THE TROPHIC AND SPATIAL ECOLOGY OF A SYMPATRIC DASYATID COMMUNITY AT A REMOTE ATOLL, SEYCHELLES

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Abstract

Batoid populations are declining globally but the paucity of information makes management or conservation efforts difficult. Additionally, batoids are mesopredators and are hypothesised to play important ecological roles, but a comprehensive understanding of these roles is also limited. Therefore, information on batoid habitat use, foraging ecology, and resource partitioning is needed. St. Joseph Atoll is a relatively pristine ecosystem that hosts a sympatric dasyatid community (*Pastinachus sephen*, *Urogymnus granulatus*, and *U. asperrimus*). Passive acoustic telemetry identified high levels of long-term site affinity by both juvenile and adult dasyatids to St. Joseph Atoll. Dispersal from the atoll by larger juveniles was also evident. Juveniles displayed restricted horizontal movements in the atoll, but the extent of these movements differed seasonally. Stomach content and stable isotope analyses identified inter-specific prey partitioning (*P. sephen* juveniles were mollusc specialists and *U. granulatus* juveniles were crustacean specialists) and intra-specific prey partitioning. Juveniles were reliant upon a seagrass-based food web, whereas adults were reliant on phytoplankton-based food web. Adults fed at higher trophic levels compared to juveniles (mean of 4.6 and 3.4 respectively). Juvenile dasyatids preferentially selected the shallow reef flat habitat of the atoll, where there was no evidence for inter-specific habitat partitioning (individuals co-occurred randomly with con- and hetero-specifics). Conversely, resident adults were more reliant on the deeper lagoon. Juveniles selected the reef flat habitat likely because it provided refuge from predation and foraging opportunities. However, juveniles were also detected in the lagoon habitat and this appeared to be necessitated by physical factors as they were detected more frequently in the lagoon at low tides and when temperatures on the reef flats became too warm or too cold. All results together suggest that St. Joseph Atoll is a nursery area for these dasyatids. Juveniles may be limited by resources as prey was partitioned between species. However, top-down control by larger sharks was likely a significant influence on habitat selection, as was the physical effects of tide and temperature. Finally, St. Joseph Atoll is suitable for the designation of a Marine Protected Area, which would likely confer high conservation benefits to this dasyatid community.

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Chapter 1

Introduction

Historically, chondrichthyans (i.e. cartilaginous fishes) have been under-represented in scientific literature. Recent decades have seen an exponential increase in the volume of shark research however, there is still limited scientific effort focused on batoids (the ‘flat-sharks’). There is a need for more research on this faunal group given both their purported ecological roles and their vulnerability, with batoids constituting five out of the seven most threatened chondrichthyan families (Dulvy *et al.*, 2014).

1.1 Batoids: Dasyatidae

1.1.1 Morphology and taxonomy

Batoids are closely related to sharks, having evolved from shark-like ancestors nearly 200 million years ago (Last *et al.*, 2016a). However, they are different to sharks in that they are dorsoventrally flattened with ventrally located gill slits. Body plans are often modified into a disc-shape through the complete or partial fusion of the pectoral fins with the head and trunk, but morphological diversity within this group is large (Aschliman *et al.*, 2012).

The batoids are the most speciose subgroup of chondrichthyans, comprising 26 families and 633 valid named species. They have long been regarded as taxonomically problematic and, although monophyly of the batoid group is widely accepted, the inter-relationships within this group are not as well defined (Last *et al.*, 2016a). The recently completed “Chondrichthyan Tree of Life project” (sharksrays.org) has produced the most taxonomically robust inventory and classification of chondrichthyans using both molecular and morphological data. Four major monophyletic batoid orders were identified through this study: Rajiformes (skates), Torpediniformes (electric rays), Rhinopristiformes (guitarfishes and sawfishes), and Myliobatiformes (stingrays and their relatives). The latter consists of 11 families, including Dasyatidae (the whiptail stingrays), a large and complex family that was mostly resolved as monophyletic (Last *et al.*, 2016b).

1.1.2 Distribution and habitat

Batoids are widely distributed, from polar latitudes to the tropics, and have evolved to occupy a variety of habitats ranging from the shoreline to depths of 3000m, in pelagic, coastal and freshwater ecosystems (Last *et al.*, 2016a). The tropics support the highest abundance and diversity of dasyatids specifically (Fowler *et al.*, 2005). They are most commonly found in shallow coastal and shelf regions, often contributing a significant proportion to the fish biomass (O'Shea, 2012). The majority of species are benthic or benthopelagic and preferentially occur in soft-sediment habitats including mangroves, sandy shores, and coral reef lagoons (Snelson, Jr. *et al.*, 1988; Thrush *et al.*, 1991; Gray *et al.*, 1997; Cartamil *et al.*, 2003; Cerutti-Pereyra *et al.*, 2014; Davy *et al.*, 2015).

1.1.3 Life histories

Biological measures, such as age and growth, are missing for many batoid species but, given the available estimates, this group is generally characterised by late age at maturity, long lifespans, low fecundity, and low natural mortality (Camhi *et al.*, 1998; Last *et al.*, 2016a). There are some exceptions to this general trend, for example, the fan ray *Platyrhina sinensis* is a relatively fast-growing species with a maximum age of 5-12 years and age at maturity of 2-3 years (Kume *et al.*, 2008). Little information is available for dasyatids specifically but maximum age has been reported between 3-28 years and age at maturity between 3-8 years (Cowley, 1997; Mollet *et al.*, 2002; Ismen, 2003; **?**). The life-history strategies of batoids are diverse, with some species laying eggs (e.g. skates) while others give birth to live young (Dulvy & Reynolds, 1997; Ebert & Cowley, 2008; Ebert *et al.*, 2008). However, overall, there is considerable maternal investment to produce small numbers of fully-developed young (Camhi *et al.*, 1998). Dasyatids have litters of 1-13 pups (Musick & Ellis, 2005) and mothers exhibit histotrophic viviparity whereby growing embryos are not only nourished by the yolk-sac, but by a protein- and fat-enriched milk secreted by the uterus. This enables faster growth rates and newborn pups do not require maternal investment after birth (Last *et al.*, 2016a).

1.2 Ecological importance of batoids

1.2.1 Role as predators

Within marine ecosystems, elasmobranchs occupy both apex- and meso-predatory trophic positions. However, the majority of species, including small- to medium-sized batoids, fulfil the latter role (Vaudo, 2011). Mesopredatory batoids are largely benthic carnivores, feeding on a variety of invertebrates and sometimes small pelagic fish. Dasyatids, in particular, feed mostly on decapod crustaceans and polychaete worms in the soft sediments over which they occur but, larger individuals/species can consume significant proportions of fish (Jacobsen & Bennett, 2013).

Apex-predatory elasmobranchs are often considered to exert top-down influence over their prey communities (Hammerschlag *et al.*, 2017b, 2018). However, some of the best evidence for elasmobranch top-down control comes from studies on mesopredatory batoids which show that certain species can regulate the structure and dynamics of benthic invertebrate communities (VanBlaricom, 1982; Thrush *et al.*, 1991; Peterson *et al.*, 2001; Wetherbee & Cortés, 2004). However, top-down effects are not universal. Elasmobranch predation may be compensatory in some situations and prey populations may be regulated by other factors (Stevens, 2000; Piraino *et al.*, 2002). Ecological roles played by species will vary with food web complexity, levels of trophic redundancy, and productivity of an ecosystem (Navia *et al.*, 2010). Unfortunately, studies examining top-down influence by elasmobranchs, and batoids specifically, are still few in number and are limited to a small number of taxa. A more expansive understanding of the predatory roles played by batoids is needed but, this is limited by the small number of studies detailing their dietary habits.

