from distance sampling was seen to be significantly higher during falling tide than rising tide (ANOVA $F=4.31$ **$P=0.041$**).



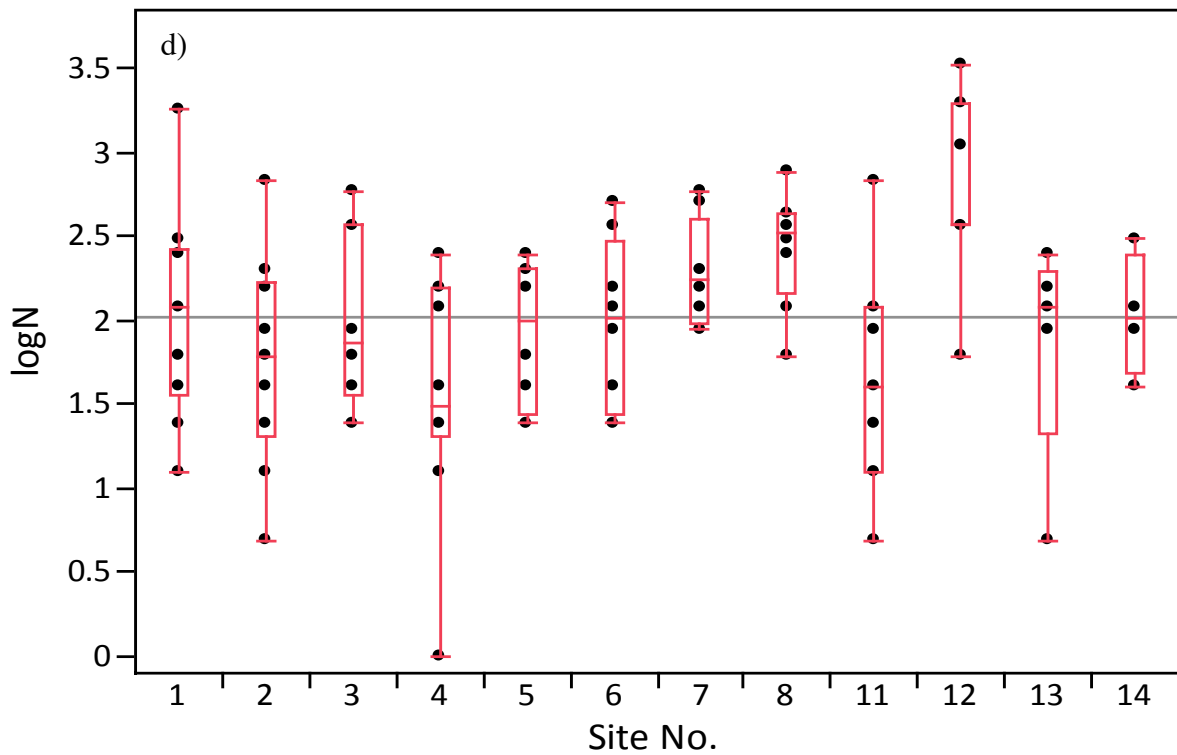
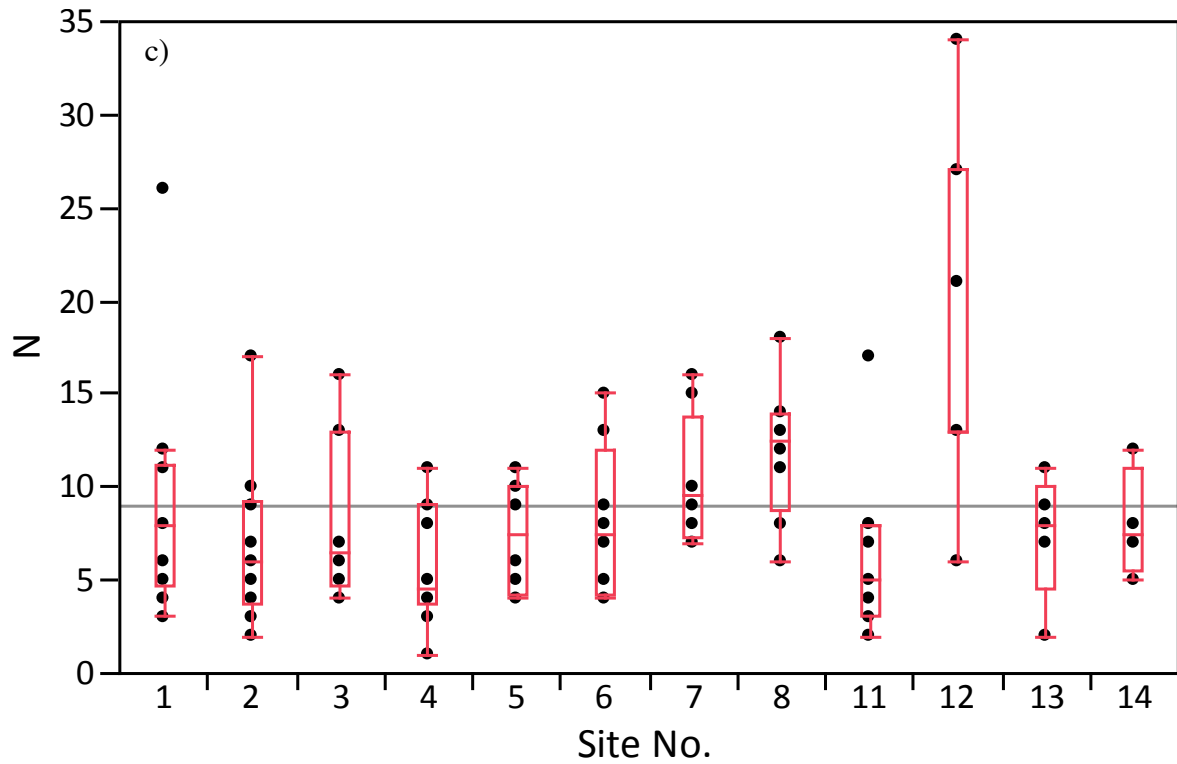


Figure 2.7: Observation frequency boxplots of *Dasyatis americana* from 95 transects conducted in the Glovers Reef lagoon margin between June 2009 and July 2010 by a) month, b) hour and c & d) site number. Red box plots represent lower, median and upper quartiles, with bars depicting 95% confidence intervals. Black dots represent frequencies for individual transects (N=95) and the grey line indicates the overall mean frequency. Sites 1-6 are within the no-fishing zone of GRMR, 7-14 are in the general use zone. No transects were conducted in the months of December and January. Note log transformed x axis in 2.7d.

Population size and density

Distance estimates for 845 rays observed in lagoon margin boat transects were reduced to estimates less than 20 m due to deviations from the line in quantile-quantile plots (Fig. 2.8). 802 rays were observed at distances of less than 20 m and used for modelling detection probability and population size for sand flat habitats. Distance observations close to zero showed significant rounding, so data was grouped into intervals and tested against various models for goodness-of-fit using Chi-square. Data was grouped into 7 intervals of distance and fitted tightly to a half uniform cosine model ($\chi^2=0.95$, $df=3$, $P=0.81$) (Fig. 2.9).

Detection probability was calculated to be 0.52 (95%CI 0.46 – 0.59) with an effective strip width of 10.4 m (95%CI 9.22 – 11.83). Estimated total stingray count for sand flat habitat sampling was projected to have been 1594 (compared to actual observed total of 802). Lagoon margin area was calculated to be 34.2 km², giving an estimated sand flat population of 8641 stingrays (95%CI 6744-11072 %CV 6.5) equating to a density of 252.5 per km² (95%CI 222.4–286.6).

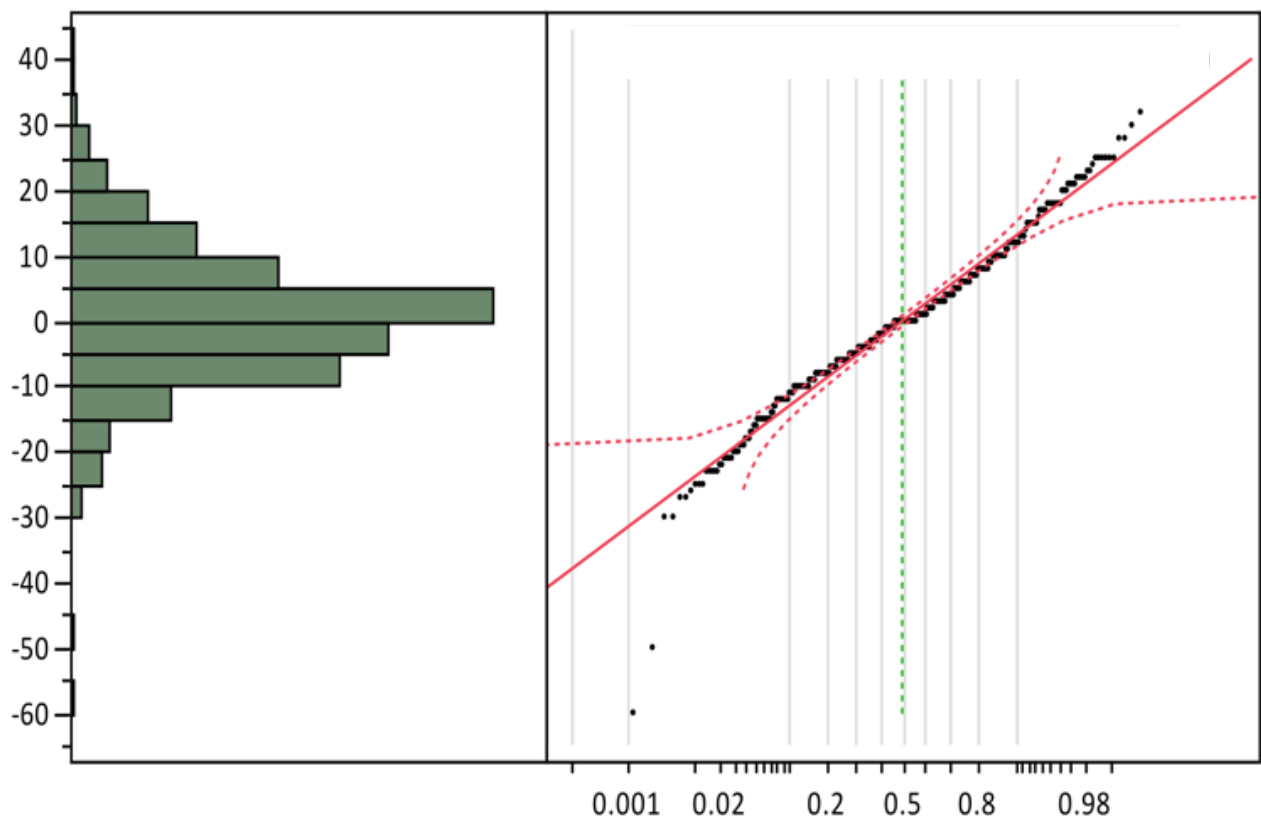


Figure 2.8: Distribution histogram of distance estimates for *Dasyatis americana* encounters over 14 sampling sites (N=846), and associated quantile-quantile plot.

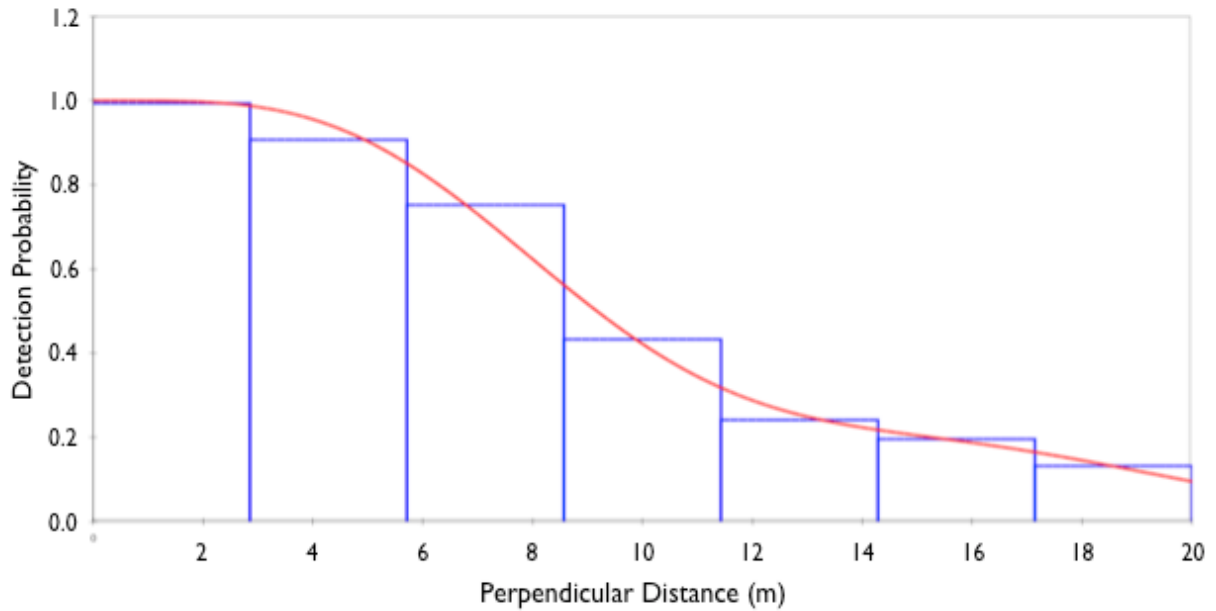


Figure 2.9: Plot of half-normal cosine detection function fitted to distance estimates for *Dasyatis americana* from boat sampling in lagoon margin habitat at Glovers reef, conducted between May 2008 and August 2010.

2.3.2 In-water elasmobranch surveys: shallow lagoon and White Sands

A total of 262 rays (179 females & 83 males) were recorded during 48 timed swims of 90 minutes (72 hours total), in two habitats, lagoon (LG) and White Sands (WS) (Table 2.1). The WS site exhibited a significantly higher sighting frequency (mean \pm SD 12.25 \pm 3.16) than LG (3.68 \pm 1.66) (ANOVA F=30.68 df=45 **P<0.0001**) (Table 2.2).

Table 2.1: Size distribution for all *Dasyatis americana* individuals recorded during timed lagoon and forereef swims at Glovers Reef between November 2009 and March 2010.

	Female	Male	Total
Lagoon	120	39	159
Small (<40 cm)	14	2	16
Medium (40-80 cm)	35	34	69
Large (>80 cm)	71	3	74
White Sands	59	44	103
Small(<40 cm)	17	0	17
Medium (40-80 cm)	33	40	73
Large (>80 cm)	9	4	13
Total	179	83	262

The mean frequency of large rays showed no difference between sites, yet small and medium rays were significantly more abundant at WS than LG (Z=4.70 P<0.0001 and Z=4.14 P<0.0001 respectively) (Table 2.2).

Overall female to male sex ratio across both sites was 2.15:1. Due to difficulties in identifying presence of claspers in neonates, small rays were excluded from further analyses of sex ratios. Sex ratio for M/L rays from WS was 1.05:1, non-significantly different from 1:1 ($P= 0.83$), whereas within the LG site, sex ratio was 2.86:1 skewed towards higher female abundance, with a ratio insignificantly different from a 3:1 ratio ($P= 0.81$).

Table 2.2: Mean frequency and standard deviation (SD) of *Dasyatis americana* recorded during timed lagoon swims by size and sampling site at Glovers Reef between November 2009 and March 2010.

Site	Small		Medium		Large	
	Mean	SD	Mean	SD	Mean	SD
LAG	0.41	0.64	1.77	1.13	1.90	1.48
WS	1.89	1.05	8.11	2.32	1.44	0.73

Ray abundance in the lagoon was negatively correlated with depth (exponential Kolmogorov $D= 0.29$ $P<0.01$) (fig. 2.10). Depth distribution analysed by size categories showed large and small rays in highest abundance in shallow water, and medium rays distributed evenly throughout the depth range. Small and medium rays were extremely rare in depths of 2 - 2.5 m, where the sand apron ends and the seagrass begins (fig. 2.11a & b). Mean depth of stingrays was significantly shallower in the afternoon (1200-1700 hours) than the morning (0700-1200 hours) (ANOVA $F=18.11$, $df = 153$, $P<0.0001$).

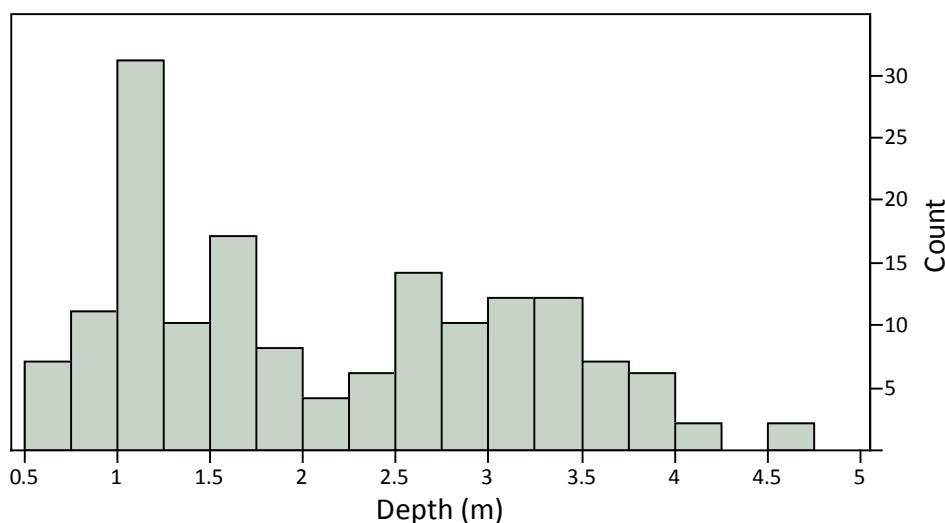


Figure 2.10: Bimodal frequency distribution histogram of southern stingray observations (N=159) by depth in the lagoon margin of Glovers Reef Atoll, sampled between November 2009 and March 2010.

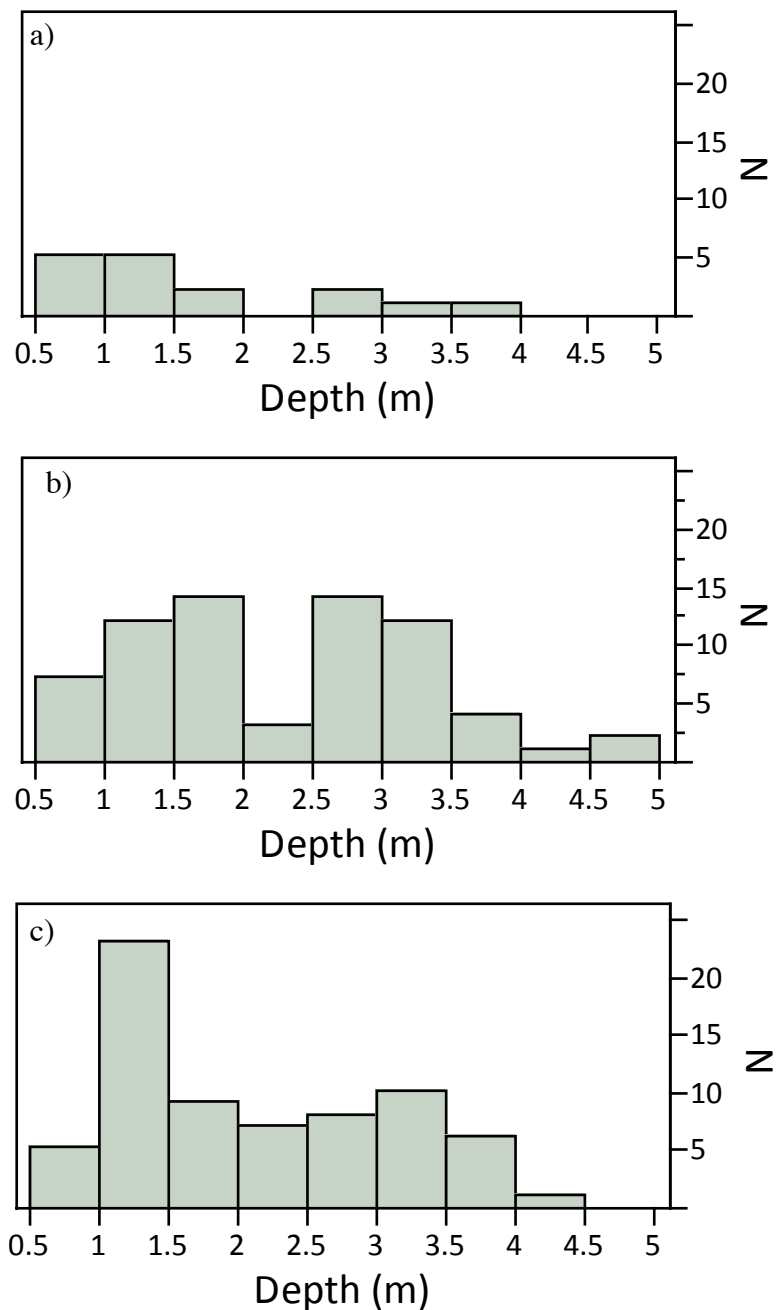


Figure 2.11: Depth distribution for (a) small (<40 cm mean = 1.64 m, n=16), (b) medium (>40<80 cm mean = 2.23 m, n=69) and (c) large rays (>80 cm mean = 2.08 m, n=74) observed in the Glovers Reef lagoon between November 2009 and March 2010. Bars are labelled with percentage of total count.

Rays were significantly shallower during flooding tide than ebbing tide but depth did not vary significantly between low and high slack tides (Fig. 2.12). When analysed by sex, female rays showed the same pattern with rising tide significantly lower than high slack (ANOVA $F=6.56$, $P=0.0004$, $P=0.001$) and low slack ($P=0.009$). Depth distribution of males showed no difference across tidal stages (ANOVA $F=2.57$, $P=0.07$).

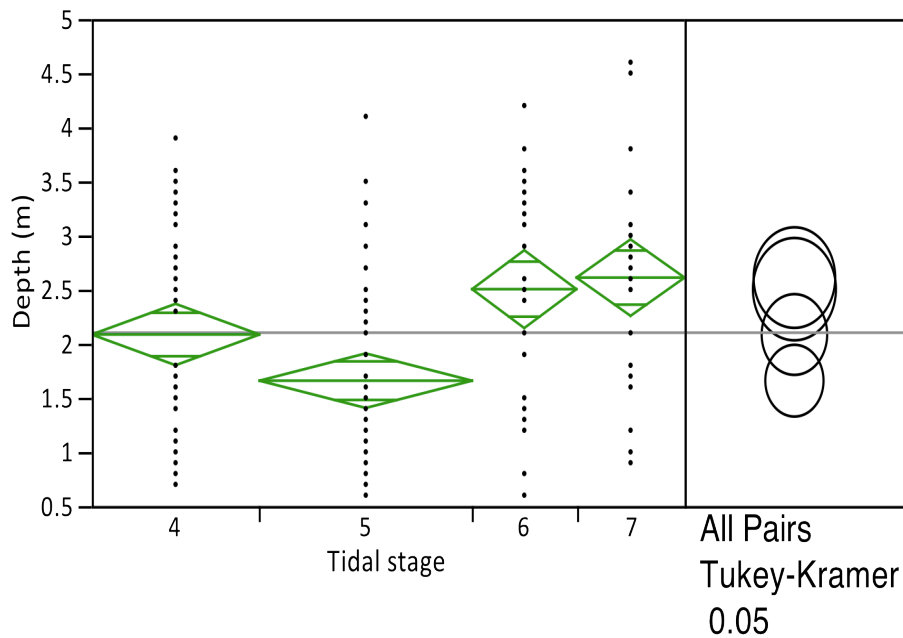


Figure 2.12: Analysis of variance between mean depth distributions of *Dasyatis americana* in the Glovers Reef lagoon margin at varying tidal stage, sampled between November 2009 and March 2010 (N=159). Data shows rays at greater depth during high slack (6) and low slack (7) tides than at falling (4) or rising tide (5). The grey line represents the overall mean.

2.3.3 Forereef in-water elasmobranch surveys

A total of 302 *Dasyatis americana*, 162 *Ginglymostoma cirratum*, 67 *Aetobatus narinari*, and 20 *Carcharhinus perezi* were recorded in 58 daytime hours in-water by 6-8 observers on the shallow forereef. *Galeocerdo cuvier*, *Urolophus jamaicensis* and *Narcine bancroftii* were not actively counted so were treated as incidental and data was not analysed. *Dasyatis americana* was significantly more abundant than all other elasmobranchs (Kruskal-Wallis $H=91.83$ $P<0.0001$ $df=3$), with an average frequency of 5.2 per site. Stingray abundance was highest during the morning hours of 0900-1100 hours (fig. 2.13), however changes in abundance with time of day were non-significant in all four species (Wilcoxon(1)= 0.03 $Z=-0.17$ $P=0.86$) (fig. 2.14).

Site location had no significant effect on abundance of all elasmobranchs grouped (Kruskal-Wallis $H=9.454$ $P=0.853$ $df=15$), or on species analysed independently[†], however *Carcharhinus perezi* showed significantly higher abundance on the Eastern (windward) sites compared to the Western (leeward) edge sites of the atoll (Wilcoxon(1)=3.8588 $Z=1.945$ $P=0.0495$). Abundance of *Dasyatis*

[†] *G. cirratum*. $H=14.60$, $P=0.48$ $df=15$; *A. narinari*. $H=12.70$, $P=0.63$ $df=15$; *D. americana*. $H=13.94$, $P=0.53$ $df=15$; *C. perezi*. $H=16.01$, $P=0.38$ $df=15$)

americana also exhibited positive correlations with *G. cirratum* and *A. narinari* frequency ($r(39)=0.36$, $P=0.022$, and $r(21)=0.58$, $P=0.004$ respectively).

Mean elasmobranch abundance of the 4 species pooled was higher in April sampling than in November (Wilcoxon(1) = 8.71 Z= -2.94 P=0.0032) (Fig. 2.15). Analysis by species showed higher April abundance of *Dasyatis americana* (Wilcoxon(1)=15.16 Z=-3.89 P<0.0001) and *G. cirratum* (U= 4.71 Z=-2.16 P=0.03), whereas differences in mean abundance for *C. perezi* (U=0.65 Z=-0.79 P=0.42) and *A. narinari* (U=0.56 Z=-0.74 P=0.46) were non-significant between April and November.

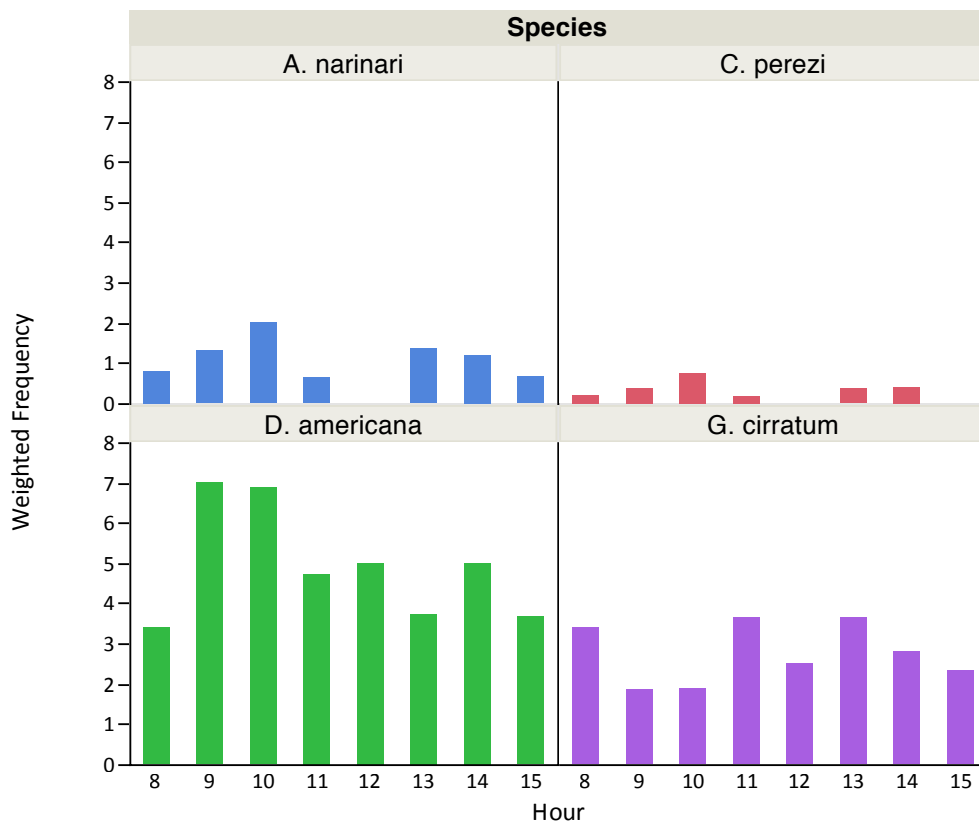


Figure 2.13: Effort-weighted hourly observation frequency of four elasmobranch species on the shallow forereef of Glovers Reef Atoll from biannual swim transects conducted between Nov 2008 and April 2010. Total hourly observation frequencies are divided by the total effort (no. of surveys).

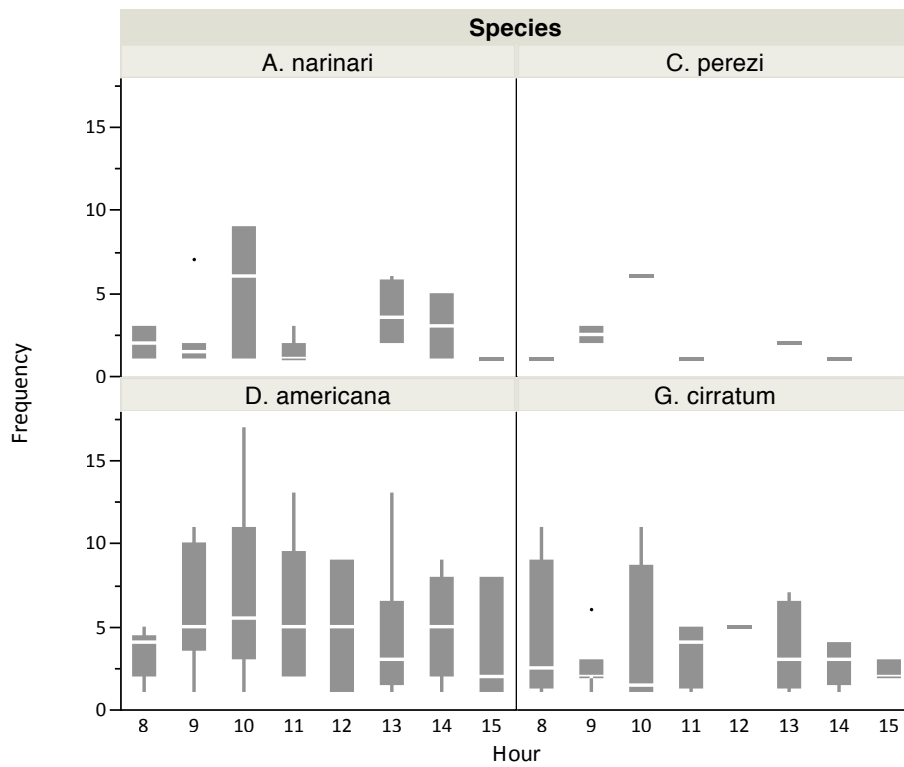


Figure 2.14: Hourly observation frequencies of four elasmobranch species on the forereef of Glovers Reef Atoll from biannual swim transects conducted between November 2008 and April 2010. Boxplots represent lower, median and upper quartiles, with bars depicting 95% confidence intervals.

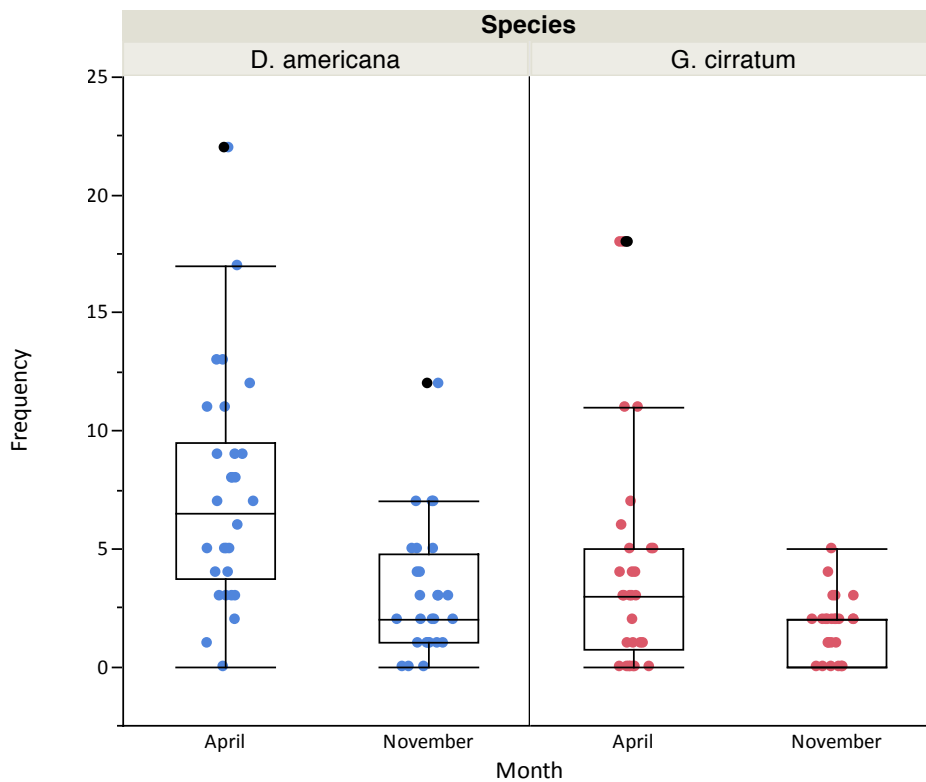


Figure 2.15: Combined observation frequency of *Dasyatis americana* (red) and *Ginglymostoma cirratum* (blue) from Glovers Reef forereef swim surveys conducted in April 2009 & 2010, and November 2008 & 2009. Red and blue points represent individual transect frequencies for each species. Box plots represent lower, median and upper quartiles, with bars depicting 95% confidence intervals.

Population size and density

Ray sightings on the forereef were not recorded for distance, so population size estimates include some major assumptions with wide confidence intervals. 302 southern stingrays were sighted in 58 surveys of ~0.22 km². Total area of forereef habitat <30 m depth was calculated as 22.7 km² using a GIS habitat map of the atoll. Assuming a detection probability (DP) of 1 (i.e. every ray present is sighted), a mean of 5.21 stingrays were seen per site; 23.25 per km² of forereef. Assuming infallible detection probability and an even distribution of rays throughout forereef habitats[‡], total daylight frequency and densities of species on the forereef were estimated to be:

<i>Dasyatis americana</i>	530 (23.25 per km ²)
<i>Ginglymostoma cirratum</i>	284 (12.47 per km ²)
<i>Aetobatus narinari</i>	118 (5.16 per km ²)
<i>Carcharhinus perezi</i>	35 (1.54 per km ²)

It is important to restate that these are not population estimates for other more transient species, but rather a calculation to compare daytime frequency of these species to frequency of *Dasyatis americana* in forereef habitats.

2.3.4 Deep lagoon elasmobranch surveys (SCUBA)

No elasmobranch species were seen during 6 hours of dive transects conducted in the deep lagoon between 2008-2010.

2.3.5 Sediment sampling - Prey species abundance

A total of 39 species in 8 classes in 7 phyla were identified over all sediment sampling (appendix 2.1). Prey density was highest in substrates covered with mobile algae. Species richness and diversity was highest in substrates of semi-dense seagrass cover (Table 2.3), and showed a positive relationship with depth (Fig. 2.16). Species richness showed a positive relationship with depth (Spearman's $r(24) = 0.43$, $P=0.019$) (Fig. 2.16a). Total number of individual prey items per site also showed a positive correlation with depth ($r_s(8) = 0.81$ $P=0.015$) (Fig. 2.16b). Number of classes by depth showed a quadratic fit ($r_s(8) = 0.88$ $P = 0.005$ F Ratio = 18.37).

[‡] DP is extremely unlikely to be 1, as this implies every elasmobranch present is sighted. Despite in-water communication between observers regarding sightings there are likely to be duplicate sightings of the same individuals, suggesting these figures may be overestimations of abundance. However, DP for this method is likely to be very high in any case due to the following factors: 1) high number of closely spaced observers 2) Limited stingray flight response prior to observation due to the depth of water between the swimmer and the rays, and 3) reduced boat engine noise compared with lagoon boat transects.

Table 2.3: Diversity index and species richness for benthic prey species found in 5 substrate categories at 8 sites in the Glovers Reef lagoon margin, sampled between June and August 2010.

Substrate	Simpson Index of Diversity	Species richness	Mean depth	Total n individuals
Algae/Sand	0.804	15	3.45	192
Sand	0.320	4	1.6	23
Sparse seagrass	0.863	14	1.8	55
Medium seagrass	0.933	21	3	62
Dense seagrass	0.909	21	3.1	77

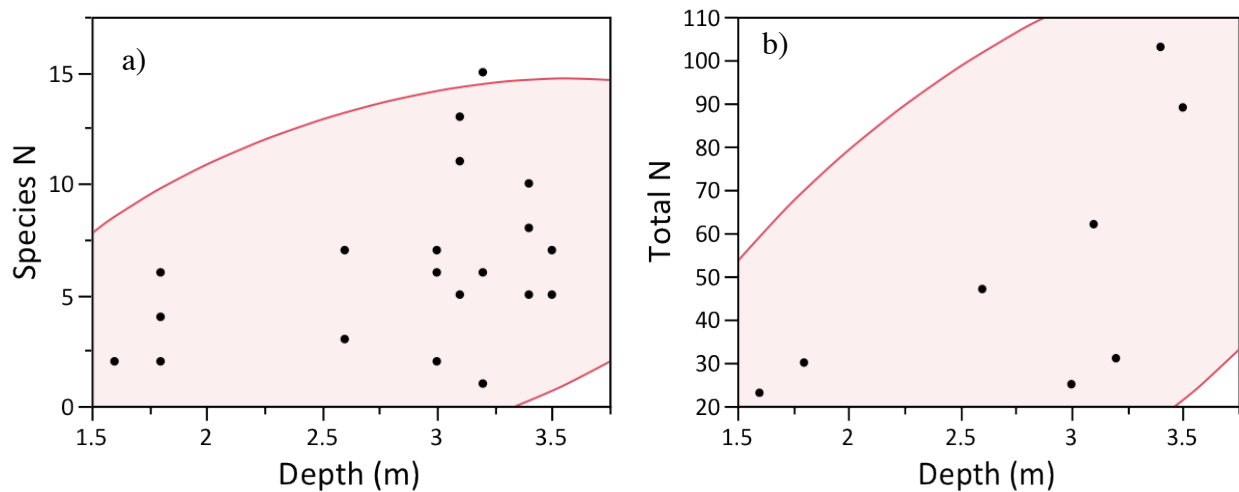


Figure 2.16: Sediment sampling for benthic prey species in lagoon margin at Glovers Reef between June and August 2010: a) Total number of benthic prey species per 1 m² quadrat by depth (m) (N=24); b) Total number of benthic prey organisms per site by depth (m) (N=8). Red shaded areas represent 95% bivariate normal confidence ellipses.

2.3.6 Environmental data

Mean hourly temperatures peaked between 1300-1600 hours year round (fig. 2.16). In the months of September to November temperatures at a depth of 5 m averaged higher than 1 m in lagoon margins.

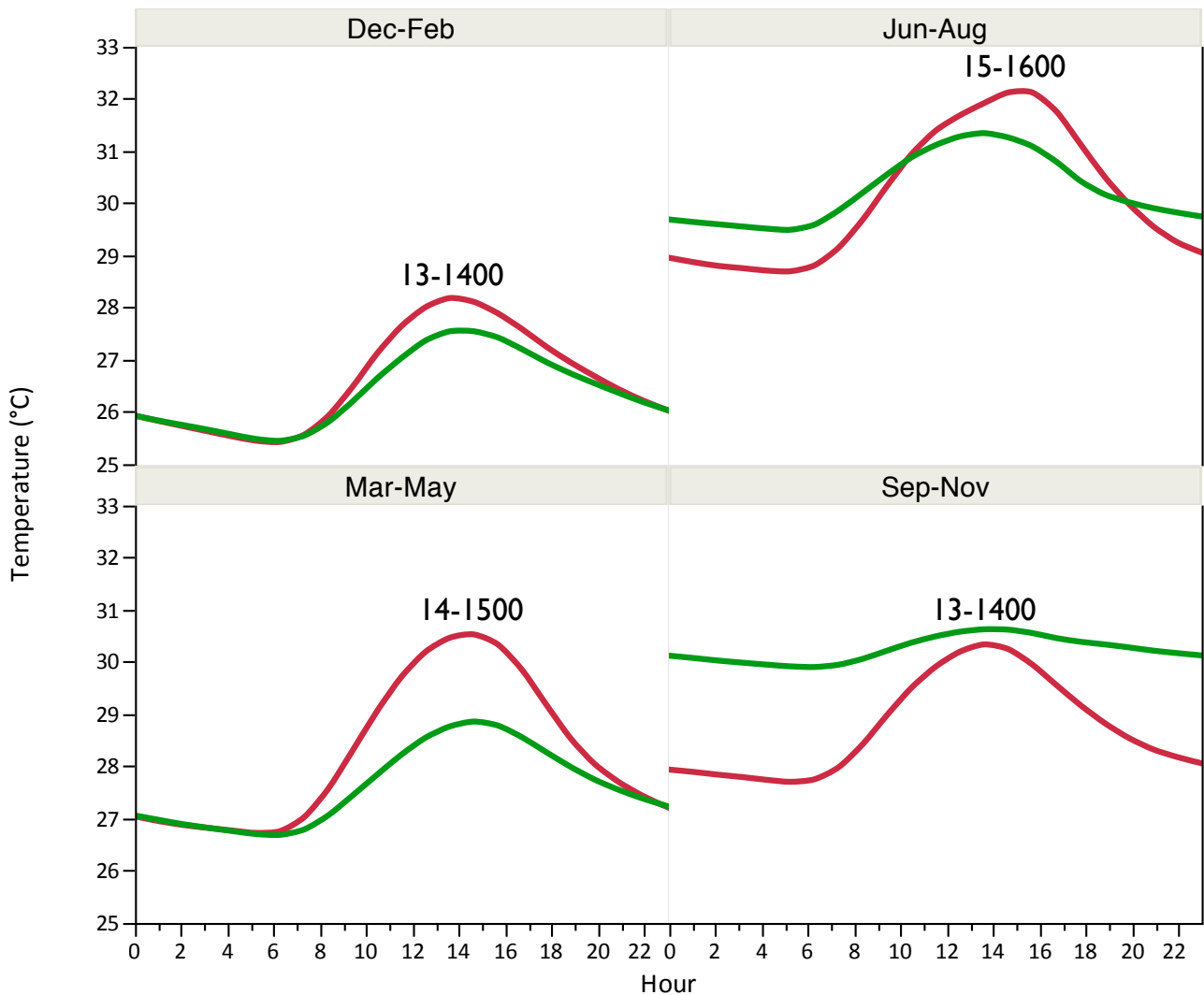


Figure 2.17: Mean hourly water temperature in Glovers Reef lagoon at 1 m (red line) and 5 m depth (green line) according to 3-month grouping. Time of temperature high per monthly category is labelled. Recorded using in situ data loggers (Onset Corp.) between February 2009 and July 2010.

2.4 DISCUSSION

Southern stingrays *Dasyatis americana* were the most abundant elasmobranch in insular forereef slope and lagoon margin habitats at Glovers Reef, followed by nurse sharks *Ginglymostoma cirratum*. The highest elasmobranch density was seen in the 600 m² forereef sand slope (White Sands) to the east of Long Caye, where the mean encounter rate (in-water) for *Dasyatis americana* was ~ 8.2 rays/hour compared with a lagoon encounter rate of 2.5 rays/hour, however greater depth and associated visibility would likely increase detection probability in this habitat. Stingray density in the rugose forereef areas was substantially lower than in the lagoon margin, which is likely to be at least in part a factor of availability of foraging habitat such as narrow sand channels between reef structures. The actual surface area of usable forereef habitat is likely to be less than one tenth of the forereef slope surveyed (i.e. ~2.3 km²). Sediment sampling was not carried out in forereef habitats so the relationship to prey availability is unknown. No elasmobranch species were observed during sampling of the deep lagoon, however sampling effort was much lower in this habitat than in all others and methods appropriate for the size, depth or visibility of this habitat were not feasible for this study. Lagoon and forereef surveys found *G. cirratum* to be the most abundant shark species, in contrast to findings from standard longline sampling at Glovers Reef where Caribbean reef sharks (*C. perezi*) were highest (Pikitch et al., 2005). However, this is likely to reflect the setting of hooks above the substrate in midwater (Chapman, pers. comm.), reducing take of predominantly demersal feeding nurse sharks.

High usage of the White Sands site by small and medium sized rays (<80 cm) of both sexes is interesting in that it shows rays can occur in numbers at greater depth (8-12 m), and that perhaps ray selection of lagoon sand flats may not be merely for shallow habitat but potentially for the substrate itself, where rays are exploiting foraging grounds of higher energetic gain, or lower predation risk (due to greater range of visibility).

Abundance in forereef habitats was similar throughout the atoll, with no substantial differences seen between windward and leeward forereef areas of sampling sites, however there were significant differences between sites sampled within the lagoon. The highest lagoon abundances were at sites in the NE of the atoll, close to the main channel out to the forereef. Increased physical disturbance through wind and wave action has been shown to positively affect benthic diversity in coral reef environments, suggesting that prey diversity should be higher on the windward edge of the atoll (Huston, 1985), however stingray abundance was not seen to be significantly different in these two environments. Lower use of the forereef is likely due to significantly reduced foraging habitat limited to reef grooves of sand substrate. Prey availability in these habitats is unknown, yet might be

assumed to be relatively high as a result of the higher production at the sand reef interface (Gratwicke & Speight, 2005).

Further work might look to sample forereef prey abundance to test the hypothesis of ray distribution according to prey, however this study only sampled prey density in lagoon margin habitats. Even distribution may indicate that suitable habitat exists throughout the atoll and stingrays are not limited by prey availability.

In lagoon margins, the mean stingray abundance was higher in the general use zone than those in the conservation zone, yet predominantly driven by high densities at two sites (8 & 12). Site 12 is a known cleaning site favoured by fishermen due to its proximity to the NE reef cut, whereas site 8 is midway up the eastern lagoon margin and in a large area where catch cleaning occurs but existence of specific sites are unknown. Supplementary feeding has been seen to have significant effects on distribution of *Dasyatis americana* (Corcoran, 2006), so higher density of rays in the general use zone may be as a result of incidental feeding by fishermen, rather than reserve effects of higher competition from teleost predators of crustaceans such as hogfish, triggerfish and snappers, whose numbers are controlled by fishing pressure in the general use zone (McClanahan et al., 2001).