1.2.2 Role as prey

The mesopredatory nature of most benthic batoids necessitates that they link upper and lower trophic levels in the food webs that they occupy. Indeed, Cortés (1999) calculated the standardised diet composition for 149 shark species and found that chondrichthyans were present in the diet of 51 shark species and made up more than 25% of the diet in 7 species (with the diets of the great hammerhead shark *Sphyrna mokarran* and broadnose sevengill shark *Notorynchus cepedianus* consisting of almost half of chondrichthyans).

Predation pressure can impact prey through direct consumptive effects, or via indirect risk effects. The fear of predation has altered prey behaviour in a variety of ecosystems (Laundre *et al.*, 2001; Wirsing *et al.*, 2008) and the strength of this effect is likely because the decision to avoid predation is the most important an animal can make (given that survival depends on it) (Brown *et al.*, 2014; Laundre *et al.*, 2014). For batoids specifically, predator-avoidance traits include i) morphological adaptations, for example venomous barbs in dasyatids, ii) behavioural responses, such as grouping behaviour seen in a *Pastinachus* species (Semeniuk & Dill, 2005, 2006), and iii) specific habitat selection, for example nursery use (see reviews by Heupel *et al.* 2007; Martins *et al.* 2018). Indeed, the effect of fear can be so pronounced in batoids that they have been known to trade-off energy for safety (Tilley, 2011; Vaudo & Heithaus, 2013).

Through these anti-predatory behaviours, it has been suggested that batoids can mediate changes brought about by apex predator loss via trophic cascades and mesopredatory release (Myers *et al.*, 2007). These phenomena have occurred in a range of terrestrial and marine environments (Ritchie & Johnson, 2009) however, latest evidence shows that batoids are unlikely to play important roles in these trophic cascades (Grubbs *et al.*, 2016). Furthermore, the roles that batoids can play as both predator and prey can be highly redundant in certain ecosystems whereby different species fulfil the same trophic role (Navia *et al.*, 2017). More studies are needed to fully understand the links that these mesopredators form in food webs.

1.2.3 Role as competitors

Not only may batoid communities be subject to top-down predatory impacts, but potentially also bottom-up effects (Hunter & Price, 1992). If resources are limiting in an ecosystem (i.e. bottom-up control), similar species will compete with each other, leading (in theory) to competitive exclusion (Hardin, 1960). The initial theory to explain species coexistence was that of niche differentiation. Particularly, species will partition resources along some ecological axis (usually food, space or time), ensuring that inter-specific competition is lower than intra-specific competition and facilitating the coexistence of sympatric species (Schoener, 1974). However, the recent hypothesis of neutrality has influenced ecologist's understanding of how species can co-exist. The idea behind this theory is that co-occurring species do not need to differentiate in resource use if they are equal in their competitive abilities, thus negating the effect of competitive exclusion (Hubbell, 2005). Today, there seems to be a growing consensus that both niche and neutral hypotheses likely explain patterns of species co-occurrence present in nature. Specifically, theoretical models highlight that species can co-exist if they are sufficiently similar or sufficiently dissimilar from each other, leading to patterns of similar species coexisting in niches (Scheffer & van Nes, 2006; Vergnon *et al.*, 2009, 2012).

When considering elasmobranch communities, competition is an important aspect to consider given that elasmobranchs often occur in abundant sympatric communities. Specifically, competition has been hypothesised to limit the abundance of less competitive skate species (Dulvy *et al.*, 2000) and to effect the distribution of shark species over a small geographic range (Papastamatiou *et al.*, 2006; Heupel *et al.*, 2018b). Most studies examining the potential effect of competition do so through investigating trophic resource partitioning. For batoids, observed patterns match these theoretical predictions as some communities exhibit inter-specific dietary differentiation while others show high dietary overlap (Marshall *et al.*, 2008; Vaudo & Heithaus, 2011; Yick *et al.*, 2011; O'Shea *et al.*, 2013; Yemken *et al.*, 2017).

1.2.4 Role as bioturbators

Finally, benthic batoids also play significant ecological roles through altering the physical benthos over which they occur. They disturb the sediment and create ray "feeding pits" through their feeding behaviours, and are thus considered bioturbators. This bioturbative behaviour causes physical disturbance and has been implicated in damage done to both eelgrass and turtlegrass beds (Orth, 1975; Valentine *et al.*, 1994; Nelson *et al.*, 2009). Bioturbation also has positive ecosystem benefits: biologically, it causes colonisation events for benthic invertebrate communities (VanBlaricom, 1982; Cross & Curran, 2000) and it may alter the geochemistry of the substrate by flushing out inorganic nutrients while oxygenating the sediment (Lohrer *et al.*, 2004).

1.3 Batoid threats and conservation

1.3.1 Population declines and anthropogenic threats

A recent paper reviewed the conservation status of chondrichthyans according to the IUCN Red List of Threatened Species (Dulvy *et al.*, 2014). This review found that 20% of batoid species were listed as threatened and 25% as Data Deficient. Dasyatids, in particular, were found to be the fifth most threatened chondrichthyan family, largely due to their shallow-water coastal nature and thus high overlap with fishing activities. As the majority of batoids have k-selected life histories, populations cannot compensate effectively for the increased mortality that fisheries impose (Camhi *et al.*, 1998; Musick *et al.*, 2000; Dulvy & Reynolds, 2002; Field *et al.*, 2009). Indeed, sharks (who share similar life history traits to batoids) have been estimated to exhibit twice the fishing extinction risk compared to teleosts (Myers & Worm, 2005). Therefore, fishing pressure is likely the largest contributor to observed population declines of batoids (Dulvy *et al.*, 2014).

Batoid fisheries date back to prehistoric times. Cave drawings indicate that dasyatids were possibly an important food resource for indigenous Australians, the leather was valued by ancient Egyptian craftsmen and artisans, and they figured prominently in ancient Greek mythology and medicine (Last *et al.*, 2016a). Today, batoids are a considerable food resource in parts of the world and are increasingly targeted by various fisheries (Smith *et al.*, 2007; Ebert *et al.*, 2008; Smith *et al.*, 2008; Bizzarro *et al.*, 2009; Jit *et al.*, 2012). However, it is difficult to estimate the total annual global batoid catch as landing estimates are unreliable. Indeed, global landed chondrichthyan catches are reported to the Food and Agriculture Organisation (FAO) of the United Nations, of which batoids contribute a significant proportion, however, there is no species-specific landings data (Dulvy *et al.*, 2014). Additionally, the true catch is likely to be 3-4 times higher than reported (Worm *et al.*, 2013) as these estimates do not include discards, a significant factor given that batoid catches are often incidental (White *et al.*, 2006; Piovano *et al.*, 2010; Clarke *et al.*, 2014).

While the major threats faced by batoids come from the fishing sector, they are also vulnerable to other anthropogenic influences. The effects of climate change may be significant (Field *et al.*, 2009), for example, batoids on the Great Barrier Reef were hypothesised to be vulnerable to increases in temperature (Chin *et al.*, 2010). Finally, batoid species that occur in shallow coastal areas are considered to be vulnerable to habitat degradation and destruction (Camhi *et al.*, 1998; Lotze *et al.*, 2006; Field *et al.*, 2009).