The highest densities of *Dasyatis americana* were seen in lagoon margin habitats where the population estimate for the atoll was 8641 with 95% confidence limits of 6744 – 11072. The encounter rate of *Dasyatis americana* on the forereef slope suggests a significantly smaller number of rays utilise this habitat, representing an additional ~530 rays to the atoll population. A population of this size in an area of ~57 km² is likely to have significant influence on prey species (Dale, 2011) and commercially important decapods (Mintz et al., 1994), and fluctuations in ray population density may have knock on effects throughout the trophic system if strong interactions exist with certain prey species (O'Gorman & Emmerson, 2009).

Dasyatis americana females were more abundant in the lagoon than males by 3:1, whereas White Sands showed no difference from a 1:1 ratio. Henningsen (2000) reported a 1:1 sex ratio in neonates born in captivity, suggesting that females are not merely in greater numbers at GR, but rather that they actively select the lagoon. Carvalho et al. (2010) found a significantly female-skewed population of *Dasyatis americana* in the relatively similar habitat of a small oceanic atoll in Brazil. Higher lagoon temperatures are likely to account for skewed sex ratio, where females actively select warmer temperature, as seen in the Atlantic stingray *D. sabina* (Wallman & Bennett, 2006). Thermal habitat properties have been seen to play an important role in elasmobranch reproduction (Economakis & Lobel, 1998), where pregnant females use enhanced temperatures to speed embryo development and gestation times (Wallman & Bennett, 2006; Di Santo & Bennett, 2011). Temperature changes at GR are relatively small within a diel cycle, yet even 1°C changes were calculated to shorten gestation

periods in *Dasyatis sabina* by up to two weeks (Wallman & Bennett, 2006). Sex ratios were only monitored from December 2009 to February 2010, so it is unclear if this is a temporal pattern, or if the same abundance of females persists in the lagoon year round. Carvalho et al. (2010) found no seasonal fluctuations in population sex ratios in Brazil. Elasmobranch species often select restricted embayments as nursery areas as a result of heightened protection and food availability for neonates (Simpfendorfer & Milward, 1993; Heupel et al., 2007; Dale et al., 2011), and occurrence of neonates and small juveniles in the GR lagoon suggests this is a nursery area for *D.americana*, *N. brevirostris*, *C. perezii* and *G. cirratum* (Pikitch et al., 2005). However, prey diversity and abundance results from sediment sampling imply the lagoon shallows of GR may be a nursery area selected for safety rather than energy gains.

Abundance of benthic invertebrate species increased with depth in the shallow lagoon margin (0.5-5 m). Deeper lagoon sediments were not sampled for invertebrate prey density due to equipment depth limitations, however in a separate study at Glovers Reef (Hauser et al., 2007), diversity and richness of bivalve fauna across the lagoon were shown to increase from deep (~15 m, characterised by silts and muds) to shallow (~4 m, fine sand and seagrass). Although this does not offer information on prey abundance, we might infer lower diversity also offers lessened foraging preference for rays given their diverse and opportunistic diet. Low prey availability offers a likely explanation for very low occurrence of mesopredatory elasmobranchs in the deep lagoon, however sediment sampling of this habitat is needed to bring greater clarity to prey availability. Analysis of ray movement within the lagoon, and proportion of time spent within different depths and habitats will be investigated in the following chapter (3).

Stingrays were seen actively foraging across all depth and substrate habitats in the shallow lagoon margin (0.5 – 5 m) with the exception of back reef pavement and coral patch reefs. Large and small stingrays showed a general daytime preference for shallow sand flat habitat over deeper lagoon margins characterised by finer sediment, seagrass beds (*Thalassia testudinum* and *Syringodium filiforme*) and patch reefs. Rays of medium size class were distributed evenly throughout the range of depth. Benthic sampling showed significantly higher prey abundance and species richness in dense seagrass, than patches of sparser seagrass and sand substrate. Prey abundance was highest in mobile algae fields of green (*Anadyomene stellata*, *Codium intertextum*, *Derbesia* spp., *Halimeda copiosa*) and brown (*Lobophora variegata*) species, than any other substrate. The location of these algae is controlled by water conditions, and algal fields can be disrupted or shifted by strong storms (A. Tilley pers. obs.). The adherence to feeding within this habitat by stingrays may indicate that their distribution is changeable with the algal field. Algae is not present in all areas and at the time of sampling, these fields only occurred in the northern sampling area, away from lagoon margins

analysed for stingray habitat use. In a predator free environment it is hypothesised that stingrays would be distributed according to the availability of their prey (Fretwell & Lucas, 1970) indicating juvenile stingrays may be foraging in shallow water as a strategy of reducing predation risk rather than prey availability. Other studies have shown juvenile rays to be distributed in shallow areas (Carvalho et al., 2010), and partitioned in habitats by age (Aguilar et al., 2009) as seen in *N. brevirostris* and *G. cirratum* at Glovers Reef, where shallow seagrass and mangrove habitats were utilised by neonates and young juveniles, and deeper lagoon habitats were preferred by larger juveniles (Pikitch et al., 2005). Results further correlate with Pikitch et al. (2005) who found lower numbers of reef sharks in the shallow lagoon areas sampled than in deep lagoon and forereef areas. Invertebrate assemblages in seagrass were predominated by mobile prey species (crabs & shrimp), seen as important prey items in other studies (Gilliam & Sullivan, 1993) whereas sand flat habitats exhibited more sessile species assemblages dominated by polychaetes as would be expected. An alternative explanation to variation in habitat use by different size classes might be ontogenic diet shift (see section 5.3.2), where larger rays are able to access more nutritious deeper burrowing prey species in sand environments. Finally, the higher temperatures in lagoon shallows in the afternoon correlated with greater abundance of rays. Thermal preference may be driving rays into shallow water during the day as a mechanism of behavioural thermoregulation, either to decrease gestation times (Wallman & Bennett, 2006), or speed metabolism for foraging such as in hunt warm-rest cool strategies (Sims et al., 2006).

Medium and small rays appear to avoid habitat edges altogether, yet greater prey density is generally higher in edge habitats between seagrass and sand patches (Macreadie et al., 2010), suggesting a possible trade off between higher prey availability/ energy gain (at patch edges) (Macreadie et al., 2010) and heightened predation risk at these interfaces (Smith et al., 2011).

Alternatively, foraging in the sand flat habitat could be as a response to habitat structure and the accessibility of prey in complex habitats. Greater habitat complexity of seagrass implies more refuging opportunities. Impacts of stingray feeding on the structure of *Thalassia testudinum* seagrass beds have been shown to be minimal, limited only to large rays (Valentine et al., 1994), whereas capacity for structuring beds of seagrass species of lighter foliage and sparser rhizome networks is significant (Short & Wyllie-Echeverria, 1996; Townsend, 1998). The greatest density of rays was observed actively feeding on the fore reef sand slope of White Sands, structured with patches of *Syringodium filiforme* of varying density (fig. 2.16). Despite the effects of depth and visibility on detection probability at this site, greater stingray abundance is also a possible result of better foraging opportunities for mobile prey. This is additionally supported by observations at White Sands of high numbers of predatory teleosts of the same trophic guild as stingrays such as snappers (*Lutjanus* spp.),

margate (*Haemulon album*), hogfish (*Lachnolaimus maximus*) and blue runners (*Caranx crysos*), known to prey upon similar mobile invertebrate species (Froese & Pauly, 2000), and even follow rays to scavenge from their excavations (A.Tilley pers. obs.) (fig. 2.18).

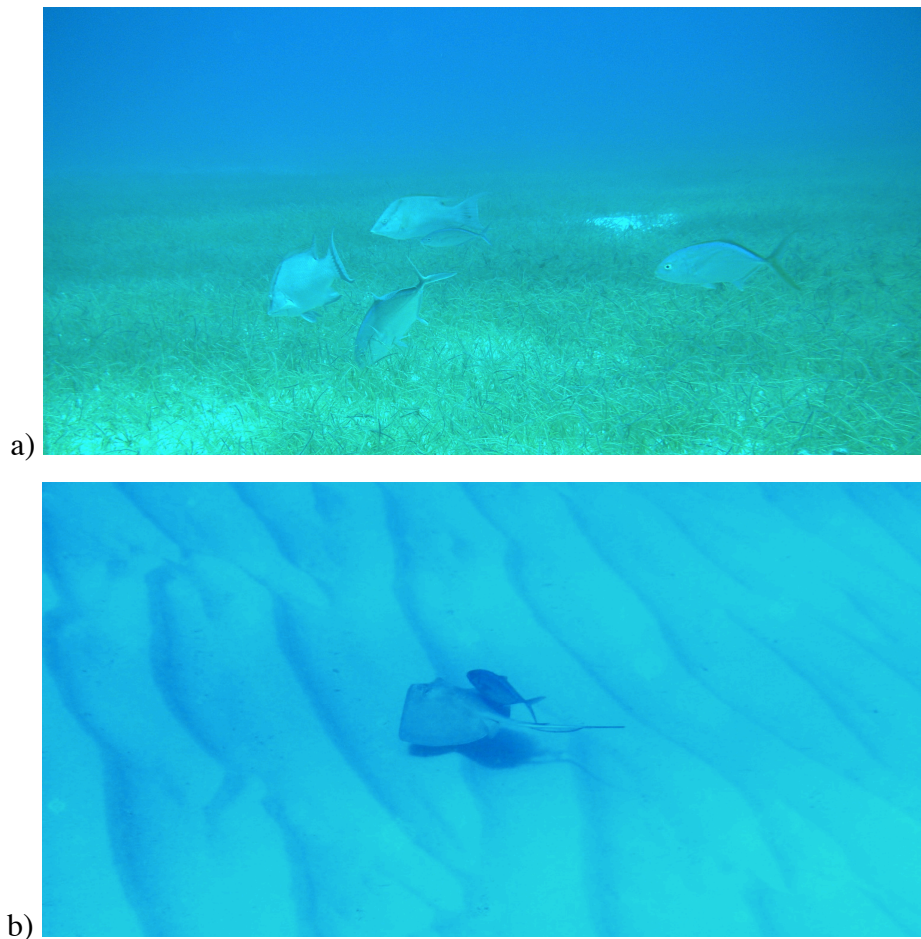


Figure 2.18: White Sands forereef sand slope in Glovers Reef Marine Reserve, Belize: **a)** Foraging hogfish (*Lachnolaimus maximus*) and blue runners (*Caranx crysos*) over seagrass bed (*Syringodium filiforme*), **b)** a southern stingray followed by a scavenging blue runner.

The main limitation to this type of study is the inconsistency of methods associated with sampling across habitat types of varying depth, visibility, size and rugosity. Techniques used to sample for rays in forereef habitat do not provide usable estimates of mobile shark populations, as depth is a limiting factor for observation based survey techniques. Abundance estimates for shark species are likely to be severely underestimated due to low detection probabilities, however this study sought to evaluate daytime elasmobranch abundance in shallow water environments (<20 m), including only the insular forereef slope. Hence it was acknowledged that estimates of reef and pelagic sharks would be low, and used only as a comparative reference for frequency of demersal elasmobranch species in shallow environments.

Sediment sampling should be widened to include White Sands in order to compare prey densities in different seagrass complexes, and sampled throughout the year to evaluate temporal trends related to temperature. Further work should also attempt to minimise method inconsistency and the associated errors this brings to population and density estimates, by introducing a consistent aspect to all habitat types such as a remote underwater video, and using this to calibrate wildlife survey techniques.

Conclusions

Elasmobranch diversity is low at Glovers Reef, with *Dasyatis americana* more abundant than any other species in shallow forereef and lagoon margin habitats. The deep lagoon sampling returned no elasmobranch sightings, yet density of rays in the lagoon margins was significantly higher than forereef habitat. Distribution of elasmobranchs was even across forereef sites, whereas lagoon margins showed areas of higher usage by rays in the north of the atoll outside of the no-fishing zone of the marine reserve. Stingrays were in their greatest density in a forereef sand slope exhibiting patchy seagrass (*Syringodium falsiformes*). In lagoon margins, medium sized stingrays (40-80 cm) utilised the entire depth range (0.5 - 5.5 m) and all habitat types during the daytime, however small juveniles (<40 cm) and large females (>80 cm) were more concentrated in shallow sand flat habitat than in deeper seagrass patches. Prey density and diversity was seen to increase with depth and substrate complexity, indicating that large female and juvenile rays are selecting habitats for reasons other than prey availability, such as reduced predation risk and increased temperature. Chapter 3 will further investigate habitat use using movement tracking to elucidate the influences of diel and tidal cycles on *Dasyatis americana* movements and home range according to individual size.

3. Diel movement of *Dasyatis americana* on Glovers Reef Atoll, Belize

Abstract

Understanding the spatial ecology of an animal according to biotic and abiotic factors allows for identification of essential habitat and provides insight into the mechanisms shaping population distribution and structure. Manual acoustic tracking was used to analyse the movement, activity space and habitat selection of southern stingrays *Dasyatis americana* throughout diel and tidal cycles in the lagoon of Glovers Reef Atoll, Belize. Stingrays of all life stages exhibited heightened crepuscular activity, with intermittent activity through day and night. Behaviour and habitat selection differed according to size, creating a diel foraging partition where juvenile rays move to shallow waters in the daytime, and shelter in deeper water by patch reefs at night, and large rays rest in deeper waters during the day then move to shallow sand flats to forage at night. Total activity space was significantly larger in daytime (mean $0.35 \text{ km}^2 \pm 0.19$) than night (mean $\pm \text{SD } 0.2 \text{ km}^2 \pm 0.11$) and increased with ontogeny, however only large rays ($>65 \text{ cm}$ disk width) showed significant night time movements, suggesting use of a size refuge. Despite very small tidal amplitude, heightened rate of movement correlated with low tide, in contrast to suggestions that rays utilise foraging grounds exposed by rising tide, but rather suggesting potential use of the thermal properties of ebbing tide, or more influential drivers of movement such as predation risk.

3.1 INTRODUCTION

A knowledge of the mechanisms underpinning the movement of fishes, and small-scale habitat use in response to ecological and environmental cues, is essential in understanding their population dynamics, distribution and community structure in heterogeneous ecosystems (Sims, 2010), and may also provide insight into behavioural and morphological evolution in marine fishes (Lowe et al., 1994; Heithaus et al., 2002; Lowe & Bray, 2006). Spatial and temporal components which guide fish movements vary considerably according to species and location, as well as the biotic and abiotic factors which affect them (Lowe & Bray, 2006). Most biological cycles in fishes are driven or maintained by exogenous cues, and studies have shown movements of sharks and rays to be driven or defined by changes in tidal stage (Huish & Benedict, 1977; Teaf, 1980; Medved & Marshall, 1983; Smith & Merriner, 1985; Silliman & Gruber, 1999); diel stage (Cartamil et al., 2003); temperature (Matern et al., 2000), light (Wolfe & Tan Summerlin, 1989) and salinity (Ortega et al., 2009). Diel rhythms are particularly important as daylight is the primary zeitgeber (cue) regulating circadian

rhythms, by which critical predictions of changes in prey availability and prey abundance are drawn (Hutchison & Maness, 1979). Circadian rhythms have been identified in horn sharks and swell sharks (Nelson & Johnson, 1970), and some degree of adherence to diel rhythms is seen in most studies of elasmobranch movements, in both vertical (Nelson et al., 1997; Chapman et al., 2007; Andrews et al., 2009) and horizontal movement planes (Klimley et al., 1988; Andrews et al., 2009), yet the question remains if these diel movements are driven by exogenous cues such as light intensity, or if these merely entrain endogenous physiological cues. In general, diel movement patterns can be attributed to predator avoidance (Holland et al., 1999; Cartamil et al., 2010), behavioural thermoregulation (Hight & Lowe, 2007) and foraging (Gilliam & Sullivan, 1993; Heithaus, 2004), and the few studies carried out suggest this to also be true of batoids (Matern et al., 2000; Cartamil et al., 2003; Vaudo & Lowe, 2006; Farrugia et al., 2011). Studies on the southern stingrays offer conflicting evidence on habitat use and the role of diel and tidal cues in driving movement patterns. A study in the Cayman Islands found evidence to suggest *Dasyatis americana* are crepuscular and nocturnal foragers, remaining predominantly buried during daylight hours (Corcoran, 2006); and in the Bahamas, the frequency of full stomachs found at high tides may indicate a preference for moving and feeding according to tidal cues (Gilliam & Sullivan, 1993)[§]. Elasmobranchs inhabiting embayments and estuaries are more often shown to cue to tidal state (Matern et al., 2000; Carlisle & Starr, 2010), as might be expected due to greater foraging area exposed by rising tides and associated vertical distribution of intertidal species. In Atol das Rocas, Brazil, fluctuations in abundance of southern stingrays was said to be influenced by tidal state (Carvalho et al., 2010), however the only active tracking study carried out thus far with *Dasyatis americana* found no influence of tide on movements (Corcoran, 2006).

Thermoregulation is a key driver of movement in ectotherms as a result of their need to regulate body temperature, and enhance or slow metabolic processes (Hutchison & Maness, 1979). Temperature is possibly the most important abiotic factor driving movement behaviour in poikilothermic fishes (Brett, 1971), as it can significantly affect physiological processes such as aerobic muscle performance (Donley et al., 2007), reproduction (Economakis & Lobel, 1998; Hight & Lowe, 2007) and feeding & digestion (Matern et al., 2000; Wallman & Bennett, 2006; Di Santo & Bennett, 2011), by shifting between habitats of varying temperature. Our understanding of temperature in elasmobranch ecology is extremely lacking, due to logistical difficulties of studying and

[§] This study only analysed stomachs of rays captured during daylight hours.

manipulating large marine animals in laboratory conditions (Fangue & Bennett, 2003), hence most documented examples of thermotaxis are anecdotal or based on observations (Wallman & Bennett, 2006). Pregnant grey reef sharks, *Carcharhinus amblyrhynchos* (Economakis & Lobel, 1998) and Leopard sharks, *Triakis semifasciata* (Hight & Lowe, 2007) have been seen to actively move to warmer waters during daytime, potentially to shorten gestation periods and speed embryonic development. This process of thermotaxis may also benefit feeding efficiency by increasing foraging rate and intensity in warm waters, and slowing evacuation rates to increase nutrient absorption in cooler waters (Wallman & Bennett, 2006; Di Santo & Bennett, 2011), so called hunt warm, rest cool strategies (Sims et al., 2006). Elasmobranch fishes have been seen to move between thermally heterogeneous habitats on vertical (Sims et al., 2006) and horizontal (Matern et al., 2000) planes, and potentially even utilise introduced sources of heat in marine environments (Vaudo & Lowe, 2006). Understanding the mechanisms that drive ray movement is also extremely important in evaluating the impact of anthropogenic factors, which can have significant influence on behaviour (Corcoran, 2006) and health of opportunistic fishes (Semeniuk & Rothley, 2008), in response to provisioned food from e.g. tourism or fishing. Very little scientific data exists on the effects of wildlife feeding, however in the most recent count, there exist 300 sites in 42 countries where wild animals are fed in order to bring them closer to tourists (Orams, 2002). In the Cayman islands, fed southern stingrays at two popular feeding sites were seen to have reversed their natural movement and foraging patterns in comparison to wild rays, and exhibit significantly reduced overall activity space in response to provisioned food (Corcoran, 2006). When a similar study was carried out for Caribbean reef sharks (*Carcharhinus perezi*) however, no behavioural shifts were observed (Maljkovic & Cote, 2011).

Foraging organisms can increase the chance of encountering and capturing prey, and not being prey themselves, through the selection of optimal foraging grounds or habitat (Stephens et al., 2007). Habitats can exhibit spatial and temporal heterogeneity in a number of factors such as temperature (Matern et al., 2000); salinity, water quality (Ortega et al., 2009), predation risk (Morrissey & Gruber, 1993a; Vaudo & Heithaus, 2009), and accessibility (Gilliam & Sullivan, 1993). Spatial heterogeneity in habitats is the basis for ecological models of patch use and favours organisms with flexible feeding behaviours (Brown, 1992).

The area minimisation principle in ecology implies that an animal will move within the smallest area that will satisfy its energetic and reproductive requirements (Mitchell & Powell, 2004). This area, or a defined percentage of it (e.g. 95%), is considered to be the home range (Grubbs, 2010). An inverse relationship existing between home range size and habitat complexity should suggest that fishes that occupy low relief or soft substratum habitats should have larger home ranges than those found over

rocky reef habitats (Lowe & Bray, 2006). Home range emergence in animal dispersion simulations has shown them to be a highly efficient foraging strategy in terms of overall fitness and bioenergetics (Börger et al., 2008), assuming that direct movement to known patches increases efficiency by reducing searching time, thereby reducing predation risk (time exposed). By remaining within a known area, it is thought that animals return to key features, habitats and patches using reference memory (Van Moorter et al., 2009). Some studies have documented home range and habitat use by sharks and rays (Morrissey & Gruber, 1993b; Holland et al., 1993; Cartamil et al., 2003), often illustrating ontogenic expansion of home range (Morrissey & Gruber, 1993b; Heupel et al., 2004; Garla et al., 2005). Due to logistical and time constraints, ‘activity space’ is substituted to represent when recorded animal movements may not represent the entire home range, but are merely the movements during the tracking phase (Grubbs, 2010). Area calculations of home range from tracking data have traditionally been estimated using Minimum Convex Polygons (MCP), which represent the smallest area encompassing a predefined percentage of location points (e.g. 95%) (Heupel et al. 2004; Collins et al., 2007).

More emphasis now lays with identifying habitat preference, where a foraging animal may select habitat disproportionately to its availability (Heithaus, 2001; Heithaus et al., 2002; Weng et al., 2007; Cartamil, 2009), or where habitats are used for different behavioural states (Economakis & Lobel, 1998; Wallman & Bennett, 2006) or life stages (Heupel et al., 2007; Dale et al., 2011). This information allows for much finer scale decision making in conservation and fisheries management (Sims, 2010). Statistical methods of Kernel Density Estimation (KDE) have been adapted for use with animal movements to smooth MCPs, and to better represent an utilisation distribution within a home range, i.e. areas or habitats used more than others (Laver & Kelly, 2008).

KDE is a data smoothing process highly dependent on a free parameter known as the smoothing factor (h), which dictates the bandwidth, or the spread of the kernel around each point. Small values of h will produce narrow, potentially under-smoothed kernels, where the utilisation distribution (UD) is characterised by many small clusters of disconnected areas. If values of h are large, the kernels may be too wide, overestimating UD and not allowing for identification of centres of activity. The optimal smoothing factor can be calculated from the distribution of points with different mathematical and subjective methods, and there is disagreement regarding the use of each for different types of location data. It has been suggested that the most commonly used mathematical process, the *Least Squares Cross Validation* (LSCV) may radically under-smooth UD of data sets containing various centres of activity or duplicate values (Park & Marron, 1990; Laver & Kelly, 2008), or overestimate linear home ranges (Blundell et al., 2001). An automated approach to evaluating h is to calculate a reference bandwidth (h_{ref}) from the optimum value of a bivariate normal

distribution (Equation 2), where the square root of the mean variance in x (var_x) and y (var_y) coordinates, is divided by the 6th root of the number of points (Worton, 1995; Rodgers & Kie, 2010). Proportions of h_{ref} (0.2, 0.4 etc) can be used to incrementally widen bandwidths (expanding UD) to estimate optimal h , by visually comparing and selecting appropriate home range size and shape (Worton, 1995; Rodgers & Kie, 2010).

$$h_{ref} = n^{-1/6} \sqrt{\frac{var_x + var_y}{2}} \quad [2]$$

Traditionally animal movement paths are said to be oversampled if autocorrelation occurs (Turchin, 1998), where the sampling frequency is such that the turn angle and step length of each path segment correlate with the previous one or more steps. Blundell et al., (2001) found that home ranges utilised by animals choosing narrow bands of habitat, or those restricted by geographical features, are not affected by autocorrelation of locations, and that traditionally used home range methods such as LSCV may significantly overestimate activity space in these cases. This suggests “oversampling” may actually be beneficial in defining home ranges defined by geographically linear movements (Blundell et al., 2001), such as in narrow spatial bands. Another recent study goes further to suggest that autocorrelated data can provide more accurate UD of animal home ranges (Benhamou & Cornéllis, 2010), where restricting or reducing sampling can sacrifice biologically significant information (Reynolds & Laundre, 1990).

Habitat preference is a choice of one habitat over another relative to availability, and detailed knowledge of a species’ habitat use and preference is crucial to understanding its functional ecology within the system, and for informing conservation decision-making (Steneck & Sala, 2005). Most studies of elasmobranch habitat use have focused on the identification of nursery grounds (Branstetter, 1990; Simpfendorfer & Milward, 1993; Castro, 1993; Heupel et al., 2007), defined as areas where sharks or rays are more commonly encountered than in others; where they have a tendency to remain or return for extended periods; and where the area is repeatedly used across years (Heupel et al., 2007). Branstetter’s hypothesis (Branstetter, 1990) states that slower growing species are born at relatively large sizes, or use protected nursery grounds, whereas faster growing species will tend to rely on rapid development rates. It is most commonly found that nursery areas are defined by factors lessening predation and enhancing energy intake (Yokota & Lessa, 2006), such as in shallow, coastal embayments (Springer, 1967), however in some cases energy is sacrificed in favour of enhanced safety (Dale et al., 2011). Juveniles may also exhibit migrations within a nursery habitat in order to exploit locally abundant resources and minimise interspecific resource competition in core areas, such as the dusky shark *Carcharhinus obscurus* (Hussey et al., 2009b).

Following Branstetter's hypothesis (Branstetter, 1990) the slow growth rates of *Dasyatis americana* seen in captivity (Henningsen & Leaf, 2010), would suggest their use of a nursery area, however to date, no nursery areas have been confirmed for *Dasyatis americana*. In Brazil neonate rays were highest in abundance around the beaches, with larger individuals found in deeper reef areas (Aguilar et al., 2009). There was also some evidence for a secondary, slightly deeper nursery habitat for larger juveniles over rocky or gravel substrate (Yokota & Lessa, 2006).

The aim of this chapter was to investigate the spatial ecology of the southern stingray, and elucidate the influence of diel phase, tide and temperature on the movement and habitat use of *Dasyatis americana* at Glovers Reef Atoll, Belize.

Hypotheses & Objectives

H₀ *Dasyatis americana* movement shows no variation with abiotic (diel stage, tidal stage & temperature) or biotic factors (individual size and sex).

H_{A1} *Dasyatis americana* shows larger nocturnal foraging preference with activity spaces that increase in size with ontogeny.

H_{A2} *Dasyatis americana* show greater movement during the night and at high and rising tides.

H_{A3} Fine scale vertical and horizontal movements of *Dasyatis americana* correlate with lagoon water temperatures.

Obj. 1. To evaluate the influence of individual size & sex and diel stage on activity space size and shape utilised by southern stingrays in Glovers Reef lagoon, using manual acoustic telemetry on stingrays of various size throughout diel cycles over a 14-month period.

Obj. 2. To investigate the influence of tide and diel stage on vertical and horizontal movement and habitat use of southern stingrays in the Glovers Reef lagoon by creating a bathymetric map of shallow lagoon margins and using manual acoustic tracking of rays through diel and tidal cycles.

Obj. 3. To examine the influence of temperature on vertical and horizontal movement and habitat use of southern stingrays in Glovers Reef lagoon, using in situ temperature data loggers and manual acoustic telemetry of rays throughout diel and seasonal time scales.

Obj. 4. To gather annual weather, water temperature and light intensity data using in situ data loggers at varying depth in macrohabitats of Glovers Reef over a two-year period.

3.2. METHODS

3.2.1 Field methods for stingray tagging and manual acoustic tracking

Manual acoustic telemetry was used to track the movements of stingrays over varying spatial and temporal scales throughout their home ranges, and diel & tidal cycles.

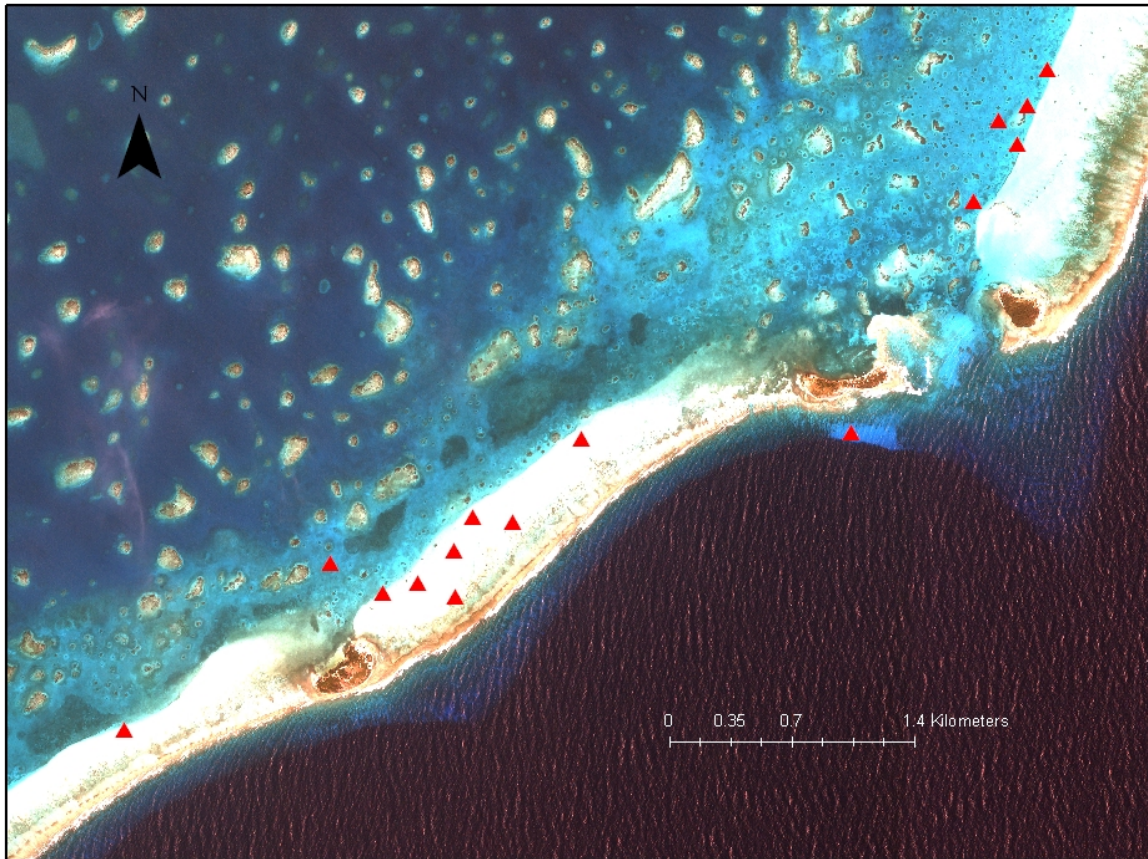


Figure 3.1: Satellite image of southeastern area of Glovers Reef Atoll depicting capture locations (red triangles) for all *Dasyatis americana* rays actively tracked between June 2009-August 2010 using manual acoustic telemetry.

Fourteen southern stingrays were captured and tagged within the lagoon of Glovers Reef Atoll, and one on the forereef sand flat of White Sands (WS) (fig. 3.1), between June 2009 and July 2010 using three varying techniques of capture and tag attachment:

i) Nine rays were captured using a 100 m x 1.5 m monofilament gill net, into which they were corralled, or entered for bait, and then became entangled. A dip net was then used to move the ray into a 1.5 m diameter onboard tank filled with regularly replenished seawater. Rays were measured by disk width (DW), sexed, biopsied for muscle tissue (if general health and size deemed suitable) and an acoustic continuously emitting tag (Vemco Systems) crimped to a monofilament loop passed through the tail musculature using a hypodermic needle (fig. 3.2, method adapted from Le Port et al., 2008). A pit tag was then injected into left side dorsal musculature to allow identification of individuals and avoid duplication. Handling times did not exceed 10 minutes and rays were released at capture site and monitored in-water after release for periods of up to 30 minutes to ensure

recovery. Capture locations were dependent upon ray sightings, but two broad areas inside the lagoon were sampled, with one ray being captured on the outer reef (Fig. 3.1).



Figure 3.2: Female southern stingray *Dasyatis americana* with V13 continuously emitting acoustic tag (Vemco systems, Canada) attached to crimped monofilament line passed through tail musculature (ringed in white)

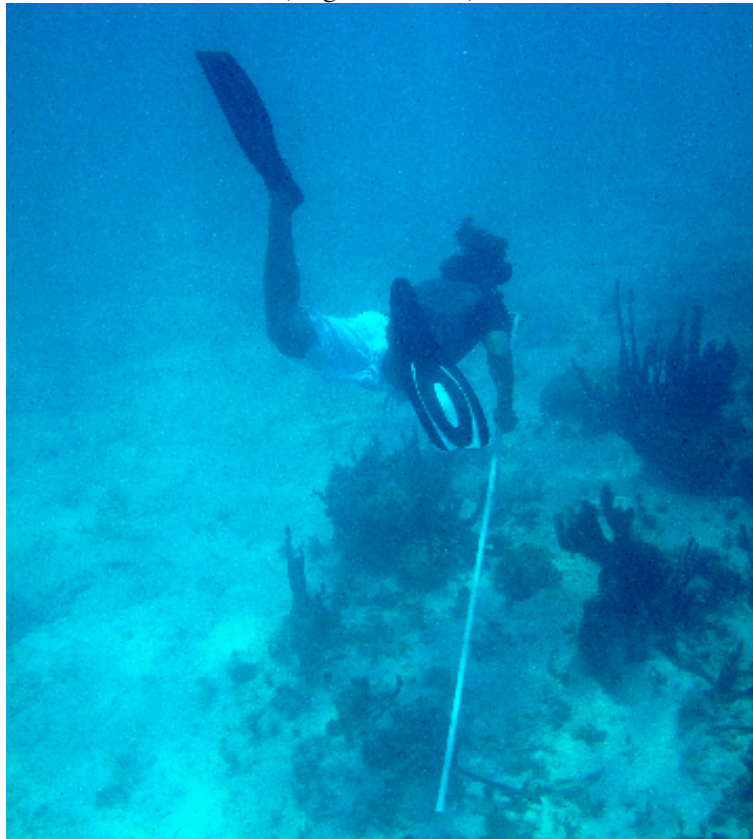


Figure 3.3: Method for in-water tagging of *Dasyatis americana* in Glovers Reef lagoon using an adapted pole spear. Swimmer would approach in blind spot above and behind the stingray and insert tag dart into the dorsal wing musculature.

ii) Six rays were tagged in-water using a modified dart tag applied by pole spear (fig. 3.3) into the dorsal musculature in the saddle areas above the flanges. Rays were located by boat or with swimmers, then while rays were engaged in foraging or cleaning activities, a snorkeler approached in a blind spot directly behind the ray, and inserted the dart tag. Size measurements were taken post capture during in-water observation, by measuring the width of feeding pit or resting “footprints” left by tagged rays during foraging (fig. 3.4).



Figure 3.4: Feeding pit “footprint” used for disk width (DW) size estimation of rays tagged and biopsied in-water.

iii) One ray was captured at the “White Sands” site on the outer reef using a barbless hook-and-line, baited with conch guts (*Strombus gigas*). Measuring and handling methods followed i) above. A hull-mounted V110 directional hydrophone connected to a VR100 or VR60** acoustic telemetry receiver (fig. 3.5, Vemco Systems, Canada) were used to track V9/V9P & V13 tags with a signalling interval of 2 seconds on one of six frequencies (60, 63, 75, 78, 81, 84 kHz) and V13P depth tags on 66 and 81 kHz. Range tests were carried out in the field with both receiver units prior to tracking with a static and moving tag in-water, to test detection range and angle accuracy according to signal

** Use of two different receiver systems was as a result of equipment damage to the VR100 unit during fieldwork. The VR60 unit was generously loaned to the project by M. Shivji of Nova Southeastern University for the final month of fieldwork. This unit did not record GPS locations, so only the handheld Garmin GPS locations were used during this phase.

strength over varying substrates and depths. Signal range did not differ significantly between tags, with a maximum detection range of 400-600 m, however detection range shortened considerably in water of less than 0.5 m. Signal strength in decibels was recorded at incrementally increasing distance to calibrate boat movements around rays during tracking to minimise disturbance of natural behaviour.

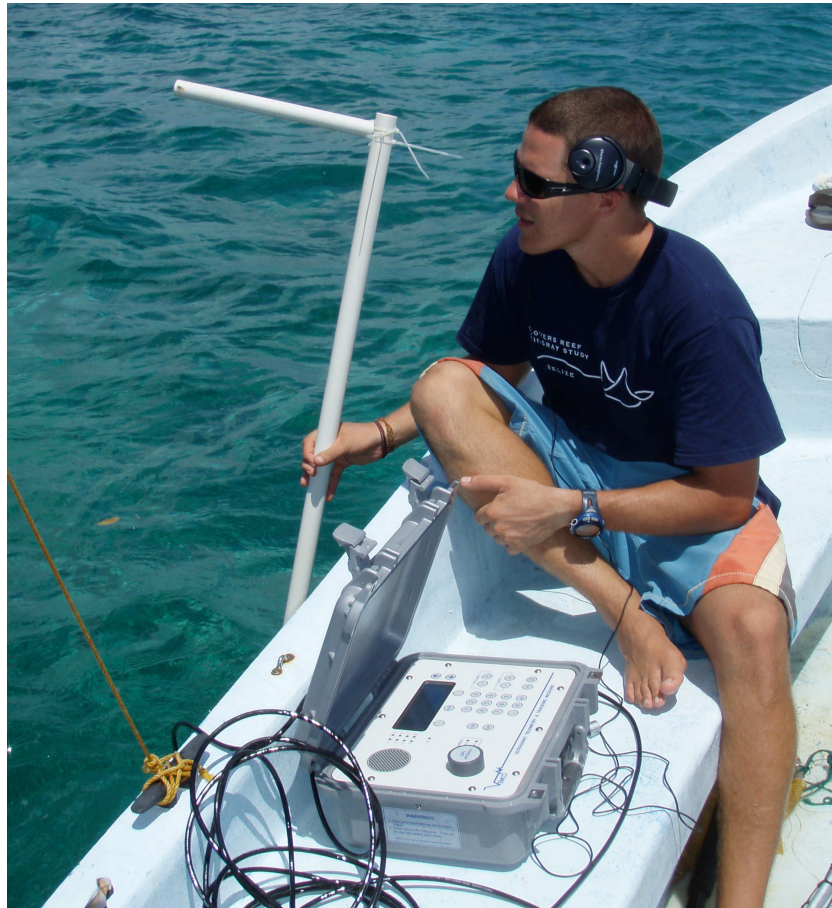


Figure 3.5: Manual acoustic tracking of southern stingrays at Glovers Reef using a V110 directional hydrophone connected to a VR100 ultrasonic telemetry receiver (Vemco systems, Canada)

Tagged rays were left to return to normal behaviour for at least 24 hours before commencement of tracking. Rays were tracked one at a time for periods of 3-24 hours, and for a total of up to 32 hours. Ray locations were recorded by the VR100 unit upon reception of a signal with built-in Global Positioning System (GPS). Handheld Garmin 76 GPS units (accuracy <15 m) were used to record locations at 5-minute intervals with the VR60 receiver unit. Results from range testing were used to judge distance from the tagged ray, and distance was kept to >10 m in order to minimize disturbance of natural movements. Bearings to the signal direction, water temperature and salinity readings were taken at 15-minute intervals or if the ray changed location significantly, along with notes on ray behaviour and local weather conditions.

3.2.2 Analysis of stingray activity space: size & aspect ratio

Stingray GPS locations along with temporal data were plotted using Arcmap (ArcGIS, ESRI 9.3) laid over an *Ikonos* satellite image (4 m resolution) to analyse habitat use. Total and diel activity spaces were calculated using Kernel Density Estimation (KDE) to produce polygons of 50% (core area) and 95% (home range) isopleths of utilisation. Additionally activity spaces were estimated using LocoH (a k-Nearest Neighbour Convex Hull estimator) (Getz & Wilmers 2007) and MCPs for comparison of area estimates with alternative techniques. Area values of activity space were non-normally distributed, so a Wilcoxon signed rank test was used to analyse differences in size of diel activity space with ray size and sex.

Utilisation distributions (UD) were calculated using proportions of an optimum reference bandwidth (h_{ref}) taken from a Gaussian kernel (Rodgers & Kie, 2010). Polygons created from 50% and 95% density isopleths using proportional h_{ref} values were compared to find the most suitable home range estimate for the distribution of location points for individual rays (fig. 3.6). Tracking data was analysed for independence of observations using Schoener's ratio for autocorrelation defined as t^2/r^2 , where t^2 is the mean squared distance between successive positions, and r^2 is the mean squared distance from the centre of activity (Home Range Tools (HRT) Ver. 1.1 (Rodgers & Kie, 2010)). Aspect ratio was calculated to return an index for the shape of activity spaces. Aspect ratio was calculated by dividing the longest diameter of activity space area by the shortest perpendicular diameter, returning a value between 0 and 1. Values close to 1 represent nearly circular activity spaces, and elongate as they near 0.



Figure 3.6: Kernel utilisation distributions from locations of an individual stingray, comparing fixed (a-e) and adaptive (f-j) kernel methods, using incremental proportions of reference bandwidth (0.2, 0.4, 0.6, 0.8, 1.0). This illustrates the *ad hoc* method of smoothing factor selection where the optimal value may be selected from the shape of distributions around the points. In this example (b) would appear the best representation of ray activity space.

3.2.3 Analysis of stingray movement

Diel phase was classified using a mean value of local sunrise and sunset times during the tracking period for each individual ray (variation of ± 4 mins). Tidal cycle was divided into four states, rising, falling, low and high. Low and high slack tides were considered as 1 hour either side of low and high tides stated in local Belize City tables (XTide, <http://tides.mobilegeographics.com>). Stingray movement characteristics were analysed across diel and tidal cycles using Rate of Movement (ROM), linearity and net displacement.

Rate of movement

Location data was reduced into 5, 10 & 20 minute intervals, and distances between successive positions (step lengths) were extracted and divided by the time interval to calculate ROM using HRT version 1.1 (Rodgers & Kie, 2010).

Due to the difference in sample size between individual rays and tracking methods, mean ROM values for all rays pooled were calculated by using frequency weighting, i.e. mean hourly rates of movement were calculated for each ray and then averaged once again for the mean ROM for all rays combined. Hourly mean ROM was then used to analyse movement patterns throughout a diel cycle. Relationships of ROM with temperature, light intensity and depth were investigated to determine the effect of environmental variables on stingray movement.

Linearity

Linearity evaluates the directness of movement paths by quantifying their straightness. This was done by dividing the path length by the direct distance (“as the crow flies”) between the start and end point), returning a value between 0 and 1. A value of 1 denotes a perfectly straight path between two points; values nearer zero denote more tortuous paths. Only paths greater than 100 m in length were chosen for analysis of linearity.

Net displacement

Net Displacement (ND) is the direct distance measurement from the start point of a path, and complements the use of ROM by distinguishing significant searching movements from oscillatory foraging within the same area. ND was calculated for each movement path, along with turn angles between successive moves and bearings (direction of moves) using Hawth’s Tools for ArcGIS (Beyer, 2004). Relationships between ND and temperature, light intensity and depth were explored and analysed using JMP 9 statistical software (SAS Institute) to determine the effect of environmental variables on stingray movement.

3.2.4 Fine scale bathymetric mapping

Spot depth measurements spaced 100 m apart were taken using a handheld sonar (Norcross Marine Products) to form two grids covering activity spaces of tracked rays in lagoon margin habitat. Grids of spot depth values and locations were plotted into ArcMap (ArcGIS Ver. 9.3) and interpolated to create a bathymetric map of the tracking area (appendix 3.1). Depth information was then extracted for tracked stingray positions and compared with depth values from a 530 mm male ray tagged with a pressure sensing V9P tag (Vemco systems, Canada).

3.2.5 Environmental data collection

Weather parameters of wind speed, air temperature, light intensity and rainfall were recorded on land throughout the field phase using a weather station (Oregon Scientific). Water temperature and light intensity at 1 m & 5 m depths were recorded using data loggers (Hobo pendant, Onset Computers). Light intensity was measured at 1 m water depth, limited to two-week periods 4 times per year due to marine fouling affecting accuracy of light sensors.

3.3 RESULTS

A total of 15 southern stingrays were tracked in sessions of variable duration between 6-24 hours. Rays tracked for cumulatively longer than 22 hours throughout a representative diel cycle were used for analysis of movement patterns (n = 12). Due to variations in tracking method and frequency of recorded position between sites, tracks were considered as two separate groups, North East (NE) (n = 4) and Middle Caye (MC) (n = 8) for analysis of movement, then combined for activity space and overall results.

Stingray response to tagging varied among individuals, however all rays swam a short distance from the boat and rested on the sand for a period of 5-30 mins. Smaller rays remained still on the sand, with heightened respiration (spiracle pumping) for some time until swimming away. Larger rays swam away sooner into deeper water to patch reefs either refuging within coral/rock patches or burying in sand nearby. Rays tagged with a pole spear in-water swam off quickly and directly, with minimal apparent distress, some returning to foraging in the vicinity almost immediately. Four out of five tags attached in this manner were retained for the duration of tracking with no visual injury or scarring other than slight rubbing of skin mucous around tag insertion. In one ray (DA13) the tag detached after ~10 hours. Site fidelity of rays allowed for the acoustic relocation of rays within 2 hours at most attempts, even with several days in between tracking periods.

All rays tagged in the lagoon remained within the lagoon for the duration of all tracks. One ray tagged on the fore reef (DA9) moved into the lagoon through a reef channel late in the evening (2100 hours) but was not followed throughout a diel cycle. Rays spent many hours during day and night unmoved, buried or resting on the sand very near to patch reefs. Juvenile rays were reliably relocated for each tracking session around the same patch reefs, indicating fidelity to a particular area or activity space at a young age (possibly where parturition occurred). Adult rays were far more wide ranging and often would be sought for >1 hour prior to tracking.

3.3.1 Stingray Activity Space

95% activity space areas using MCP ranged between 0.17 to 0.83 km² with a mean (\pm SD) of 0.38 \pm 0.21 km²; Using LocoH convex hull, 95% values ranged from 0.05 to 0.3 km² with a mean of 0.17 \pm 0.12 km²; and using KDE isopleths of 95% ranged from 0.16 to 0.79 km² with a mean of 0.42 \pm 0.19 km². Total activity space for all rays showed a positive relationship with individual size (disc width) ($r^2 = 0.50$ $p = 0.10$) (Fig. 3.7). Sex had no influence on total activity space (Wilcoxon(1) $Z = -0.83$ $P = 0.40$); or on day (Wilcoxon (1) $Z = -0.97$ $P = 0.33$) or night time activity space (Wilcoxon (1) $Z = 0.49$ $P = 0.63$).