1.3.2 Conservation efforts and the importance of spatial ecology data

Unfortunately, despite population declines, there is limited appropriate management and conservation strategies in place for batoids. Indeed, both the number of protected species and the number of countries that provide protection for batoids are very low (Last *et al.*, 2016a). Effective management and conservation relies on scientific information including the distributional patterns, biological characteristics, genetic diversity, foraging ecology, as well as movement patterns and habitat use of species (Camhi *et al.*, 1998; Simpfendorfer *et al.*, 2011; Shiffman *et al.*, 2012; Henderson *et al.*, 2018). The latter is especially pertinent so that potential management strategies can occur at appropriate spatial and temporal scales (Simpfendorfer *et al.*, 2010; Lea *et al.*, 2016; Henderson *et al.*, 2018). Management interventions are known to be less successful when the spatial ecology of a species is not accounted for (Thirgood *et al.*, 2004; Moffitt *et al.*, 2009).

For batoids, this information is very limited and simple knowledge regarding the where, when and why they occur is lacking. Some species are known to form seasonal aggregations, suggesting they are capable of both site fidelity and large-scale movements (Collins *et al.*, 2008; Campbell *et al.*, 2012). However, the popular assumptions of batoid philopatry and increased activity at night proved to be unfounded from recent literature reviews (Flowers *et al.*, 2016; Hammerschlag *et al.*, 2017a). This highlights the lack of understanding regarding batoid spatial ecology and the need for more focused research on this topic.

1.4 SWIO, Seychelles, and St. Joseph Atoll

The site that was chosen to perform an ecological study on dasyatids was St. Joseph Atoll, a remote ecosystem in the outer islands of Seychelles, South West Indian Ocean (SWIO). This ocean is considered to have among the highest marine species richness globally (Tittensor *et al.*, 2010) however, this diversity is threatened by fisheries and limited effective management (Mora *et al.*, 2009). Indeed, a recent review of fisheries in this region by Temple *et al.* (2018) highlighted that unregulated and poorly documented small-scale fisheries were dominant, accounting for 75-85% of marine landings. Additionally, interactions between these fisheries and elasmobranchs were found to be common (as both incidental and targeted catch). Statistics from the review showed that in Seychelles specifically, even though fishing for elasmobranchs had been ongoing since the 1920s (primarily for fins), elasmobranchs only accounted for 1% of total small-scale fishery landings in 2014 (this catch includes dasyatids). However, as with the entire region, these catch statistics are likely underestimated and unreliable given the under-reporting of landings, large-scale illegal fishing and discards (Temple *et al.*, 2018). St. Joseph Atoll is largely exempt from these fisheries given it's isolation and it hosts a relatively pristine and abundant community of dasyatids. It is important to study communities under these baseline conditions as the ecological effect of a species may be dependent on the density of individuals (Estes *et al.*, 2003).

Three species are commonly encountered in this atoll, the broad cowtail ray *Pastinachus ater*, the mangrove whiptail *Urogymnus granulatus* (formerly *Himantura* *granulata*), and the porcupine ray *Urogymnus asperrimus*. As with dasyatids as a whole, research is limited on these species and available information is equivocal given the confusion surrounding species identification and the lack of genetic information. For example, the genus *Urogymnus* was historically thought to be monotypic, containing only *U. granulatus*. However, most recent genetic analyses have included four other species into this genus, including *U. granulatus* (Last *et al.*, 2016b). There has also been considerable revision in the *Pastinachus* genus. Historically, this genus was also thought to be monotypic, containing only *P. sephen*. However, this species is now considered to occur only in the northwestern Indian Ocean, and the larger *P. ater* (formerly *P. atrus*) is considered more widespread throughout the Indo-Pacific Ocean (Last *et al.*, 2016b). The posited available information and demographics for these species are summarised in Table 1.1.

My MSc thesis investigated the trophic and spatial ecology of *U. asperrimus* in St. Joseph Atoll (Elston, 2016). I found that the atoll was probably a nursery area for this species, given the high levels of site affinity displayed by juveniles. Habitat specificity in the atoll suggested predator avoidance was one of the major drivers of spatial behaviour, while thermoregulatory behaviours were identified through diel patterns of acoustic detections. Juveniles were also found to be specialist feeders on abundantly occurring polychaetes. The present thesis is an expansion of this work to incorporate data collected from all three dasyatid species and to expand the ecological understanding to a community level.

Table 1.1: Available information for three species of dasyatid occurring in St. Joseph Atoll (taken from Last *et al.*, 2016a). All size estimates are disc-widths (cm).

Species	**Range**	**Size-at-birth**	**Size-at-maturity**	**Maximum size**	**Prior literature**	**IUCN Red List status**
Pastinachus ater	Indo-Pacific ocean	18		200	Semeniuk & Dill, 2005 Semeniuk & Dill, 2006 Vaudo & Heithaus, 2009 Vaudo & Heithaus, 2011 Vaudo & Heithaus, 2012 O'Shea *et al*., 2013	Least concern
Urogymnus granulatus	Indo-Pacific ocean	14 - 28	55 - 65 (male)	141	Davy *et al*., 2015	Vulnerable
Urogymnus asperrimus	Indo-Pacific ocean		100 (female) 90 (male)	115	Theiss *et al*., 2010 O'Shea *et al*., 2013 Cerutti-Pereyra *et al*., 2014 Chin *et al*., 2014	Vulnerable

1.5 Thesis aims and structure

The overall aim of this thesis is to advance the understanding of the spatial and trophic ecology of a sympatric dasyatid community using data collected from the relatively pristine location of St. Joseph Atoll. This information can be utilised to gain a better understanding of the ecological roles of dasyatids when they occur sympatrically, while also providing relevant information for potential management or conservation initiatives. The chapters are set out as follows:

Chapter 2 provides a description of the study site and general methods and materials used to avoid repetition throughout the thesis.

Chapter 3 utilises a long-term passive acoustic telemetry data set to determine which ontogenetic stages of two dasyatid species (*P. ater* and *U. granulatus*) are resident to St. Joseph Atoll, whether dispersal is evident, and the extent of space that is utilised within the atoll and how this varies seasonally. This is used to provide support for the hypothesis that St. Joseph Atoll is a dasyatid nursery.

Chapter 4 utilises the complementary techniques of stomach content and stable isotope analyses to determine the dietary compositions and trophic levels of *P. ater* and *U. granulatus*. Data from *U. asperrimus* is included to determine whether diet differs significantly intra- and inter-specifically, providing insights into potential trophic resource partitioning. Important carbon pathways for dasyatid food chains are also identified.

Chapter 5 investigates whether dasyatids display any habitat selectivity within the atoll and whether they differ in their habitat utilisation inter- and intra-specifically. This informs whether dietary differences identified in the previous chapter are potentially driven by habitat partitioning.

Chapter 6 investigates whether daily residency patterns to the atoll and habitat use within the atoll are effected by physical environmental variables such as temperature, rainfall and tidal height.

Chapter 7 provides a general discussion to synthesise the information from every chapter and to discuss the significance of these findings in terms of ecology and conservation.

Chapter 2

General methods and materials

This chapter provides an overview of the methods and materials that were used across multiple chapters. Included are: a description of the study site, preliminary estimates of dasyatid abundance, an explanation of the capture and handling of dasyatids, a summary of the acoustic receiver array and transmitters used as well as individuals tagged, and finally an overview of shared statistical analyses.

2.1 Study Site

All fieldwork for this thesis was conducted at the remote St. Joseph Atoll, Republic of Seychelles, South West Indian Ocean (5.43° S, 53.35° E) (Figure 2.1). The atoll forms part of a small chain of outer islands known as the Amirantes Group, situated on the Amirantes Bank. The bank is a relatively shallow plateau with an approximate area of 3220km^2 and is characterized by depths of mostly less than 40m. It is surrounded by open ocean with depths in excess of 2000m. St. Joseph Atoll (area of $\sim$22.5km^2) comprises 16 small uninhabited islands (1.4km^2) atop an uninterrupted reef flat (17.7km^2) that completely encloses a central shallow lagoon (4.8km^2) of 3 - 9m depth (Stoddart *et al.*, 1979). The reef flats are dominated by large areas of sand flats and are scattered with seagrass beds (largely *Thalassia hemprichii* and *Thalassendron ciliatum*) (Figure 2.2). The lagoon is mostly comprised of a sandy benthos with numerous large poriitid and flaviid coral outcrops that rise to the surface.

This tropical region experiences two seasons: the south-east (SE) monsoon from April - November and the north-west (NW) monsoon from December - March. The SE monsoon experiences strong persistent south-easterly winds with lower precipitation and temperatures. The NW monsoon has only light north-westerly winds but with higher temperatures and precipitation. A mixed semi-diurnal tidal cycle is present with two high tides and two low tides of different heights every lunar day. Within the atoll there is a maximum tidal range of approximately 2.2m at spring tide and a minimum of 1.6m at neap tide. There is no tidal channel in the atoll rather, water spills in and out over the entire expanse of reef flats as the tide rises and ebbs. Thus, at low tides the flats may be exposed or be covered with very shallow water (10-30cm depth), while at high tides the flats can be covered by up to 2m of water.

A variety of detailed local environmental variables were recorded for the duration of this study. A weather station (Davis Vantage Pro2) was situated on the neighbouring D'Arros Island (approximately 1km west of St. Joseph Atoll) to record weather parameters. Each acoustic receiver (see details of array below) was coupled with a sea temperature logger (HOBO Water Temperature Pro v2 Data Logger, Onset, Bourne, USA) to record ocean water temperatures. Loggers were set to record the temperature at 10 minute intervals. A pressure logger (HOBO Water Level Data Logger, Onset, Bourne, USA) was deployed in the lagoon of the atoll to record water pressure for a six-month period. The tidal height in meters was then calculated using the known depth of the logger. Tidal cycles were modelled using the Oregon State University Tidal Model Driver (Egbert & Erofeeva, 2002) using the harmonics for St. Joseph Atoll. The model predicted accurate tidal heights for the atoll in 10 minute intervals for the entire study period (predictions differed only by 1.96% compared to the logger recordings) (Lea, 2017).

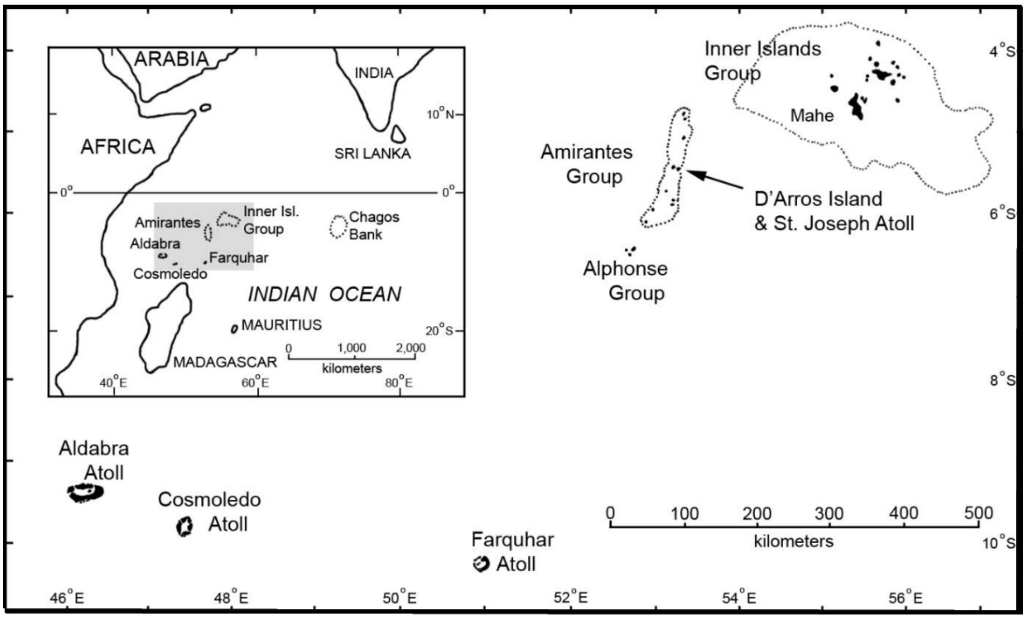

Figure 2.1: Geographic location of St. Joseph Atoll and neighbouring D'Arros Island, Republic of Seychelles, South West Indian Ocean (after Filmalter *et al.* 2013)