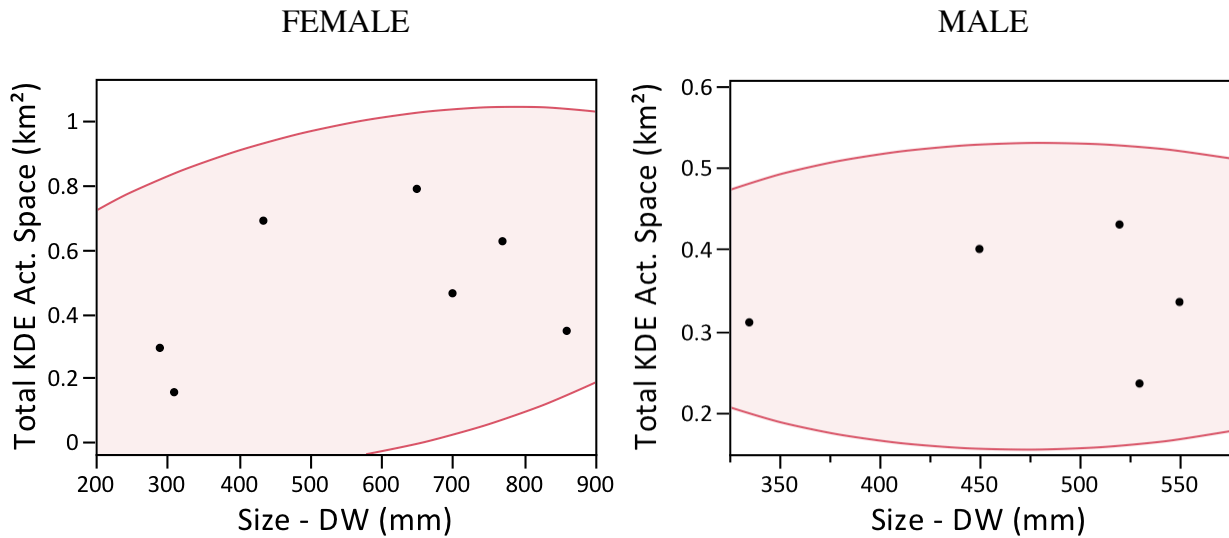
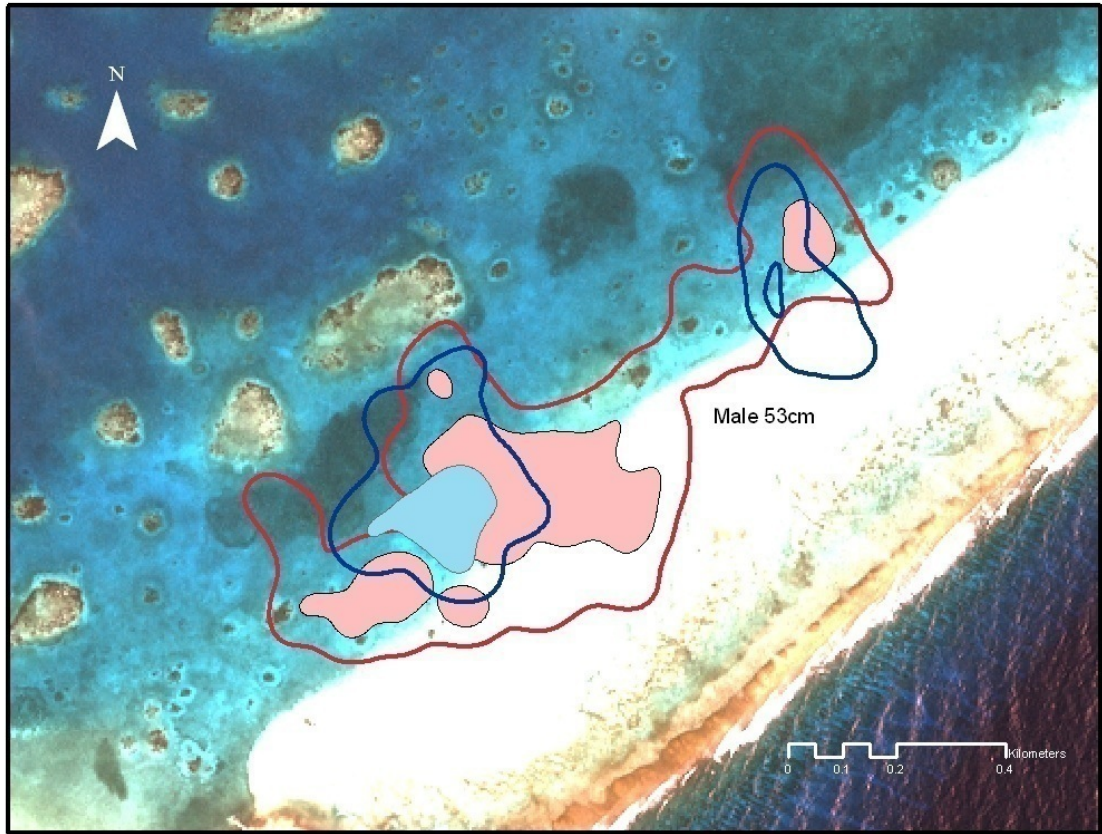
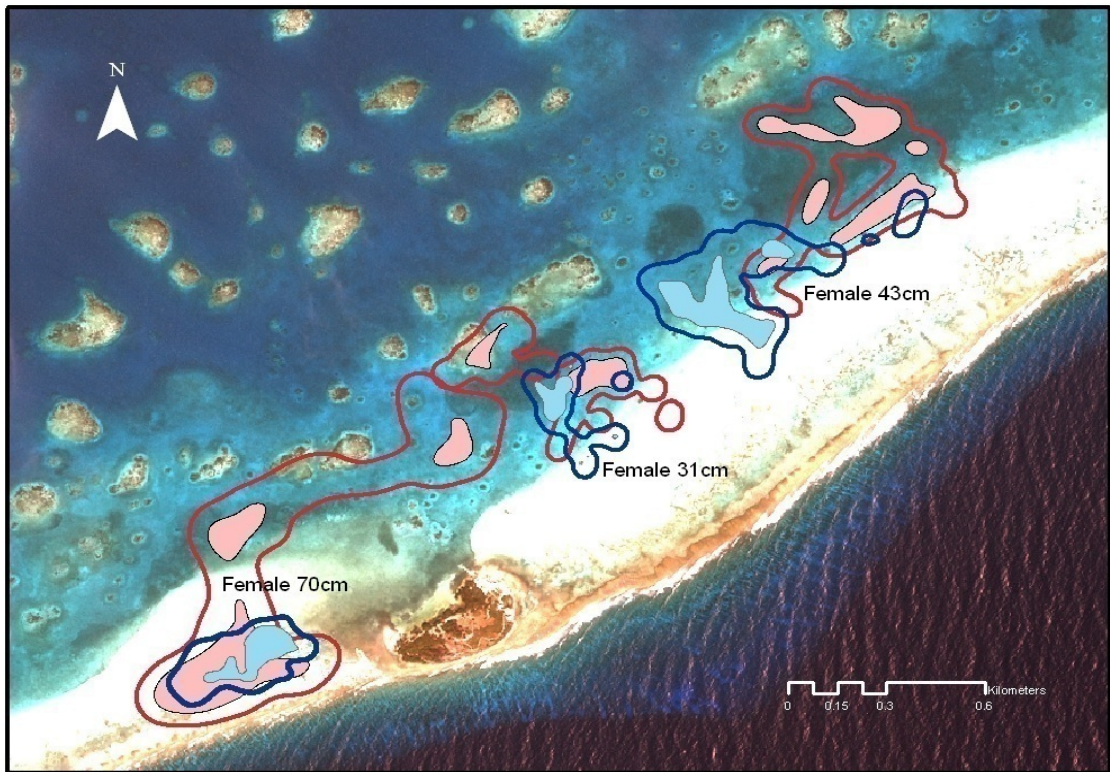


Figure 3.7: Total activity spaces (km²) of *Dasyatis americana* individuals by size (disk width) and sex (n = 12) using kernel density estimation. (Spearman's $r^2 = 0.4965$ $p = 0.1006$)

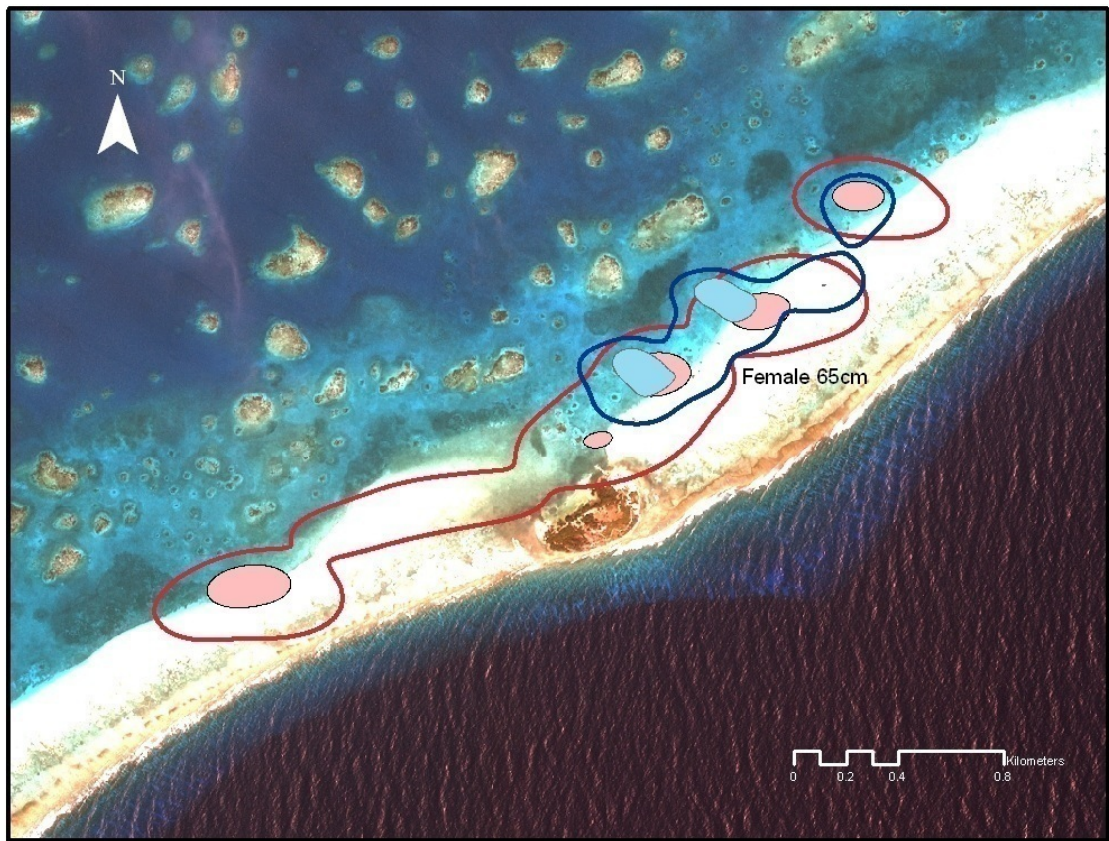
Mean night time activity space (95%) was significantly smaller for all rays combined (Wilcoxon(1) $Z=1.94$ $P<0.05$). Core activity spaces (50%) showed no significant difference in size with day and night (Wilcoxon(1) $Z=-1.59$ $P=0.06$) and accounted for a very small proportion of mean total area (\pm SD) ($17.1 \pm 10.7\%$), indicating large amounts of time spent stationary, or in small patches for fine scale feeding or cleaning (fig. 3.8). Mean core activity space for rays was 0.07 ± 0.04 km² (see Table 3.1).



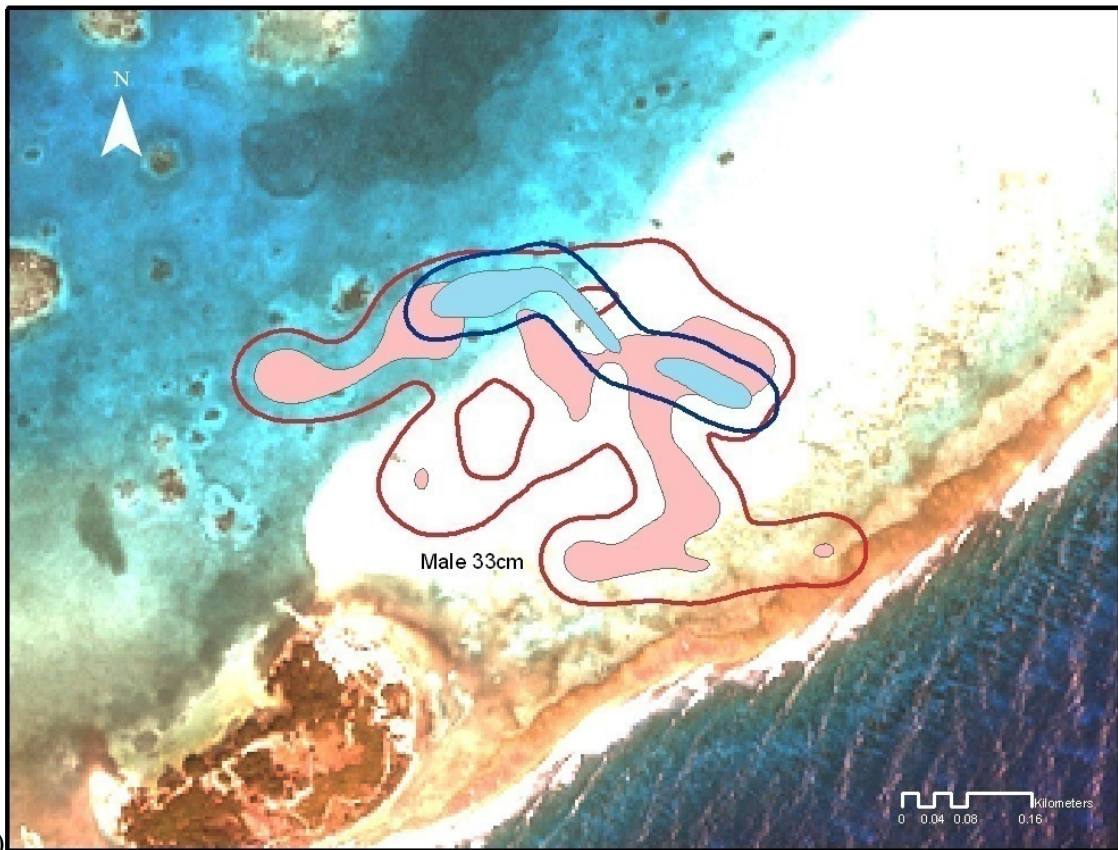
a)



b)



c)



d)

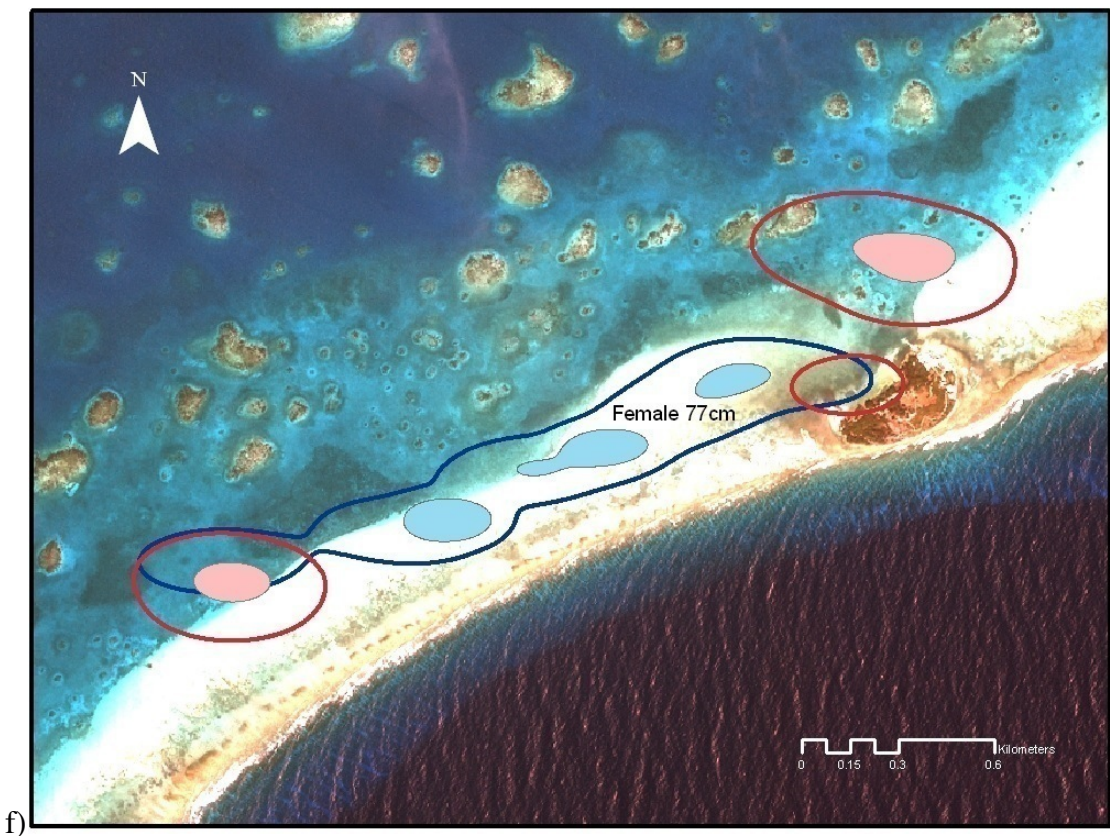
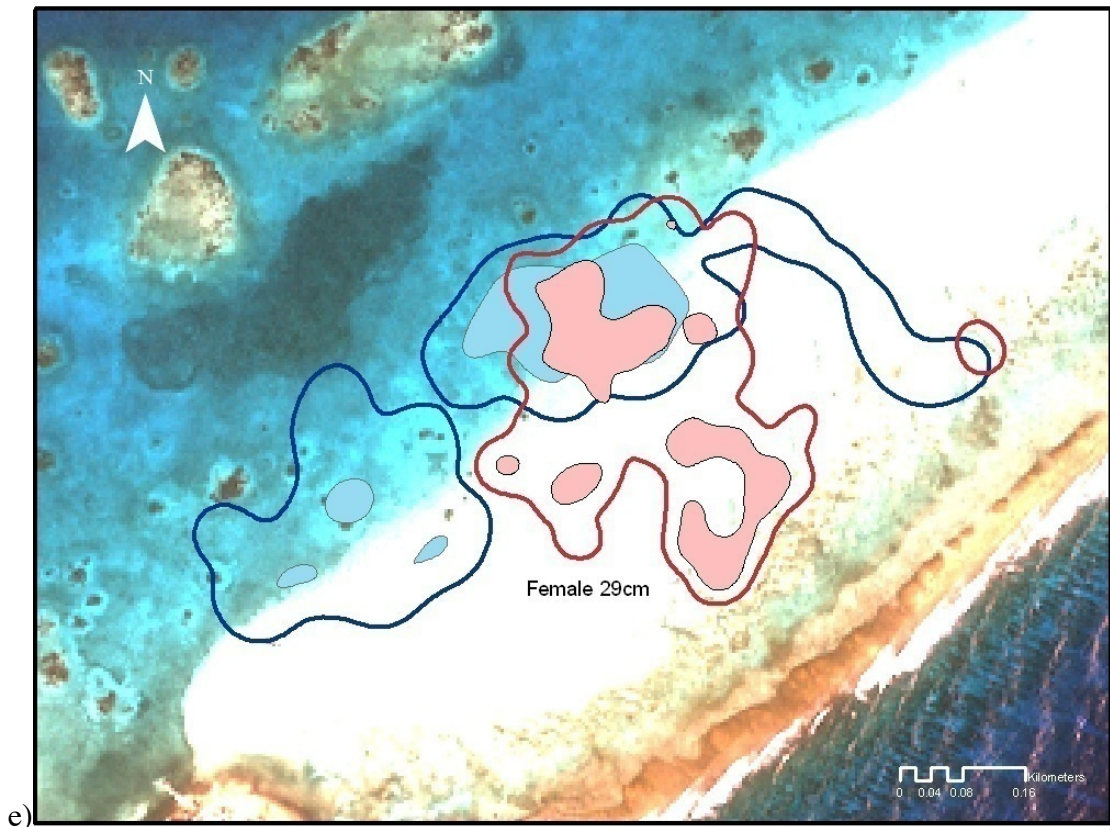


Figure 3.8: 6 satellite images of the south east area of Glovers Reef Atoll, Belize overlaid with night and day activity spaces for 8 *Dasyatis americana* individuals tracked acoustically between June 2009 and May 2010. Image (b) depicts the activity spaces for three female rays. Dark blue outlines indicate 95% night areas with core 50% areas shaded light blue. Daytime 95% areas are outlined in red with core 50% use areas in pink.

Table 3.1: Information of all acoustically tracked rays at Glovers Reef between June 2009 and May 2010. Activity space was calculated only for southern stingrays tracked for >22 hours. Area values are given in km². ‘All’ values refer to 95% and 50% total activity space accounting for overlaps of day and night areas. Day and Night reflect the proportion of the total area (All) represented by day and night values. Size represents disk width in mm.

Ray ID	Date tagged	Hours tracked	Sex	Size	95% All	50% All	Proportion	95% Day	50% Day	95% Night	50% Night
DA1	12-Jun-09	13	Female	584	-	-	-	-	-	-	-
DA2	23-Aug-09	24	Female	310	0.155	0.014	9%	0.137	0.0234	0.07	0.0118
DA3	23-Aug-09	30	Female	650	0.788	0.098	12%	0.849	0.168	0.308	0.069
DA4	09-Sep-09	24	Female	770	0.625	0.084	13%	0.441	0.084	0.262	0.05
DA5	11-Oct-09	24	Female	435	0.689	0.086	13%	0.393	0.048	0.284	0.072
DA6	11-Oct-09	27	Male	335	0.31	0.154	50%	0.192	0.022	0.038	0.006
DA7	15-Nov-09	24	Male	530	0.235	0.04	17%	0.341	0.096	0.107	0.02
DA8	12-Feb-10	26	Female	290	0.293	0.048	16%	0.188	0.055	0.253	0.052
DA9	02-Mar-10	16	Female	700	-	-	-	-	-	-	-
DA10	16-Apr-10	32	Female	700	0.463	0.045	10%	0.355	0.055	0.089	0.021
DA11	05-Jul-10	22	Male	520	0.43	0.057	13%	0.241	0.045	0.348	0.078
DA12	14-Jul-10	26	Male	450	0.4	0.075	19%	0.327	0.082	0.322	0.088
DA13	22-Jul-10	10	Male	420	-	-	-	-	-	-	-
DA14	24-Jul-10	24	Female	860	0.346	0.05	15%	0.446	0.095	0.156	0.033
DA15	26-Jul-10	22	Male	550	0.335	0.061	18%	0.244	0.064	0.263	0.058

Aspect Ratio

Aspect ratio of home ranges, calculated using MCPs, ranged from 0.11 to 0.97 (mean=0.50, median=0.54, SD=0.31). Aspect ratio showed a negative correlation with size (Pearson’s $r(12)=0.40$, $P=0.03$) with a strong relationship with female size (Pearson’s $r(7)=0.83$, $P<0.01$), but non-significant with male size (Pearson’s $r(5)=0.08$, $P=0.65$) (Fig. 3.9), however no differences were seen in aspect ratio magnitude according to sex (ANOVA $P=0.7184$).

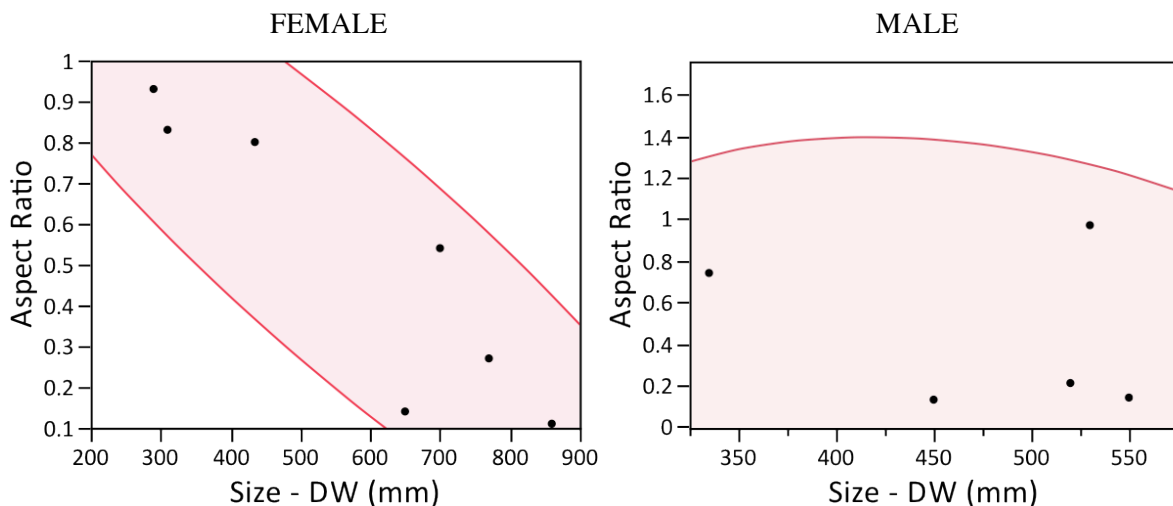


Figure 3.9: Aspect ratio of *Dasyatis americana* activity space by individual size (n = 12). An aspect ratio of 1 represents a range with equal width and length (*sensu* circular), 0 represents significantly elongate activity space. Red shaded areas represent 95% bivariate normal confidence ellipses.

3.3.2 Stingray rate of movement & linearity

Tracking location data were found to be significantly autocorrelated in all 5, 10 and 20 minute intervals likely due to the relatively restricted, linear nature of stingray movements interspersed with long periodic rests. ROM showed considerable individual variation throughout diel cycles. Stingray inactivity during burial or resting periods skewed mean hourly ROM data heavily towards zero. For acoustically-tracked rays, ROM values of less than 10 m/minute were treated as zero to eliminate false movement associated with boat sway and GPS positional accuracy. Mean ROM (\pm SD) for all 12 rays was 0.80 km/h \pm 0.25 (0.2 m/sec). Mean hourly ROM values for each individual ray are shown in Table 3.2. Mean ROM differed significantly according to diel stage, with lowest values being seen at night, and highest at dusk (Kruskal-Wallis H=24.80 P<0.0001 df=3). Daytime values were significantly higher than nighttime (Wilcoxon(1) Z=4.66 P<0.0001), however dusk ROM was not statistically different from daytime values (Wilcoxon(1) Z=0.64 P=0.52). ROM for rays were seen to be significantly higher at low slack tide than all other stages in the tidal cycle (Kruskal-Wallis H=32.71, df=3 P<0.0001)(Fig. 3.10). This trend was seen to be particularly important during crepuscular periods. ROM values at all tidal stages for each group are listed in Table 3.3.

Table 3.2: ROM (m/sec) and path distance (metres) for 12 *Dasyatis americana* individuals tracked acoustically at Glovers Reef between June 2009 and May 2010. Hours tracked represents cumulative time tracked, made up of 2-6 tracking phases. Size represents disk width in mm.

Ray	Hours Tracked	Sex	Size	Path Dist.	Mean ROM	Mean Night time ROM	Mean daytime ROM
DA2	24	Female	310	4917	0.019	0.42	0.63
DA3	30	Female	650	12601	0.433	1.02	1.00
DA4	24	Female	770	10552	0.251	0.45	0.73
DA5	24	Female	435	7831	0.341	0.59	0.59
DA6	27	Male	335	3472	0.086	0.07	0.41
DA7	24	Male	530	11293	0.353	0.45	0.48
DA8	26	Female	290	6226	0.121	0.42	0.50
DA10	32	Female	700	11996	0.434	0.58	0.77
DA11	22	Male	520	7457	0.110	0.15	0.13
DA12	26	Male	450	6226	0.132	0.09	0.20
DA14	24	Female	860	6470	0.115	0.08	0.18
DA15	22	Male	550	5135	0.109	0.13	0.12

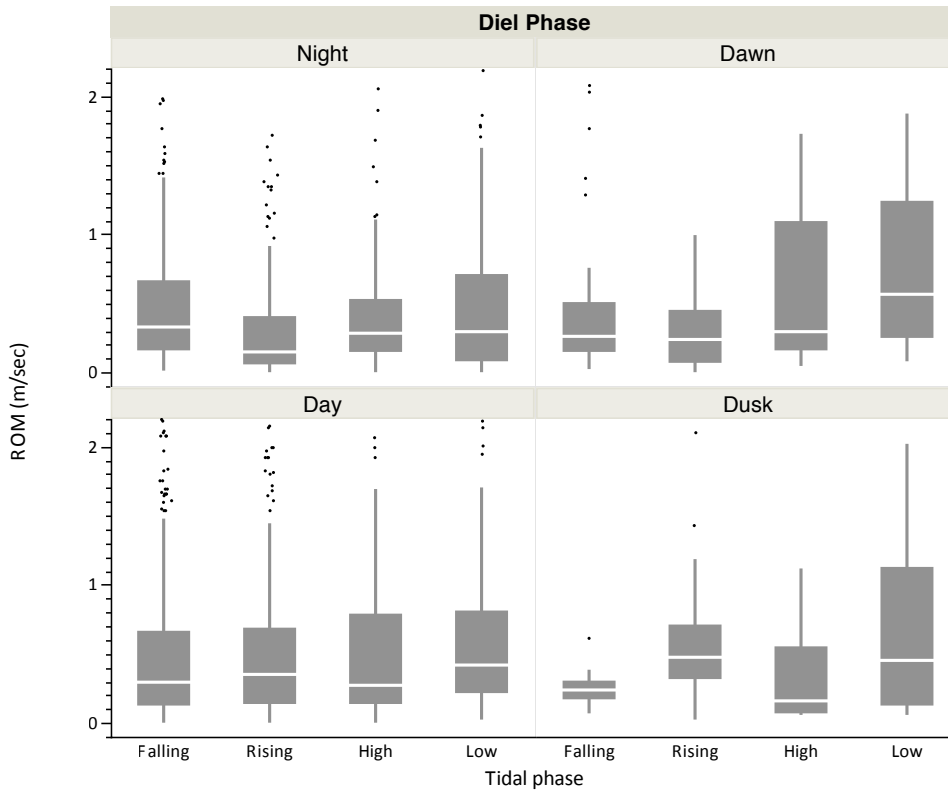


Figure 3.10: ROM (m/sec) of acoustically tracked *Dasyatis americana* at Grovers Reef according to tidal and diel phases (n=12). Boxplots represent lower, median and upper quartiles, with bars depicting 95% confidence intervals.

Table 3.3: Mean rate of movement (m/sec) of all acoustically tracked *Dasyatis americana* (n=12) at Grovers Reef according to tidal stage. Slack tides represent 1 hour either side of the high and low tides.

Tidal Stage	Mean ROM (m/s)	SD	Variance	df
Falling	0.343	0.227	0.051	11
Low Slack	0.375	0.213	0.045	11
Rising	0.282	0.194	0.038	11
High Slack	0.278	0.279	0.078	11

ROM exhibited a positive linear relationship with size (Spearman’s $r(11)=0.16$, $P=0.005$), and females were seen to have a higher mean ROM than males (Wilcoxon(1) $P<0.023$). The significant difference of ROM across hour categories for MC rays and combined groups (Kruskal-Wallis $P<0.001$) showed the highest peaks of ROM during early morning and early afternoon (0700-0800 hours, 13-1400 hours), with another peak of activity at sunset 1700-1800 hours (Fig. 3.11). This is supported by mean net displacement by hour, showing the highest displacement during the early morning around sunrise (0500-1000 hours), with additional large movements at mid afternoon (1300-1400 hours) and evening (2000-2100 hours) (fig. 3.12).

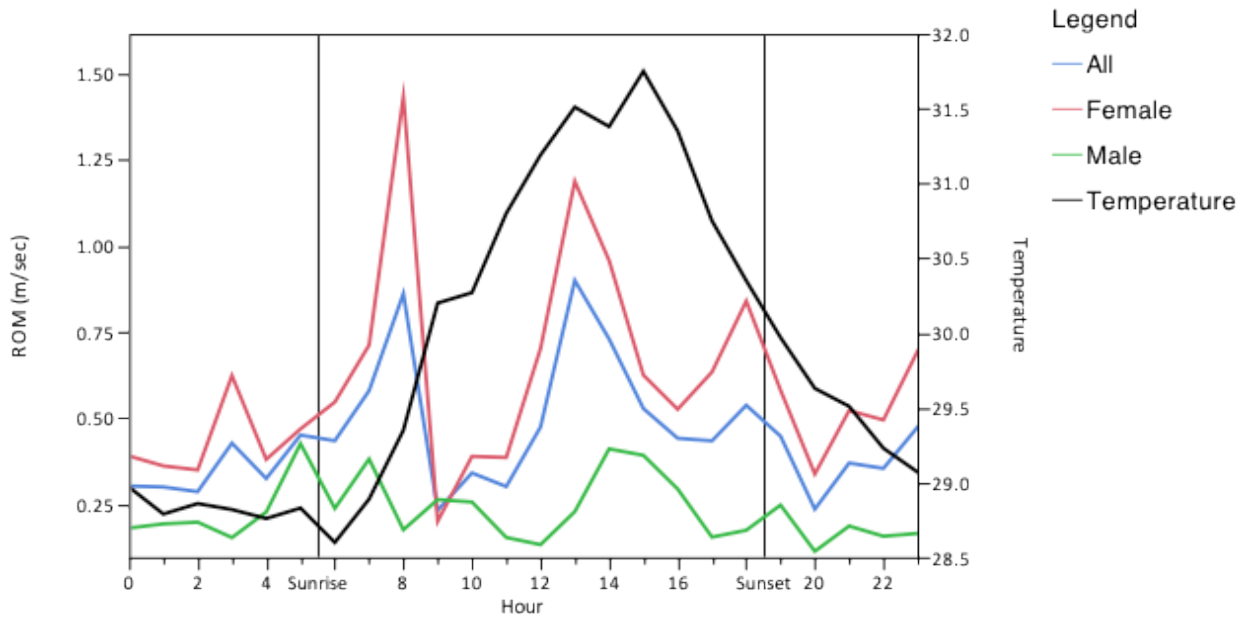


Figure 3.11: Mean hourly rates of movement for 12 *Dasyatis americana* stingrays acoustically tracked at Glovers Reef. Lines represent all rays combined (blue), only females (red) and only males (green), and temperature is in black

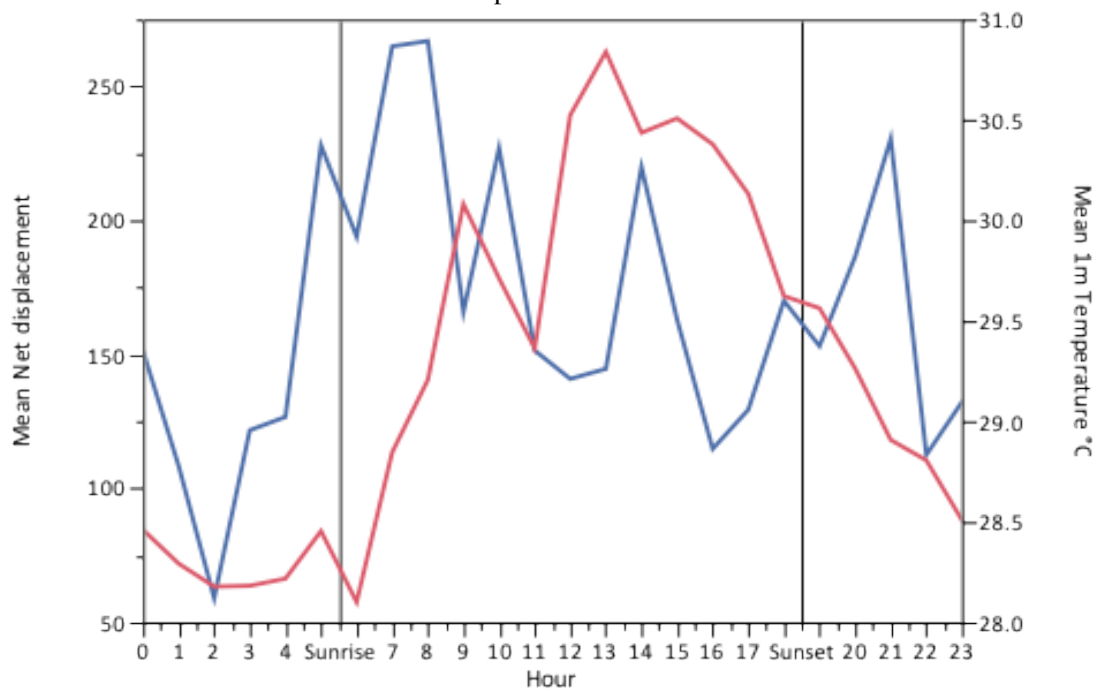


Figure 3.12: Mean hourly net displacement for 12 *Dasyatis americana* stingrays acoustically tracked at Glovers Reef (blue line). Temperature is shown in red.

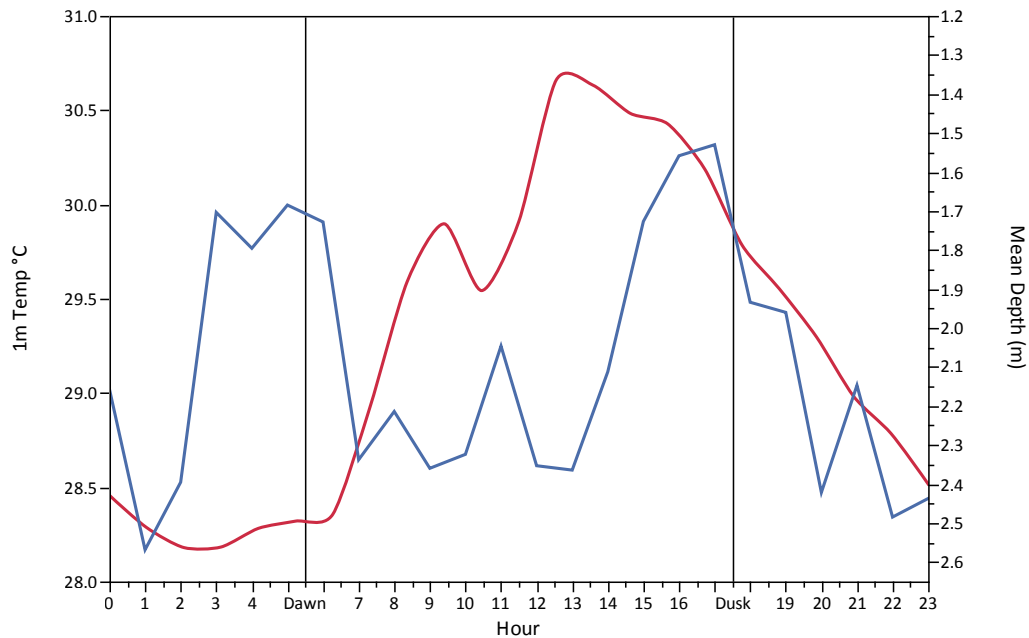


Figure 3.13: Combined mean depth distribution (blue line) of 12 *Dasyatis americana* individuals acoustically tracked at Glovers Reef and mean 1 m temperature (red line) by hour. Note inverted right axis to more easily visualise depth.

Tracking produced no clear patterns of movement according to daily temperature cycles, however the highest daily temperatures between 13-1500 hours correlated with a daytime peak of activity, with rays showing heightened ROM (fig. 3.11) and net distance travelled (fig. 3.12). Mean monthly temperatures in 1 m water depth ranged from a low of 25.6°C in January to a high of 31.3°C in September. January and September also exhibited the largest and smallest variation in temperature with a range of 11.6 °C and 1.9 °C respectively (Fig. 3.14). Mean rate of movement did not change significantly with mean monthly temperature, with the highest mean ROM recorded in April (Fig 3.14).

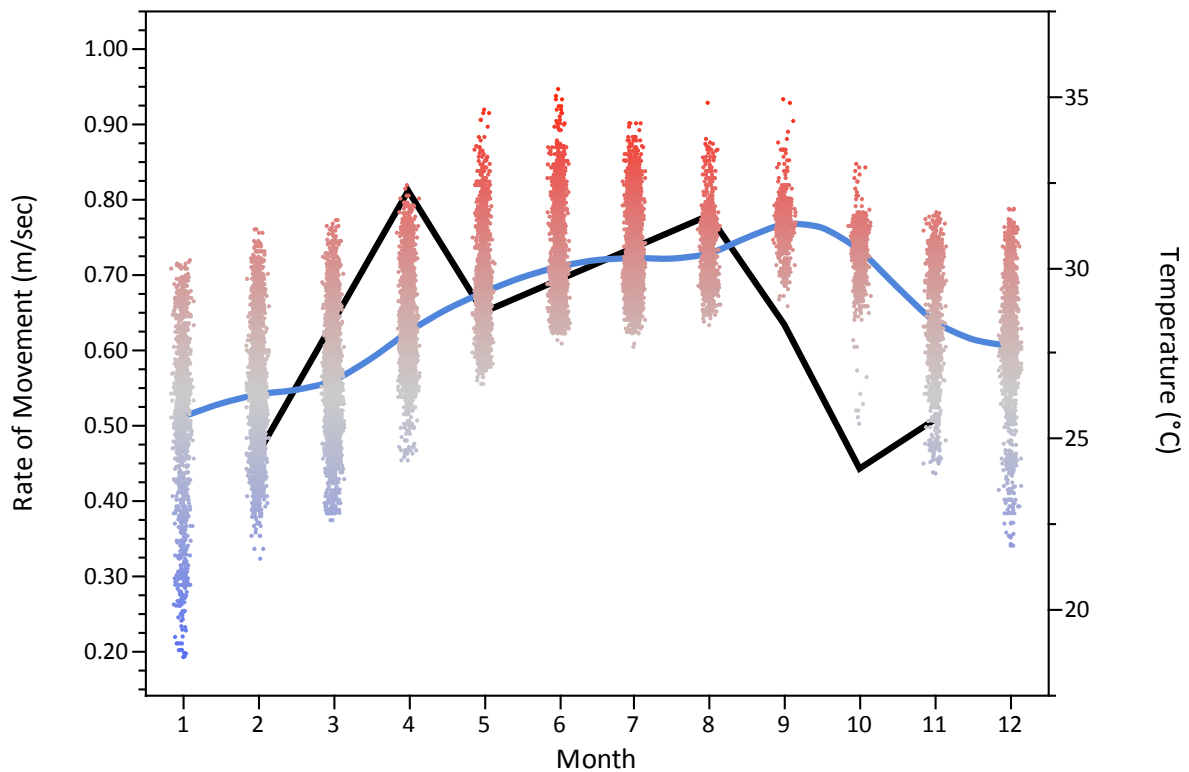


Figure 3.14: Annual 1 m lagoon water temperatures by month (1-12) at Glovers Reef Atoll, Belize against mean monthly Rate Of Movement (ROM) (m/sec) of *Dasyatis americana*. The blue line represents mean monthly temperature and black line represents mean ROM values for those months sampled (N.B. ROM data is not continuous throughout months, but is depicted as a line for ease of interpretation). Low values in May are unreliable due to small sample size. N.B.

Linearity ranged from 0.02 to 0.98 (mean=0.4 ± 0.27, median=0.37). Analysis of path linearity supported findings of ROM, where paths were significantly straighter during low slack tide and falling tide than high and rising tides for both diel periods (Kruskal-Wallis H=158.3, df=3, P<0.0001). Overall daytime movements had significantly straighter paths than nighttime (H=28.1, df=1, P<0.0001). Females had significantly straighter paths than males (Kruskal-Wallis H=66.5, df=3 P<0.0001) As with rate of movement, linearity showed a positive relationship with individual size (Spearman's $r(12)=0.22$, P<0.0001) with larger rays showing straighter movement paths.

3.3.3 Habitat and depth preference of stingrays

The depth range utilised by rays tracked in the lagoon was between 0.3 - 5.5 m. Accurate depth data was logged from one tracked 550 mm male ray using a pressure sensing tag (Vemco V13P). This data showed rate of movement correlating negatively with increasing depth (Spearman's $r(878)=-0.21$, P<0.0001), supporting data obtained from *in situ* bathymetric field mapping where ROM for all rays pooled correlates negatively with depth to the same degree (Spearman's $r(1566) = -0.21$, P<0.0001) (Fig. 3.15).

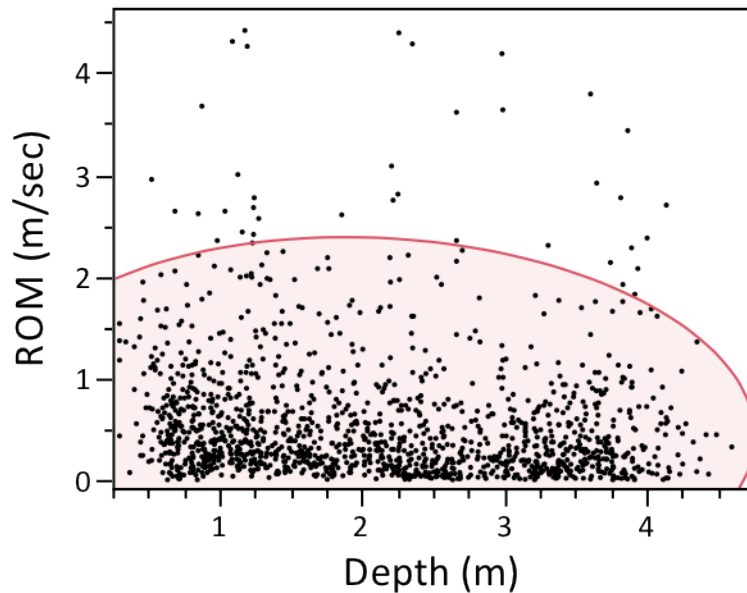


Figure 3.15: Bivariate plot of Rate of Movement (ROM m/sec) by depth (m) for all rays tracked ($n = 12$) showing a negative relationship (Spearman's $r(1456) = -0.08$ $P=0.0008$). Depth data was extracted from a bathymetric map created in ArcGIS from field sampling. Red shaded areas represent 95% bivariate normal confidence ellipses.