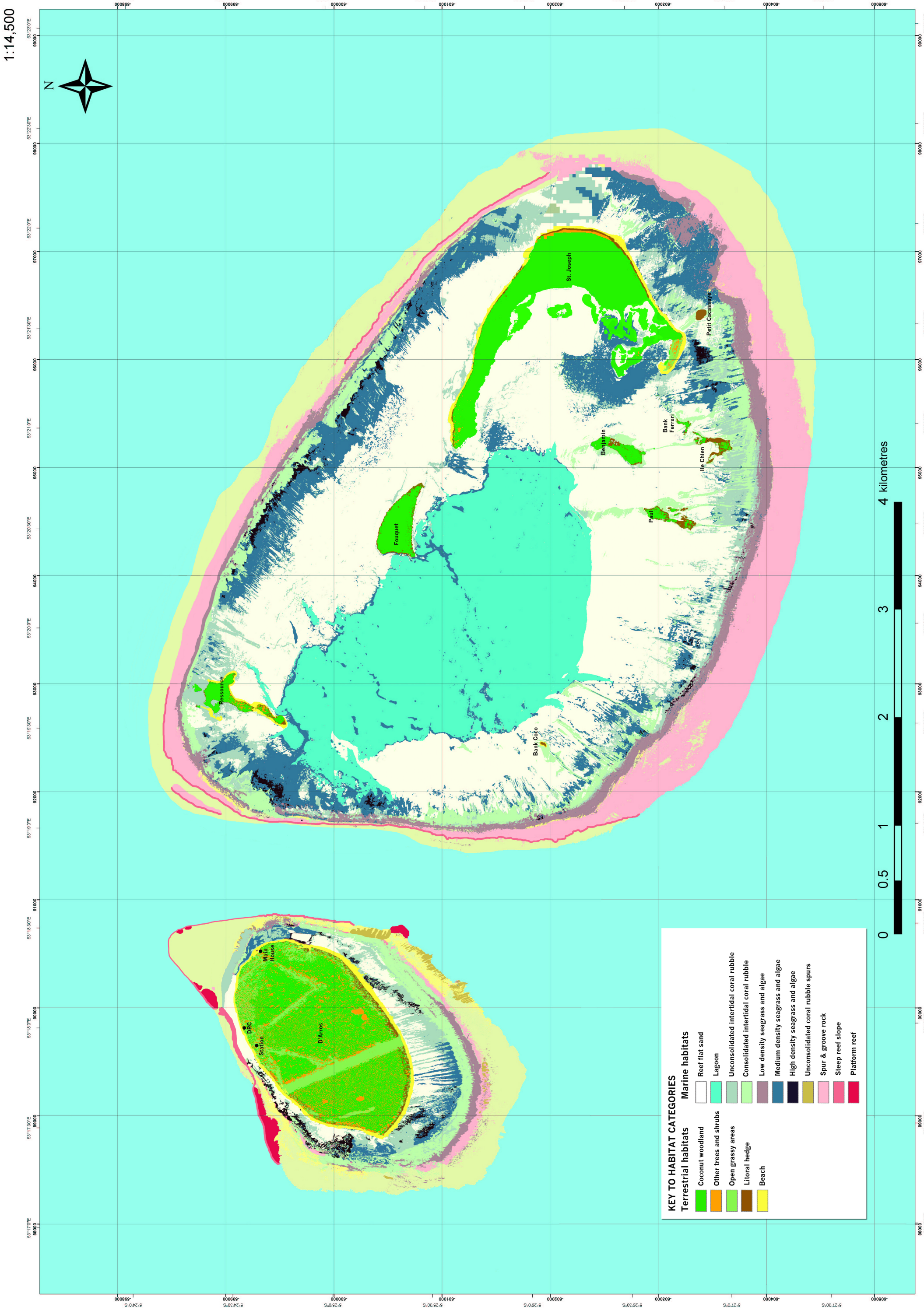

Figure 2.2: Detailed habitat map of St. Joseph Atoll and neighbouring D'Arros Island. Data collected by the Cambridge Coastal Research Unit and produced by Spencer *et al.* (2009)

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2.2 Preliminary dasyatid abundance

Prior to this study, only anecdotal evidence suggested that dasyatids were abundant at St. Joseph Atoll. Therefore, surveys were conducted to provide preliminary estimates of dasyatid abundance. Six equidistant parallel transect lines were delineated running from north to south across the expanse of the atoll (Figure 2.3). Two surveys were conducted whereby the transects were traversed by a small motorized skiff in August 2015. Transects were completed during mid- to high-tide to allow the skiff to maneuver across the reef flats. Transects were restricted to the reef flats of the atoll given their shallow nature and high water visibility that allowed for the easy spotting and identification of a batoid from the skiff. In addition, individuals could be easily identified before the initiation of their flight response. If a batoid was encountered within 10m either side of the transect line, a GPS waypoint was taken, the water depth was measured using a depth stick, the species identification was recorded as well as the approximate size (disc-width DW) of the individual (small $<$ 50cm DW, 50cm $<$ medium $>$ 100cm DW, large $>$ 100cm DW).

A total of 64 and 61 dasyatids were encountered on the first and second surveys respectively, resulting in a mean density of 342 dasytids/km^2. Multiplying this density with the area of the reef flats results in an approximate abundance of 6000 dasyatids occurring in this habitat. Only three species were encountered - *P. ater*, *U. granulatus* and *U. asperrimus*. In both surveys *U. granulatus* were encountered most frequently and *U. asperrimus* with the least frequency (an outcome that may be attributed to their camouflaged colour). Dasyatids were encountered on every transect line, barring the eastern most line on the fringe of the atoll, suggesting that they occur throughout the reef flats (Figure 2.3). Medium-sized individuals were the most common for all three species (92%, 98% and 63% for *U. asperrimus*, *U. granulatus*, and *P. ater* respectively). For *U. asperrimus*, no individuals over 100cm (adults) were encountered while for *U. granulatus*, only one large individual and no small individuals were encountered. Dasyatids were encountered in a range of depths from 30cm - 130cm (mean of 70cm).

The density of dasyatids calculated from these surveys is slightly higher, but similar, to densities of southern stingrays *Dasyatis americana* found at a Caribbean atoll (Tilley & Strindberg, 2013) (342 individuals/km^2 and 245 individuals/km^2, respectively). Regardless, it is important to note the preliminary nature of the results presented here; more transects would be needed at different tidal heights, at different times during the day and in each of the two seasons before estimates can be reliable. However, the similarity of results for both the transects, and to previously published atoll densities, suggests a level of accuracy sufficient enough for the purposes of deducing that a healthy population of dasyatids were present in the atoll, making it an ideal location to study the trophic and spatial dynamics of a sympatric dasyatid community.

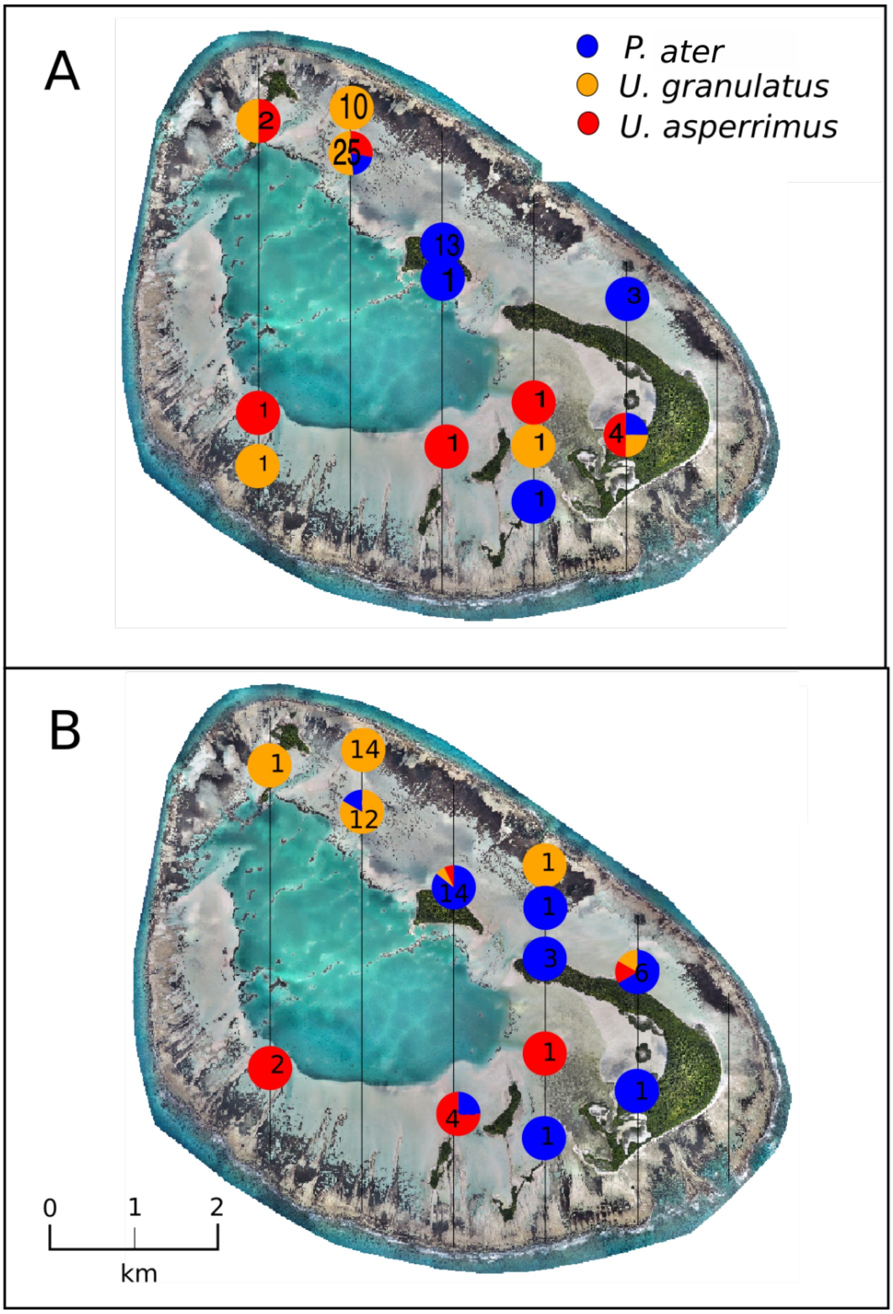

Figure 2.3: Dasyatid abundance recorded on the first (A) and second (B) transect surveys in August 2015. Black lines represent the transect lines. Numbers within the pie charts represent the total number of dasyatids encountered in each location.