Analysis of depth distribution against individual size by diel phase (night, dawn, day, dusk), illustrated a tendency for large rays to be distributed more in shallow areas at night and dawn (Fig 3.16a-b), whereas small rays were in shallow waters during the daytime and dusk (Fig 3.16c-d). Night time and dawn depth distributions showed negative relationships with disk width (Spearman's $r(845) = -0.16$ $P < 0.0001$ and $r(107) = -0.22$ $P < 0.022$ respectively) where larger bodied rays were distributed in shallow water, whereas the inverse situation was shown during daytime ($r(1055) = 0.25$ $P < 0.0001$) and dusk ($r(82) = 0.41$ $P < 0.0001$) where shallow areas were populated by small rays and large rays remained predominantly in deeper water (fig 3.16).

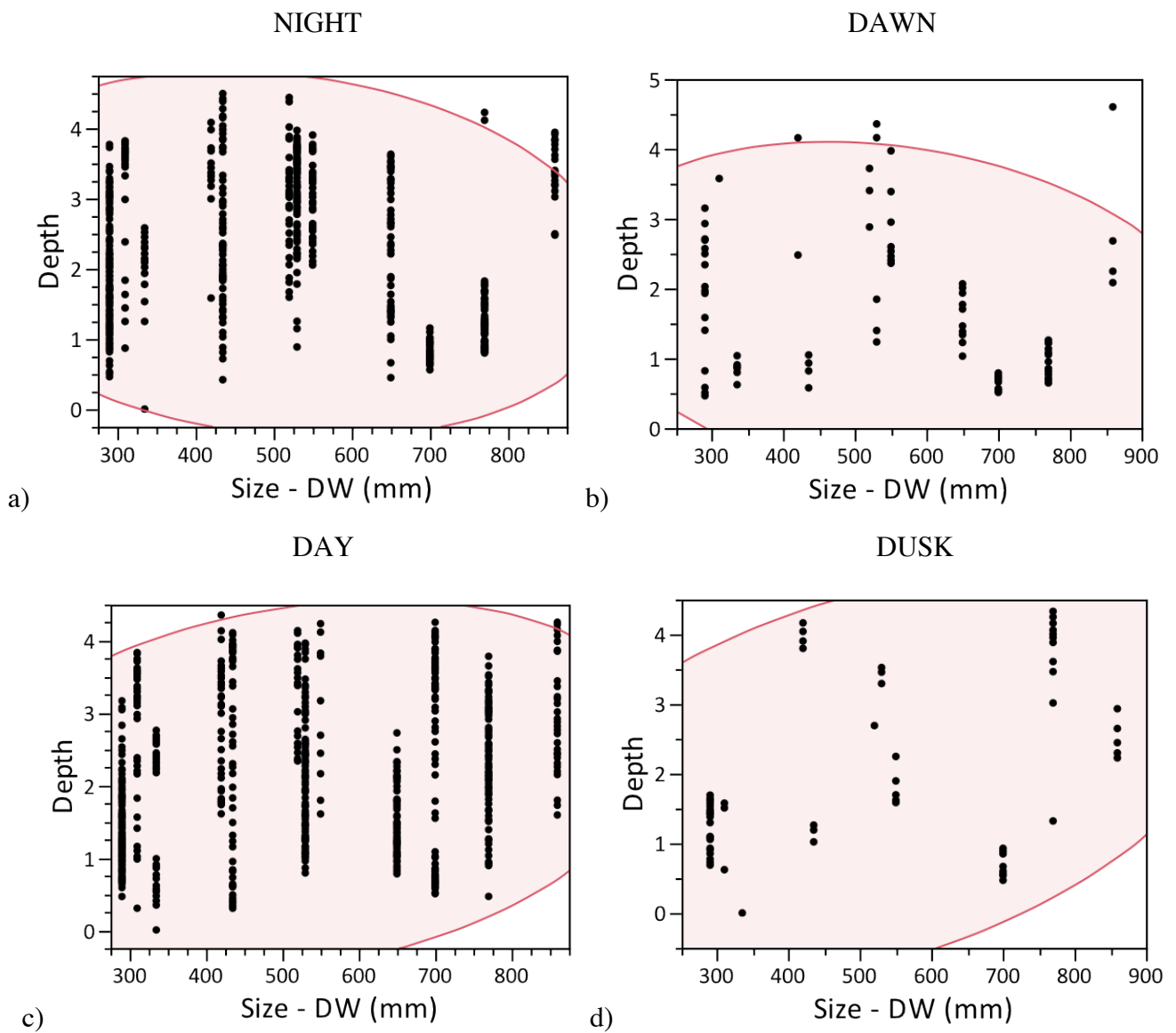


Figure 3.16: Diel depth distribution of *Dasyatis americana* (n = 12) according to individual size (mm disk width - DW) over a) night time, b) dawn, c) daytime and d) dusk periods. Red shaded areas represent 95% bivariate normal confidence ellipses.

Neonates and small juvenile rays (<40 cm DW) showed a small variance in depth preference according to hour, whereas larger rays showed much larger individual variations (fig 3.17).

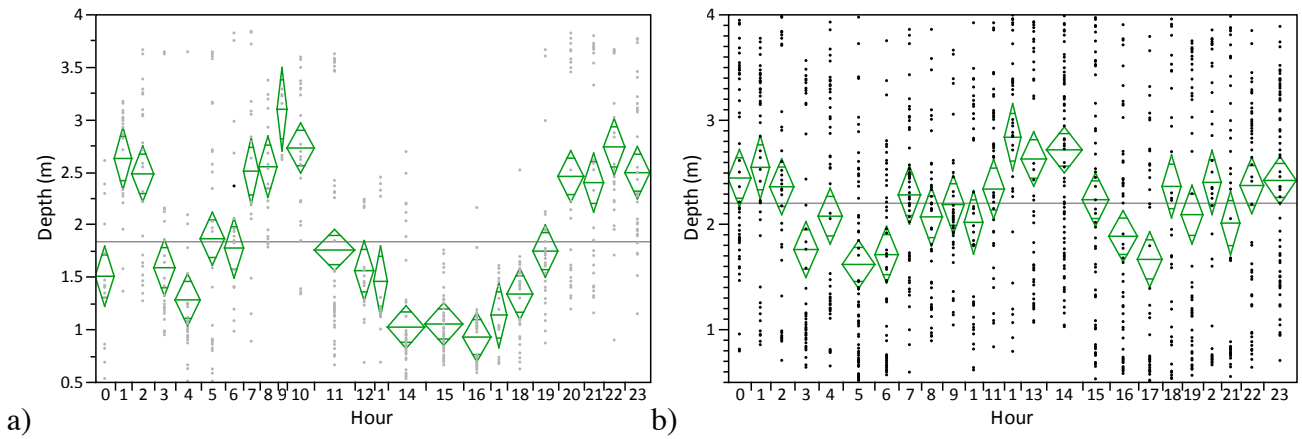


Figure 3.17: Depth distribution of two size categories of *Dasyatis americana* a) <40 cm disk width (n=3) and b) >40 cm disk width (n=9) acoustically tracked in Glovers Reef lagoon. Grey horizontal line represents overall mean depth.

Light measured at a depth of 1-1.5 m (variable with tide) showed higher mean light intensity in July & August than all other months sampled. September and October were not sampled for light intensity (fig. 3.18), yet were the highest ROM values.

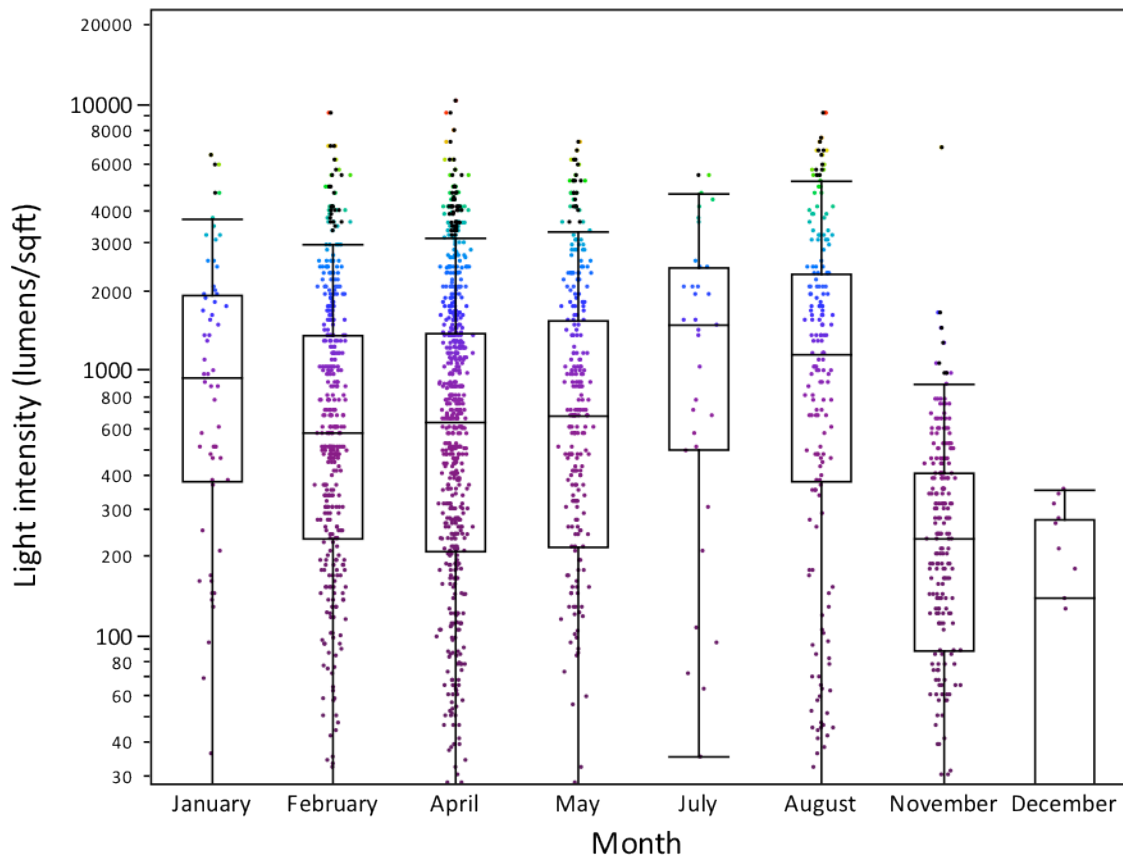


Figure 3.18: Monthly light intensity at 1 m depth in Glovers Reef atoll lagoon. Light intensity (lumens/ft²) values are plotted on a log scale (coloured dots). The boxplots represent lower, median and upper quartiles, with bars depicting 95% confidence intervals. Only sampled months represented – the hottest months of September and October are missing.

3.4 DISCUSSION

Southern stingrays at Glovers Reef (GR) displayed significantly higher rates of movements and larger activity spaces in the daytime than at night. Almost all studies on elasmobranch movement have found some element of altered behaviour according to diel changes (Speed et al., 2010), yet for the most part coastal sharks and rays show preference for night time foraging (McKibben & Nelson, 1986; Holland et al., 1993; Cartamil et al., 2003; Vaudo & Lowe, 2006; Chapman et al., 2007; Collins et al., 2007). Field observations confirm active feeding is carried out during the day (Tilley, pers. obs.), and rates of movement and net displacement imply some stingrays do continue to forage periodically during night time periods, as seen by Sazima (2006). These movements may be in response to sporadic prey encounters, reflecting the opportunistic feeding strategy of *Dasyatis americana*, as seen in *Sphyrna lewini* pups (Holland et al., 1993), and these occasional forays for food may explain expansion of night time areas from core refuge positions and observed localised movements in some individuals. Corcoran (2006) found no significant effects of diel cycle on *Dasyatis americana* in the Cayman Islands, yet observed higher activity at night (Corcoran, 2006) likely explained by foraging movements. Corcoran (2006) sampled wild southern stingrays (n=7) of 84.9 cm mean disk width (± 18.2 SD), indicating a population of predominantly adult rays (Henningsen, 2000). In contrast, mean disk width of the GR rays tracked (53.3 ± 18.3), represents predominantly juveniles and young adults. Greater nocturnal foraging was seen in two of the larger rays in the GR sample (DA4 & DA10) that displayed continued movement throughout the night in shallow water, while smaller rays remained relatively inactive in deeper water (Fig. 3.5 a & f). This suggests that nocturnal foraging may be utilised by stingrays that have reached a size refuge, which in the Glovers Reef sample appears to be approximate to that of female maturity (~ 70 cm DW) (Henningsen, 2000). In contrast small rays (<65 cm) at GR remained mostly stationary during the night in slightly deeper water in the clear sand areas surrounding patch reefs, which may suggest these topographical resources within the lagoon margin may represent important structures to rays in refuging behaviour, potentially by reducing angles on vertical and horizontal planes from which a predator might attack (see Heithaus et al., 2002). Large rays rested for the majority of the daylight hours while smaller rays forage, potentially utilising lighter conditions to minimise predation risk through visual vigilance (McComb & Kajiura, 2008). Gut content studies indicate that *Dasyatis americana* is an opportunistic and continuous forager (Gilliam & Sullivan, 1993), which may explain a lack of clear distinction between diel feeding periods. ROM data from GR suggests rays of all sizes increase activity levels during crepuscular periods, likely in response to increased prey availability during these periods, or increased predation risk driving movements of smaller rays from foraging to refuging habitats.

Diel changes in activity can be attributed to varying factors such as prey abundance, behavioural thermoregulation and predator avoidance, yet are likely to be driven by a combination of them. It is possible that movements between shallow sand flat habitat and deeper lagoon margin serve an additional thermoregulatory purpose for *Dasyatis americana* in controlling physiological processes. No seasonal pattern emerged between ROM and temperature, with similar mean ROM values observed for all months, however hourly mean ROM for GR rays peaked between 1300-1400 hours, corresponding to the mean hottest period of the day in months September-February. Increased ROM may imply that rays may be using temperature enhanced metabolic rates to fuel heightened foraging intensity such as in hunt warm-rest cool strategies (Sims et al., 2006). Daytime depth of rays <40 cm showed a negative relationship with 1 m temperature values, indicating rays are moving towards warmer shallow waters in the middle of the day and remaining there until after dusk. This may reflect thermotaxis as been seen in bat rays *Myliobatis californica*, which moved between warm waters for feeding and cooler for digestion (Matern et al., 2000), and round stingrays *Urolophus halleri* where movements positively correlate with increasing water temperature (Vaudo & Lowe, 2006). However, mean daytime depth patterns for larger rays (>40 cm) showed the opposite response to rising temperatures, suggesting a use of temperature for heightened foraging activity is unlikely. Low prey encounter rates in sand flat habitat (seen from low prey abundance in section 2.3.5) and limited movements observed for small rays, imply the effects of higher temperatures in speeding metabolism and evacuation rates may be deleterious for young rays, significantly hampering energy intake and hence growth rates. This further supports that young rays select shallow waters as a trade off of energy for safety.

Intermittent feeders for whom feeding represents a heightened predation risk such as rays, may benefit from a hunt warm-rest cool strategy by increasing the efficiency of food uptake, rather than speeding evacuation times (Di Santo & Bennett, 2011). During rest periods, stingrays will most often bury in the sand, which may be a dual process of minimising predation risk, and actively regulating temperature and metabolic processes. Thermal properties of cryptic burial in sand is unknown, yet reduced temperatures within the benthos may further enhance the rays ability to control metabolism, as some fish are seen to bury within soft substrates to facilitate thermoregulation (Crawshaw, 1974). Slowing digestion rates for a cryptic animal is likely to be important in minimising time spent moving and hence reducing predation risk. This will be more crucial for smaller, juvenile rays as reflected in their daytime feeding strategies.

Stingray ROM was highest during low tide and falling tide. Typical temperature variation within a tidal cycle at Glovers Reef was 3-5°C, and given the potential relationship seen with temperature, ray

movements with falling and low tide may be a response to higher temperatures brought about by an ebbing tide. Two studies undertaken with *Dasyatis americana* suggest their movements are influenced by the tidal cycle, due to high tides exposing shallow foraging grounds (Gilliam & Sullivan, 1993; Carvalho et al., 2010), however if increased ROM is related to increased foraging effort, then stingrays at GR are not utilising expanding areas of accessible foraging grounds at high tide. As might be expected, tidal flow is shown to have a strong influence on coastal elasmobranch species (Huish & Benedict, 1977; Medved & Marshall, 1983) that feed in intertidal habitats such as sand and mudflats (Hines et al., 1997; Montgomery & Walker, 2001) which *Dasyatis americana* certainly does. However, maximum tidal amplitude is very small in Belize (± 20 cm), so even if rays were responding to tidal cues, the response is likely to be diminished due to minimal inundation of additional foraging grounds. Prey abundance at Glovers Reef increases with depth (section 2.3.4), so stingray foraging in shallow sand flats at GR reflects a habitat preference that is not based solely on prey density (i.e. an ideal free distribution). Prey accessibility could play a part in restricting ray exploitation of shallow intertidal back reef areas, as substrate tends to be a sand and coarse rubble mix, which may limit prey extraction. Alternatively, rays may not exploit shallow areas available at high tide due to enhanced predation risk or low prey abundance, however this seems counter intuitive given their flattened morphology allowing for access to extremely shallow water. Furthermore, juvenile lemon sharks (*Negaprion brevirostris*) in the Bahamas were said to select shallower lagoon habitat in order to escape predatory sharks in the deeper lagoon (Morrissey & Gruber, 1993a). Two female stingrays (>700 mm DW) were tracked foraging and feeding in very shallow water (<0.5 m), while all others remained in water exceeding 0.75 m aside from brief forays into shallower sand flats during foraging. Juvenile stingray reduction in movement during the night may signal a response to increased predation risk, where larger rays continue to forage utilising a size refuge. Male Caribbean reef sharks were shown to move up into shallower lagoon waters at night at Glovers Reef (Chapman et al., 2007), which may signal similar behaviour in other known shark predators of batoids such as bull *Carcharhinus leucas* (Cliff & Dudley, 1991), tiger *Galeocerdo cuvier* (Lowe et al., 1996) and great hammerhead sharks *Sphyrna mokarran* (Strong et al., 1990; Cliff, 1995); the latter of which has been identified inside the lagoon at Glovers Reef (Pikitch et al., 2005), attacking and consuming batoid prey (W. Lomont pers. comm., Glovers Atoll Resort). It appears from in-water field observations and tracking data that rays utilise clear areas around patch reefs for foraging and cryptic burial. Patch reefs are likely to provide multiple benefits to rays in terms of minimising predation risk, increasing foraging opportunities due to heightened productivity at the reef/sand interface (Gratwicke & Speight, 2005). In Hawaii *Sphyrna lewini* juveniles were also seen to exploit these areas (Lowe, 2002). Rays were rarely seen refuging actually within or on top of the patch structure,

except for during cleaning bouts or when directly threatened. The use of topography or complex structures for refuge mirrors findings by Strong et al., (1990), where rays sought refuge of a submerged wreck in the presence of a great hammerhead, and field observations from GR (Chapman, pers. comm.) where a ray was seen to refuge on top of a patch reef in the presence of a Caribbean reef shark.

Juvenile rays were predictably found over multiple days of tracking in the same area, but night time refuging showed no fidelity to specific patch reefs or locations over consecutive night tracks. 90% of night time activity space used by juvenile rays fell within daytime activity spaces, either fully or partially, suggesting extremely restricted night time movements. A positive linear relationship between activity space and individual stingray size (disk width) reflects ontogenic expansion as seen with many elasmobranch species (Morrissey & Gruber, 1993b; Heupel et al., 2004; Weng et al., 2007), and is thought to be associated with the increased energetic requirements of growing and reproducing, combined with the reduced predation risk derived from larger body size (Speed et al., 2010). Ontogenic expansion of activity space in GR rays corresponded with straighter movements and reduced aspect ratios, indicating a linear expansion characteristic of coral reef environments. In an atoll environment in Brazil *Dasyatis americana* was found to inhabit shallow beach areas during the juvenile stage, with large stingrays moving to exploit deeper reef environments (Aguiar et al., 2009). Very large rays (>90 cm DW) were noticeably absent from surveys (Chapter 2) and captures at Glovers Reef, so conclusions cannot be drawn as to whether the *bigger-deeper* phenomenon holds true for this species. No rays tracked in this study ventured out of lagoon margin habitats into the deep lagoon, however one female (70 cm DW) was tracked moving between forereef and lagoon habitats at night.

At GR significant differences in activity space were seen between males and females, possibly due to sexual dimorphism with females attaining much larger size than males, and the associated bioenergetics requirements of increased size and reproduction requirements. Provisioned stingrays in the Cayman Islands have been seen to reduce activity space to maximise accrual of food resources, suggesting that spatial parameters are adaptive and potentially a function of food availability (Corcoran, 2006). Morphological changes with ontogeny are also likely to affect activity space, home range and habitat use by enhancing prey extraction in deeper or more complex substrates (seagrass or rubble), or increasing gape allowing for manipulation of larger prey items. Additionally, in non-destructive feeding of prey patches, memory and experience may shape an animals' home range (Van Moorter et al., 2009), where infaunal repopulation of feeding pits (Thrush et al., 1991) drives frequent and relatively more efficient re-visitation. This may certainly be the case with patch reef –

sand flat interfaces, representing areas of higher productivity; topographic benefits in terms of predator avoidance; and recognition, driving rays to orientate between patches within their home ranges.

The primary limitation to this study was a low sample size of very large rays. The relationship between size and nocturnal foraging would be more robust with tracking data from individuals greater than 80 cm throughout a diel cycle. The dart tag method used in this study would facilitate the in-water selection of only very large individuals, and minimise the difficulties and dangers of handling large rays in the boat. Additionally, acquisition of temperature measurements along with tracking information would allow for higher resolution analysis of movement according to thermally heterogeneous habitats.

Conclusions

Southern stingray movement shows peaks of activity interspersed with long resting periods, consistent with early conclusions of stingray behaviour. Foraging activity is reliant on diel cues in timing of movements and habitat use in lagoon margin habitat, with most intense foraging occurring during crepuscular periods. Stingray size affects timing and spatial characteristics of foraging movements, where small rays refuge in shallow water to feed opportunistically during the day, and remain buried deeper at night, whereas large ray movements are less constrained by diel stage once within a size refuge. The scale of ray movements increases with size, displaying an ontogenic expansion in activity space where home range becomes elongated according to the nature of the lagoon margin habitat. Temperature was not seen to drive movement on seasonal or diel timescales. Despite small tidal amplitude at the study site, a significant rise in activity was seen at low tide. Patch reefs are very important focal resources for rays in the lagoon margin as resting and burying locations. Chapter 4 will analyse these movement tracks at a structural level, to evaluate how ray movements and search strategy indicate changes according to spatial scale, and investigate to what extent rays can orientate their movement towards known goals such as these patch reefs.

4. Scales of movement, orientation & patch use of the southern stingray *Dasyatis americana*

Abstract

Analysis of movement path structure enables the identification of spatial scales at which animals respond to their habitat, and enhances understanding of the mechanisms of search behaviour and community dynamics. Dispersal characteristics of stingray movement paths from Glovers Reef, Belize, were compared with random walk models to evaluate orientation distance, and path structure was studied using fractal analysis to measure scales of response to a heterogeneous environment. Southern stingray movements were more dispersed than a correlated random walk to scales of ~100 m, yet showed no super-diffusive characteristics of a Lévy walk, indicating rays use directed walks orientated towards goals or features at this scale. A transition was also identified between two domains of stingray movement structure at 100 m, indicating two distinct scales of habitat to which rays respond, and use differently. No transitions were seen at spatial scales smaller than 90 m patch size indicating an absence of dense prey patches and associated lack of systematic searching, supporting classification of *Dasyatis americana* as an opportunistic predator. Narrow confidence intervals suggest all rays move with similar patterns, however larger rays showed more linear movement paths. Results suggest that stingray movement within or through foraging patches is directed, but selection of these prey patches is random. Home range behaviour may account for the ability to orientate at scales up to 100 m as a result of experiential learning of landmarks and key topographical features. This implies that rays may use the distribution of lagoon coral reef patches at Glovers Reef as a network of refuges, connected by pathways of potential foraging areas as seen in some terrestrial animals, and has important consequences in understanding habitat use and dispersal.

4.1 INTRODUCTION

The movement responses of animals in heterogeneous landscapes provide a key insight into their influence on ecological dynamics (Turchin, 1998), such as community structure, biodiversity (Tilman, 1994), population viability (Laidre et al., 2004) and metapopulation dynamics (Hanski, 1998; Turchin, 1998). Spatial ecology can have as equivalent an effect on populations as mortality, predation and competition (Hanski, 1998), and also dictate their dispersal pattern in heterogeneous environments and according to prey density (Humphries et al., 2010).

Natural selection favours the most efficient forager (Morrison, 1978); one that can achieve optimal allocations of time and energy expenditure (MacArthur & Pianka, 1966). The movement pattern an

animal uses while foraging is crucial to their success and efficiency in terms of overall fitness. A predator searching for prey distributed in a homogenous, stable environment is likely to utilise a search pattern such as a random walk or correlated random walk (Stephens & Krebs, 1986), but in heterogeneous environments of patchy prey distribution, or on a larger scale where foragers have no knowledge of prey location and availability, the optimal forager will aim to maximise chance patch or prey encounters (Sims et al., 2007) through random patterns and processes derived from probabilistic laws (Bartumeus, 2011) such as Lévy walks.

A powerful tool in analysing movement paths is viewing them as a discrete series of step lengths and turn angles (Turchin, 1998) (Fig.4.1), which can be compared to statistical distributions and random walk models (Bartumeus et al., 2005). The classical random walk, is a Brownian diffusion process where particles move at random (Preisler et al., 2004; Smouse et al., 2010) with a Gaussian distribution of step lengths and a uniform distribution of turn angles. Due to cephalo-caudal polarisation and bilateral symmetry in animals, there is a natural tendency to move forward (Bovet & Benhamou, 1988), hence random walk processes in animals are biased in a particular direction, known as directional persistence, where turn angle distribution is non-uniform and correlated, adhering to a wrapped Gaussian distribution (Bartumeus et al., 2005).

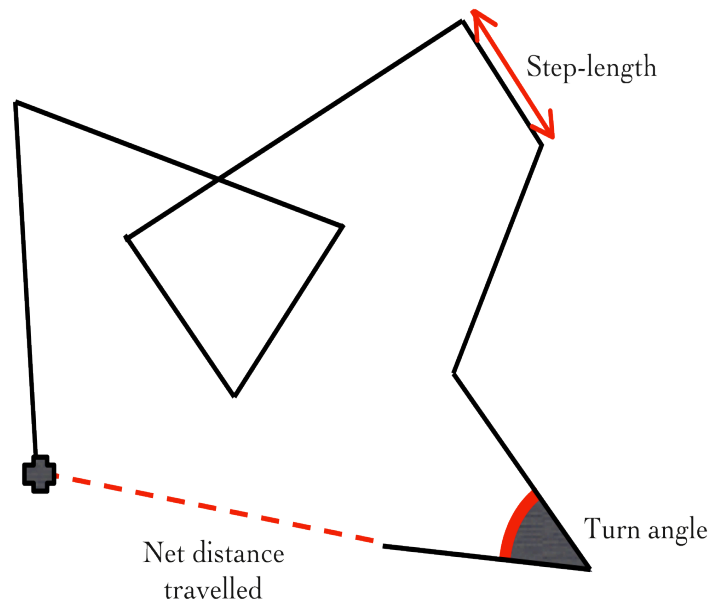


Figure 4.1. Diagram of the elements of path structure

Correlated Random Walks (CRW) were identified in early studies with insects and explained animal movement structures over short spatial and time intervals (Bovet & Benhamou, 1988; Crist et al., 1992) in areas of variable prey density and availability (Humphries et al., 2010). A CRW is a model of probabilistic discrete steps (Bartumeus et al., 2005) producing frequency distributions of turn

angles and step lengths, against which empirical animal movements (squared net distance from a start point) can be compared. This proves useful in trying to dissect orientated movement paths (where an animal moves towards a known goal), from a CRW with a directional persistence, as orientation of moving animals is difficult to assess if their goal is unknown. Random walks in cases of animals with a repeated point of reference, such as a burrow or den, exhibit a mean-reverting Ornstein–Uhlenbeck process, where individuals drift randomly, but are attracted back to an average point (Smouse et al., 2010). Some random walk movement paths exhibit a looping pattern at certain scales, as in cockroaches (Bell & Kramer, 1979) or elephants (Dai et al., 2007); or take alternating turns, giving the impression of orientation due to generally linear movements, such as in pipevine swallowtail butterflies (Kareiva & Shigesada, 1983). Presumably, this type of false orientation may also be true of organisms within a bounded habitat such as a reef, where to stay in relatively constant environment of habitat type, depth and temperature they must move laterally along it. However, it is possible that while foraging, organisms use cumulative experience of their environment to select optimal habitat patches (Heithaus et al., 2002; Papastamatiou et al., 2009) or features, and hence are moving in a particular direction. Animals can orientate towards prey or other targets (mates, refuge) when they are in range of their sensory systems, yet an animal shown to move towards a known goal at greater spatial scale than sensory range, would be said to move with a directed walk (Papastamatiou et al., 2011).

Animal search patterns may reflect a combination of movement processes (Bartumeus et al., 2005), reflecting foraging within, and at scales greater than the range of sensory perception (Papastamatiou et al., 2011). Thus orientation is expected of animals at small scales, when prey are within detection range of sensory mechanisms (sight, olfactory, electrosensory), whereas orientated movements at larger scales, where the location of patches (areas of heightened energetic or protection to foragers) are known through memory and experiential learning, are likely characterized by directed walks. A cognitive map of a home range or of key features within it are likely to increase foraging efficiency (Van Moorter et al., 2009), conserving energy and lessening predation risk, as commute time between patches can be faster and more direct (Papastamatiou et al., 2011). Due to the difficulties in knowing the intended destination of an animal, directed walks have been identified for very few species. Three species of wide ranging sharks were found to orientate at scale of up to 6-8 km, yet the only reef species in the study showed no large scale orientation (Papastamatiou et al., 2011). By analysing the comparison between an animal's net displacement (from a start point) and a CRW at various spatial scales, it can be determined if movement paths are orientated, and to what scale. If paths are initially positive (more diffused than a CRW) but then quickly diminish to less than that of a CRW, then orientation is likely limited to within the range of sensory capabilities. However, if

paths remain more diffused than a CRW at large spatial scales, then it is suggested the movement is orientated, and the animal is using a directed walk (Nams, 2005).

The frequency and magnitude of step lengths and turn angles of a discretized movement path defines its shape or tortuosity, whereby the shortening of step lengths and increasing turn angle magnitude and/or frequency, increases path tortuosity (Bartumeus et al., 2005). Fractal geometry is a mathematical process that analyses the repeating statistical patterns maintained over multiple scales in various natural systems (Mandelbrot, 1983; Bascompte & Vila, 1997), and has been used in various capacities such as physiological architecture (Shlesinger & West, 1991), bacterial growth patterns (Matsushita & Fujikawa, 1990), and forest patch shape (Rex & Malanson, 1990). Fractal analysis of movement paths measures the tortuosity using the fractal dimension (D) on a scale between 1 and 2, where 1 is a straight line and 2 is a path so tortuous as to cover a 2D plane (Nams, 1996). The existence of fractal characteristics in a movement path indicates that D is scale invariant thereby implying that either 1) the animal is moving through a homogenous environment in which prey abundance is equal at all scales, and a classic random walk or CRW suffices for energetic requirements, or 2) a probabilistic random process is at work such as a Lévy walk, characterized by super-diffusive movements and a power law tail (Viswanathan et al., 1996).

Lévy flight models (made up of Lévy flights and Lévy walks) attempt to describe super-diffusive animal movements, where animals foraging over uncertain territory may have the ability to optimise their search for prey by adopting step lengths from a probability distribution with a power-law tail, rather than random step lengths (from a Gaussian distribution) (Viswanathan et al., 1996; Bartumeus et al., 2005). Lévy flights are said to be scale invariant and super-diffusive because the ‘flyer’ will be displaced over much greater distance over said time (Viswanathan et al., 1999). Lévy ‘flyers’ have been seen to undertake long linear movement stages (steps), and as a result are said to describe movements of animals with no prior knowledge of their environment, or those in search of sparse or unpredictable prey patches (Humphries et al., 2010; Smouse et al., 2010). These movement patterns occur in systems of isolated patches of prey, where patches occur at a range greater than the sensory perception of the organism. Lévy flights are instantaneous jumps, whereas Lévy walks are straight-line paths taken by organisms moving at a slower pace (that cannot translocate immediately to another destination). Lévy walks have been shown to be a strategy utilised by organisms as diverse as dinoflagellates (Bartumeus et al., 2003), elephants (Dai et al., 2007) and humans (Brown et al., 2006) for optimal foraging in environments of random, unknown distribution of prey (Humphries et al., 2010).

The absence of a fractal tendency in movement paths implies that animals respond to different domains of scale, or scales of their environment at which they vary their movement mechanism

(Wiens, 1989), such as between and within high density prey patches. In ecological terms, behavioural aspects such as foraging intensity, navigational ability, and habitat selection would affect tortuosity. Scales at which tortuosity changes are known as transitions, and highlight potential size limits for prey patches utilised by a foraging animal (Wiens, 1989). By testing for variance of D at differing spatial scales, domains of animal movement in its heterogeneous environment can be identified (Nams, 1996).

The analysis of movement structure is relatively novel in the marine environment, and has increased with technological advancement of tracking equipment. Manual acoustic tracking can achieve high resolution spatial data necessary to elucidate foraging changes and patch use in marine species with relatively small home ranges, such as in blacktip reef sharks *Carcharhinus melanopterus* in a tropical reef system (Papastamatiou et al., 2009). No studies examining the movement structure or directionality of any batoid species have yet been undertaken.

The overall aim of this chapter was to investigate how stingrays respond to spatial scales in their heterogeneous marine environment, and how this links to their foraging ecology and dispersal patterns.

Hypotheses & Objectives

H₀ *Dasyatis americana* movements show no variation in structure at all spatial scales and dispersal fits a Brownian random walk model.

H_{A1} *Dasyatis americana* shows orientated movement to small scales within patch size, equivalent to the range of sensory mechanisms, with larger scale dispersal fitting models of a correlated random walk.

H_{A2} *Dasyatis americana* show response to spatial domains, at scales less than and greater than foraging patch size.

H_{A3} Size of foraging patches used by *Dasyatis americana* corresponds to spatial distribution of habitat resources at Glovers Reef.

Obj. 1. To investigate if stingrays orientate to known goals, and if so at what scale, using fractal analysis of movement paths from manually tracked stingrays in Glovers Reef lagoon.

Obj. 2. To determine if stingray search behaviour fits a correlated random walk or Lévy walk model.

Obj. 3. To analyse southern stingray movement path structure for spatial domains indicating patch use.

Obj. 4. To quantify the spatial distribution of habitat resources and topography within Glovers Reef lagoon to compare with scales of response in stingray movement path structure.

4.2 METHODS

Movement path data were obtained from manual acoustic tracking of 15 southern stingrays for periods of 3-24 hours. Additional tracks were generated from 25 stingrays^{††}, followed in-water (FW) using a handheld GPS unit for 1 hour periods. For full methods on capture, tagging and tracking, see section 3.2.

Manually tracked rays were analysed in two groups according to the method and frequency used to record locations, and in-water follows were analysed separately due to differences in overall scale of movements. GPS locations were plotted into ArcMap (ArcGIS - ESRI) and converted into XY coordinates for use in fractal analysis.

4.2.1 Stingray movements compared to a Correlated Random Walk model

To determine if stingrays were moving using CRW the net displacement of moving stingrays from their starting point was compared to that of computer-generated CRW values using the CRW_{diff} statistic described by Kareiva & Shigesada (1983) adapted by Nams (2006):

$$CRW_{diff} = \frac{1}{k} \sum_{n=1}^k \frac{\overline{R_n^2} - E(R_n^2)}{n^2 \bar{l}^2 - E(R_n^2)}$$

Where R_n^2 represents the observed mean (net distance)² for each number of consecutive moves; E is the expected mean (net distance)², l is the mean step length, and k is the turn angle concentration. Multiple computations were carried out along various step lengths within the track and a single overall estimated value for CRW_{diff} was generated. If CRW_{diff} is less than zero, this suggests that movements are more constrained than a CRW. All analyses were carried out using Fractal software Version 5 (V. Nams, Nova Scotia Agricultural College - NSAC).

^{††} Field collection of in-water data was carried out with the assistance of volunteer Marie Smedley. Numerous volunteers assisted with acoustic tracking over the fieldwork period.

4.2.2 Stingray movements compared to a Lévy Walk model

Movement paths of 12 rays were grouped into bins of 5, 10 & 20 minute intervals, and step lengths were calculated for each interval. The Lévy exponent (μ) for each time interval was calculated following Sims et al. (2007). The frequency distribution of step lengths was divided into 5 percentile bins (in the place of logarithmic bins, fig. 4.2), and the frequency of steps lengths was calculated for each bin. This frequency was divided by the bin width, then by the total number of steps, then log transformed (Log-10). These values were plotted against transformed (Log-10) bin widths, to return a straight-line fit with slope $-\mu$. Log-log plots of frequency vs. step length were used to calculate the Lévy exponent (μ) for each interval.

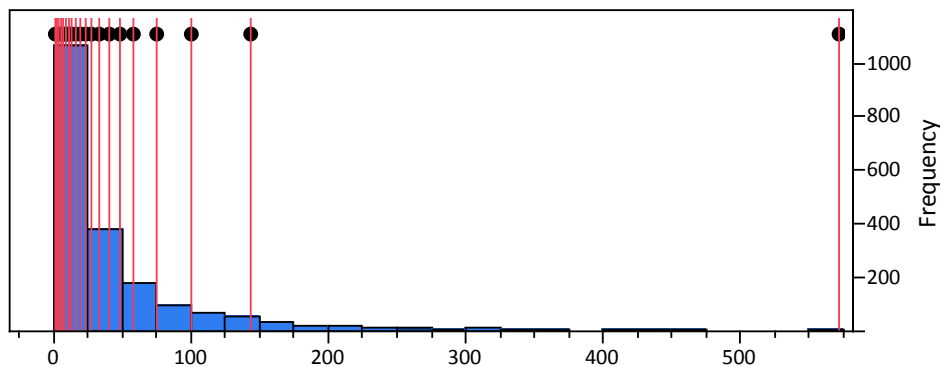


Figure 4.2: Diagram illustrating the process of dividing step lengths into bins of 5 percentiles, used in the place of logarithmic binning (adapted from Sims et al., 2007).

4.2.3 Analysis of orientation of stingray movements

CRW_{diff} was also used to analyse if rays were orientating towards certain goals, and if so, at what scale, using a scaling test of orientation (Fractal Ver. 5, Nams NSAC). This test assumes that a ray orientating to a feature would have greater displacement at large spatial scales than a CRW, because it is moving towards the goal location as directly as the environment will allow. If CRW_{diff} is less than or equal to zero at all spatial scales, then the ray is not orientating towards a particular location at any scale. Movement paths were analysed at 200 spatial scales in order to elucidate how path structure changed with scale (following Papastamatiou 2011). The minimum step size for the analysis was set at 10 m in to allow for level of inaccuracy of location data, and bootstrapping was set to a mean of 1000 replications.

4.2.4 Stingray patch use and response to spatial scale

The CRW_{diff} methodology for calculating deviation from a CRW assumes homogeneity along the movement path. It is possible however, that an animal moving with varying tortuosity in a patchy environment can disperse at the same rate as a CRW, reducing the power of the test. The CRW_{diff} test is not sufficient to determine if there is patch use, and at what scale (Nams, 2005), but the tortuosity

of a path will vary depending upon the scale at which it is viewed. Hence, *FractalD* is used as a measure of tortuosity on a scale of 1 to 2, where a decline in *FractalD* equates to a straightening of movements paths (Nams, 2005). Mean *FractalD* was calculated for individuals and analysed with stingray size and sex using JMP ver. 9 (SAS Inc.).

FractalD was measured along the length of the movement paths, sampled using dividers of different lengths to assess tortuosity at increasing scales. If the animal is using patches of varying prey abundance during foraging, then movement path structure inside and between those patches will differ. The divider method uses three tools to highlight changes in path structure: *FractalD*; correlation among cosines of successive turn angles; and variance of *FractalD*. When movement paths illustrate spatial heterogeneity the following features can emerge:

- Variance shows peaks at transitions. Peaks in variance of tortuosity can illustrate boundaries of spatially distinct patches. When *FractalD* is sampled at a smaller or larger scale than patch size, variance will be low, however when *FractalD* is sampled across the spatial boundaries of the patch, variance will be high (Fig. 4.3).

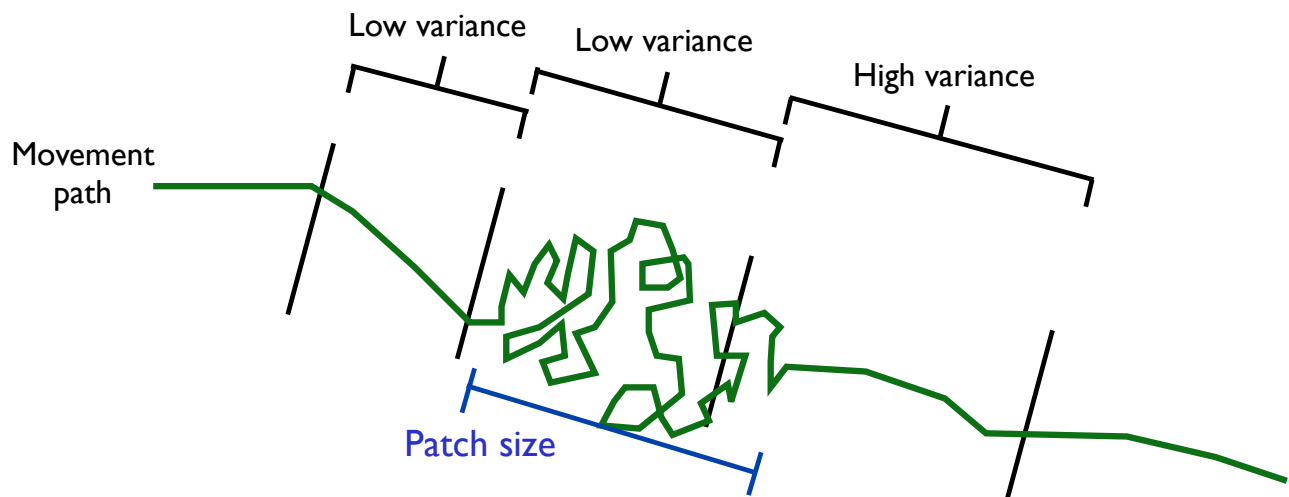


Figure 4.3: Diagram of divider method analysing variance of *FractalD* over increasing spatial scale. Contrasting scales depicting high and low variances may indicate a transition between spatial domains.

- *FractalD* shows a discontinuity at transitions between domains of scale (Nams, 2005). If *FractalD* (tortuosity) increases dramatically at a certain spatial scale followed by a decrease or levelling of *FractalD*, paths are becoming straighter which may imply the sampled scale is greater than that of the domain size.
- If moving animals are utilising patches, analysis of correlation may show significantly positive values at scales smaller than patch size (i.e. turn angles are correlated as movement paths are straight), decreasing to negative values at patch size (when turn angles reflect more random

searching) then increasing back to zero at scales larger than patch size. The correlation compares successive turn angles over a range of spatial scales. A steep rise (above zero) indicates similarity between *FractalD* at that spatial scale compared with the previous, whereas a decline from positive to negative may indicate the boundaries of patch size (Nams, 2005). No patch use would return correlation values of zero throughout, irrespective of the movement type (random walk, CRW etc.). If movement paths contain no spatial heterogeneity, *FractalD* and variance continuously increase with scale. As spatial scale increases, precision of *FractalD* estimates decrease because of decreasing sample size (Nams, 1996). All analyses of movement path structure were carried out using the VFrac tool of Fractal ver. 5 (V. Nams, NSAC).

4.2.5 Habitat Analysis

An *Ikonos* satellite image (4 m resolution) of the study area was used to analyse habitat availability and spatial characteristics of the south eastern portion of Glovers Reef lagoon using ArcGIS. Nearest neighbour distance analysis (Hawth's tools extension for ArcMap) was used to assess patch reef spacing within the cumulative activity space utilised by all acoustically tracked stingrays in both lagoon sites Middle Caye (MC) & North East (NE) (see section 3.3.1). Patch reefs >5 m were analysed for distance from their closest three neighbouring patches within the cumulative stingray activity space, and the mean distance was used to compare stingray movements with resource spatial distribution.

4.3 RESULTS

4.3.1 Stingray movements compared to a Correlated Random Walk model

Paths from 13 rays tracked acoustically were combined into two groups of 8 (MC rays) and 5 (NE rays) due to differences in location sampling frequencies. In addition 25 tracks from rays followed in-water (FW) were grouped for analysis of movement path structure at fine spatial scales. When rays were combined, all groups showed a significantly greater displacement than a CRW (Table 4.1).

Table 4.1: Mean CRW_{diff} values for grouped rays (Middle Caye = MC, North East = NE and In-water Follows = FW). Note: Degrees of freedom reflect number of tracks analysed, not number of individuals.

	CRW_{diff}	P	df
MC (8 rays)	0.0684	0.0042	68
NE (5 rays)	0.14602	0.0004	12
FW (25 rays)	0.696	0.0002	24

Individual rays showed a large degree of variation in CRW_{diff} , with ~54% of rays exhibiting significantly more dispersed movement paths than CRW (Table 4.2).

Table 4.2: CRW_{diff} values for individual southern stingrays of Middle Caye (MC) and North East (NE) groups. Size represents disk width in mm. Significant values indicated with *.

Sex	Size	CRW_{diff}	SE	95% C.I.	P	df
Female	310	0.099	0.200	-0.456, 0.654	0.646	4
Female	650	0.092	0.421	-1.076, 1.260	0.838	4
Female	770	0.355	0.067	0.069, 0.642	0.033*	2
Female	435	0.634	0.075	0.310, 0.959	0.014*	2
Male	335	0.400	0.060	0.247, 0.553	0.001*	5
Male	530	1.014	0.249	0.222, 1.806	0.027*	3
Female	290	0.276	0.129	-0.134, 0.686	0.122	3
Female	700	0.324	0.087	0.110, 0.537	0.010*	6
Female	700	1.220	0.323	0.475, 1.965	<0.005*	8
Male	520	0.152	0.027	0.066, 0.239	0.011*	3
Male	450	0.044	0.080	-0.302, 0.390	0.641	2
Female	860	0.048	0.053	-0.178, 0.275	0.457	2
Male	550	0.081	0.076	-0.891, 1.051	0.483	1

4.3.2 Stingray movements compared to a Lévy Walk model

Mean step lengths according to time intervals, standard deviation and Lévy exponent (μ) values are shown in Table 4.3. Lévy exponents were very similar for all time intervals, reflecting minimal effects of a power law tail, indicating rays are not utilising Lévy walks in foraging movements (fig. 4.4).

Table 4.3: Mean movement step lengths for southern stingrays, with standard deviation and Lévy exponent (μ) values.

Interval (mins)	Mean Step Length	SD	Levy exponent (μ)
5	53	1.8	1.301
10	92	3.6	1.297
20	174	7.6	1.295

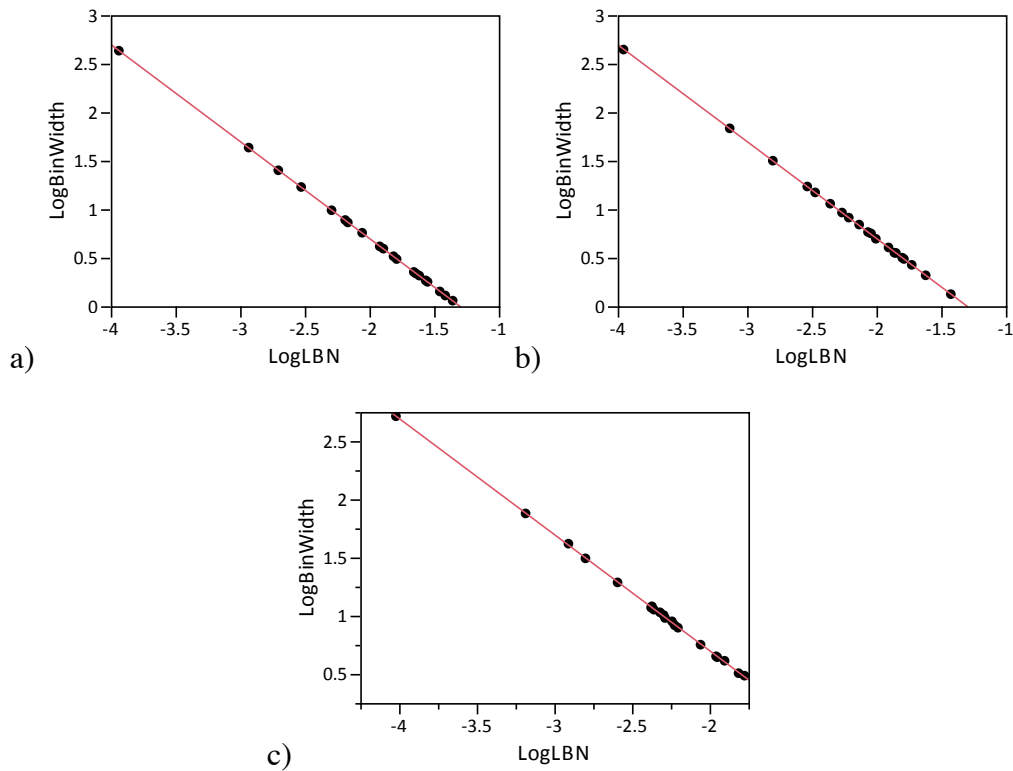


Figure 4.4: Histograms of a) 5 minute b) 10 minute and c) 20 minute interval movement step lengths for southern stingrays after dividing 5-percentile frequency by bin width and total n . The slope of the fit line represents the negative Lévy exponent: a) $\mu = 1.301$ b) $\mu = 1.297$ c) $\mu = 1.295$.

4.3.3 Analysis of orientation of stingray movements

The change in CRW_{diff} over spatial scale gives an indication of whether rays are orientating to certain goals or not. Ray movement paths analysed according to sampling groups MC and NE show significantly positive values of CRW_{diff} at spatial scales up to 100 m (Fig. 4.5b & c). At spatial scales larger than 100 m, dispersal decreases to negative values compared to a CRW, suggesting more random movements, yet confidence intervals are too wide to make inferences.

Rays followed in-water (FW) were tracked for 1 hour periods at small scales up to a maximum dispersion of 65 m allowing for greater resolution of fine scale movements. FW rays showed initially positive CRW_{diff} values up to a spatial scale of 18 m, whereupon movements were indistinguishable from a CRW. This appears congruent with a general trend seen in 58% of acoustically tracked rays analysed individually for CRW_{diff} , where initial values are positive, fall below zero, then increase again to significantly positive values at larger scales (e.g. Fig. 4.6b, e & n).

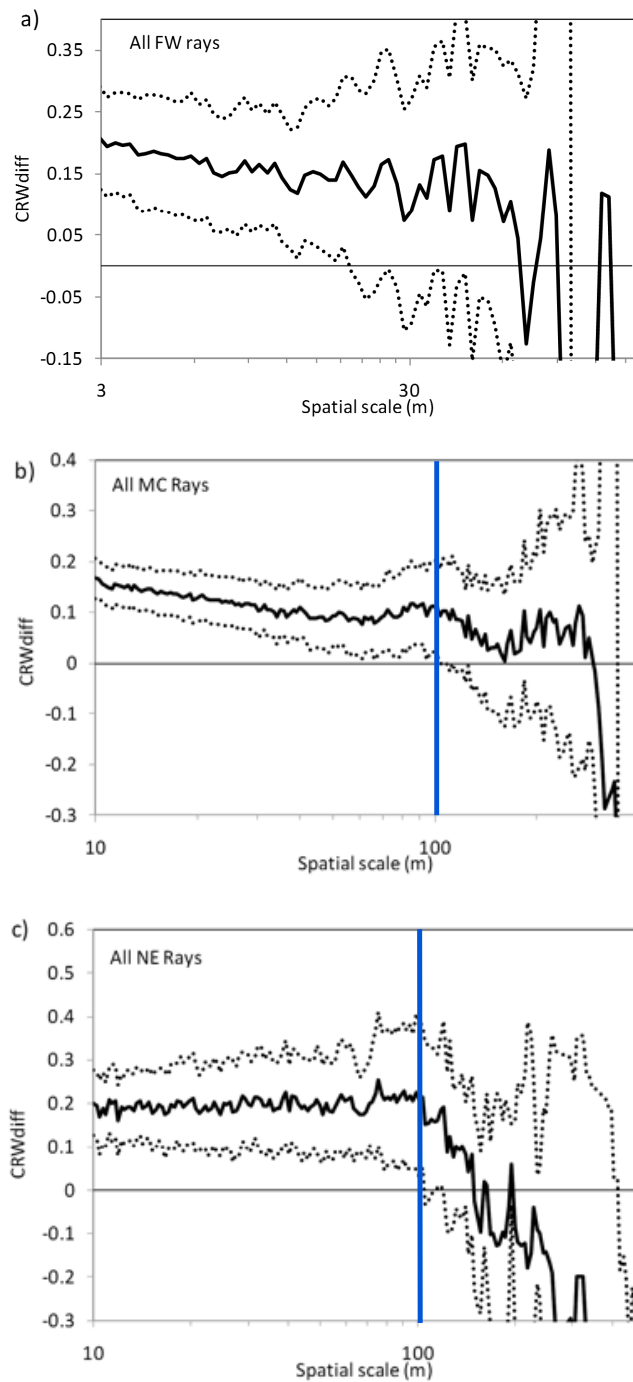
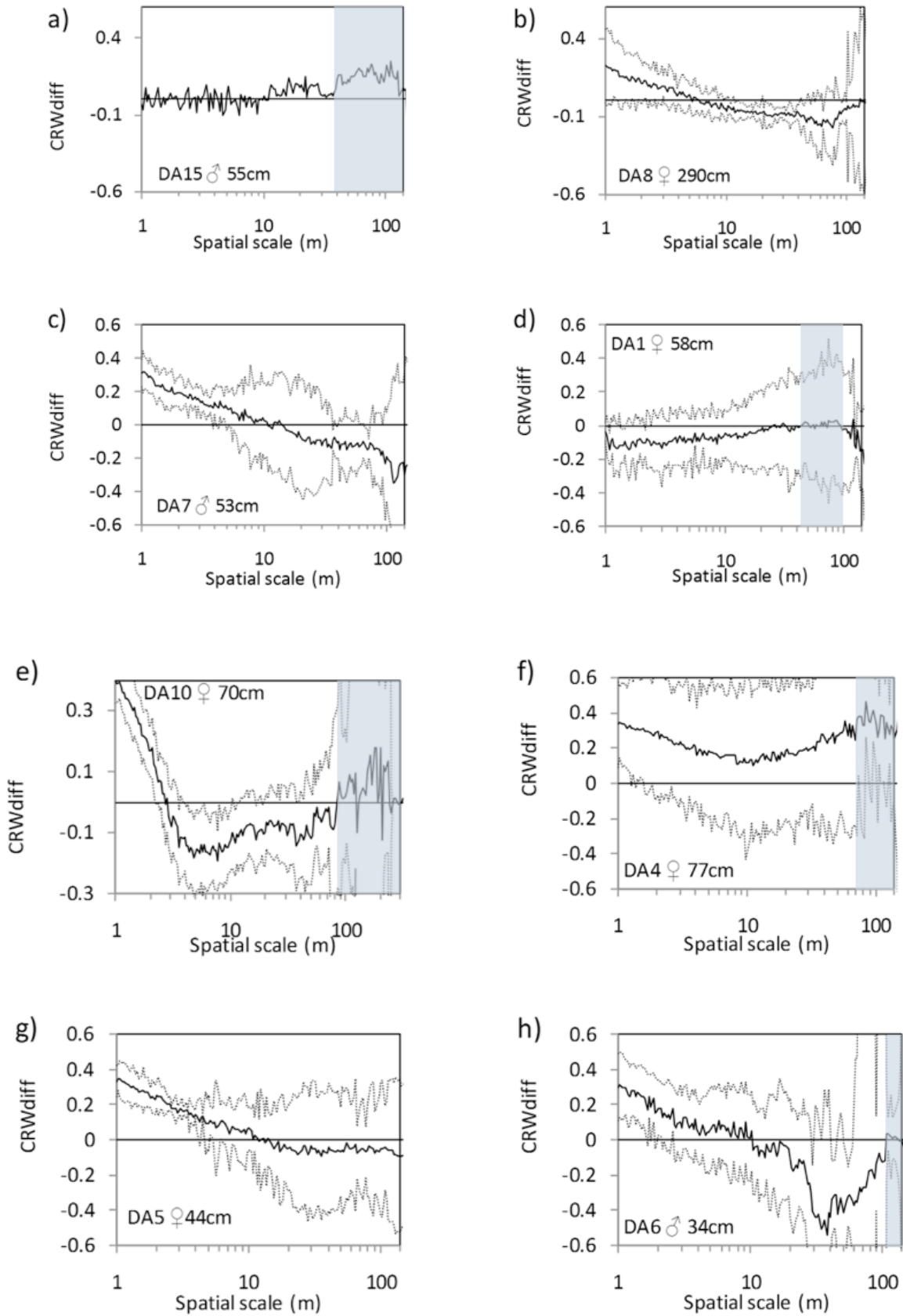


Figure 4.5: Combined analysis of orientation for three groups of southern stingrays: a) FW (N=25) b) MC (N=8) c) NE (N=4). Spatial scale is a logarithmic scale in metres. Orientation is seen to a scale of ~100 m in MC & NE groups (b & c) denoted by the blue line. Note varying spatial scale magnitude between graph a) and b) & c), the short duration of FW tracks limited distance travelled.

Individual rays showed a large degree of variation in diffusion patterns over spatial scale (Fig. 4.6). CRW_{diff} was positive at scales of up to 100 m for 71% of individual rays tracked, but wide confidence intervals at this spatial scale restrict significant conclusions. Only three rays show statistically

significant greater diffusion than CRW, all of which were relatively large females >650 mm in size (Fig. 4.6f, m & n). Two rays showed no sign of orientation at any stage (Fig. 4.6c & g).



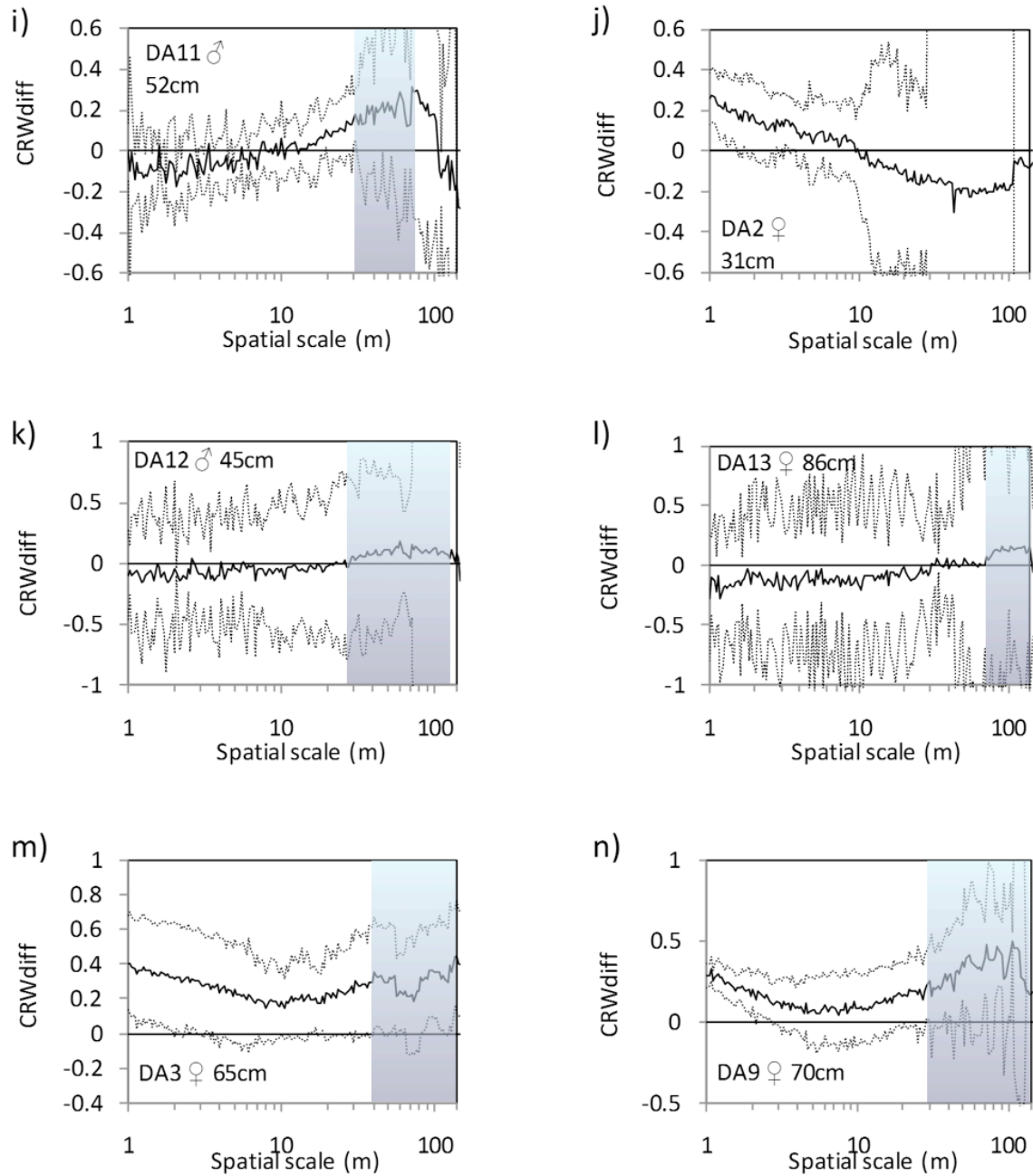


Figure 4.6: Scaling test of orientation, showing changes in CRW_{diff} with spatial scale for all 14 acoustically tracked southern stingrays from MC (a-j) and NE (k-n) rays. Dotted lines indicate 95% confidence interval.^{‡‡} Shaded boxes illustrate scales at which movements increase to greater than CRW (significant in (f), (m) & (n)).

^{‡‡} Analyses with absent confidence intervals are from animals with only one movement path, or where divider width is larger than half the total path length, resulting in only one value for that spatial scale (i.e. no error estimation).

4.3.4 Fractal mean

The mean *FractalD* value was calculated for individual rays in order to investigate possible relationships with individual size and sex (Table 4.4). All rays pooled showed a non-significant negative correlation between *FractalD* and size (Pearson's $r(13)=0.09$, $P=0.306$), yet was significant when analysed with only female rays (Pearson's $r(8)=0.54$, $P=0.025$). Male rays (Pearson's $r(4)=0.11$, $P=0.579$) showed a non-significant positive trend, however with very small sample size (fig. 4.7).

Table 4.4: *FractalD* and patch size for acoustically tracked southern stingrays at Glovers Reef (n=14).

ID	Sex	Size DW (mm)	<i>FractalD</i>	95% CI	Patch Size (m)
DA1	Female	584	1.574	1.274-1.873	52-120
DA2	Female	310	1.489	1.349-1.628	45-70
DA3	Female	650	1.256	0.959-1.553	50-90
DA4	Female	770	1.264	0.882-1.647	55-85
DA5	Female	435	1.493	1.115-1.872	65-95
DA6	Male	335	1.189	1.091-1.288	50-85
DA7	Male	530	1.438	1.177-1.700	60-80
DA8	Female	290	1.555	1.322-1.788	50-80
DA9	Female	700	1.263	1.161-1.365	67-90
DA10	Female	700	1.444	1.313-1.575	65-115
DA11	Male	520	1.228	1.178-1.278	130-200
DA12	Male	450	1.268	1.043-1.493	40-80
DA13	Female	860	1.303	1.114-1.491	65-87
DA15	Male	550	1.184	0.082-2.286	35-100

Analysis of rate of movement showed no positive correlation with *FractalD* (Pearson's $r(348)=0.0004$, $P=0.72$), nor when grouped by hourly means (Pearson's $r(23)=0.008$, $P=0.677$). However, when plotted on an hourly scale, peaks of ROM appear to align roughly with most troughs of tortuosity as might be expected (Fig. 4.8). Correlations may be skewed considerably by the zero values of ROM, brought about by long resting periods of crypsis.

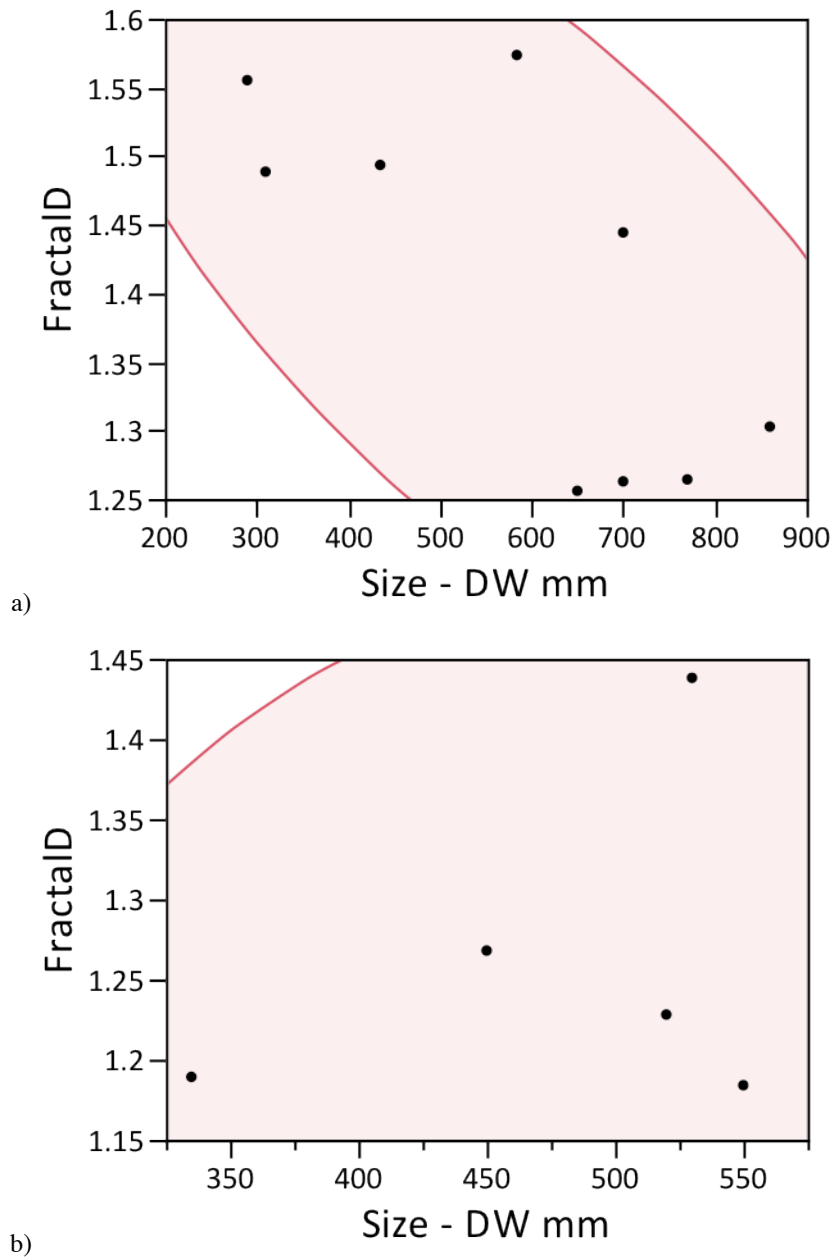


Figure 4.7: Tortuosity of movement paths (*FractalD*) versus size for individual southern stingrays tracked acoustically at Glovers Reef (n=14). Tortuosity of female movements (a) showed a significant negative correlation with size (Pearson's $r(8)=0.54$, $P=0.025$); male movement paths (b) showed no significant relationship.

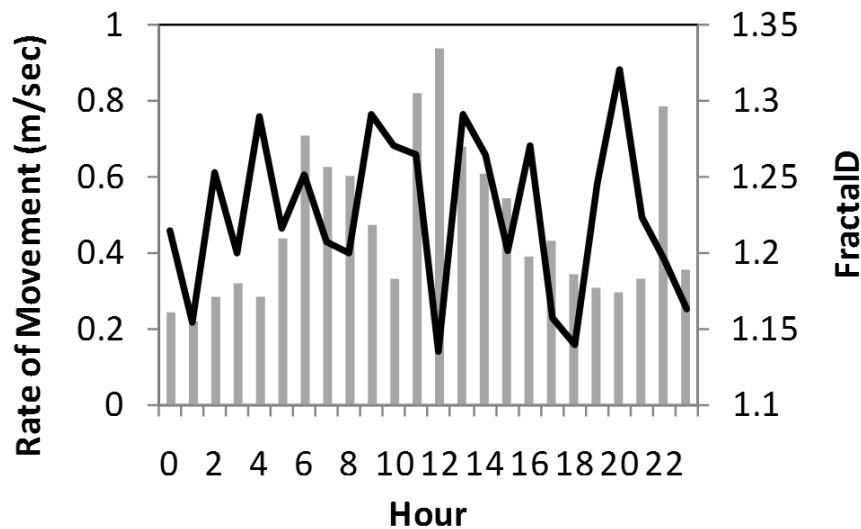


Figure 4.8: Histogram of rate of movement by hour (grey bars) overlaid with mean hourly *FractalD* for all rays combined (solid line). Note peaks in ROM align roughly with troughs of *FractalD*.

4.3.5 Scaling and Patch Use

Analysing changes in *FractalD* with spatial scale allows for identification of the spatial domains and transitions to which rays respond to their heterogeneous environment. Analysis of *FractalD*, variance of *FractalD*, and correlation among cosines of successive turn angles ('correlation' hereafter) were analysed for tracked rays (fig. 4.9) & followed rays (fig. 4.10), and individuals (fig. 4.11).

All acoustically tracked rays combined showed scale invariance in *FractalD* up to 90 m, where there is a discontinuity indicating a domain (Fig. 4.9a). Variance peaks at 117 m indicating a transition (Fig. 4.9b). Narrow confidence intervals suggest all rays move with similar patterns. Correlation of turn angles remains positive until a decline to negative values at 120 m, indicating a transition above patch size. No transitions were seen at spatial scales smaller than patch size in acoustically tracked rays.

On a smaller scale rays followed in-water (FW) exhibited relatively invariant tortuosity until 36-42 m, with a discontinuity in *FractalD* and a spike in variance at 40 m. Correlation exhibited a negative trend at 25-32 m (fig. 4.10).

Analysis of tracks from individual rays (fig. 4.11 & appendix 4.1-4.6) showed wide confidence intervals, allowing for only limited resolution. However, some rays showed changes in tortuosity and peaks in variance at a scale of ~100 m. The lower two bivariate plots represent the actual movement path in 2D space in metres.

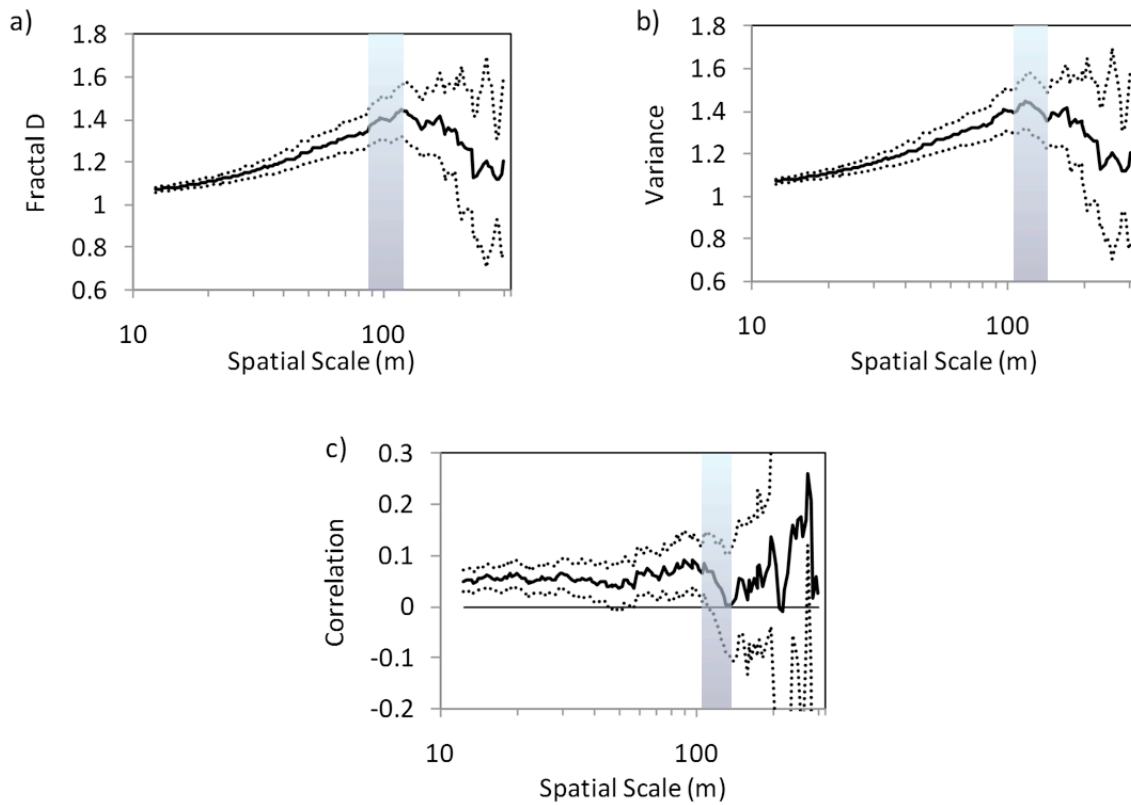


Figure 4.9. Analysis of *FractalD*, variance of *FractalD* and correlation over spatial scale for all acoustically tracked rays (MC & NE groups) combined (N=12). Dotted lines indicate 95% CI. Shaded boxes indicate scale of domains of movement structure. Peaks in *FractalD* and variance at 115 m indicate a transition between two spatial domains.

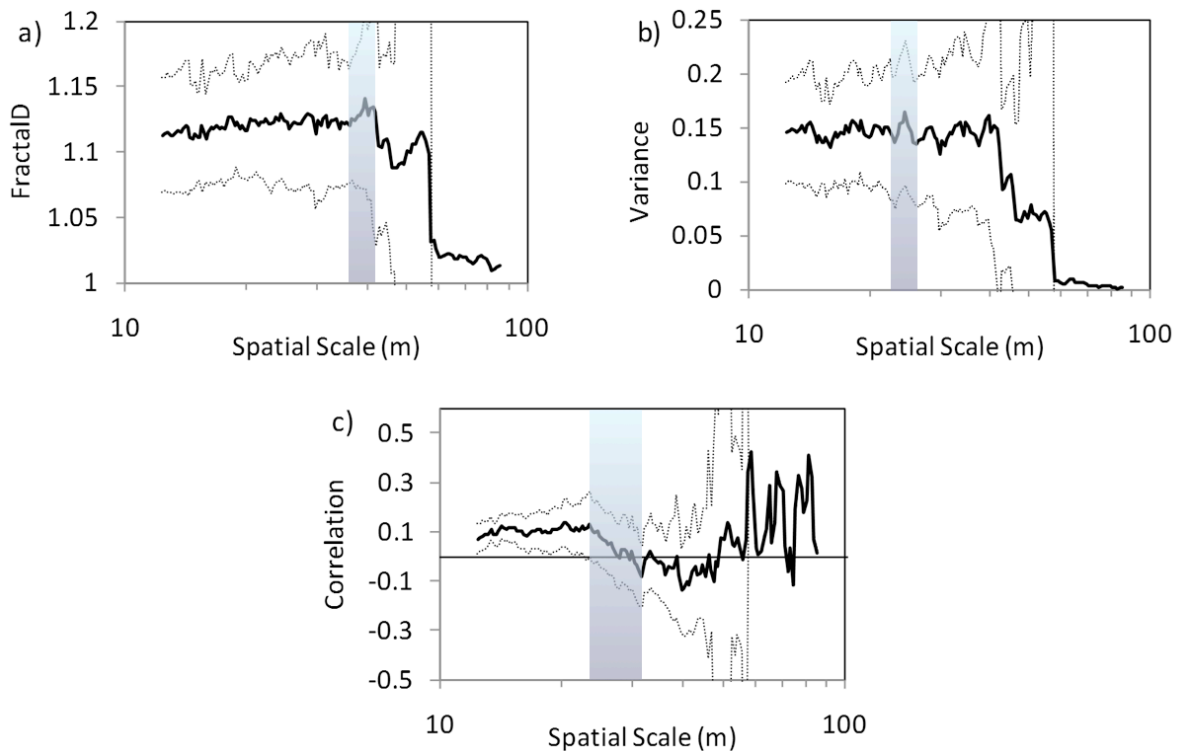


Figure 4.10: Analysis of *FractalD*, variance of *FractalD* and correlation over spatial scale for combined movement paths from southern stingrays (N=25) followed in-water for 1 hour periods at Glovers Reef between November 2009 and March 2010

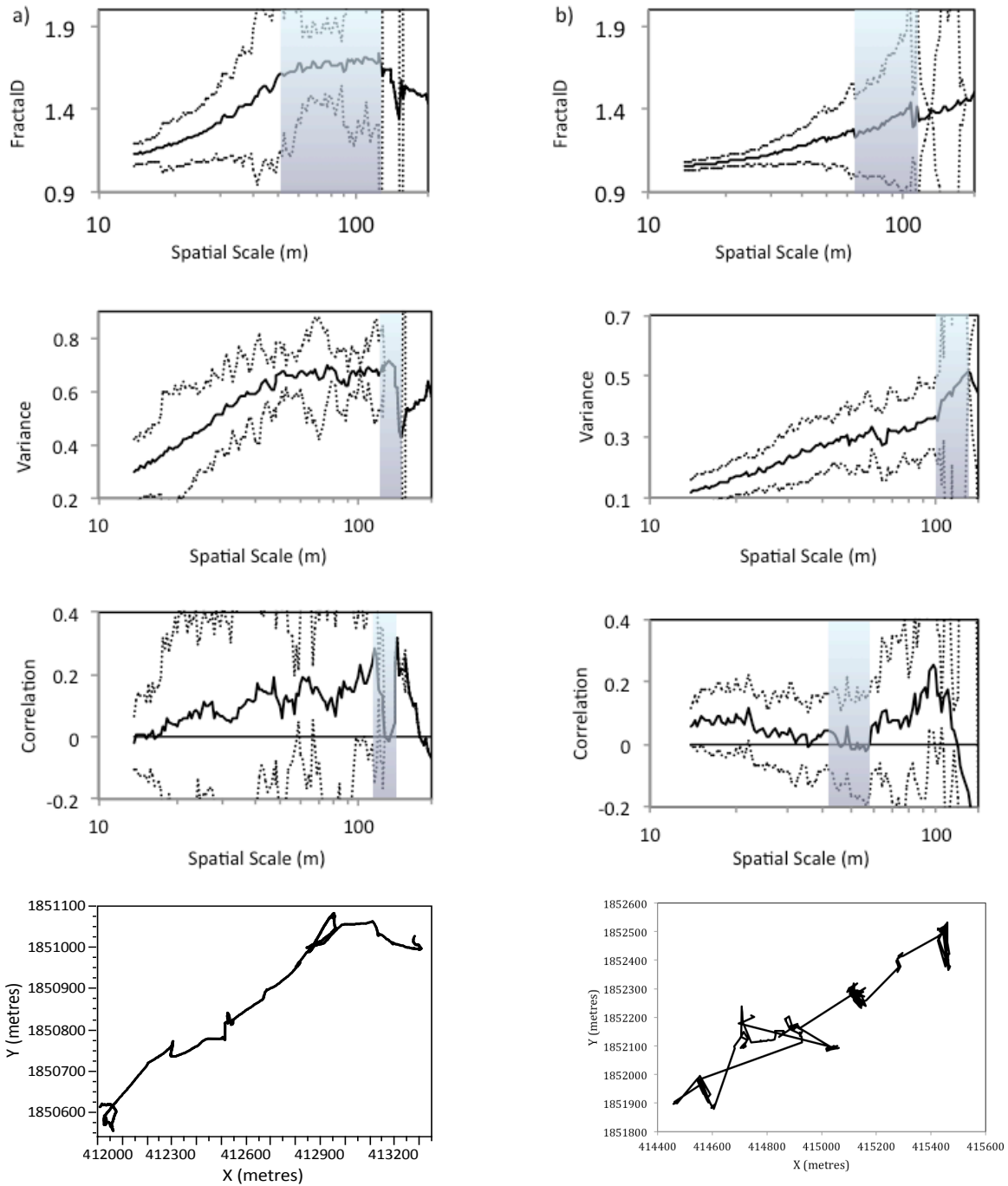


Figure 4.11: Analysis of *FractalD* (tortuosity), variance of *FractalD* and correlation of successive turn angles over spatial scale for two stingrays tracked using manual acoustic telemetry at Glovers Reef: (a) DA1 (584 mm female ray) and (b) DA4 (770 mm female ray). Two final plots represent xy graphs of individual paths.

4.3.6 Habitat Spatial Analysis

The mean distance (\pm SE) between patch reefs and the three nearest neighbours was 100 m (\pm 4.5). Nearest neighbour distance ranged from 33 – 293 m (fig. 4.12).

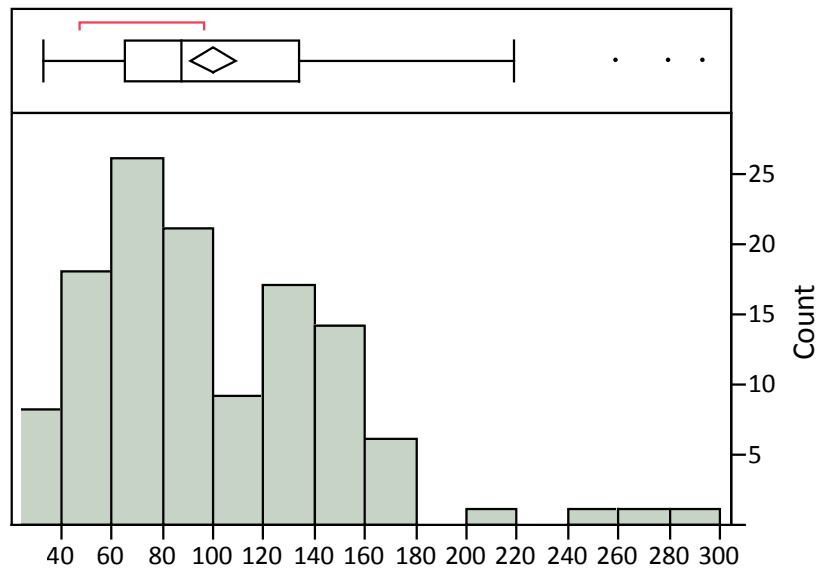


Figure 4.12: Frequency distribution of nearest three neighbour distances between patch reefs within cumulative stingray activity space during manual acoustic tracking in Glovers Reef lagoon between May 2009 and August 2010. The boxplot represents lower, median and upper quartiles, with bars depicting 95% confidence intervals and diamond reflecting the mean distance value.

4.4 DISCUSSION

Fractal analysis enables the identification of the spatial scales to which stingrays respond to their environment and hence enhance our understanding of their habitat use and foraging ecology. The patterns and structure of stingray movement paths were analysed for evidence of orientation and scales of response to a heterogeneous environment. Southern stingray movements showed significantly greater displacement than predicted from a CRW to scales of up to ~100 m. In addition, movement structure did not exhibit super diffusive tendencies as characterise a Lévy walk model (Viswanathan et al., 1996), implying rays are utilising directed walks towards known or perceived goals at this scale. Orientation at very small scales (<10 m) is likely to be in range of direct sensory mechanisms such as sight (McComb & Kajiura, 2008), olfaction, and electroreception, however location data for tracked rays at GR would not allow for resolution of data at this scale, so this is speculative. At scales greater than 100 m, ray movement was not significantly different from a CRW suggesting that movement within and between patches is directed, but selection of foraging patches is more random, as seen with mice in Benhamou (1991).

Environmental circumstances significantly affect sensory ability, yet it is understood that under appropriate conditions olfaction, hearing and vision operate at relatively large distances, whereas mechano-sensory and electro-sensory systems are of much shorter range (Montgomery & Walker, 2001) at less than 0.5 m (Haine et al., 2001). Elasmobranchs are renowned for remarkable olfactory ability, which like teleosts is sensitive to amino acids at extremely low concentrations (Meredith & Kajiura, 2010). Blacktip sharks were found to use directed walks within patch size, but move randomly between patches (Papastamatiou et al., 2009), indicating they are orientating while foraging, i.e. to prey stimuli. Olfactory cues driving orientation in rays from a distance of ~100 m is feasible if the stimulus is a continually diffusing source, such as opportune fish/conch remains, however naturally emitted odorants (Kotrschal, 2000) from common prey species decapods, bivalves, crustaceans (Randall, 1967; Gilliam & Sullivan, 1993) are unlikely to be sufficient for rays to trace from distance in a wave disturbed environment.

Stingrays are probably visually vigilant due to their prominent dorsal eye position. The Atlantic stingray *Dasyatis sabina* possesses near panoramic vision of approximately 326°, with an anterior binocular overlap of 72° (McComb & Kajiura, 2008), maximising predator detection probability while allowing visual detection of prey items in front of it. Even if visual perception extends to 100 m in rays, water clarity is likely to frequently inhibit visibility (Semeniuk, 2004) at this range, which suggests rays may orientate using memory of their home range from experiential learning, combined with inherent environmental cues such as depth and vertical habitat diversity. Without precise

knowledge of stingray visual and olfactory abilities, and range of perception, it is difficult to conclude the role of direct sensory cues as a driver of orientation.

The scale of orientation and the two spatial domains of stingray movement, correspond to spatial attributes of the lagoon habitat dotted with comparatively rugose patch reefs with a mean (\pm SE) spacing of 100 m (\pm 4.5). This may be coincidental, yet implies that relatively rugose patch reefs represent an important facet of a stingray's ecological and spatial environment, perhaps as areas of increased prey productivity related to complex habitat structures (Gratwicke & Speight, 2005); for refuge from predators by minimising potential attack angles (Semeniuk & Rothley, 2008); or other behavioural interactions such as cleaning (Snelson et al., 1990). Due to the linearity of reef environments, and in turn the elongating aspect ratio of stingray activity space (see section 3.3.1) another possible explanation is that orientation is achieved merely by utilising spatially structured depth gradients. Rays were seen to move in a very narrow depth band of 1-5 m, in the lagoon margins (see Chapter 3). Movement along this apron edge could be used for orientation throughout home ranges 3 km in length, but this would not account for a diminished orientation by rays at greater scale than 100 m as found when compared to a CRW. However, this may be a factor of home ranging behaviour itself, as it has been suggested that the boundaries of an animal's home range could elicit movement paths of lower displacement than a CRW on large scales (Börger et al., 2008).

Stingray use of patch reefs for orientation within their home range would support recent suggestions that home range formation increases foraging efficiency, and that memory systems are key in the emergence of home range behaviour (Van Moorter et al., 2009). Moreover, there is evidence to suggest that memory builds home range from heterogeneous patches (Van Moorter et al., 2009), rather than animals first selecting home ranges, then choosing the habitats to use therein, as has been previously suggested (Sallabanks, 1993). The decreased organisation of movements to a CRW at scales >300 m indicates that rays randomly select foraging patches. Home range behaviour may account for the ability to orientate at scales up to 100 m as a result of experiential learning of landmarks and key topographical features, as illustrated with homing wood mice (Jamon & Benhamou, 1989). This implies that rays may use the distribution of patch reefs as a network of refuges, connected by pathways of potential foraging areas.

Individual size of rays was seen to correlate negatively with mean path tortuosity, meaning that larger, older rays used straighter movement paths (lower *FractalD*). This again represents sensory perception vs. memory as the facilitator for orientation, as straighter movement paths by adult rays could suggest more developed sensory mechanisms (i.e. greater perception range), or merely greater experience of their environment leading to better reference memory (Van Moorter et al., 2009). As this is a mean value for entire paths, this correlation could be a factor of the ontogenic expansion of

stingray activity space in a linear environment, where large rays travel greater distances so represent a greater sample size at larger spatial scales. However, the mechanics of fractal geometry dictate that *FractalD* of paths naturally increases with the scale at which they are viewed (Nams, 2005), where longer paths (if scale invariant) will display a gradually higher value of *FractalD* (i.e. more tortuous) than shorter, scale invariant paths.

Variance and correlation of turn angles of all rays combined showed a singular transition between domains at 120 m. The lack of significant transitions less than 100 m is likely a factor of high variation in foraging patch size. The reason for such a clear transition at 120 m is potentially due to foraging patches not exceeding this size threshold, i.e. rays are orienting up to a scale of ~100 m between coral patches, and foraging in prey patches of varying size along the way.

Further work outside the scope of this study should be to identify relationships between the spatial distribution of resources and habitats (e.g. patch reefs) and home-range size (i.e. with variations in spacing of patch reefs, and in fore reef habitats exhibiting only limited sand substrate) in order to understand more regarding the dispersal of these important marine mesopredators in heterogeneous environments. Additionally greater understanding of the sensory capabilities of stingrays with regard to range of vision and olfaction would allow for greater insight into the dependence of these senses in foraging behaviour. To understand the relationship between stingray movement structure and home range, it will be necessary to conduct experimental tests with rays outside of their natural home range such as in Bovet & Benhamou (1989). Finally, greater sampling of large rays >90 cm will clarify the role of ontogeny in shaping stingray search behaviour.

Conclusions

Southern stingrays use directed or orientated walks up to a scale of 100 m, suggesting they may utilise a reference memory of topography within their home range to navigate between resources, thereby increasing foraging efficiency and lessening predation exposure whilst moving. Random movements of rays at scales greater than 100 m imply selection of foraging patches is random and that rays move in two spatial domains in their environment. This is ecologically significant, as it implies rays are not targeting specific patches more than others (as a result of higher prey density), but rather moving randomly thereby regulating disturbance levels and recolonisation times for benthic communities. Chapter 5 will further investigate the effects of southern stingrays on benthic communities by analysing interactions with prey, diet composition and trophic niche.

5. Trophic ecology of *Dasyatis americana* on Glovers Reef Atoll, Belize

Abstract

The trophic ecology of southern stingrays is not well understood in terms of the effects they have as benthic predators, and partitioning of habitat and prey partitioning with sympatric elasmobranchs is virtually unknown. This study modelled the diet composition of *Dasyatis americana* using stable isotope analysis of ^{15}N & ^{13}C in muscle and skin tissue, to study the effects of various proposed Tissue Enrichment Factors (TEF) and compare proportions of prey types to ranked stomach contents data from the literature. Trophic niche of rays was approximately twice the width of sympatric nurse and reef sharks, showing very little overlap, indicating limited competition for resources. However stingray $\delta^{15}\text{N}$ values also showed a positive relationship with individual size, indicating an increase in prey size with ontogeny and suggesting more potential for resource competition from large rays. Stingrays showed the largest variation in $\delta^{15}\text{N}$ suggesting diverse prey selection at various trophic levels in the prey community, whereas $\delta^{15}\text{N}$ values in shark species were comparatively very stable. Isotope mixing models were robust to changing TEF magnitude, suggesting bivalves and annelids are proportionally more important in the stingray diet than crustaceans and teleosts at Glovers Reef. However, considering additional gut contents information from the literature, TEF values of $\Delta^{15}\text{N} \approx 2.7\text{‰}$ and $\Delta^{13}\text{C} \approx 0.9\text{‰}$ are suggested to be most appropriate for use with stingray muscle isotope studies. Results highlight the ecological importance of rays to system stability through numerous prey interactions helping to absorb trophic perturbations, however evidence suggests that changes to stingray habitat preference brought about by a release from predation pressure would significantly change benthic community structure through diet specialisation.

5.1 INTRODUCTION

Dasyatis americana are said to be opportunistic foragers, with diet dominated by benthic and infaunal invertebrates and demersal teleosts (Bigelow & Schroeder, 1953; Gilliam & Sullivan, 1993). Diet composition is highly variable according to location, where in Florida and Bahamas primary prey species were crustaceans (Snelson & Williams, 1981; Gilliam & Sullivan, 1993), whereas in Puerto Rico and the Virgin Islands annelids made up the highest proportion of diet (Randall, 1967). It is not known what effects stingray size has on prey selection, yet rays have been shown to be highly selective (either passively or actively) of the size of prey items consumed (Stokes & Holland, 1992). This ability to select and manipulate specific prey items by their characteristic morphology has also

been seen with cownose rays feeding on bivalves, where thick shells are separated from flesh, but thin shells are consumed whole (Smith & Merriner, 1985). Rays have a number of morphological adaptations allowing for manipulation, processing and consumption of hard benthic prey, such as pavement-like dentition and strong stabilising ligaments (Summers, 2000), yet with ventral mouth & jaw morphology and inertial suction feeding comes an inherent reduction in consumable prey types (Dean et al., 2007).