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2.3 Dasyatid capture and handling procedure

Dasyatids were captured during daylight hours on the reef flats of St. Joseph Atoll using one of three methods. Larger *U. granulatus* and *P. ater* individuals (>50cm DW) were spotted from a motorised boat or on foot and were foul-hooked using a weighted treble-hook and line. Smaller individuals of these two species (<50cm DW) were caught using dip and cast nets in shallow (<30cm) water. Once an individual was captured, a person would carefully grab the tip of the tail and then place the other chainmail-gloved hand around the tail and spine to hold the latter flat against the former. This person would hold the spine against the tail for the duration of the dasyatid handling to ensure the safety of those working on the dasyatid. Given that *U. asperrimus* lack a spine on their tail, they were captured by hand. Individuals were approached from behind until close enough to lunge and place a gloved hand around the base of the tail.

Once individuals were caught and and spines restrained (if necessary), they were placed ventral-side up on a water-filled working surface (a sit-on-top recreational kayak with plugged drainage holes was used) to ensure continued respiration and minimal stress throughout procedures. Individuals were sexed based on presence of claspers and DW was measured to the nearest cm using callipers. Maturity was assessed based on levels of clasper calcification in males and on available estimates of size-at-maturity. Date, time and locality (using a hand-held GPS) of each capture was also recorded.

Acoustic transmitters (see below for details) were surgically implanted into the abdomens of individuals by making a small (∼3cm) incision with a scalpel through the lining of the abdominal cavity. Incisions were subsequently closed using two to three independent sutures (Supramed). The average surgery time was three minutes.

All captured individuals were released as close as possible to capture locations, and were monitored to determine the short-term post-release health of the individual. All individuals resumed normal behaviour upon release and no predation events were witnessed.

2.4 Passive acoustic telemetry

To meet the objectives of chapters 3, 5 and 6 of this thesis, passive acoustic telemetry was used to track the movements of dasyatids. Acoustic tracking has numerous advantages: acoustic signals are ideal in the marine realm as they can transmit with low energy loss through seawater (Nelson, 1976), equipment costs are low, and movements can be tracked over varying spatial and temporal scales (Heupel *et al.*, 2006). Passive acoustic tracking, i.e. the automation of tracking through the use of *in situ* receivers that continuously listen for signals, is advantageous compared to active tracking as multiple individuals can be monitored simultaneously for long periods of time with minimal labour (Heupel *et al.*, 2006). Therefore, arrays of permanent listening stations have become popular as they can detect the presence of tagged individuals within the detection radius, and the locations of multiple receivers can be used to reconstruct animal movements retrospectively (Voegeli *et al.*, 2001).

An array of 88 acoustic receivers located in St. Joseph Atoll, on reefs surrounding the atoll, and further along the Amirantes Bank was used to passively monitor the movements of dasyatids over a four-year period (Figure 2.4). In deeper waters, VR2W acoustic receivers (Vemco Ltd, Bedford, Canada) were moored to concrete blocks using steel chain and line and were held 1-2m off the ocean bottom with a float. On the reef flats, receivers were attached to concrete blocks and moored on the substrate. Each receiver was retrieved every few months to download detection data and batteries were replaced annually. Seventy of these receivers were deployed between August and November 2012, prior to the start of this study. The remaining receivers were deployed a few months subsequent to the first tagging trip, between August and November 2014. The addition of these receivers was shown to effect the results obtained from analysing the movement data for *U. asperrimus* (Elston, 2016) therefore, only detection data recorded subsequent to November 2014 was analysed throughout the thesis, unless otherwise stated.

Detections of acoustic transmissions by receivers are often affected by the distance between the receiver and the transmitter, physical factors such as temperature and wind speed, and biological sources such as alphaeid shrimps (Heupel *et al.*, 2006).

Given the shallow nature of the reef flats of the atoll, the effects of physical factors on receiver efficiency were likely to be more pronounced. Therefore, prior range testing has been conducted in this habitat, see Elston (2016) for details. In brief, results revealed that the probability of detection decreased with increasing distance from the receiver, but detections up to 300m were still recorded. Increasing wind speeds significantly reduced the probability of detection, tidal heights only influenced detection probability at distances greater than 200m, and temperature had negligible effects on detection probability (Elston, 2016).

Depending on the type of acoustic transmitter, battery life can span from days to years. A mixture of Vemco V9, V13 and V16 transmitters (depending on the size of the tagged individual) with various nominal delays (Vemco Ltd, Bedford, Canada) were used in this study, resulting in battery lives ranging from 196 days to 10 years (Table 2.1). Sixty dasyatids were surgically equipped with acoustic transmitters, 20 from each of the three species, during two field trips (March 2014 and May 2015) (Figure 2.4). These individuals consisted of both males and females and spanned the range of sizes present in the atoll (consisting largely of juveniles and a few adults) (Table 2.1). Telemetry data collected for *U. asperrimus* had already been analysed (Elston, 2016), but this data was included in chapters 5 and 6 to provide new community-level ecological insights.