In general it has been suggested that stomach contents analyses may over-represent hard-to-digest prey items, such as those with exoskeletons (Wetherbee & Cortés, 2004). Also, stomach contents represent a snapshot of diet, and may not take account of changes in consumer diet or feeding mode with ontogeny, such as carnivory to herbivory (Carseldine & Tibbetts, 2005) or planktivory to piscivory (Werner, 1984). Isotope ratio mass spectrometry (IRMS) allows for the analysis of stable isotope ratios of carbon and nitrogen (and less commonly sulphur, hydrogen and oxygen) in tissues to elucidate an organism's trophic level, and gain an insight into their diet composition (Peterson & Fry, 1987; Post, 2002; Michener & Kaufman, 2007). Isotope ratio analysis can also highlight seasonal and temporal variability in diet (Michener & Kaufman, 2007); ontogenic changes in diet (Arthur et al., 2008); and track temporal movements in migrant populations by their diet (Hobson, 1999).

Compositions of ^{13}C and ^{15}N change relatively predictably as elements cycle through ecosystems, as a result of the trophic interactions of species (Peterson & Fry, 1987). Delta (δ) represents the ratio of ^{13}C or ^{15}N relative to their lighter isotopes ^{12}C and ^{14}N in tissues. Carbon is conserved through trophic systems, and $\delta^{13}\text{C}$ values are used to determine the source of carbon from primary producers (Caut et al., 2009), such as differentiating between ocean (phytoplankton) and coastal (algae, seagrass & detritus) systems (Post, 2002). ^{15}N is enriched through the trophic system, with consumers typically having $\sim 3.2\%$ higher percentage mass of ^{15}N than the mean value of their prey species (Peterson & Fry, 1987; Caut et al., 2009).

Due to the close link between isotopic ratios of consumers and their prey, isotope analysis can provide a means of quantifying the ecological niche an animal occupies within their trophic system by calculating the area of 2D space occupied on biogenic axes of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Jackson et al., 2011). This enables the visualisation of the ecological role of the species within their community, as its niche will incorporate all trophic interactions with prey species.

Isotope mixing models can be used to estimate consumer diet composition by comparing the $\delta^{15}\text{N}$ & $\delta^{13}\text{C}$ values of the prey species with those of the consumer tissue (once adjusted with trophic enrichment factors) (Phillips & Gregg, 2001; 2003). The experimental quantification of species-specific Tissue Enrichment Factors (TEF) enable more accurate interpretation of stable isotope data, of particular importance for determining diet contributions using stable isotope mixing models, and

for estimating trophic position (Hussey et al., 2010). TEF values represent the ratio of an isotope between trophic levels (predator and prey) (Sulzman, 2007, Hussey et al., 2009a) and are represented by a capitalised delta (Δ):

$$\Delta^{15}\text{N} = \delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{prey}}$$

$$\Delta^{13}\text{C} = \delta^{13}\text{C}_{\text{consumer}} - \delta^{13}\text{C}_{\text{prey}}$$

Studies have commonly utilised standardised values of TEF calculated from values of prey groups (Caut et al., 2009, Cortés, 1999): Mean values for $\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$ of $\sim 3.2\text{‰}$ and $\sim 1.0\text{‰}$ respectively have been determined to be appropriate for general use in ecological isotopic frameworks (Hobson, 1999; Post, 2002), however, with an increase in the use of isotope discrimination to formulate isotope mixing models, it is becoming clear that there is a need for scientists to elucidate discrimination factors experimentally for specific species, as small variations may lead to marked variation in outputs (Phillips, 2001; Ben-David & Schell, 2001; MacNeil et al., 2006).

Very few studies have elucidated TEF for elasmobranchs, however recent work found that the commonly used TEF value of 3.4‰ for $\delta^{15}\text{N}$ in aquatic systems (Vander Zanden & Rasmussen, 2001) was an overestimation for sand tiger sharks (*Carcharias taurus*) and a lemon shark (*Negaprion brevirostris*), where ^{15}N TEFs were shown to be $2.29\text{‰} \pm 0.22$ (mean \pm SD) based on lipid extracted shark and prey muscle tissue (Hussey et al., 2009a). In contrast, work with spiny dogfish (*Squalus acanthias*) and coastal skates (*Leucoraja* spp.) found TEF values to be very close to estimated values for teleosts fish (Logan & Lutcavage, 2010). Recent studies of elasmobranch diet illustrate that tissue type greatly affects uptake and elimination rates of stable isotopes (MacNeil et al., 2006) and that values cannot be assumed equal across tissues (Hussey et al., 2011). Muscle tissue shows extremely long durations to equilibrium with diet, whereas blood and liver are significantly quicker having been shown to reach equilibrium with diet in less than 1 week (MacNeil et al., 2006). This has a critical relevance for studies of wild populations where isotopic values in muscle tissue may represent diet composition from some months before, possibly from a spatially or temporally distinct zone (in highly migratory species), or in studies of scavenging or omnivorous species. It is suggested that multi-tissue sampling will produce more robust analysis of trophic position for individual species, however results may be confounded if values from muscle and fin are analysed together (Hussey et al., 2011).

The aim of this study was to investigate the trophic ecology and prey interactions of *Dasyatis americana* compared to sympatric shark species, through analysis of diet composition and trophic niche using stable isotope analysis.

Hypotheses & Objectives

- H₀** Diet composition and trophic position of southern stingrays are similar to sympatric shark species, and shows no variation with ontogeny.
- H_{A1}** Trophic position of southern stingrays aligns with that of mesopredators (~3 - 4.5)
- H_{A2}** Southern stingray skin and muscle tissues will exhibit disparate stable isotopic compositions
- H_{A3}** $\delta^{15}\text{N}$ values in stingray tissues show a positive relationship with individual size.
- H_{A4}** Variation of $\delta^{15}\text{N}$ values in stingray tissues illustrates a wider trophic niche than sympatric shark species.
- H_{A5}** Diet composition reflects consumption of more soft-bodied prey items than suggested by gut contents studies.
- H_{A6}** Suggested $\Delta^{15}\text{N}$ values for rays will be closer to tissue enrichment factors found experimentally for teleost fish than predatory sharks.

Obj. 1. To calculate trophic position of *Dasyatis americana* with information from stomach contents studies in the literature

Obj. 2. To examine the feasibility of multiple tissue sampling in stingrays, by comparing $\delta^{15}\text{N}$ & $\delta^{13}\text{C}$ isotopic compositions of skin and muscle tissue from wild southern stingrays sampled at Glovers Reef.

Obj. 3. To assess the effects of ontogeny on diet by analysing stable isotope values in muscle tissue of southern stingrays over a range of individual size.

Obj. 4. To investigate dietary niche size in southern stingrays and compare with sympatric shark species using stable isotope analysis of muscle tissue from *Dasyatis americana*, *Ginglymostoma cirratum* and *Carcharhinus perezi*.

Obj. 5. To compare the isotopic distribution of prey species and stingrays adjusted with TEF values from experimental values in the literature, in order to estimate appropriate TEF values for use with demersal elasmobranch predators.

Obj. 6. To model diet composition of Glovers reef southern stingrays from values of $\delta^{15}\text{N}$ & $\delta^{13}\text{C}$ in muscle tissue and prey species using Bayesian mixing models.

5.2 METHODS

5.2.1 Stingray trophic position from diet studies

Modern techniques of estimating the trophic position of consumers utilise stable isotope composition of stomach contents. Working within a marine protected area restricted the sampling of stingray stomach contents, so diet composition was reconstructed using four literature studies where stomach contents of *Dasyatis americana* had been identified at least to phyla and ranked: Randall (1967), Snelson & Williams (1981), Gilliam & Sullivan (1993), Bowman et al. (2002).

Mean trophic levels of four prey phyla (Mollusca, Crustacea, Chordata & Annelida) were used to calculate the trophic level of *Dasyatis americana* (TL_k) following (Cortés, 1999):

$$TL_k = 1 + \left(\sum_{j=1}^6 P_j \times TL_j \right)$$

Where P_j is the proportion of each prey category (j) in diet analyses, and TL_j is their trophic level. Following analysis using stable isotope mixing models, trophic level was reassessed considering findings from this diet.

5.2.2 Field sampling of elasmobranch and prey species tissues

Southern stingrays (*Dasyatis americana*) and sharks (*Ginglymostoma cirratum*, *Carcharhinus perezi*, *Carcharhinus signatus*, *Carcharhinus falciformis*) were sampled between June 2009 and August 2010 on Glovers Reef Atoll, Belize. Rays captured for tagging purposes (section 3.2.1) were biopsied using a 5 mm diameter medical muscle punch, taking a plug (~1 g) of white muscle tissue and skin from the dorsal musculature. Individual sex and size (DW) measurements were recorded prior to release. Further rays were sampled using an adapted muscle biopsy punch attached to the end of a pole spear^{§§}. Size and sex measurements for these individuals were recorded by the researcher in-water prior to sampling (see section 3.2.1). All rays were captured using gill net or long-lines in the lagoon, except one caught by hook and line on the forereef. Sharks were captured using standard long lines as part of a wider research project into shark abundance and population at Glovers Reef, conducted by Stony Brook University. Muscle and skin plugs (~1 g) were taken from dorsal musculature below the dorsal fin. Where skin of *G. cirratum* was too tough for the biopsy punch, fin clips were taken as a substitute. Additionally one Cuban night shark and one Caribbean reef shark

^{§§} Two additional rays found dead were sampled for various tissue types. One ray died as a result of swallowing a hook during capture, and the other washed up in the study site, suspected to have been killed by fishermen.

died during longline sampling. These four fish were used opportunistically to categorise gut contents, and gather different tissue samples for isotope analysis.

Muscle tissue was separated from skin and sinew in all biopsy samples and stored frozen prior to drying in foil cups in a solar oven. Potential prey species (teleosts & invertebrates) were collected during sediment sampling (see section 2.2.2) and opportunistically during fieldwork between June 2008 and Aug 2010.

Stable Isotope Ratio Mass Spectrometry

Animal tissue samples (~1 mg) were analysed using automated continuous-flow isotope ratio mass spectrometry (Preston & Owens, 1983) by the Boston University Stable Isotope Laboratory. The samples were combusted in an elemental analyser (EuroVector) and N₂ and CO₂ gases were separated on a GC column, passed through a GVI (GV Instruments) diluter and reference gas box, and introduced into the GVI IsoPrime isotope ratio mass spectrometer; water was removed using a magnesium perchlorate water trap. Ratios of ¹³C/¹²C and ¹⁵N/¹⁴N were expressed as the relative per mil (‰) difference between the samples and international standards (Vienna PDB carbonate and N₂ in air, respectively) where:

$$\delta^{15}\text{N} = \left(\frac{{}^{15}\text{N}_{\text{sample}}}{{}^{15}\text{N}_{\text{standard}}} - 1 \right) \times 1000 \text{ (‰)}$$

and

$$\delta^{13}\text{C} = \left(\frac{{}^{13}\text{C}_{\text{sample}}}{{}^{13}\text{C}_{\text{standard}}} - 1 \right) \times 1000 \text{ (‰)}$$

In addition to carbon and nitrogen isotopes from the same sample, continuous flow also reported ‰ C and ‰ N data. Urea was not removed from the muscle tissue samples for processing. Isotope ratio mass spectrometry precision was ~0.1‰ for carbon and ~0.2‰ for nitrogen.

5.2.3 Multiple tissue analysis and ontogenic diet shifts

$\delta^{13}\text{C}$ & $\delta^{15}\text{N}$ values from *Dasyatis americana*, sharks, teleost fish and invertebrates from Glovers Reef were tested for normality, and data was analysed for differences between tissue types, species and correlation with individual size using JMP 9 (SAS Institute). Where significant differences existed between tissue types, they were treated independently in all analyses thereafter.

5.2.4 Trophic niche width

$\delta^{13}\text{C}$ & $\delta^{15}\text{N}$ values from tissues of *Dasyatis americana*, *Ginglymostoma cirratum* and *Carcharhinus perezi* were used to compare their isotopic niche sizes. The R package ‘Stable Isotope Analysis in R’ (SIAR) (Parnell et al., 2010) was used to generate Bayesian ellipses of isotopic space for the three elasmobranch species. Standard ellipse areas (SEA) were corrected (SEAc) for low sample size using

SEAc = $SEA(n-1)(n-2)^{-1}$ (Ibid.). Values were also analysed with Layman's metrics (Layman et al., 2007) using convex hulls of niche space for comparison of results with the novel Bayesian ellipses.

5.2.5 Stable Isotope Mixing Models & Tissue Enrichment Factors

$\delta^{13}\text{C}$ & $\delta^{15}\text{N}$ values from *Dasyatis americana* and benthic prey species from Glovers Reef were plotted in 2D isotopic space. Tissue enrichment factors from experimental and modelling studies in the literature (Table 5.1) were then applied to prey source values and plotted in bivariate space to analyse overlap with values from *Dasyatis americana* muscle tissue. The TEF used from Caut et al. (2009) was calculated using their equation for source values for fish. Due to small sample sizes for individual prey species, mean $\Delta^{15}\text{N}$ & $\Delta^{13}\text{C}$ values were calculated for each prey species and then combined to form one mean value for use in mixing models. Additionally a TEF was calculated for rays from $\delta^{15}\text{N}$ values using the linear regression compiled from various studies in Robbins et al. (2010): $y = 5.02 + 0.77x$.

Table 5.1: Tissue Enrichment Factors from the literature used to adjust prey species values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for use in stable isotope mixing models of stingray diet composition. All values are in per mil (‰). A $\Delta^{13}\text{C}$ value (or SD values) was not published in Robbins et al. (2010), so the median value of 0.39 from all studies was used in these plots.

Label	$\Delta^{15}\text{N}$	SD	$\Delta^{13}\text{C}$	SD	Source
a)	3.39	± 3.03	-0.22	± 2.33	(Calculated from Caut et al., 2009)
b)	3.7	± 0.4	1.7	± 0.5	(Kim et al., 2011)
c)	2.29	± 0.22	0.9	± 0.33	(Hussey et al., 2009a)
d)	3.4	± 0.98	0.39	± 1.3	(Post, 2002)
e)	3.49	± 0.13	0.05	± 0.36	(Vander Zanden & Rasmussen, 2001)
f)	2.75	± 0.22	0.9	± 0.33	(Vanderkluft & Ponsard, 2003)
g)	2.57	-	(0.39)	-	Robbins et al. (2010)

$\delta^{13}\text{C}$ & $\delta^{15}\text{N}$ values of prey species identified from stomach contents studies in the literature (Randall, 1967; Smith & Herrnkind, 1992; Stokes & Holland, 1992; Gilliam & Sullivan, 1993; Bowman et al., 2000) were plotted to assess prey groupings in 2D isotopic space, for use as prior information in enhancing the accuracy of Bayesian mixing models. Anomalous prey values with large variation from other values were removed to improve the accuracy of the model. Teleost fish and crabs showed a large degree of isotopic overlap, so were combined as a prey category for mixing models. Other prey categories used were bivalve, annelid, conch, decapod.

The diet compositions of *Dasyatis americana* and *Ginglymostoma cirratum* were modelled using SIAR, a Bayesian stable isotope mixing model that generates probability distributions for proportions of prey items based on their relationship with consumer tissue values (Parnell et al., 2010). SIAR models were generated using $\delta^{13}\text{C}$ & $\delta^{15}\text{N}$ values for stingray, shark and prey items sampled from Glovers Reef, and run using TEFs (\pm SD) for prey species found within the literature (Table 5.1).

5.3 RESULTS

Isotope samples were gathered from 14 *Dasyatis americana* of size range 290 - 770 cm (DW); 8 *Ginglymostoma cirratum* (49 - 300 cm) & 9 *Carcharhinus perezi* (90 - 200 cm) were caught in lagoon and forereef habitats. 2 *Carcharhinus signatus* and 1 *Carcharhinus falciformis* samples were gathered from captures on standard long lines in deeper forereef habitat on the southern edge of GR. One smooth dogfish (*Mustelus canis insularis*) was donated for analysis by a local fisherman. Of two rays analysed for gut contents, one was empty and the other contained 1 shrimp (2.5 cm) and one portunid crab (1 cm) and some unidentifiable material containing crustacean matter.

5.3.1 Stingray trophic position from diet studies

Crustaceans, predominantly crabs were the most proportionately significant prey group from gut contents studies in the literature (Table 5.2). Trophic level (TL) calculation for southern stingrays was considerably dependent on the TL attributed to teleost fish prey. Use of a TL value reflective of herbivorous teleost prey returned a value of 3.39, whereas inclusion of a value reflecting more carnivorous fish (e.g. \sim 3.4 following Cortes (1999)) returns a value of 3.65. Without more detailed information on prey to species level, a mean value of 2.8 from *Fishbase* was used for teleost fish (Froese & Pauly, 2000). TL was calculated to be 3.52 (\pm 0.31) for *Dasyatis americana*, corroborating the figure reported for this species in *Fishbase* (Froese & Pauly, 2000). Variation in the proportion of annelid and hemichordate prey (TL \sim 2.5) in diet as between findings of Gilliam & Sullivan (1993) and Randall (1967) had no influence on trophic level, likely due to the proportion of crustaceans at similar trophic level placement (TL \sim 2.52).

Table 5.2: Major prey identified from stomach analysis studies of *Dasyatis americana*, according to lowest taxonomic group. Numbered in order of decreasing proportion (%Volume). Unnumbered prey indicates prey proportions were not ranked. Number of rays sampled in each study is in column *n*, number in brackets denote number of empty stomachs if stated). (A detailed table to species level is compiled in Appendix 5.1).

Prey group	Location	n	Author(s)
1) Portunids 2) Teleosts 3) Caridea 4) Stomatopods	Exuma Cays Land and Sea Park, The Bahamas	18	(Gilliam & Sullivan, 1993)
Portunids Caridea Teleosts	Indian River Lagoon, Florida	3	(Snelson & Williams, 1981)
1) Sipunculids/Polychaetes 2) Teleosts 3) Brachyurans 4) Bivalves 5) Caridea	Puerto Rico & Virgin Islands	25 (2)	(Randall, 1967)
Decapods Stomatopods Portunids Caridea	Florida Bay, FL	5	(Smith & Herrnkind, 1992)
1) Portunids 2) Teleosts	Cape Hatteras, NC	2	(Bowman et al., 2000)
Stomatopods Shrimps Crabs Worms Fish	Bimini, The Bahamas	15	(Bigelow & Schroeder 1953)

5.3.2 Multiple tissue analysis and ontogenic diet shifts

Different tissue types sampled from the same elasmobranch species showed more variation in $\delta^{13}\text{C}$ than $\delta^{15}\text{N}$ (fig 5.1). $\delta^{15}\text{N}$ values were not significantly different between skin and white muscle tissue from *Dasyatis americana* (ANOVA $F=4.01$, $P=0.057$), however $\delta^{13}\text{C}$ values for skin were significantly higher than those for white muscle (Wilcoxon(1) $Z=2.70$, $P<0.007$) (fig 5.2).

$\delta^{15}\text{N}$ values in stingray skin tissue showed a positive tendency with individual size, (Spearman's $r(8)=0.62$ $p=0.099$) (fig. 5.3a) however the relationship with muscle tissue was non-significant (Spearman's $r(11)=0.36$, $p=0.28$) (fig. 5.3b).

$\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values showed no significant differences according to calendar month sampled (ANOVA $F=1.72$, $P=0.246$ and Wilcoxon(6) $Z=10.781$ $P=0.094$ respectively).

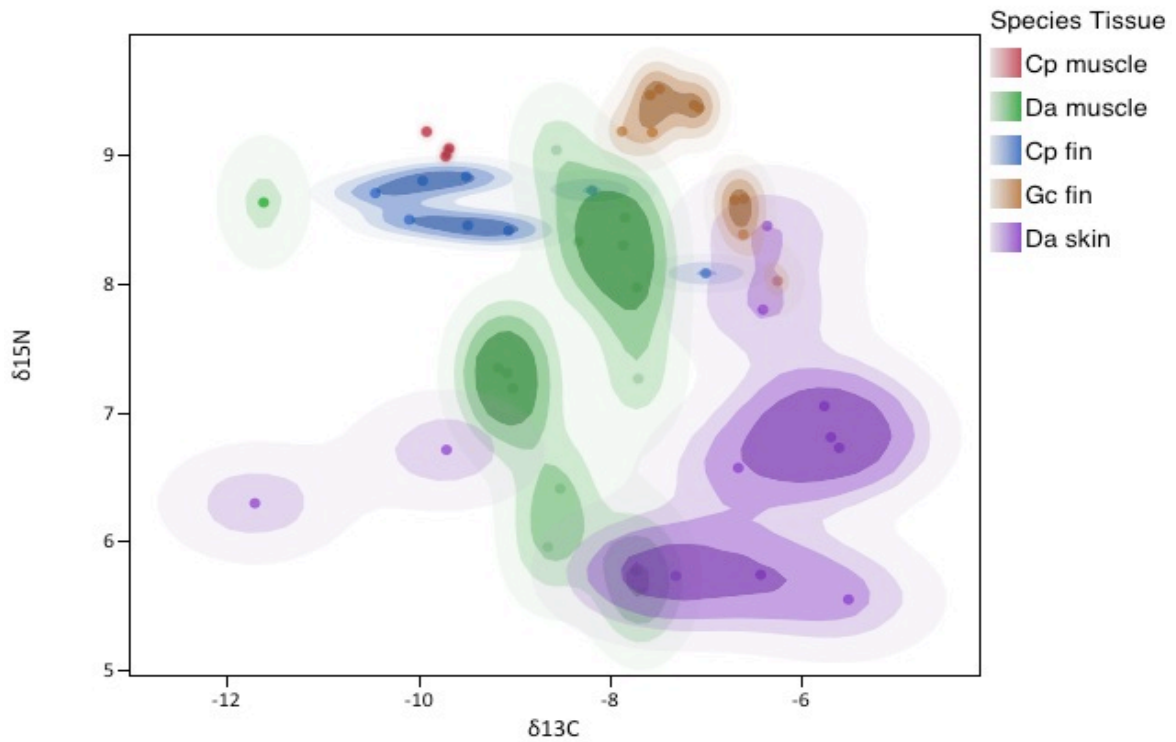


Figure 5.1: Density contour plot of $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$ values for Caribbean Reef Shark (*Cp* fin and muscle), nurse shark (*Gc* fin) and southern stingray (*Da* skin & muscle) sampled from wild populations at Glovers Reef Atoll, Belize.

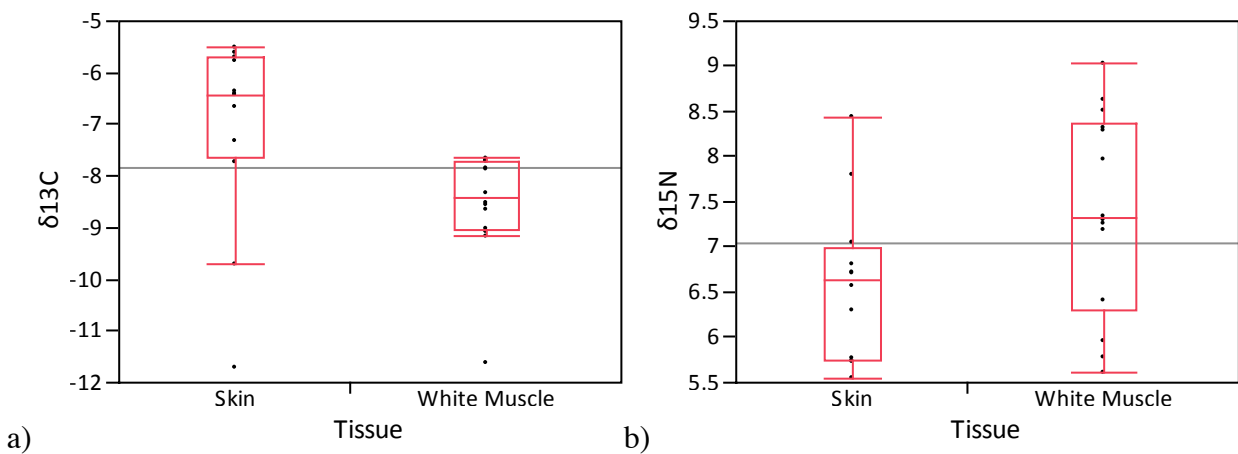


Figure 5.2: Comparison of $\delta^{13}\text{C}$ (a) and $\delta^{15}\text{N}$ (b) values from skin and white muscle biopsy samples from *Dasyatis americana* (black dots). Red boxplots represent lower, median and upper quartiles, with bars depicting 95% confidence intervals.

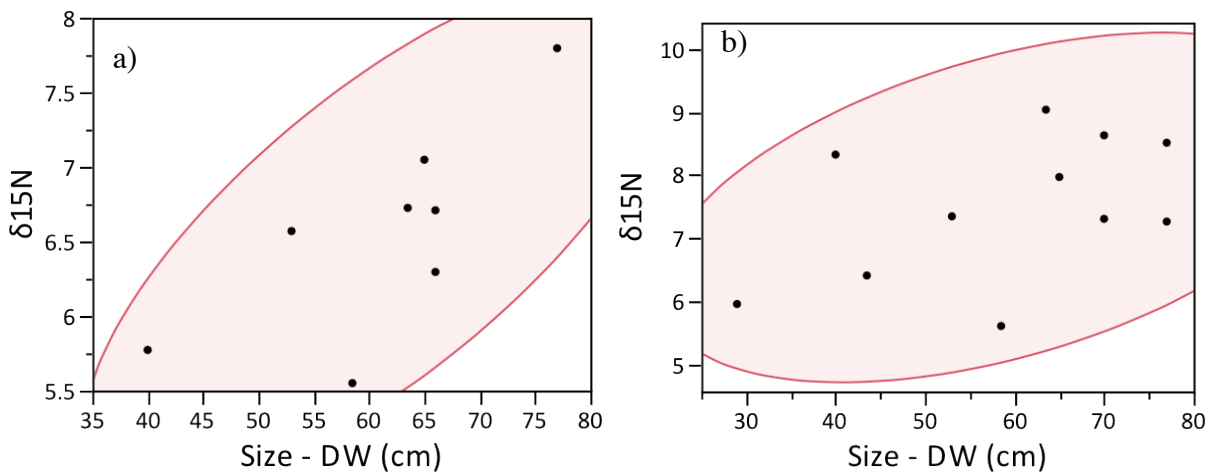


Figure 5.3: a) *Dasyatis americana* skin and (b) muscle $\delta^{15}\text{N}$ values (black dots) by individual size (disk width). Red shaded areas represent 95% bivariate normal confidence ellipses for correlative relationships
 a) Spearman's $r(8)=0.75$ $P<0.034$ b) Spearman's $r(11)=0.47$, $P=0.15$.

5.3.3 Trophic niche width

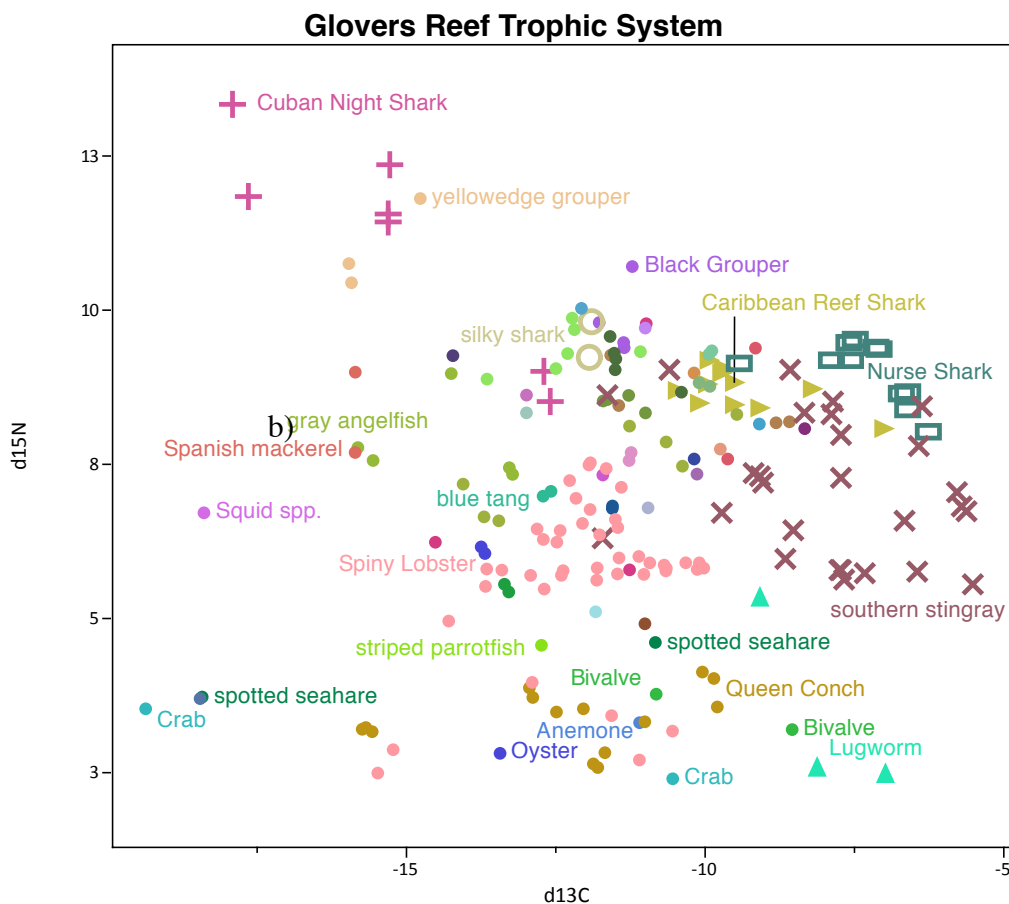


Figure 5.4: Bivariate plot of $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$ values for all marine animal tissue samples collected at Glovers Reef between 2008-2010.

Deep-water species (Cuban night shark and yellow-edged grouper) displayed the highest levels of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, with the lowest values seen in filter feeding annelids (lugworms) and bivalves, both

illustrative of their respective feeding behaviour and environment at either end of the Glovers Reef trophic spectrum (fig. 5.4).

Mean $\delta^{15}\text{N}$ ($\pm\text{SD}$) of skin tissue was $7.40\text{‰} \pm 1.05$ for *Dasyatis americana* and $9.03\text{‰} \pm 0.46$ for *Ginglymostoma cirratum* illustrating a significant difference (Wilcoxon(1) $Z = 4.66$ $P = <0.001$) (fig. 5.5a). Mean $\delta^{13}\text{C}$ values showed no significant difference (*D. americana* $-8.32\text{‰} \pm 2.62$; *G. cirratum* $\delta^{13}\text{C} -8.53\text{‰} \pm 1.12$; Wilcoxon(1) $Z = 1.57$ $P = 0.22$) (Fig. 5.5b).

Isotopic niche space of *Dasyatis americana* was larger than sympatric species using both analytical measures of area calculation. Convex hulls presented a niche space for southern stingrays of 3.15, with 1.91 & 1.51 for nurse sharks and reef sharks respectively (fig 5.6a). Corrected ellipse areas calculated southern stingray niche space as 2.18 and nurse sharks and reef sharks as 0.88 & 1.23 respectively (fig 5.6b). Stingrays exhibited a much wider range of $\delta^{15}\text{N}$ values (3.42‰) than nurse sharks (1.48‰) and reef sharks (1.09‰). The opposite was true for the range of $\delta^{13}\text{C}$ values, with nurse sharks (3.18‰) and reef sharks (3.45‰) exhibiting higher values than stingrays (1.51‰).

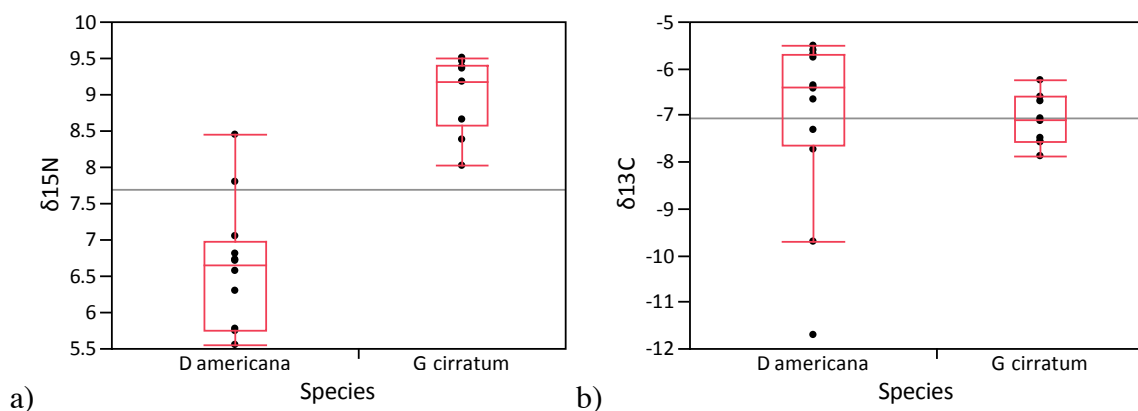


Figure 5.5: Comparison of a) $\delta^{15}\text{N}$ and b) $\delta^{13}\text{C}$ values (black dots) in skin samples of *Dasyatis americana* and fin clips of *Ginglymostoma cirratum* collected during field sampling at Glovers Reef between 2008-2010. Red boxplots represent lower, median and upper quartiles, with bars depicting 95% confidence intervals.

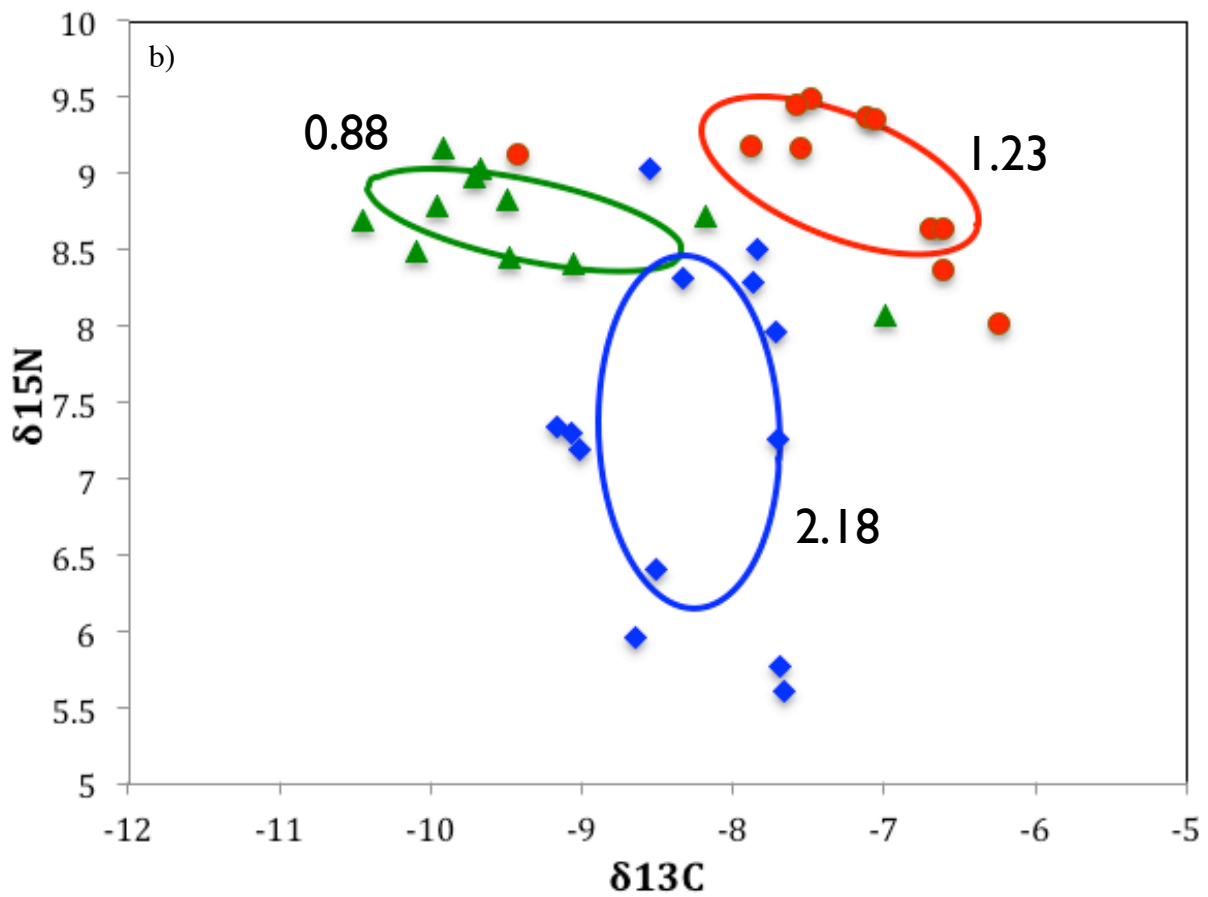
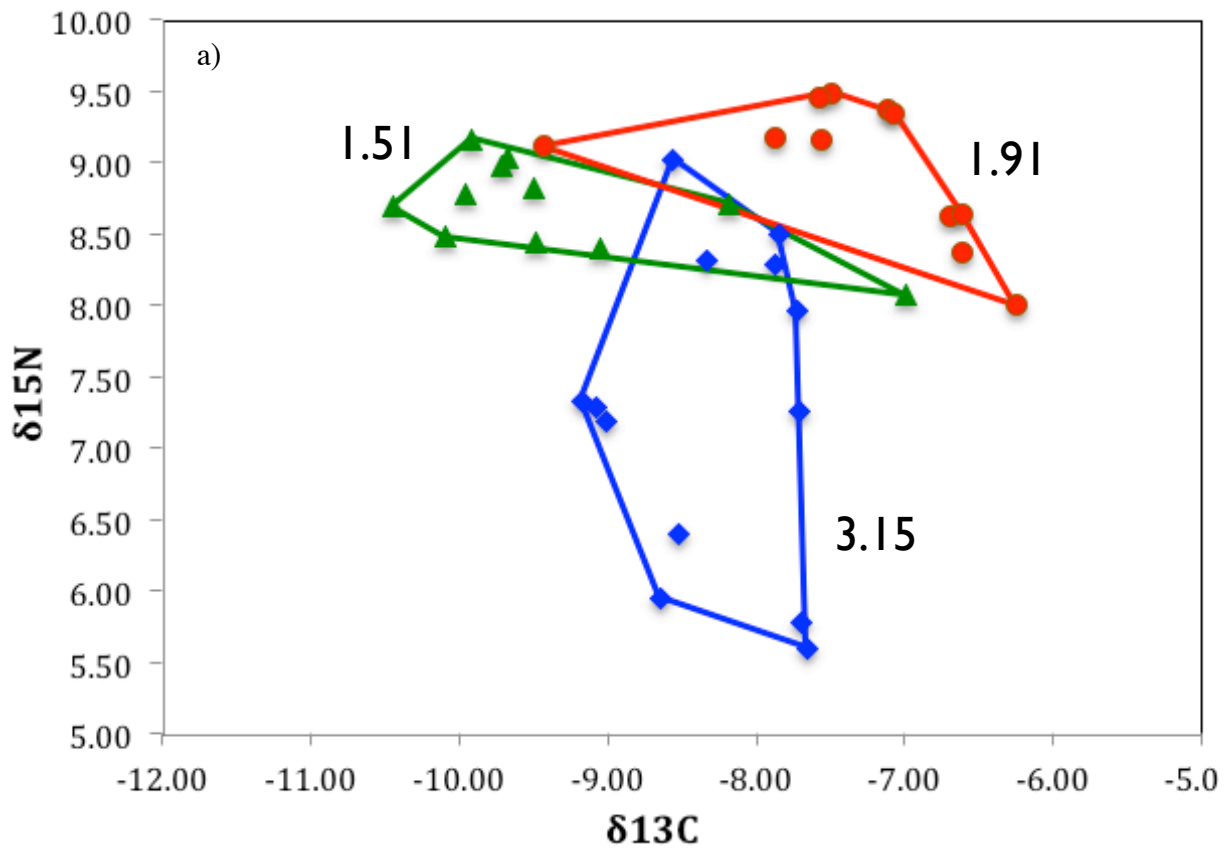
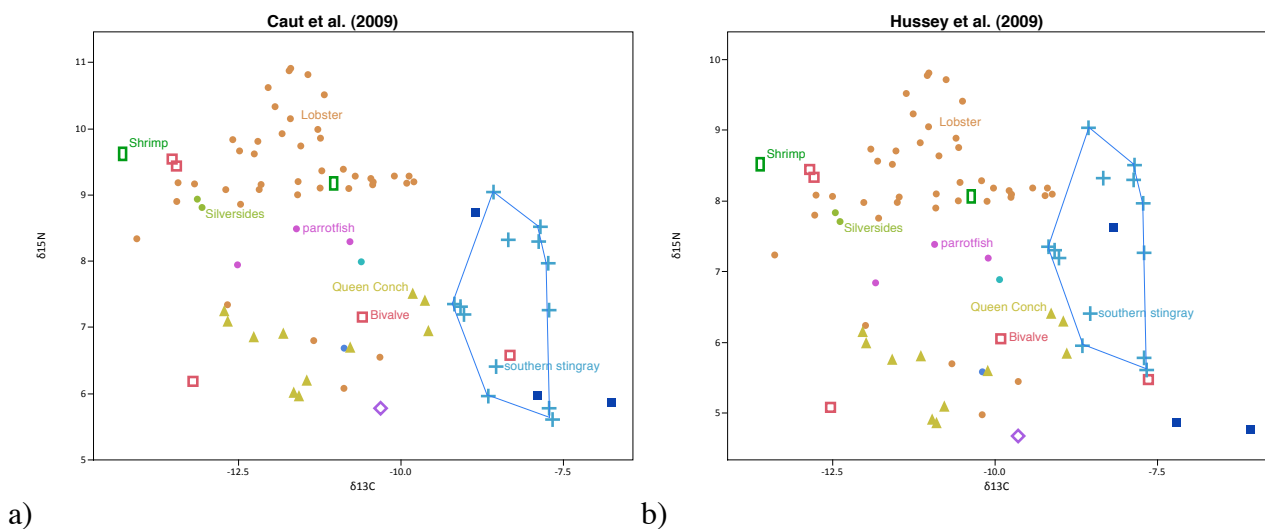


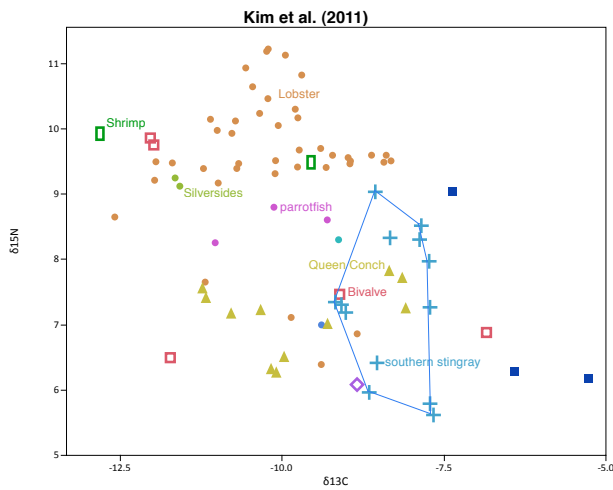
Figure 5.6: Bivariate plots of isotopic space depicting niche areas for muscle tissue of *Dasyatis americana* (blue), *Ginglymostoma cirratum* (red) and *Carcharhinus perezii* (green), using a) convex hull areas as described in Layman et al. (2007), and b) bayesian ellipses as described in Jackson et al. (2011).

5.3.4 Stable isotope mixing models and Tissue Enrichment Factors

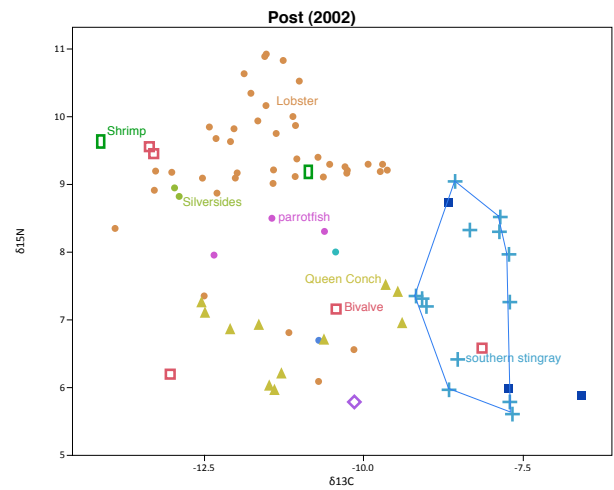
As expected, the different discrimination factors (TEFs) used in bivariate plots and Bayesian modelling with SIAR produced various results for diet composition (fig. 5.7). Shrimps and lobster showed overlapping areas in bivariate plots so were grouped as decapods for modelling. Crab isotope values were highly variable from samples taken at Glovers Reef, however the mean value was equivalent to data from another study carried out by la Moriniere et al. (2003) in Curaçao. Crab values overlapped with teleost fish into the group “Fish_crab” as prior information for mixing models.

Bivalves and lugworms were consistently a high proportion of prey for *Dasyatis americana* with all levels of TEF in mixing models. The lowest magnitude of TEF (from Hussey et al., 2009a) (fig. 5.8c) influenced the emergence of shrimp as an important dietary item, whereas the highest experimental TEF value (from Kim et al., 2011) increased the probability of higher proportions of conch (*Strombus gigas*) in stingray diet (fig. 5.8b). Combined mixing model results with all TEF values determined smaller median contributions of crab (and teleost fish) to stingray diet than suggested by stomach contents (Randall, 1967; Snelson & Williams, 1981; Smith & Herrnkind, 1992; Gilliam & Sullivan, 1993).

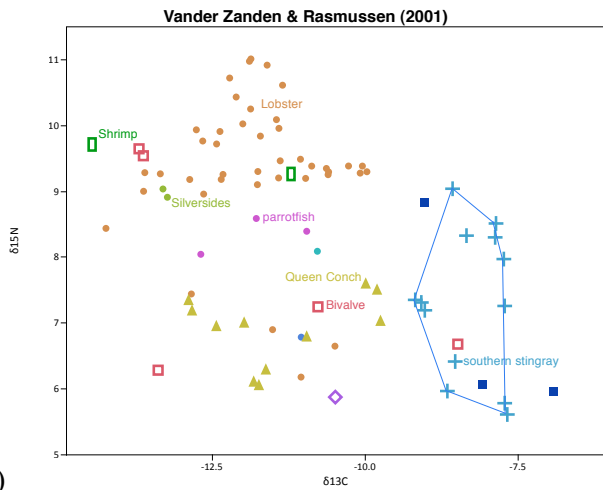




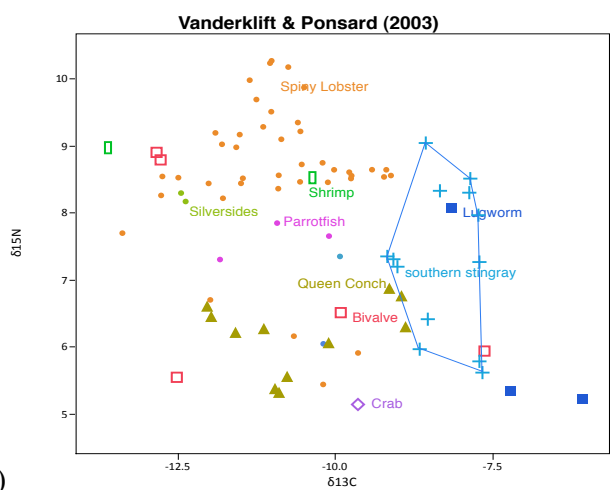
c)



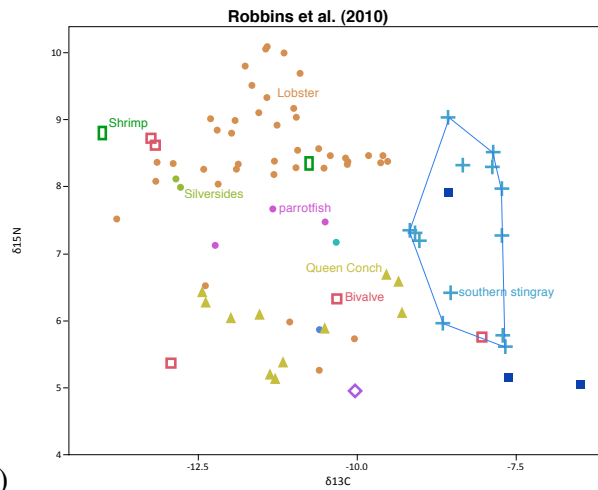
d)



e)



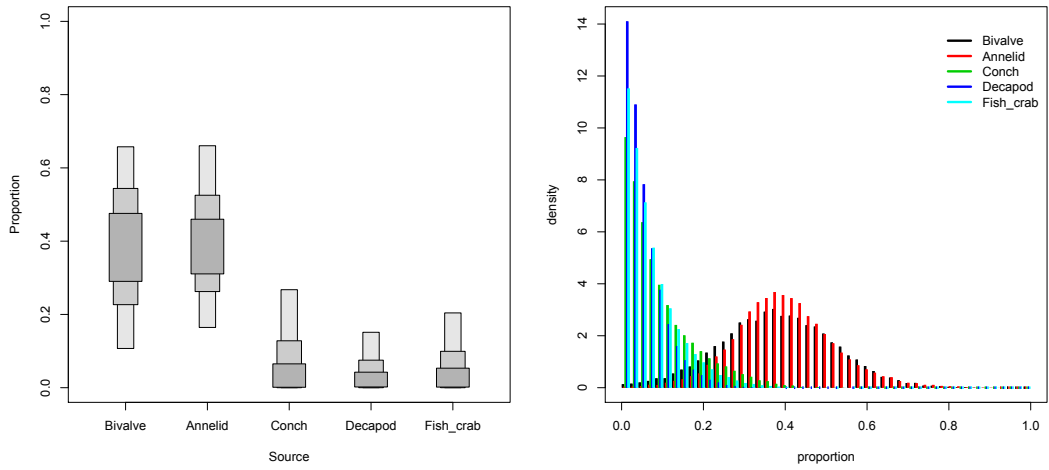
f)



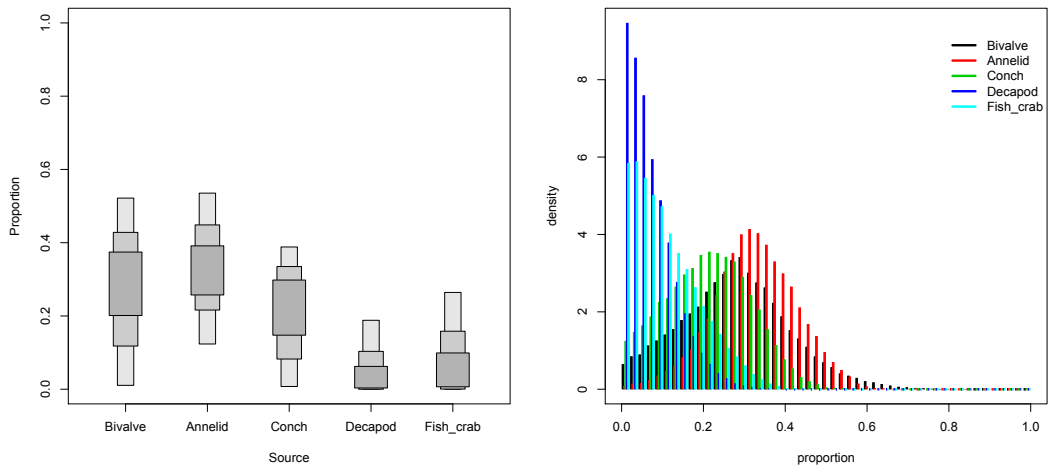
g)

Figure 5.7: Isotopic space bi-plots comparing $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$ values from southern stingray muscle tissue (blue polygon) with labelled prey items adjusted with tissue enrichment factors from the literature (detailed in plot titles). All tissues sampled from wild populations at Glovers Reef, Belize between May 2008 and August 2010.

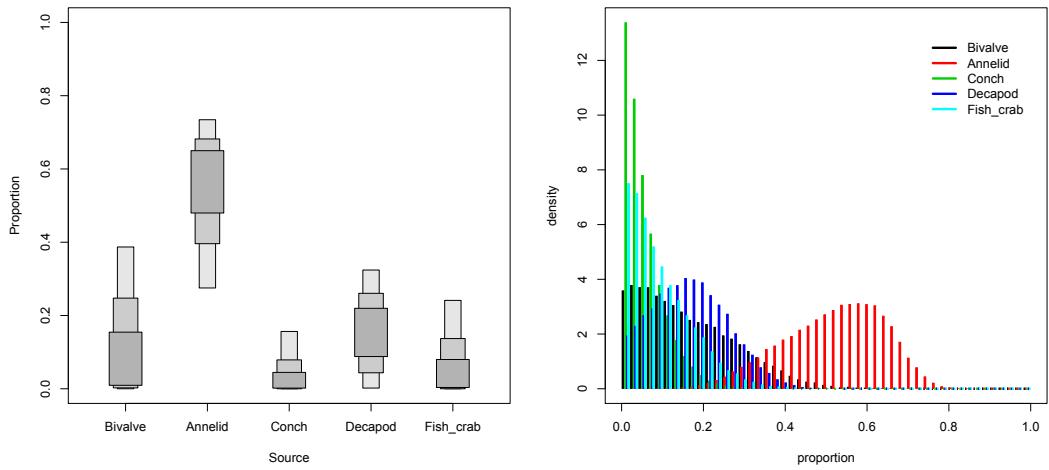
A)



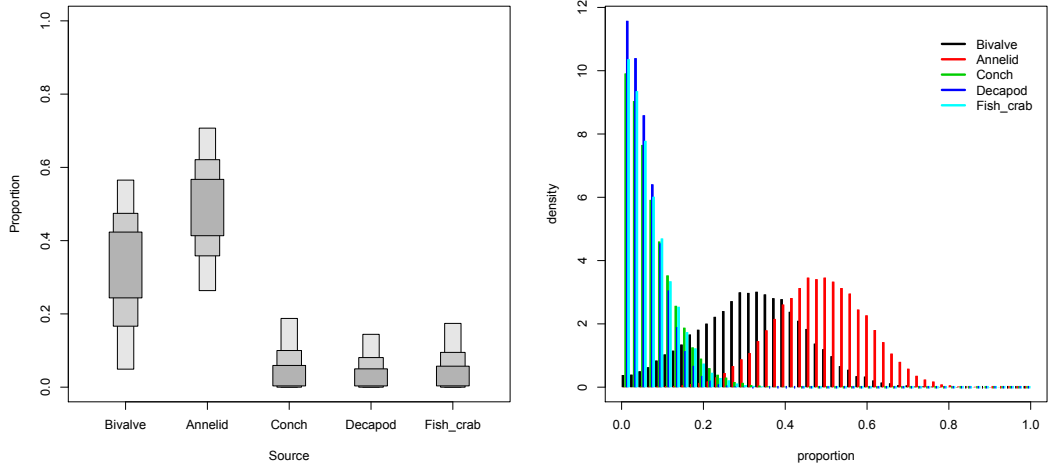
B)



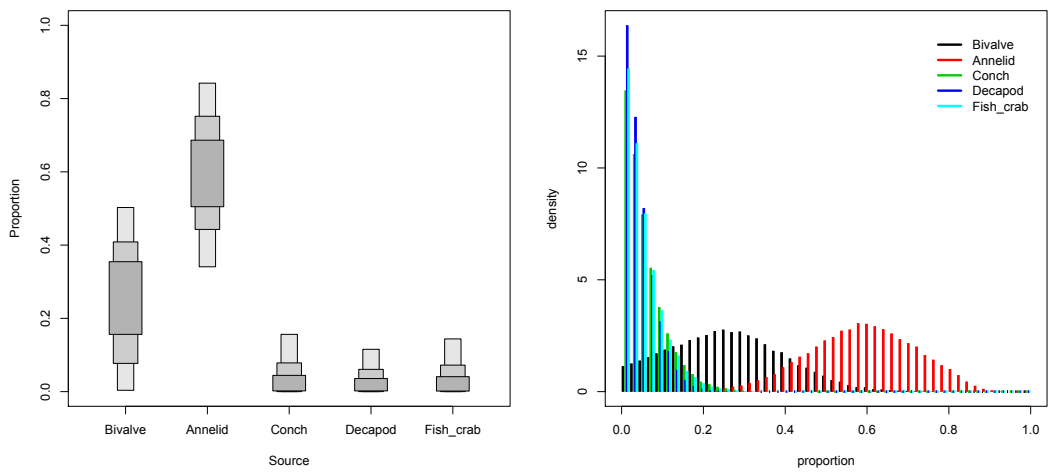
C)



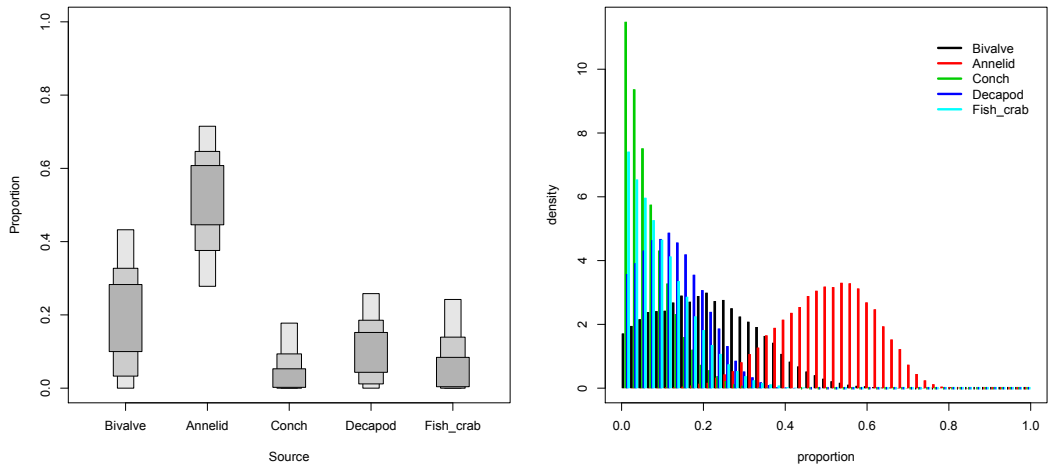
D)



E)



F)



G)

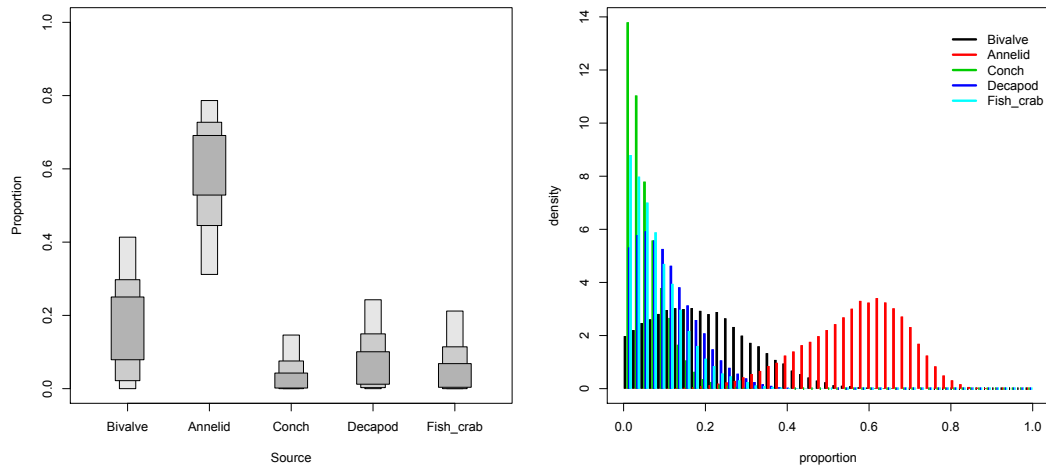


Figure 5.8: SIAR mixing models of probability distributions of prey items (including teleosts) in the diet of *Dasyatis americana* (muscle tissue), utilising TEF values from relevant literature: A) Caut et al. (2009) B) Kim et al. (2011) C) Hussey et al. (2009) D) Post (2002) E) Vander Zanden & Rasmussen (2001) F) Vanderklift & Ponsard (2003) G) Robbins et al. (2010).

5.4 DISCUSSION

Feeding modes of *Dasyatis americana* are very similar to those described in *Dasyatis chrysonata* (Ebert & Cowley, 2003) and confirmed for *Dasyatis lata* (Dale et al., 2011). *Dasyatis americana* utilise a fast, pouncing method when encountering mobile prey, yet are far more commonly observed in a ‘hovering’ search mode, sensing for buried prey in the sediment with back and forth movements, before either commencing excavation, or moving on continuing to forage (Tilley, pers. obs). This behaviour likely utilises electrosensory mechanisms for detecting buried prey, but potentially also a highly sensitive tactile mechanism described for *Dasyatis brevicaudata* sensing the weak water jets expressed by filter feeding bivalves (Montgomery & Skipworth, 1997).

Three out of the four diet studies using ranked stomach contents for *Dasyatis americana* showed diet to be predominated by crustaceans and teleosts (Smith & Herrnkind, 1992; Gilliam & Sullivan, 1993; Bowman et al., 2000)(Appendix 5.1), with one study finding annelids and hemichordates as the largest contributor to *Dasyatis americana* diet (Randall, 1967). The trophic level calculated for *Dasyatis americana* $3.52 (\pm 0.31)$ from existing information on prey items, was very similar to the published value for the species in *Fishbase* (Froese & Pauly, 2000), and lower than a general mean value estimated at 3.65 for chondrichthyan fish (Cortés, 1999). When compared with other published figures (Froese & Pauly, 2000), this mean value places *Dasyatis americana* at the lower end of the genus *Dasyatidae* in terms of trophic level. Recent findings with brown stingrays (*Dasyatis lata*) in Hawaii (Dale et al., 2011) found trophic level to vary from 3.2 to 4.5, based upon individual size, and

isotopic niche width of stingrays at Glovers Reef seems to also suggest there may be a large degree of individual variation in trophic level. This variation is often found to relate to ontogeny (Ward-Campbell & Beamish, 2005; Cummings et al., 2010), with larger predators feeding on larger prey items, or even a completely distinct prey set (Werner, 1984). Smaller gape size in juvenile rays may limit them to small prey items with lower $\delta^{15}\text{N}$ values, whereas greater foraging experience, wider gape, and better excavation skills of larger rays allow for capture of larger prey of higher $\delta^{15}\text{N}$. Dale et al. (2011) suggested limited gape may explain the higher proportion of small rays found with empty stomachs, as they are restricted to feeding on small prey items. Extensive stomach analysis was not possible at Glovers Reef, but given stingray preference for small prey items, it is unlikely to be prey size restricting juvenile feeding, but rather selection of depauperate habitat for greater safety that primarily influences feeding frequency and behaviour.

Alternatively, changes in $\delta^{15}\text{N}$ levels have also been attributed to a shift in habitat to systems with a different base nitrogen level (Michener & Kaufman, 2007; Dale et al., 2011), but this is unlikely to explain the variation at GR due to the nature of the atoll environment, where any movement to feed off the atoll would result in higher nitrogen and lower carbon baseline values characteristic of an 'offshore' system of phytoplanktonic primary producers. Levels of $\delta^{13}\text{C}$ in stingray tissues at Glovers Reef imply they are reliant upon a seagrass system, and remain within this same system for the duration of their lives, however, greater sampling of very large rays would allow for greater confirmation of this conclusion. Southern stingrays are generally only known from coastal or reef and lagoon habitats, yet one study recorded two (~85 cm) specimens caught offshore of North Carolina (Bowman et al., 2000). These two rays were shown to have a diet almost entirely comprised of decapod crustaceans (98.7%). Isotopic values were analysed from Glovers Reef rays over a range of sizes from 29-77 cm disk width, and found muscle and skin tissue values to have positive relationships with individual size, of which only skin was statistically significant. However, the concentration of size samples around the mean may imply a wide and diverse trophic niche for stingrays ~ 60 cm in size, rather than a true ontogenetic increase in $\delta^{15}\text{N}$. Findings of varied habitat use for medium sized rays in chapter 2 would support this hypothesis, where rays of this age (size) are moving between various habitats feeding on a diverse prey base to maximise energy gains for growth. Increasing the sample size of muscle tissue from Glovers Reef stingrays would allow greater resolution of ontogenic changes, including those of stingrays >80 cm disk width. Given the increasing gape size of rays with growth, and improving foraging capacity, the positive relationship seen between individual size and ^{15}N could suggest that larger rays will show even greater N values, indicating possible competition with sympatric nurse sharks. However, from the generally small size of stingray prey items we might infer that gape is not restrictive, but rather the ability to sense prey

effectively and excavate to sufficient depth were more crucial to foraging behaviour. Gilliam & Sullivan (1993) found that rays consume 65 prey types, and suggest that a mean number of 30 prey items per stomach of various prey types throughout the day denotes continuous and opportunistic feeding. This is further supported by the size relationship between rays and their prey types. Southern stingrays exhibited a much wider trophic niche than nurse sharks and Caribbean reef sharks, indicating a generalist diet characterised by a large number of strong and weak prey interactions. Reef sharks exhibited a restricted niche implying specific diet preferences likely to be characterised by a few strong interactions. Diet studies on nurse sharks suggest they prey on teleosts and larger lobster (Cortés & Gruber, 1990), and their jaw morphology & suction feeding mechanism imply predation of more teleosts than rays, due to stingray mouth and jaw structure limiting prey interactions to benthic feeding. The largest ray sampled in this study was 77 cm DW, however the largest ray recorded in gut contents studies was 136 cm (Randall, 1967), which may reflect important dietary information regarding very large rays. Randall (1967) found soft-bodied burying ‘worms’ (sipunculids, polychaetes and hemichordates) to constitute the largest proportion of diet, which may be a function of greater excavating abilities of larger rays. Further studies investigating the diet and isotopic composition of large rays (>90 cm DW) will be important in confirming ontogenic changes in diet and prey partitioning in relation to resource competition with nurse sharks.

A range of TEF values from the relevant literature were tested in isotope mixing models to compare effects on probability distributions of prey proportions in diet. All values between 2.29 – 3.7‰ produced similar results, supporting the primary importance of bivalves and annelids (& hemichordates) in stingray diet. The only known study of stingray trophic position utilised an assumed TEF of 2.7 (Dale et al., 2011), equating to that proposed by Vanderklift & Ponsard (2003). Recent studies suggest that the magnitude of TEFs relate to the nitrogen properties of the diet, where prey items containing a high percentage of quality protein, will result in smaller TEFs (Robbins et al., 2005; Caut et al., 2009; Robbins et al., 2010). Thus it seems likely that rays have a slightly higher TEF than large sharks as naturally most of their prey will have slightly lower $\delta^{15}\text{N}$ values. Some suggest that trophic enrichment values for teleosts can be accurately employed for large sharks (Logan & Lutcavage, 2010), whereas experimental feeding studies on sharks have shown highly variable TEFs for nitrogen ranging from 2.2‰ (Hussey et al., 2009a) to 3.7‰ (Kim et al., 2011). From bivariate plots and mixing models studying the effects of TEF values it appears that a TEF for nitrogen in southern stingrays would not exceed 3‰, and that carbon TEF is relatively high ~1‰. Additional information on stingray diet studies from the literature make the TEF values suggested by Vanderklift & Ponsard (2003) the most appropriate, which also present a close fit to regression lines presented in Robbins et al. (2010).

Given the prevalence of crabs and teleosts in stomach contents studies of *Dasyatidae*, one would expect them to feature as key prey in mixing models. Their absence may be explained by anomalous prey isotope values from field samples, where sampling of whole prey, rather than white muscle tissue could bring about anomalous values. However, this was tested with the use of alternative published figures for decapod crustaceans from Curaçao (la Moriniere et al., 2003) which returned the same results from SIAR mixing models, with proportions of decapod crustaceans in diet remaining low using all differing TEFs. Rather, model results supported findings of Randall (1967) who describe annelids in a higher proportion of stomachs than more recent estimates from Gilliam & Sullivan (1993) and Bowman (2000). These differences may reflect natural variation between sampling sites, in terms of habitat complexity & prey availability, or could be the response to ecological forces of predation and competition controlling the foraging intensity of rays according to habitat type.

Differences in diet have been seen to differ significantly by season in other *Dasyatidae* such as the common stingray *Dasyatis pastinaca* in the Black sea (Saglam et al., 2010), suggesting that diet variations recorded in *Dasyatis americana* could be responses to different sampling period, however no variation with sampling month was observed at Glovers Reef. Concerns have been raised that stomach analysis studies may drastically over represent hard-shelled prey items (Saglam et al., 2010). The relatively low contribution of crabs to diet in mixing models may be explained by an underrepresentation of soft-bodied, rapidly-digested prey such as annelids and bivalves in previous gut contents studies, yet may also be a response of predator size or temporal factors affecting crab abundance. Bivalves (*Tellina* spp., *Iphigenia brasiliiana*) were also prominent in model results, second to annelids. Bivalves may have played a greater importance in gut contents if rays ingest shells along with muscle tissue – suggesting rays may be manipulating prey after crushing to facilitate discarding of shell fragments. Work with Cownose rays suggests this to be the case, where ingestion of entire shells occurs only with thin-shelled prey, whereas thick shells are pried from tissue before consumption (Smith & Merriner, 1985). However, even if *Dasyatis americana* possess fine prey manipulation abilities in separating flesh from shell, it does not explain the lack of shell fragments in gut contents, as crushed bivalves are likely to be ingested along with attached adductor muscles. It is possible that the small proportion of bivalves occurring in gut contents could merely be a thin-shelled sub sample of all consumed.

The larger proportion of lugworms and bivalves in mixing models of isotope data may suggest these are a consistent prey item, consumed regularly and in quantity, whereas crustaceans and teleosts are more opportunistic prey (perhaps of less energetic effort when they are found). Buried prey can be sought out and previous work suggests the ventral surface around the mouth of stingrays is highly

sensitised to weak water jets emitted from sessile infauna (Montgomery & Skipworth, 1997), and possibly even adapted. This may imply important ecological information if rays are released from predation as specialisation may occur with more mobile prey such as crustaceans, rather than worms for which greater energetic investment is required to excavate them. Stomach contents studies illustrate many crabs are taken at one time (Gilliam & Sullivan, 1993), indicating intense foraging when opportunistically encountered.

The diversity of prey items consumed by stingrays as opportunistic generalists may complicate and confound the use of stable isotope analysis in studying diet, as location and sampling season may cause significant variations. Significant differences in isotopic characteristics of skin and muscle tissues from stingrays highlight the need for sampling consistency, yet may also represent important ecological information as skin exhibit significantly higher levels of $\delta^{13}\text{C}$, and would be expected to have a faster isotopic turnover rate than muscle (Hussey et al., 2011). Further analysis using different tissue types from rays may allow for comparison of long and short-term dietary preferences. A high degree of variation in the importance of stingray prey items may be explained by natural geographic variation in prey population structures throughout the Caribbean, where rays are feeding based on availability of prey items, and hence diet composition is a direct indication of prey abundance and benthic community. Evidence of *Dasyatis americana* feeding exclusively on lancelets in Florida (Stokes & Holland, 1992) would support this theory, as well as the idea that rays can readily select and manipulate prey items based on size characteristics, expelling small prey as energetically unprofitable. Cownose rays have been seen to favour bivalve prey to such an extent as to contradict theoretical ideas of density dependent foraging in scallop beds of the Chesapeake bay (Peterson et al., 2001). Alternatively, ray diet may fluctuate with temporal trends and population dynamics of prey species as seen with *Dasyatis pastinaca* in the Mediterranean (Saglam et al., 2010). Rays cue to temporal trends on a daily basis and are likely to alter feeding behaviour relative to prey abundance over longer timescales relative to season and associated environmental factors. The differences in diet composition may be a function of location or predation risk, or both, or may be highly sensitive to individual size samples. Thus, rays may perceive greater predation risk at GR and so are driven to prey on more buried prey in depauperate sand flats, whereas in other locations predation risk may be reduced allowing for stingrays to prey on more mobile species in more complex substrate habitat. This has implications when considering impacts of shark fishing and resulting population declines, as results from this study suggest that a release of stingrays from predation risk would significantly alter their habitat use, causing potentially much greater pressure on mobile prey species and seagrass habitats with diet specialisation.

Greater sampling of rays smaller than 40 cm and larger than 80 cm will allow for more robust analysis of ontogenic changes in diet, to clarify if rays of all sizes exhibit a wide trophic niche. Additional limitations to the study were the sample sizes of prey groups, and their isotopic sampling as whole prey rather than specific tissue types, as this may confound results from mixing models of diet composition when compared to specific tissue types of consumers. The ideal situation is combined stomach contents and stable isotope analysis to study diet composition, so perhaps further sampling of GR stingrays with non-lethal methods would support isotopic findings. Further experimental work investigating prey preference in rays will be important in refining our understanding of the effects of stingray population changes and the knock on effects to benthic communities. Finally a comparison of stingray tissues from different locations would prove an interesting study in analysing the effects of location and predation risk on stingray habitat use and diet.

Conclusions

Southern stingrays at Glovers Reef exhibit an ontogenic increase in trophic level and a much wider trophic niche than sympatric nurse and reef sharks. Nurse sharks and reef sharks feed at a trophic level equal to that of the largest rays sampled, indicating that competition may occur with the largest adults as their diet choice shifts to more proteinaceous prey. The range of trophic levels at which stingrays feed reflects the diet of an opportunistic generalist with many prey interactions, defining them as a key mesopredator and highlighting their key role in stabilising the system to trophic perturbations.

Diet composition from stable isotopes suggested soft-bodied prey items may be underestimated in stomach content studies, with rays preying heavily on annelid worms in addition to the crustaceans and teleosts found in stomach contents. This prey choice may be as a result of predation risk driving the use of relatively depauperate habitats as seen in previous chapters.

6. General Discussion

Understanding of the ecological role of predatory sharks is predominantly limited by the studies conducted on their prey species (Heithaus et al., 2010). Basic ecological information on prey species is crucial in optimising simulations of system trophic cascades, to model the effects of changing populations of predators (Christensen & Walters, 2004), and will only provide accurate results given sufficient information on the ecological mechanisms involved between predators and their prey communities. Studies of pristine systems can generate crucial information for use as an ecological baseline from which to assess impacted systems or gauge conservation and restorative measures. Research at a remote and protected site as Glovers Reef Marine Reserve (GR) allowed for ecological findings to be isolated from fishing pressure and anthropogenic impacts related to coastal development, increasing its implementation in applied ecology. Due to very little work having been carried out on *Dasyatid* rays in general, there are many gaps in understanding which this study sought to fill. Information on the density and size of stingray populations, and their distribution across macrohabitats at GR (Chapter 2) provide insight into carrying capacity of shallow reef systems. The diel movement patterns and home ranging behaviour of southern stingrays (Chapter 3) increase understanding of the effects of predation risk on habitat selection and dispersal patterns. The mechanisms of stingray foraging and search behaviour (Chapter 4) provide insight into population dispersal and redistribution, and analysis of diet and prey preference (Chapter 5) allow for greater accuracy in modelling the effects of fluctuations in stingray populations on benthic communities. This final chapter explores how these new findings expand current understanding of the ecology of demersal predators; help to assess current conservation status of southern stingrays; and inform ecological modelling and broader elasmobranch conservation challenges.

STINGRAYS AS PREY

Tropical marine systems generally exhibit high trophic complexity, and the existence of strong and weak predator-prey interactions have been shown to strengthen stability and resilience of systems to trophic disturbances (Connell, 1978; Sheader, 1993; O'Gorman et al., 2010b). Further, increasing diversity of predators has been shown to increase secondary production (O'Gorman et al., 2008). The Caribbean Sea has far lower biodiversity compared to tropical areas of the Pacific Ocean (Bellwood et al., 2004), and as such lacks breadth in trophic interactions at the top level. Elasmobranch species diversity at Glovers Reef was found to be very low in shallow reef and lagoon habitats (Chapter 2), where only 3 batoid species and 2 shark species were recorded.

The interaction strength between predators and prey can be considered as a measure of influence of one another, and also informs on the impact it has on system stability. Omnivory, or the utilisation of the same basal resource by a predator and their prey, is said to stabilise systems to trophic cascades (Bascompte et al., 2005; Heithaus et al., 2010). In systems where large sharks are preying on large bodied prey (turtles, dolphins, dugongs, stingrays) there is far more likelihood of cascading impacts from loss of sharks, as no other predator group (other than humans and perhaps killer whales) will fill this trophic niche, releasing large bodied prey from predation and causing increased impacts to their own prey communities and primary producer habitats (Heithaus et al., 2008). Large teleost predators such as scombrids have been shown to feed at similar trophic levels to sharks, and hence may stabilise systems in their absence by filling the trophic niche (Heithaus et al., 2010). Scombrids are relatively common in deep water off of the insular forereef slope at Glovers Reef, however these are unlikely to venture into shallow lagoon waters to prey on juvenile stingrays, or to target larger stingrays due to size restraints.

In a study in the Caribbean it was found that sharks were involved in 48% of strong tri-trophic interactions, where interactions are spread across three trophic levels (Bascompte et al., 2005). Many of these shark interactions are buffered by omnivory, where sharks feed on the same basal resource as their prey species, however this is not the case for sharks and stingrays as their trophic niches are substantially different, relying on dissimilar morphological feeding mechanisms. Predatory shark species known to prey on stingrays, do not also prey on benthic invertebrates in general, and the teleost prey of stingrays would be of too small a size class for large predatory sharks. This implies that ray populations may increase and exhibit less habitat restraint in response to a release from predation. Recent studies from time-series analyses of fisheries data have found correlations between declines in shark catch rate and increasing batoid catch rate (Myers et al., 2007), fuelling debate over mesopredator release. However, finding causation relies on a broad understanding of the ecological mechanisms linking sharks and batoids, and much of the contention surrounding these analyses is based on a misalignment of basic ecology and natural history of the species involved, such as rates of reproduction, distribution and feeding ecology (Heithaus et al., 2010).

At Glovers Reef, *Dasyatis americana* is the dominant species with a high density of ~162 rays per km², equating to an atoll population of >5000 rays. No other studies detailing the density of stingray populations are known, hence this is important information on the carrying capacity of these systems, and may be used as baseline by which to compare other systems. It is not presumed that this estimate for Glovers Reef represents a pristine baseline, as despite being a marine reserve, parts are still heavily fished for finfish, lobster and conch, however this does allow for comparisons with impacted areas or studies quantifying predator abundance. Such high density at Glovers Reef implies direct

predation events are rare, however even in their absence predators influence the movement and habitat selection of animals through fear (Brown et al., 1999). In the same way, predators mediate resource competition within and between mesopredator species by controlling their habitat selection, as predator abundance is not a direct indicator of risk. Different organisms select habitats based on their suite of defences evolved through millennia, such as cryptic coloration, swimming speed, burial and grouping behaviour (Lima & Dill, 1990). Juvenile stingray use of shallow sand flat habitat against the gradient of prey availability indicates preference for a depauperate habitat as a nursery area, supporting previous suggestions of a shallow nursery area for this species (Yokota & Lessa, 2006; Aguiar et al., 2009; Carvalho et al., 2010). Dale et al. (2011) suggest that high frequency of juvenile rays found with empty stomachs in Kanoeh Bay, Hawaii was due to low encounter rates with prey small enough to consume (smaller than gape size), however at Glovers Reef shallow sand flats present generally limited prey interactions with prey, as mobile species tend to refuge in seagrass and algae, and sessile prey bury to depths that rays would need to excavate. Given the low foraging frequency seen in juvenile rays, it is likely they are commonly in a state of starvation.

Tracking data from Caribbean reef sharks at Glovers Reef showed vertical nocturnal foraging movements into the lagoon at night (Chapman et al., 2007), indicating heightened predation risk for rays during nocturnal periods. Stingray acoustic tracking indicated that juvenile rays switched to deeper habitat and reduced their movement during the night, remaining buried next to patch reefs. Use of safer shallow sand flats in the daytime suggests there is still inherent risk to juvenile rays foraging during this time. Initial catch and tracking of female reef sharks within the GR lagoon suggests they might be remaining within the lagoon throughout a diel cycle (Chapman, unpublished data), potentially presenting an on-going threat to foraging rays in the daytime. Larger juvenile rays showed less restricted movements, and likely continue to minimise predation risk through the use of direct movements between patch reef refuges (Chapter 4). Kinney and Simpfendorfer (2009) suggest that predation on these larger juveniles venturing outside nursery areas may have the greatest influence on population levels. Ontogenic expansion of home range observed at Glovers Reef indicates that stingrays gain experience and expand home range to satisfy energetic requirements, inevitably reaching a size refuge whereby energetically profitably nocturnal foraging can be carried out with reduced risk, as seen with some of the largest rays tracked.

Various studies suggest that prey species have innate abilities of assessing risk and adapting accordingly on very small time scales (see Lima & Dill, 1990 for a review), however little is known about the duration of memory regarding the effects of predation risk on prey behaviour, as presumably it would be difficult to assess experimentally. If one considers that a long-lived stingray species such as *Dasyatis americana* may retain memory and experience of predation events for their

whole life (~25-30 years) then effects of mesopredator release would only be seen on a scale of generations, in addition to reproductive limitations. However, if prey species possess the ability to sense danger, and anti-predator behaviour decreases according to rate of predator encounters, the effects of release might be much faster and limited more by reproductive strategy and resource availability, especially as *Dasyatis americana* in captivity are seen to double reproduction to twice per year (Henningsen, 2000) compared to once in wild populations (Grubbs et al., 2006).

STINGRAYS AS PREDATORS

If ray populations expand and habitat use changes in response to declining predation, increased disturbance may impact benthic community structure. Most consumers, especially at higher trophic levels rely on multiple prey species, and the degree of specialisation is considered to be their trophic or dietary niche. A wide trophic niche represents a number of prey interactions, potentially stabilising trophic system to trophic perturbations caused by environmental damage or over fishing (O'Gorman & Emmerson, 2009). Results from dietary analysis of stingray diet (Chapter 5) in combination with spatial ecology and habitat use (Chapters 3 & 4) enhance understanding of the influence of stingrays on benthic prey communities, and allow for more accurate modelling of the effects of mesopredator release. Stable isotope analysis of stingrays showed a wide trophic niche (Chapter 5), which may offset low elasmobranch diversity by limiting strong prey interactions that could otherwise jeopardise system stability to cascading effects of predator declines. Nurse sharks showed a more restricted range, approximate to that of the largest rays, suggesting resource competition is limited between sympatric elasmobranch species of the same dietary guild, however greater sampling of large rays and smaller nurse sharks will evaluate this relationship more thoroughly. Mixing models showed bivalves and invertebrate worms are proportionately more important in diet compared to crustaceans and teleost fish, which supports our new understanding of the distribution and habitat use of rays at Glovers Reef (Chapters 2 & 3). Relative importance of sessile prey in the diet likely reflects their ability of detecting weak water jets of siphon feeding organisms (Montgomery & Skipworth, 1997), and the consumption of mobile prey (crustaceans and teleosts) occurs opportunistically when encountered in more complex habitats. Combined results from this study suggest that stingray population size is limited by both predation and prey availability, as predators restrict the search strategy and frequency of juvenile stingray foraging, and their movements to depauperate habitats, and hence that a release of predation pressure would change benthic community dynamics significantly.

Rays exhibit home range behaviour, and the size of their home ranges indicates that rays are resident at the site and do not forage in offshore habitat. This is also confirmed by carbon isotope values that

suggest reliance on a seagrass system, as deep waters (>1000 m) surround GR with no other shallow habitat available for ~17 miles. As a consequence it may be inferred that increasing ray populations at GR would climb rapidly due to very little emigration away from the site and no targeted fishing, however more work needs to be undertaken regarding the movement of rays over longer timescales to strengthen this finding. The structure of stingray movement paths was seen to differ at two spatial scales (Chapter 4) suggesting the use of patch reefs is key to stingrays outside of the nursery areas and perhaps those commuting to and from safer shallows. Rays move between patches with orientated movement paths, so may be directing themselves to patches in seeking refuge after feeding, or merely foraging between them as sites of higher productivity for feeding and cleaning associations^{***}.

Considering the *resource distribution hypothesis*, if prey are diffusely distributed, irregularly available and readily depleted, stingrays should systematically avoid regions that have been previously exploited, however the random structure of movement paths at scales larger than 100 m implies rays are not utilising spatial memory to avoid revisiting recently investigated patches. Furthermore, this movement strategy has implications for the role of demersal predators in controlling and structuring benthic communities through *disturbance*, where rays turn over sediment through their excavations of prey. This bioturbation of sediments is a crucial process in resuspension of organic nutrients into the water column (Yahel et al., 2008), as well as providing habitat for early colonist species of amphipods that are not found in more structured sediments (Hines et al., 1997); yet too frequent disturbance of dense prey patches may result in the destruction of habitat and prey communities (Peterson et al., 2001). Excavation of prey items by very large southern stingrays has been shown to disrupt rhizomes of turtle grass beds (*Thalassia testudinum*) (Valentine et al., 1994). The impact of *Dasyatis americana* foraging on seagrass beds of lower complexity and shallower root systems such as *Syringodium faliforme* and *Halodule wrightii* are substantial however (Fonseca et al., 1998), and high stingray foraging pressure seen in these habitats at GR suggests that use of more structured *Thalassia testudinum* habitat for prey capture may be restricted by inhibition of prey excavation and prey capture. The effects of mesopredator release on these processes can only be speculated at this stage, yet it seems likely that a release will negatively influence the diversity of prey species in sand flat habitats through reduced disturbance, as rays shift towards an *ideal free distribution* and focus foraging effort towards prey availability rather than the safety of depauperate

^{***} Cleaning behaviour is documented for *Dasyatis americana* (Snelson et al., 1990), and cleaning stations were identified at Glovers Reef during fieldwork. Cleaning behaviour was studied and documented but does not form part of this thesis.

sand flats. Additionally a decline in predation pressure may cause an increase in the use of normally riskier edge habitat of seagrass beds, causing a gradual erosion of habitat through bioturbation. Although this hypothetical situation of rays released from predation would affect entire benthic communities at Glovers Reef, the impact would likely be felt initially with the decline in recruitment of commercially important species such as Spiny lobster (*Panulirus argus*) and Queen conch (*Strombus gigas*). The role of the no-take fishing zone as a source of recruits to adult populations in the general use (fishing) areas of the MPA could fall short and the carrying capacity of the fishery would decrease rapidly. A crash in the Chesapeake Bay scallop fishery was attributed to rising populations of cownose rays in the bay, calling for suggestions of ray culling or introducing schemes such as “eat a ray, save the bay” (Berlin, 2011), aimed at reducing their destruction of shellfish beds. Conservation managers in Belize and the Caribbean region will need to exercise caution in assessing the reasons for falling stocks of commercial species, as rays will appear a popular and populous target due to their natural high densities. Although the wide trophic niche of rays may buffer the consequences of predation release somewhat, the resulting *ideal free distribution* may drive diet specialisation in rays, where efforts focus on more energetically efficient prey species, sought out without fear of predation.

CURRENT CONSERVATION STATUS AND THREATS

The limited evidence found for stingray by-catch amongst fishermen^{†††}; the estimated population size of rays at Glovers Reef of over 5000 rays; and the similar densities of rays found throughout general use and no take zones of the reserve, all indicate that rays are not currently threatened at the site. Stingrays are not known to be targeted anywhere in Belize, though artisanal scale fisheries are known to exist in neighbouring countries of Mexico (CONAPESCA-INP, 2004) and Guatemala (Graham, pers. comm.). However, due to the site fidelity of stingrays to a very localised area illustrated through manual tracking (Chapter 3), there is little cause for immediate concern regarding impacts of external ray fisheries on Belizean populations, and given their low market value it is unlikely they would be a target species for the illegal raids from neighbouring countries reported for commercial fishes, lobster and conch (Belize Fisheries Dept., pers. comm.). Distribution and habitat use findings from this study (Chapters 2 & 3) and from two offshore sites in Brazil (Aguiar et al., 2009; Carvalho et al., 2010) highlight the importance of near shore habitats for rays, which is of significant conservation concern

^{†††} One 70 cm stingray was found washed up with multiple stab injuries, suspected to be from a fisher. In-water observations of individuals with missing or truncated tails may indicate evidence of fishers chopping off the tail to prevent injuries from stingray caudal spines while handling and extracting hooks.

in these areas where targeted fisheries exist. The use of near shore habitats for refuging juveniles and reproducing females makes them extremely accessible to artisanal fisheries with unsophisticated gear such as beach seines and gill nets. Combined with low resilience to fishing pressure (Smith et al., 2008), low fecundity (Henningsen, 2000) and expanding population and development in coastal areas, stingray populations are extremely vulnerable to exploitation. Additionally, as global temperatures rise with climate change, thermal stress may inhibit the use of near shore waters for reproduction and refuge, potentially causing population level changes in habitat use, dispersal patterns and survival.

Evidence of higher abundances of rays at certain sampling sites outside the no-take zone of GR suggests rays are aggregating near to areas popular with local fishers for cleaning their catch, attracted to discarded guts^{†††}. This may imply a higher vulnerability to fishing should targeted fisheries emerge, due to opportunistic foraging tendencies of rays, and the likelihood of attraction to, and consumption of provisioned or baited food (Corcoran, 2006). Crucially this also demonstrates that traditional conservation management measures of MPAs may not be suitable, especially if designated areas are small. Furthermore, the suggestions of ray culling or harvesting as a strategy of reducing destruction of commercial shellfish beds is not currently a concern for Dasyatid rays, yet as with all batoids their slow growth, late maturity and low fecundity make them extremely vulnerable to exploitation.

The cascading effects of predator removal through trophic webs have been demonstrated in simple systems for more than 50 years, yet our understanding of the complex interactions underpinning tropical marine systems is still limited. This study finds evidence in support of the hypothesis that apex sharks may exert significant indirect control on mesopredator populations, and that demersal mesopredators can significantly influence benthic community structure. Therefore, despite probable low direct mortality, a release of stingrays from predation pressure is likely to have knock-on effects to benthic prey communities. That said, coastal stingray species are representing increasingly larger proportions of catches in targeted commercial and artisanal elasmobranch fisheries in the Latin America region (CONAPESCA-INP, 2004; Grijalba-Bendeck et al., 2007; Garro et al., 2009), as a currently abundant source of muscular protein in the absence of traditionally valuable (and overfished) teleost fish, hence findings from this study re-emphasise the importance of conservation

^{†††} Field observations were made of high numbers of stingrays and eagle rays circling underneath and in the vicinity of these boats as they cleaned their catch at the northern and western sampling sites at GR.

managers identifying and protecting nursery habitats for these vulnerable species. Isolated shallow reef systems such as Glovers Reef Atoll may see increasing effects of trophic cascades due to low levels of immigration and external recruitment, hence studies of such systems represent crucial harbingers of environmental decline. Sharks are not directly protected in Belize waters, however the restrictions of certain gear types (long lines and gill nets) at GR are crucial in protecting sharks at this site, and present a viable management example of using a suite of protection strategies to conserve biodiversity and fisheries stocks at various scales and trophic levels. For coastal-pelagic, semi-oceanic tropical species of predatory sharks, this provides a greatly reduced threat of fishing while within the boundaries of GR, however there are no restrictions on gear use outside of protected areas when migrating between sites, so their populations continue to be threatened. The effects of population declines of large bodied predatory sharks may have the greatest effects on stingray populations at GR, especially that of the supposed ray specialists the sphyrnids (hammerhead sharks). Hammerhead populations are extremely vulnerable to fishing and their populations are listed as endangered having declined by >50% in the last 10 years in the Gulf of Mexico and Atlantic (Denham et al., 2007). It will be crucial for Belize and the Caribbean region to expand protected areas to include a larger zone where gear restrictions are imposed and enforced in order to protect more mobile species, however resources for this enforcement remain under-supported. The recent introduction of restricted fishing licenses for fishers at GRMR will be important in reducing increased pressure on an already declining fisheries with increasing human population and coastal development, however as pressure on finfish, lobster and conch continue, the take of large bodied elasmobranchs is likely to increase substantially.

FUTURE DIRECTIONS

Stingrays represent a study species that with due care can be easily handled and utilised to elucidate vital ecological information about upper trophic levels. Further work should focus towards analysing the difference in stingray behaviour and habitat use between pristine and heavily impacted systems of similar environmental and geographic features. Additionally, data on predator abundance, especially with regards to seasonal changes in abundance and species of predators will allow for greater resolution of stingray behaviour and habitat use under the risk of predation. On a finer scale, further work into *behavioural optimisation theory* will elucidate the effects of physiological condition on foraging behaviour i.e. if a ray is starving or injured it will likely take more risks in foraging than one in healthy condition.