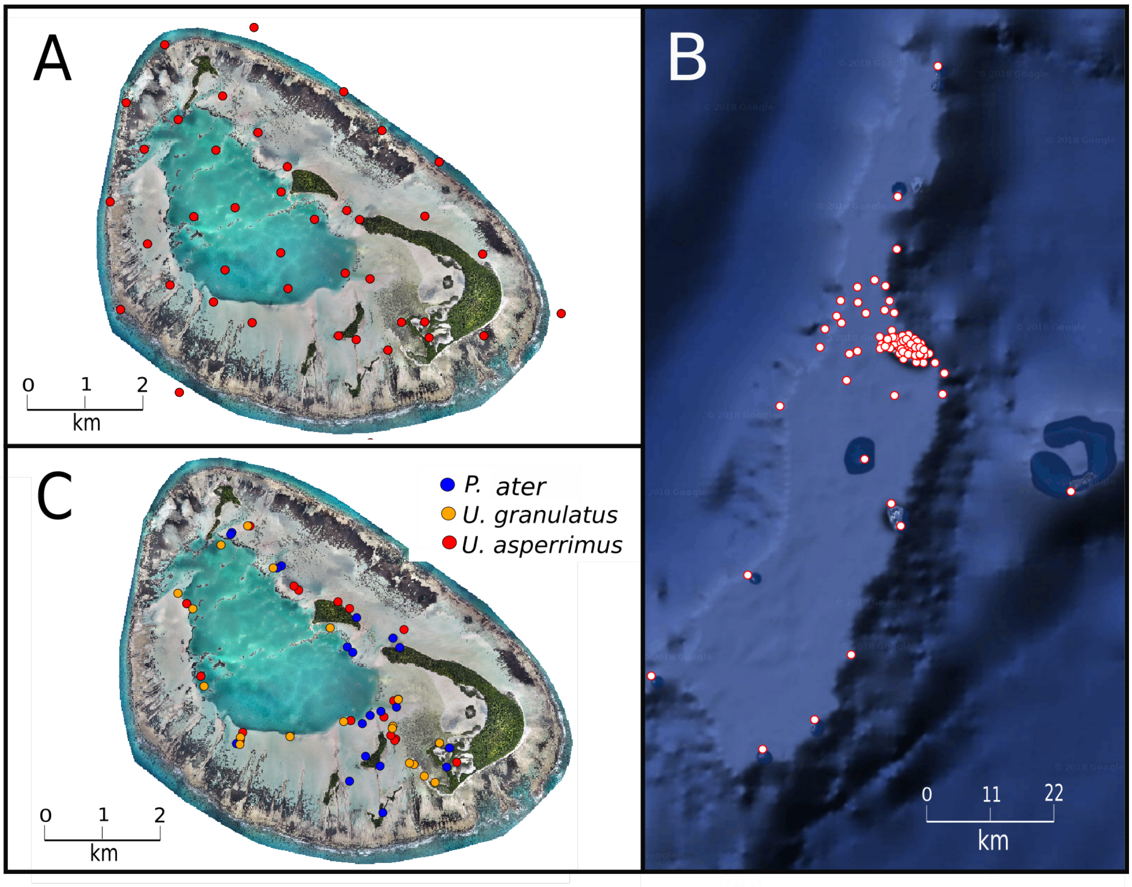

Figure 2.4: Maps of the distribution of acoustic receivers in St. Joseph Atoll (A) and across the Amirantes Bank (B), and of the acoustic tagging location of 60 dasyatids in St. Joseph Atoll where colour of the nodes represents each species (C)

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Table 2.1: Details of acoustically tagged dasyatids

Ray no.	Tag type	Tag life (days)	Tag ping frequency (secs)	Species	Date tagged	DW (cm)	Sex	Life history stage
1	V13	1582	80-160	*P. ater*	13/05/2015	49	f	Juvenile
2	V13	1582	80-160	*P. ater*	20/05/2015	54	m	Juvenile
3	V13	1582	80-160	*P. ater*	13/05/2015	55	f	Juvenile
4	V13	1582	80-160	*P. ater*	14/05/2015	56	m	Juvenile
5	V13	1582	80-160	*P. ater*	15/05/2015	58	m	Juvenile
6	V13	1582	80-160	*P. ater*	19/05/2015	60	m	Juvenile
7	V13	1582	80-160	*P. ater*	12/05/2015	61	m	Juvenile
8	V13	1582	80-160	*P. ater*	14/05/2015	61	m	Juvenile
9	V13	1582	80-160	*P. ater*	14/05/2015	62	f	Juvenile
10	V13	1582	80-160	*P. ater*	13/05/2015	66	f	Juvenile
11	V16	3650	60-120	*P. ater*	13/05/2015	73	m	Juvenile
12	V16	3650	60-120	*P. ater*	19/05/2015	73	m	Juvenile
13	V16	3650	60-120	*P. ater*	12/05/2015	74	m	Juvenile
14	V16	3650	60-120	*P. ater*	12/05/2015	76	f	Juvenile
15	V16	3650	60-120	*P. ater*	18/05/2015	77	f	Juvenile
16	V16	3650	60-120	*P. ater*	18/05/2015	81	f	Juvenile
17	V16	3650	60-120	*P. ater*	20/05/2015	84	f	Juvenile
18	V16	3650	60-120	*P. ater*	19/05/2015	89	f	Juvenile
19	V16	3650	60-120	*P. ater*	19/05/2015	103	f	Adult
20	V16	3650	60-120	*P. ater*	21/05/2015	104	m	Adult
21	V9	196	20-60	*U. granulatus*	15/03/2014	30	m	Juvenile
22	V9	196	20-60	*U. granulatus*	16/03/2014	33	f	Juvenile
23	V9	196	20-60	*U. granulatus*	16/03/2014	38	m	Juvenile
24	V13	151	30-60	*U. granulatus*	16/03/2014	46	m	Juvenile
25	V13	1118	80-160	*U. granulatus*	10/03/2014	47	m	Juvenile
26	V13	151	30-60	*U. granulatus*	16/03/2014	47	m	Juvenile
27	V13	1118	80-160	*U. granulatus*	10/03/2014	55	f	Juvenile
28	V13	1118	80-160	*U. granulatus*	10/03/2014	58	m	Juvenile
29	V16	2800	40-80	*U. granulatus*	11/03/2014	63	m	Juvenile
30	V13	1118	80-160	*U. granulatus*	14/03/2014	67	f	Juvenile
31	V13	1118	80-160	*U. granulatus*	16/03/2014	67	m	Juvenile
32	V16	3650	60-120	*U. granulatus*	19/05/2015	68	m	Juvenile
33	V16	3650	60-120	*U. granulatus*	19/05/2015	70	f	Juvenile
34	V13	1118	80-160	*U. granulatus*	14/03/2014	71	m	Juvenile
35	V16	2800	40-80	*U. granulatus*	11/03/2014	73	f	Juvenile
36	V16	3650	60-120	*U. granulatus*	18/05/2015	73	m	Juvenile
37	V16	2800	40-80	*U. granulatus*	14/03/2014	74	f	Juvenile
38	V16	2800	40-80	*U. granulatus*	14/03/2014	77	m	Adult
39	V16	2800	40-80	*U. granulatus*	11/03/2014	80	m	Adult
40	V16	2800	40-80	*U. granulatus*	14/03/2014	81	m	Adult
41	V13	1582	80-160	*U. asperrimus*	14/05/2015	53	f	Juvenile
42	V13	1582	80-160	*U. asperrimus*	15/05/2015	56	f	Juvenile
43	V13	1118	80-160	*U. asperrimus*	10/03/2014	58	m	Juvenile
44	V13	1118	80-160	*U. asperrimus*	11/03/2014	59	f	Juvenile
45	V13	1118	80-160	*U. asperrimus*	11/03/2014	59	m	Juvenile
46	V13	1118	80-160	*U. asperrimus*	14/03/2014	59	m	Juvenile
47	V16	3650	60-120	*U. asperrimus*	14/05/2015	59	m	Juvenile
48	V13	1582	80-160	*U. asperrimus*	12/05/2015	59	m	Juvenile
49	V16	1018	50-130	*U. asperrimus*	11/03/2014	60	f	Juvenile
50	V16	1018	50-130	*U. asperrimus*	10/03/2014	61	f	Juvenile
51	V16	1018	50-130	*U. asperrimus*	11/03/2014	62	m	Juvenile
52	V16	1018	50-130	*U. asperrimus*	11/03/2014	63	m	Juvenile
53	V16	1018	50-130	*U. asperrimus*	10/03/2014	63	m	Juvenile
54	V16	1018	50-130	*U. asperrimus*	11/03/2014	63	f	Juvenile
55	V16	3650	60-120	*U. asperrimus*	12/05/2015	65	m	Juvenile
56	V16	1018	50-130	*U. asperrimus*	10/03/2014	66	m	Juvenile
57	V16	3650	60-120	*U. asperrimus*	13/05/2015	67	m	Juvenile
58	V16	1018	50-130	*U. asperrimus*	10/03/2014	69	m	Juvenile
59	V16	1018	50-130	*U. asperrimus*	10/03/2014	73	f	Juvenile
60	V16	3650	60-120	*U. asperrimus*	14/05/2015	114	f	Adult

2.5 Statistical analyses

All statistical analyses throughout the thesis were conducted in R (R development core team 2013, version 3.1.4). A significance level of $p < 0.05$ was adopted for all relevant analyses.