Conclusions

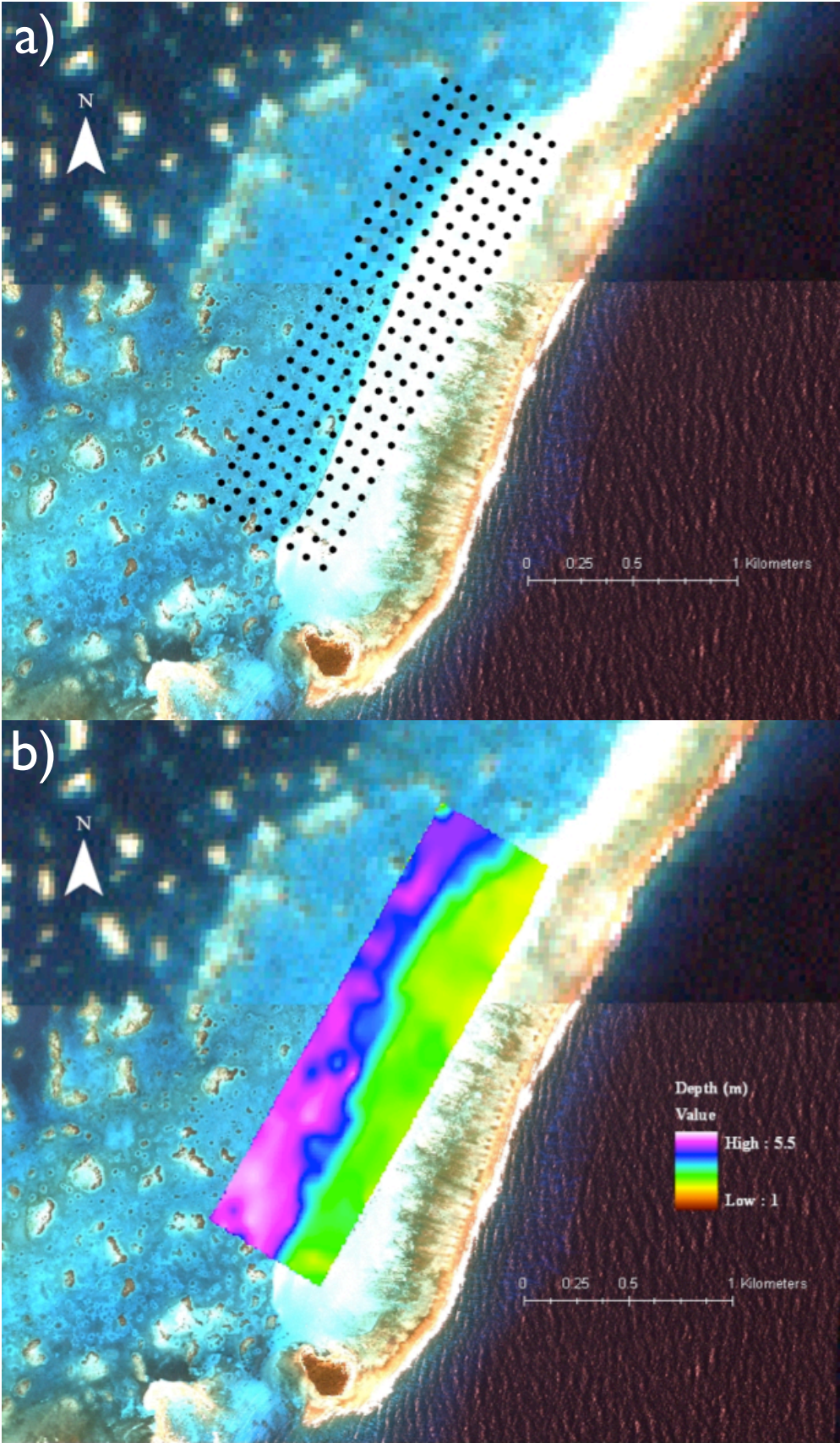
The overall aim of this study was to investigate the functional ecology of the southern stingray and assess the influence of predation risk in driving stingray spatial and trophic behaviour at Glovers Reef on different temporal and spatial scales, according to physical and biotic factors. This research presents novel information suggesting that the trophic and spatial ecology of juvenile southern stingrays at Glovers Reef is shaped by predation risk, driving the use of more depauperate habitats, and influencing search mechanisms in response to diel phase and individual size. This study illustrates an example of the control top predators can exert on the systems beneath them, in affecting habitat use and intraguild competition in lower trophic levels, and as such provides a greater tool for modelling the cascading effects of their decline and extirpation.

Appendices

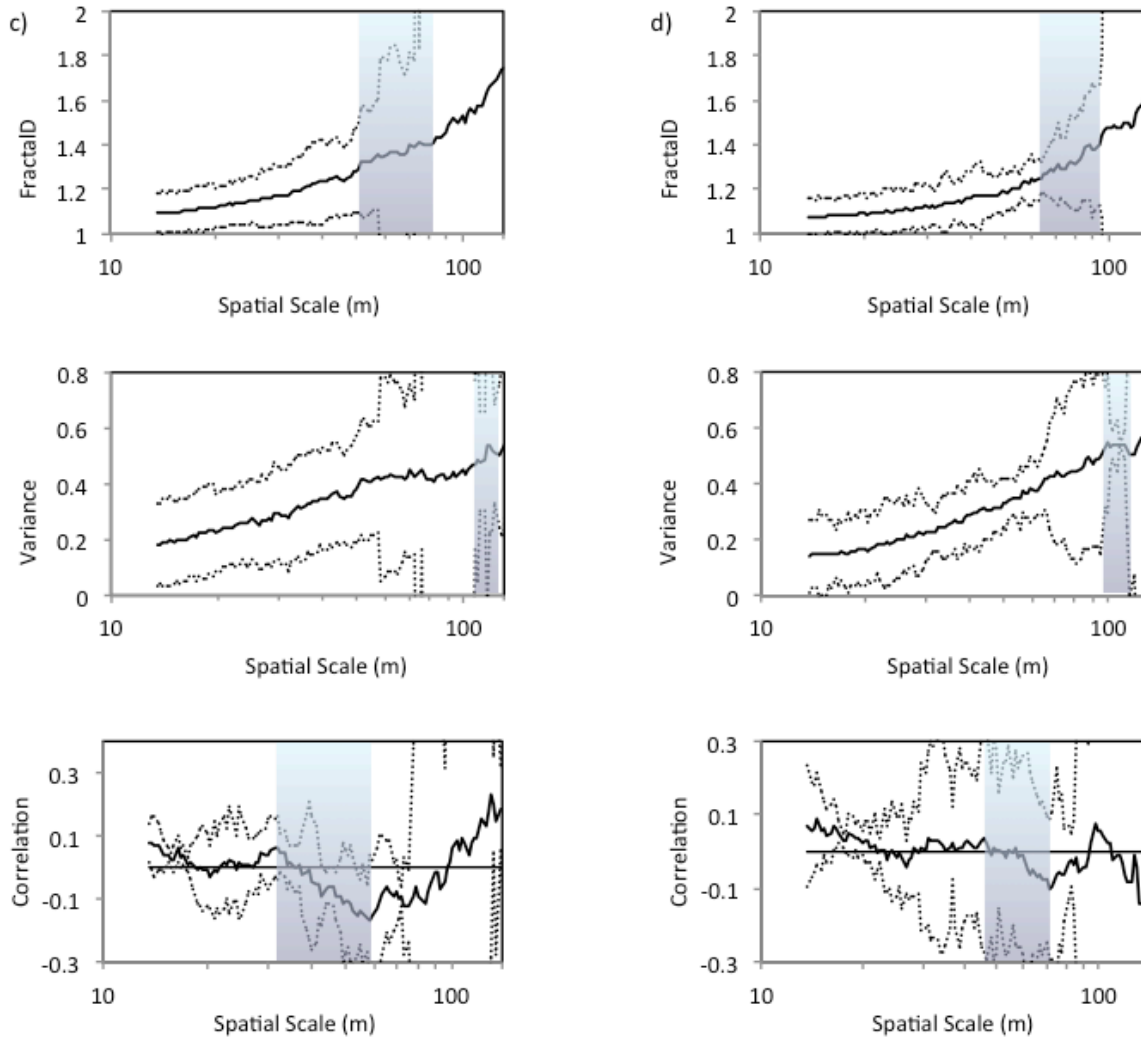
Appendix 2.1: Frequency table of benthic prey species found during sediment sampling of lagoon margin substrates at Glovers Reef Atoll between June and August 2010.

Common Name	Species	Order	Family	Total
Phylum Platyhelminthes – Class Turbellaria				1
Biocoloured Flatworm	<i>Pseudoceros bicolor</i>	Polycladida	Pseudocerotidae	1
Phylum Annelida – Class Polychaeta				33
Fireworm	<i>Chloeia</i> spp.	Aciculata	Amphinomidae	1
Lugworm	<i>Arenicola</i> spp.	Not assigned	Arenicolidae	19
Southern Lug Worm	<i>Arenicola cristata</i>	Not assigned	Arenicolidae	13
Phylum Mollusca – Class Bivalvia				70
Giant false coquina	<i>Iphigenia brasiliana</i>	Heterodonta	Donacidae	64
Sunrise Tellin	<i>Tellina radiata</i>	Veneroida	Tellinidae	6
Phylum Mollusca – Class Gastropoda				106
Angulated Wentletrap	<i>Epitonium angulatum</i>	Neotaenioglossa	Epitoniidae	5
Atlantic Grey Cowrie	<i>Cypraea cinerea</i>	Neotaenioglossa	Cypraeidae	3
Atlantic Hairy Triton	<i>Cymatium pileare</i>	Neotaenioglossa	Ranellidae	2
Dark Cerith	<i>Cerithium atratum</i>	Neotaenioglossa	Cerithiidae	1
Glowing Marginella	<i>Prunum pruinosum</i>	Neogastropoda	Marginellidae	5
Cuba Frog Shell	<i>Bursa granularis</i>	Neotaenioglossa	Bursidae	2
Miniature Trumpet Triton	<i>Pisania pusio</i>	Neogastropoda	Buccinidae	4
Music Volute	<i>Voluta musica</i>	Neogastropoda	Volutidae	3
Longspine Starsnail	<i>Astraliu phoebium</i>	Archaeogastropoda	Turbinidae	1
Orange Marginella	<i>Prunum carneum</i>	Neogastropoda	Marginellidae	2
Silky Tegula	<i>Tegula fasciata</i>	Archaeogastropoda	Trochidae	1
Stocky Cerith	<i>Cerithium litteratum</i>	Neotaenioglossa	Cerithiidae	34
West Indian chank	<i>Turbinella angulata</i>	Neogastropoda	Turbinellidae	19
Blackberry Drupe	<i>Trachypollia nodulosa</i>	Neogastropoda	Muricidae	3
White-spot Marginella	<i>Prunum guttatum</i>	Neogastropoda	Marginellidae	21
Phylum Arthropoda - Subphylum Crustacea – Class Malacostraca				187
Ciliated False Squilla	<i>Pseudosquilla ciliata</i>	Stomatopoda	Pseudosquillidae	2
Green Clinging Crab	<i>Mithraculus sculptus</i>	Decapoda	Mithracidae	15
Longtail Grass Shrimp	<i>Periclimenes longicaudatus</i>	Decapoda	Palaemonidae	3
Peppermint Shrimp	<i>Lysmata wurdemanni</i>	Decapoda	Hippolytidae	1
Red Ridged Clinging Crab	<i>Mithraculus forceps</i>	Decapoda	Mithracidae	35
Snapping Shrimp	<i>Alpheus</i> spp.	Decapoda	Alpheidae	26
Spider Crab	-	Decapoda	Majidae	97
Two claw Shrimp	<i>Brachycarpus biunguiculatus</i>	Decapoda	Palaemonidae	1
Velvet Shrimp	<i>Metapenaeopsis goodei</i>	Decapoda	Penaeidae	7
Phylum Echinodermata – Class Ophiuroidea & Echinoidea				18
Chocolate Brittle Star	<i>Ophioderma cinereum</i>	Ophiurida	Ophiodermatidae	1
Reticulated Brittle Star	<i>Ophionereis reticulata</i>	Ophiurida	Ophionereididae	16
Sea Urchin	<i>Diadema antillarum</i>	Diadematoidea	Diadematidae	1
Phylum Chordata – Class Actinopterygii				2
Chestnut Moray	<i>Enchelycore carychroa</i>	Anguilliformes	Muraenidae	1
Pallid Goby	<i>Coryphopterus eidolon</i>	Perciformes	Gobiidae	1

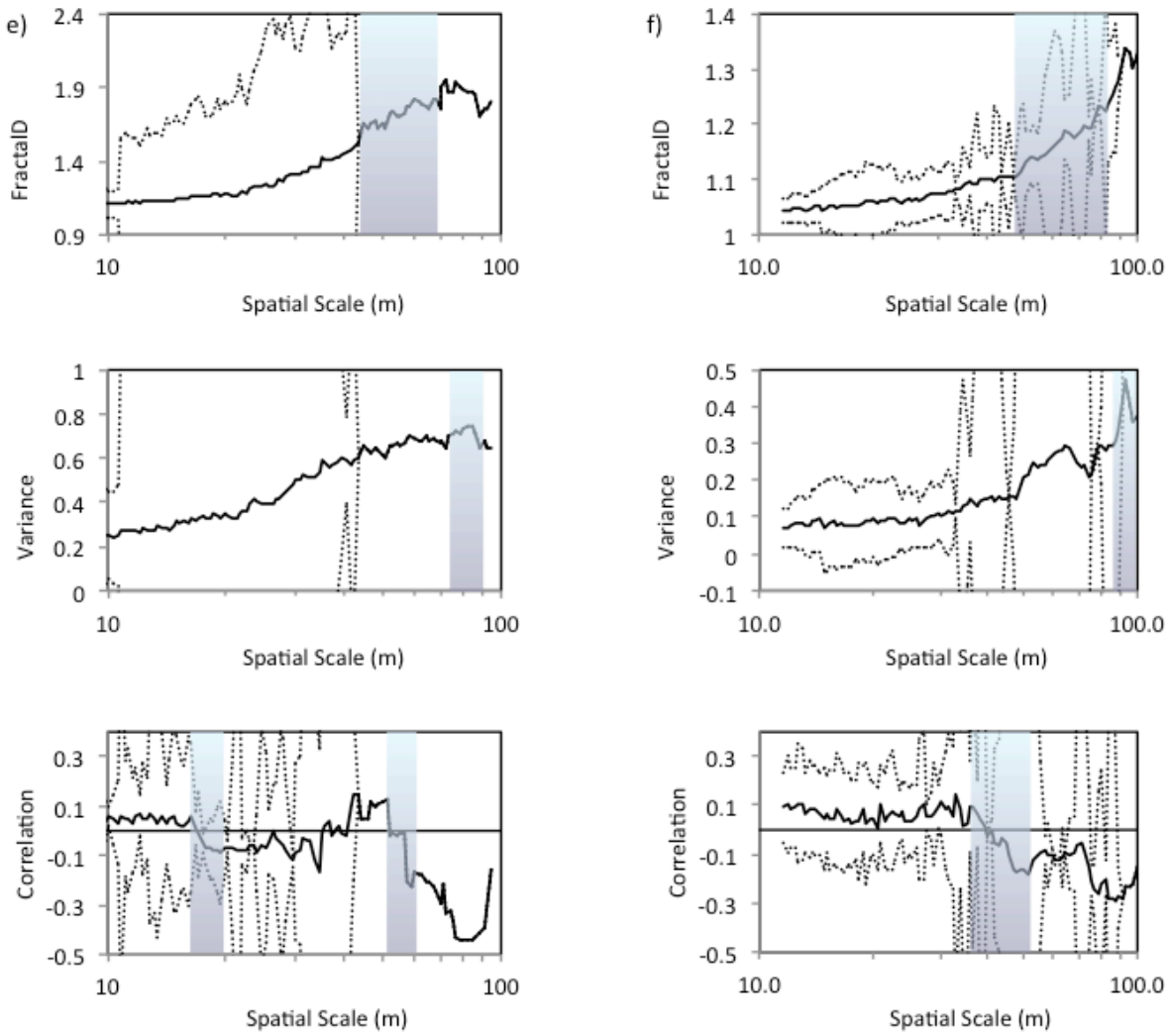
Appendix 3.1: Maps depicting a) the grid of spot depth measurements taken at the MC site using handheld sonar (Norcross Marine Products, USA) in the south eastern area of Glovers Reef lagoon margin, and b) the resulting bathymetric map created by interpolating spot depth measurements in ArcMap (ArGIS ver. 9.3, ESRI).



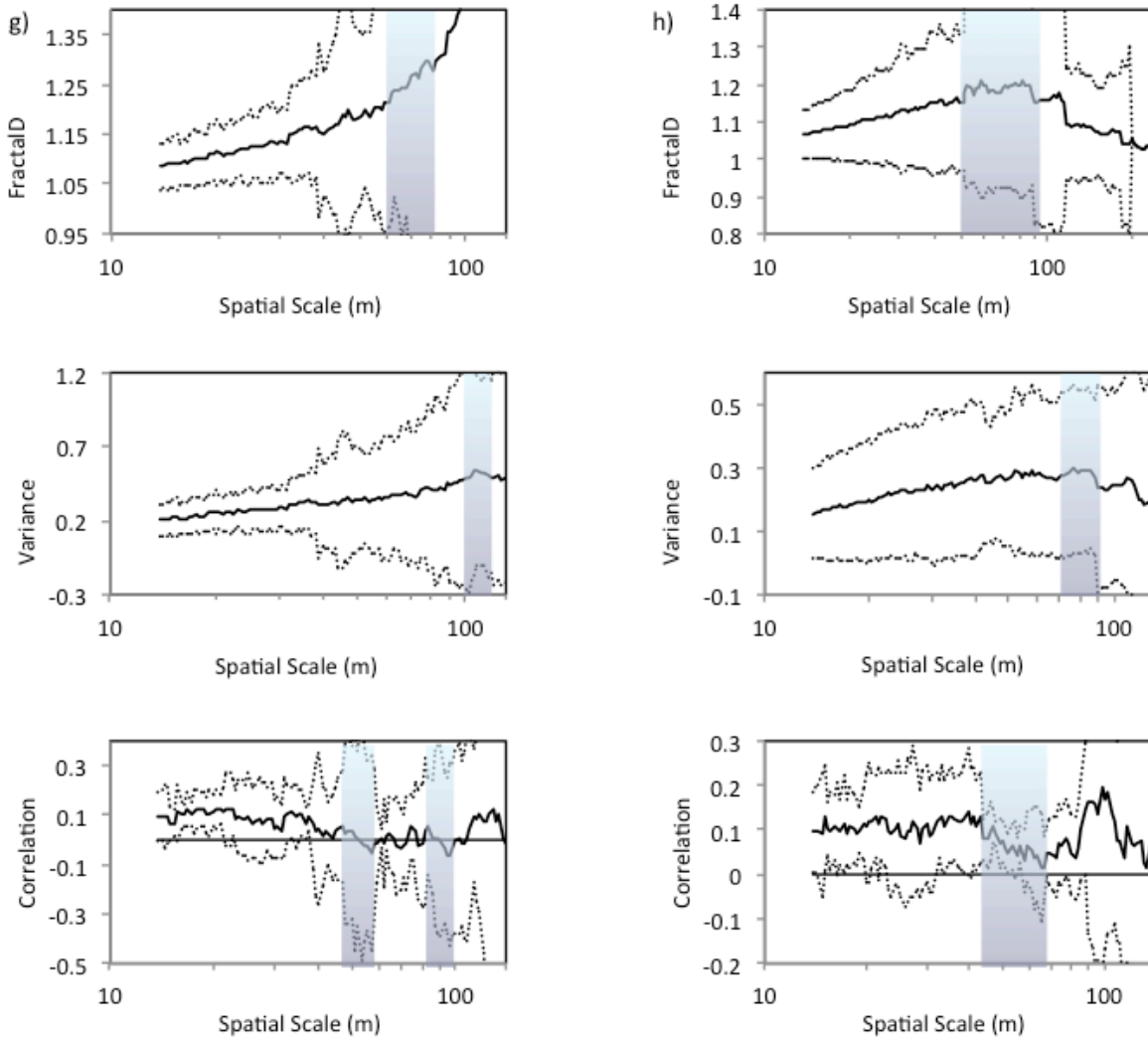
Appendix 4.1: Individual analyses of tortuosity (*FractalD*), variance and correlation of successive turn angles of southern stingray movement paths at increasing spatial scale (log), from 2 individuals tracked acoustically in Glovers Reef Atoll lagoon between June 2009 and August 2010. Shaded boxes indicate scale of domains of movement structure. c) DA8 Female 290 mm d) DA5 Female 435 mm



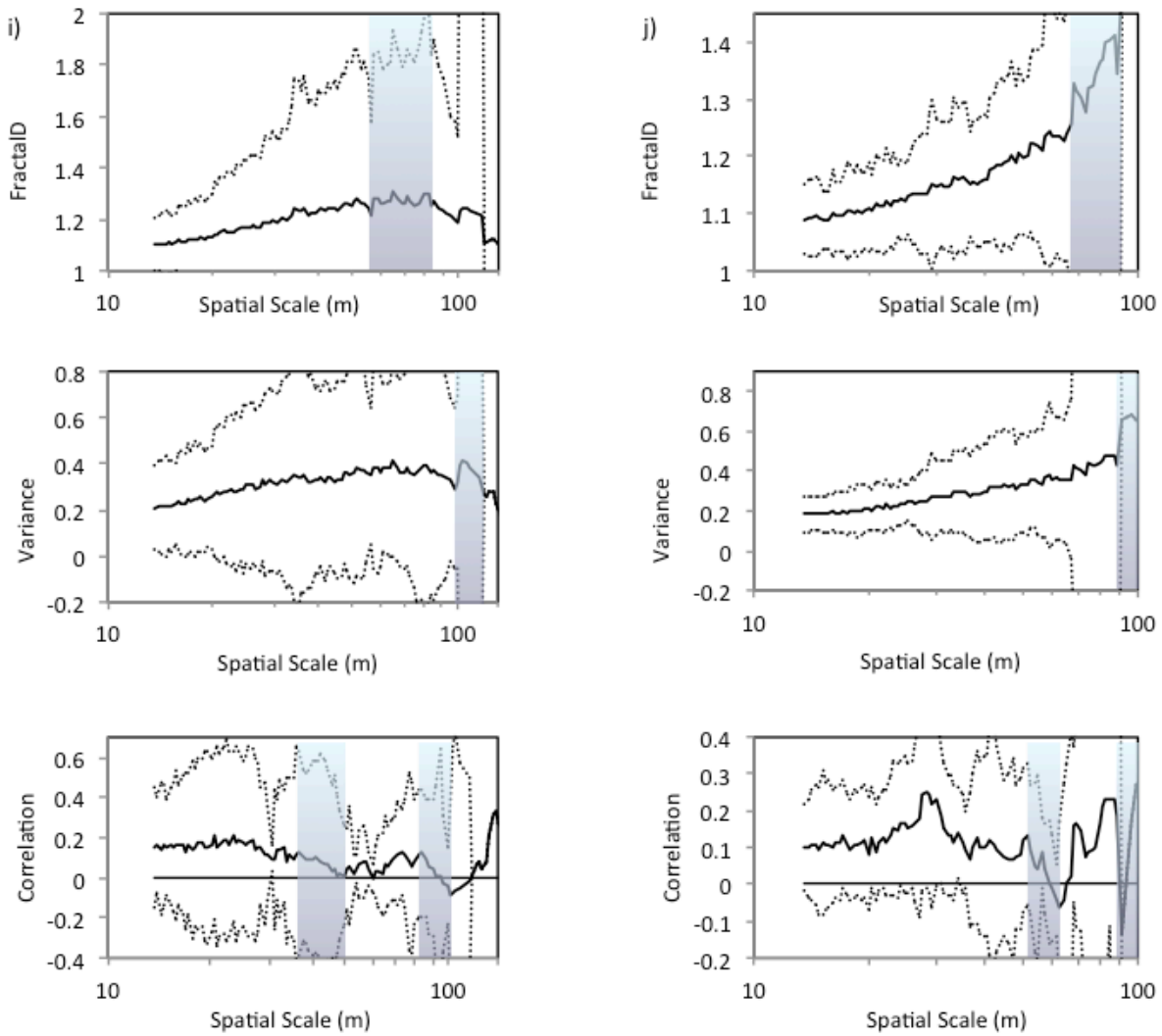
Appendix 4.2: Individual analyses of tortuosity (*FractalD*), variance and correlation of successive turn angles of southern stingray movement paths at increasing spatial scale (log), from 2 individuals tracked acoustically in Glovers Reef Atoll lagoon between June 2009 and August 2010. Shaded boxes indicate scale of domains of movement structure. e) DA2 Female 310 mm f) DA6 Male 335 mm



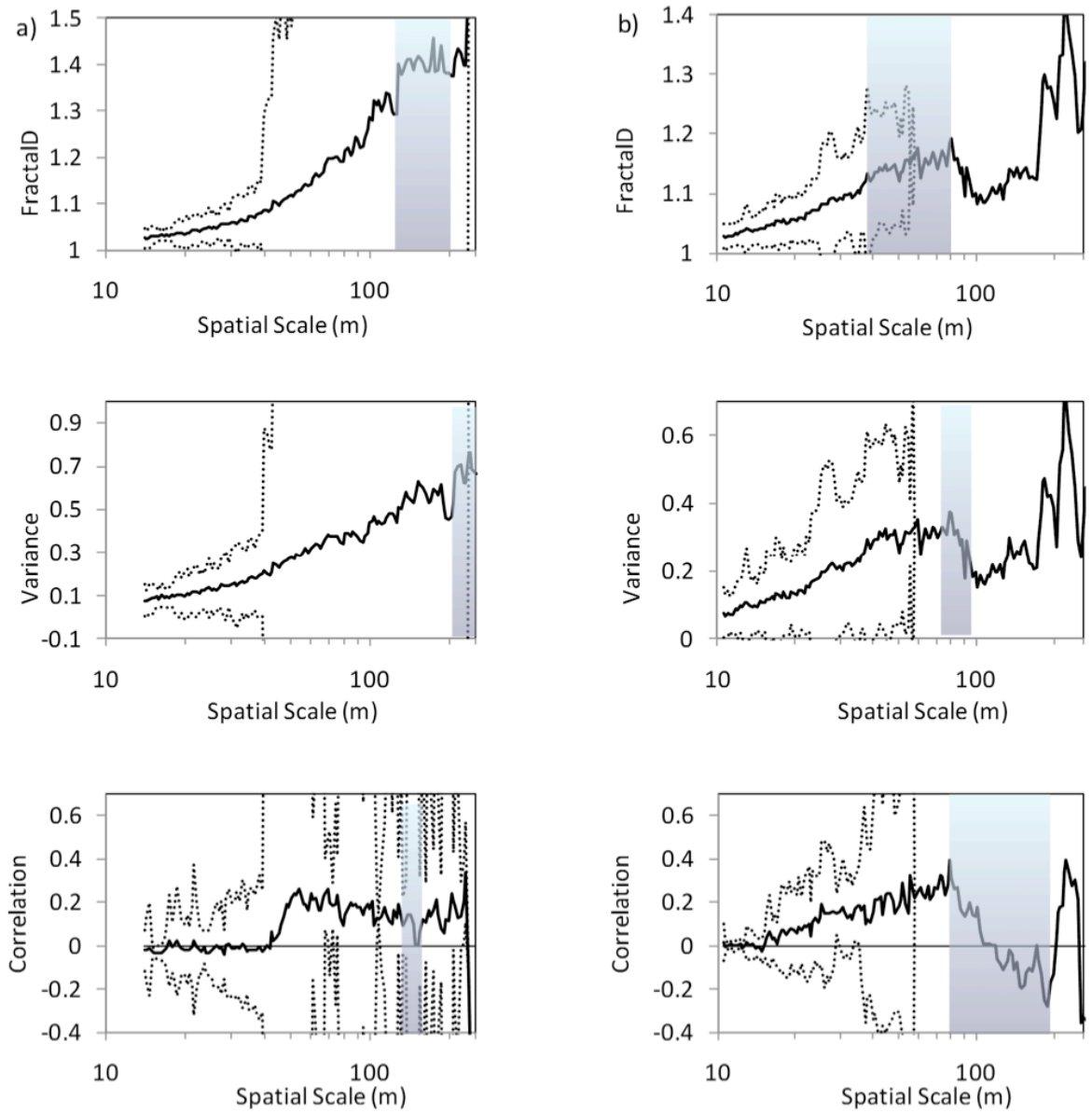
Appendix 4.3: Individual analyses of tortuosity (*FractalD*), variance and correlation of successive turn angles of southern stingray movement paths at increasing spatial scale (log), from 2 individuals tracked acoustically in Glovers Reef Atoll lagoon between June 2009 and August 2010. Shaded boxes indicate scale of domains of movement structure. g) DA7 Male 530 mm h) DA3 Female 650 mm



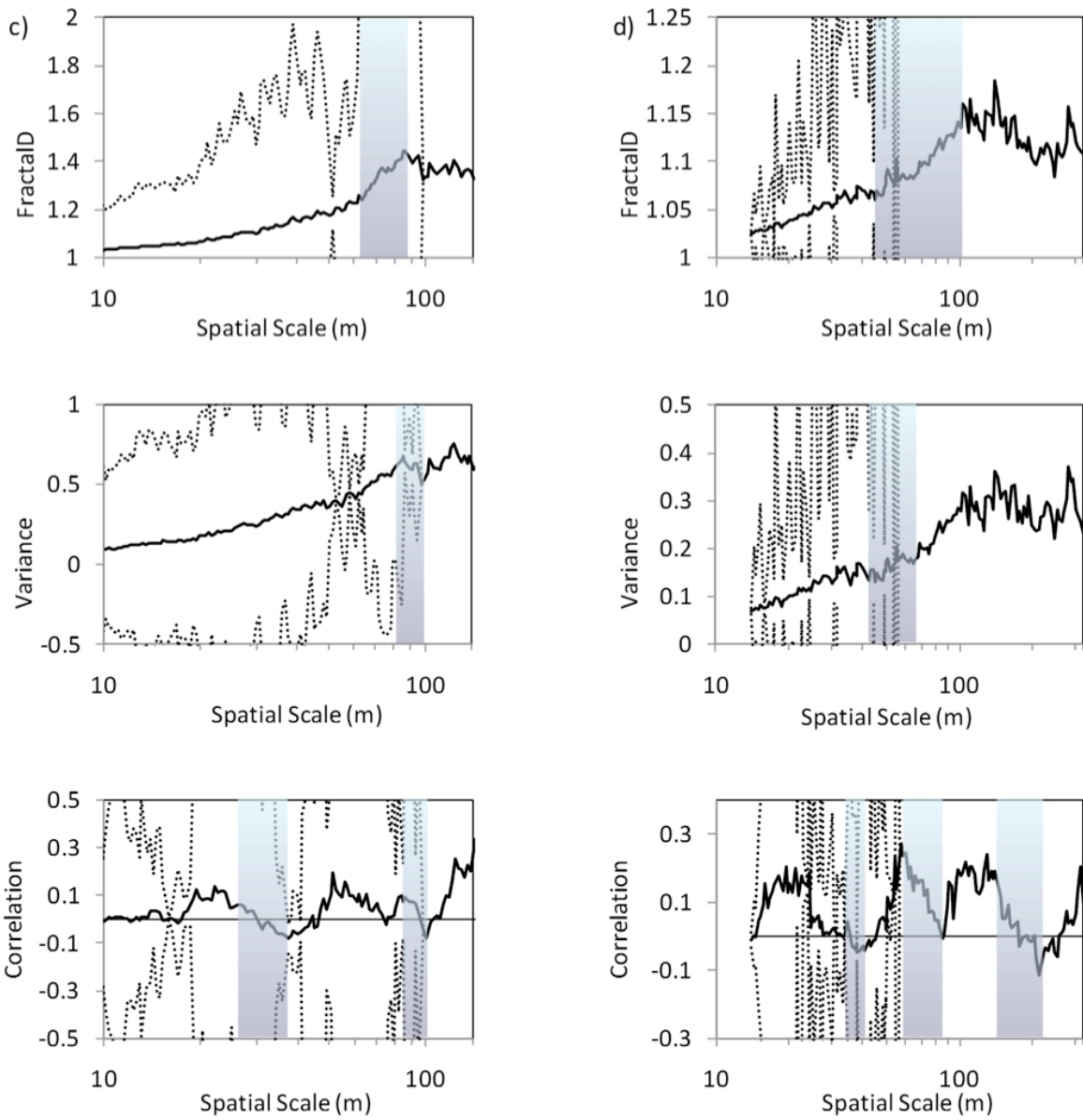
Appendix 4.4: Individual analyses of tortuosity (*FractalD*), variance and correlation of successive turn angles of southern stingray movement paths at increasing spatial scale (log), from 2 individuals tracked acoustically in Glovers Reef Atoll lagoon between June 2009 and August 2010. Shaded boxes indicate scale of domains of movement structure. i) DA4 Female 770 mm j) DA9 Female 700 mm



Appendix 4.5: Individual analyses of tortuosity (*FractalD*), variance and correlation of successive turn angles of southern stingray movement paths at increasing spatial scale (log), from 2 individuals tracked acoustically in Glovers Reef Atoll lagoon between June 2009 and August 2010. Shaded boxes indicate scale of domains of movement structure. a) DA12 Male 450 mm b) DA11 Male 520 mm



Appendix 4.6: Individual analyses of tortuosity (*FractalD*), variance and correlation of successive turn angles of southern stingray movement paths at increasing spatial scale (log), from 2 individuals tracked acoustically in Glovers Reef Atoll lagoon between June 2009 and August 2010. Shaded boxes indicate scale of domains of movement structure: c) DA14 Female 860 mm d) DA15 Male 550 mm



Appendix 4.7: CRW_{diff} values for movement paths of all 25 southern stingrays followed in-water in Glovers Reef lagoon between November 2009 and March 2010. Significant values are in bold type and negative values in red. Sites are coded as LAG for stingrays followed in the lagoon site, and WS for those followed at the forereef White Sands site.

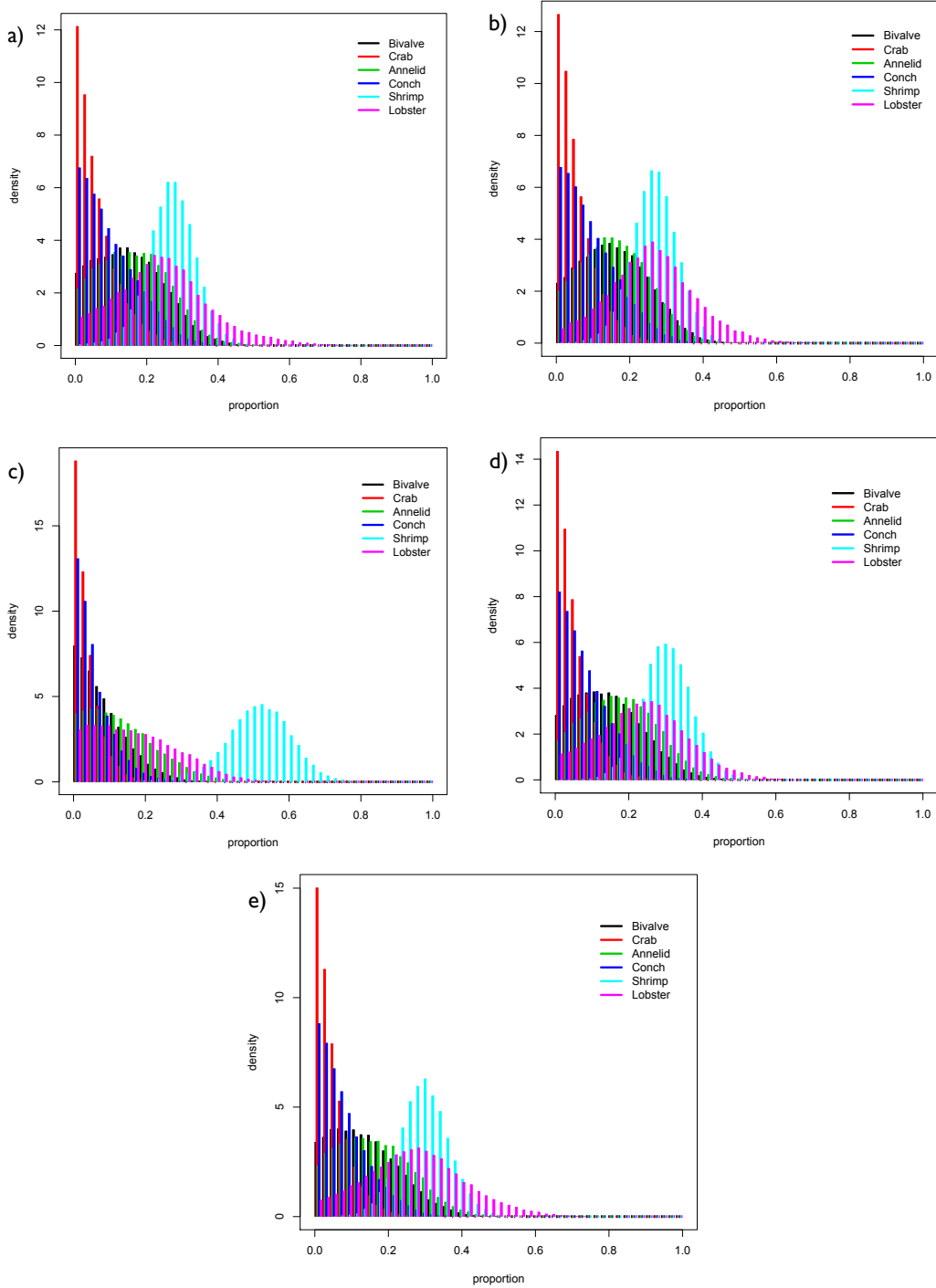
Sex/Size (cm)	Site	CRW _{diff}	SE	P	CRW2diff	SE	P
F 57	LAG	-0.880	0.123	0.000	-0.395	0.050	0.000
M 35	LAG	0.521	0.193	0.003	0.504	0.169	0.007
F 60	WS	1.025	0.388	0.001	0.932	0.290	0.008
M 40	WS	2.093	0.880	0.000	0.568	0.134	0.017
F 33	LAG	0.463	0.219	0.016	0.441	0.183	0.034
M 35	LAG	1.993	0.942	0.031	1.286	0.595	0.034
F 67	LAG	-0.390	0.198	0.105	-0.206	0.127	0.048
M 35	WS	0.297	0.153	0.014	0.290	0.118	0.053
M 35	LAG	1.812	0.965	0.020	0.978	0.420	0.061
F 60	LAG	0.890	0.502	0.052	0.768	0.395	0.076
M 40	LAG	0.375	0.216	0.038	0.355	0.171	0.082
F 51	LAG	2.011	1.261	0.088	1.618	0.947	0.111
M 38	WS	0.796	0.526	0.046	0.591	0.296	0.130
F 37	LAG	0.974	0.676	0.075	0.858	0.481	0.150
M 45	LAG	1.253	0.882	0.046	0.540	0.270	0.156
F 31	LAG	0.980	0.796	0.188	0.777	0.590	0.218
M 35	WS	0.114	0.114	0.137	0.133	0.089	0.318
M 33	WS	1.479	1.484	0.268	0.351	0.317	0.319
F 51	LAG	0.191	0.206	0.225	0.200	0.165	0.353
M 40	LAG	1.319	1.924	0.405	0.709	0.851	0.493
F 35	WS	-0.282	0.441	0.276	0.095	0.087	0.523
M 40	WS	0.088	0.546	0.369	0.249	0.277	0.872
F 40	WS	0.019	0.128	0.358	0.084	0.091	0.882
M 33	LAG	-0.046	0.997	0.884	-0.073	0.504	0.963

Appendix 5.1: Table of *Dasyatis americana* prey items recorded in diet studies using stomach analyses. Numbers represent overall proportion of diet from *n* individuals. ‘x’ represents presence where rank data is unavailable.

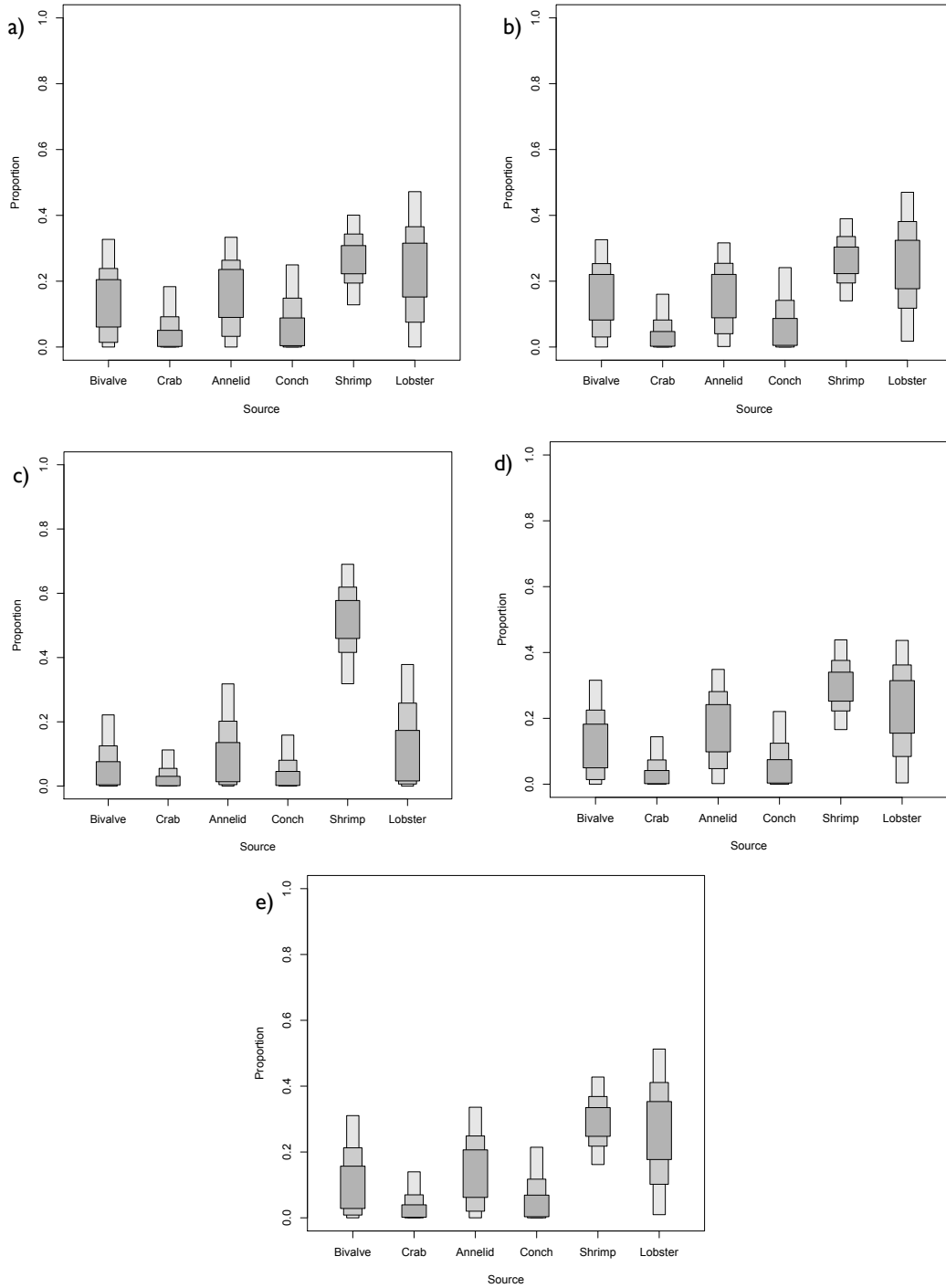
Stomach content	Guillian & Sullivan (1993) <i>n</i> =18	Randall (2004) <i>n</i> =23	Smith & Herrnkind (1992) <i>n</i> =5	Snelson & Williams (1981) <i>n</i> =3	Bowman, et al. (2000) <i>n</i> =2	Stokes & Holland (1992) <i>n</i> =1	Bigelow & Schroeder (1953) <i>n</i> =15
TELEOSTEI	17.5	21.8			0.70		x
Labridae	7.2						
Gobiidae	1.5						
Scaridae	2.8						
Blenniidae							
<i>Chasmodes saburrae</i>				10.0			
Batrachoididae							
<i>Opsanus tau</i>				10.0			
Acanthuridae							
<i>Acanthurus</i> sp. (juv.)		x					
Engraulidae		x					
Percoidei							
<i>Opistognathus</i> sp.		x					
Scorpaenidae							
<i>Scorpaena plumieri</i>		x					
Unidentified	6.0			20.0			
CRUSTACEA	61.1	17.6					
Decapoda	44.8				98.70		
Calappidae		x					
Hippidae		x					
Xanthidae		x					
Palinuridae							
<i>Panulirus argus</i>			x				
Portunidae	30.9	x		30.0			x
<i>Callinectes</i> sp.			x				
<i>Portunus</i> sp.		x	x				
<i>Portunus depressifrons</i>	13.5						
<i>P. ordwayi</i>	13.2						
<i>P. anceps</i>	3.1						
Processidae	0.1						
<i>Processa guyanae</i>	0.02						
Hippolytidae	0.02						
<i>Tozeuma carolinense</i>	0.02						
Pasiphaeidae	0.02						
<i>Leptochela carinata</i>	0.02						
Albunaeidae	0.96						
<i>Albunea gibbesii</i>	0.81						
<i>Albunea paretii</i>	0.42						
Raninoidae	0.42						
<i>Ranilia muricata</i>	0.04						
Majidae	0.04	x					
<i>Mithrax hispidus</i>	0.04						
Caridea		7.6					x
Penaeidae	7.6	x		10.0			
<i>Metapenaeopsis goodei</i>	7.4						
<i>Trachypenaeus constrictus</i>	0.2						
<i>Penaeus</i> sp.			x				
Alpheidae	1.0	x		20.0			
<i>Aplpheus schmitti</i>	1.0						
Solenoceridae							
<i>Solenocera</i> sp.		x					
Stomatopoda	9.3	2.0					x
Squillidae	5.3						

<i>Alima hyalina</i>	5.3						
Gonodactylidae							
<i>Gonodactylus</i> sp.			x				
<i>Gonodactylus oerstedii</i>	0.6						
<i>Pseudosquilla ciliata</i>	3.4						
Unidentified	7.0				0.30		
MOLLUSCA	7.1						
Bivalvia	2.4	10.8				x	
<i>Asaphis deflorata</i>		x					
Gastropoda	3.2						
Strombidae	3.2						
<i>Strombus gigas</i>	3.2						
Cephalopoda	1.5						
Octopus	0.02						
<i>Octopus joubini</i>	1.5						
ANNELIDA	6.0						x
Polychaetes		17.3			0.30		
CEPHALOCHORDATA						x	
<i>Branchiostoma floridae</i>						x	
HEMICHORDATA		2.3					
SIPUNCULA		20.6					
<i>Aspidosiphon</i> sp.		x					
PLANTAE	0.4						
Unidentified material	7.4						

Appendix 5.2: Proportional plots from isotope mixing model plots of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values for *G. cirratum* using experimental TEFs from the literature.



Appendix 5.3: Proportional plots from isotope mixing model plots of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values for *G. cirratum* using experimental TEFs from the literature.



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