A modelling approach was used in some of the telemetry data analyses. Given that acoustic detections are recorded for the same individuals through time, this violates the assumption of independence required for many statistical analyses. Generalised linear mixed models (GLMMs) are able to cope with this non-independence by including the individual as a random factor within models (Bolker *et al.*, 2009). An information theoretic model selection procedure was followed. In this approach, p values are not used to determine whether models are significantly better than the null model, rather models are compared against each other to highlight the best fit models. This approach is beneficial because it penalises more complex models, it allows for the simultaneous comparisons of multiple competing models, and it allows models to be ranked (which provides the basis for model averaging if top models perform similarly well) (Bolker *et al.*, 2009). In each instance, a global model was developed that included all relevant effects of interest using the 'glmer' function from the lme4 library (Bates, 2010). The 'dredge' function in the MuMIn package was then used to create subsets of models with all possible combinations of the chosen factors. Akaike's information criterion corrected for small-sample bias (AICc) were then used to rank the models. If top performing models had a ΔAICc < 2, model averaging was performed to produce average parameter estimates based on the top models. Cumulative AICc weights were used to evaluate the strength of evidence for each covariate within averaged models, and covariates with cumulative AICc weights > 0.5 were considered to be significant factors. Model assumptions were checked by plotting residuals and determining if models displayed over- or under-dispersal. If models were overdispersed, QAICc were then used to rank models to account for the overdispersion.

Chapter 3

An evaluation of the nursery role of St. Joseph Atoll

3.1 Introduction

The distribution of a species is largely determined by their movement and habitat use patterns and individuals will actively select habitats that provide favourable conditions (van Moorter *et al.*, 2016). What constitutes a favourable habitat may vary throughout the life history of a species (Dahlgren & Eggleston, 2000; Grubbs, 2010). For example, juveniles of species with slow reproductive cycles often make use of nursery areas to offset mortality (Branstetter, 1990) (as juvenile survival is important to their population persistence (Cortés, 2002)).

Elasmobranchs generally display k-selected life history traits (Camhi *et al.*, 1998; Dulvy *et al.*, 2014) and their use of nursery habitats has been frequently identified (reviews by Heupel *et al.* 2007; Martins *et al.* 2018) (although this is not a universal phenomenon (Knip *et al.*, 2010; Oh *et al.*, 2017)). Juveniles will select for productive and protected shallow nearshore areas in which to spend the first few months/years of their lives (Beck *et al.*, 2001). Adults, however, will have different requirements and will select for different habitats in which to exploit larger prey, occupy optimal thermal niches during pregnancy, and/or find conspecifics for mating (Gray *et al.*, 1997; Sims, 2003; Vaudo & Lowe, 2006; Wallman & Bennett, 2006; Dudgeon *et al.*, 2008; Le Port *et al.*, 2012; Jewell *et al.*, 2013). Indeed, one of the underlying principles of nursery area use is that juveniles and adults of the same species reside in and utilise different habitats (Beck *et al.*, 2001). This necessitates dispersal away from these natal areas by larger individuals and reduces competition for food resources with smaller conspecifics, which is evolutionarily beneficial (Dale *et al.*, 2011; Carrier *et al.*, 2010).

It has also been hypothesised that nurseries may provide a thermally advantageous habitat to juveniles as shallow warm waters can promote faster digestive, metabolic and growth rates (Economakis & Lobel, 1998; Dowd *et al.*, 2006). Sharks often utilise nursery areas on a seasonal basis, taking advantage of these habitats in warmer summer months (Simpfendorfer & Milward, 1993; Yokota & Lessa, 2006; Heupel, 2007; Rangel *et al.*, 2018). The limited number of studies that investigated seasonal nursery use in dasyatids showed variable trends, with both an increase and a decrease in nursery utilisation in warmer months (Vaudo & Heithaus, 2009; Cerutti-Pereyra *et al.*, 2014). Thus, there is still a lack of understanding on whether and how dasyatids use nursery areas on a seasonal basis.

This is indicative of the overall trends in elasmobranch nursery research, which is highly biased, both in terms of the species and habitat types identified (Heupel *et al.*, 2018a). The understanding of batoid nursery use is much more limited compared to that of sharks (Martins *et al.*, 2018) and, for dasyatids specifically, only a handful of studies have identified nursery areas (Yokota & Lessa, 2006; Vaudo & Heithaus, 2009; Dale *et al.*, 2011; Cerutti-Pereyra *et al.*, 2014; Davy *et al.*, 2015). This is because of a multitude of factors including the overall lack of information available on batoid populations compared to sharks, juveniles are small and benthic which contributes to their cryptic nature (Heupel *et al.*, 2018a), and they have the capacity to use complex habitats (such as mangroves) which further makes observations difficult (Davy *et al.*, 2015).

Furthermore, shallow and productive coastal areas (such as estuaries, coral reefs and sandy coastal bays) are most often the habitats identified as nurseries (Heupel *et al.*, 2018a) given their easy access for researchers. Conversely, tropical offshore atolls are often isolated and difficult to access but, they may also provide suitable nursery habitat. They frequently have productive seagrass beds (Green, 2003) which host communities of invertebrates that, in turn, can support higher trophic level animals (Heck Jr. *et al.*, 2003; Pollard & Greenway, 2013). Indeed, seagrass beds have served as elasmobranch nursery areas (Powter & Gladstone, 2009). Atolls can also provide suitable refuge habitat via shallow reef flat areas. Despite this, research in these ecosystems remains limited and only a few atoll shark nurseries have been identified (Wetherbee *et al.*, 2007; Papastamatiou *et al.*, 2010) and none yet for dasyatids.

Research that broadens the scope of elasmobranch nursery use through identifying more species that use nurseries and, the habitats that are utilised for such purposes, is required for a more comprehensive understanding of how this faunal group benefits from nursery areas (Heupel *et al.*, 2018a). Additionally, identifying and protecting these habitats may have distinct conservation benefits for vulnerable species (Kinney & Simpfendorfer, 2009).

Urogymnus asperrimus juveniles were found to be resident to St. Joseph Atoll (Elston, 2016) however, it was difficult to confirm whether the atoll was indeed a nursery habitat given the uncertainty of size-at-birth for this species and subsequent uncertainty surrounding the age of the juveniles tagged. According to Martins *et al.* (2018) the presence of specifically newborn ($<$ 1 year old) individuals is an important requirement for the classification of a nursery. The overall aim for this chapter was to evaluate the role of St. Joseph Atoll as a nursery for *Pastinachus ater* and *U. granulatus*. To delineate a batoid nursery, newborn individuals need to be commonly encountered in a specific area for extended periods of time (Martins *et al.*, 2018). The specific objectives of this chapter included:

- Calculating the long-term residency levels for different ontogenetic stages of *P. ater* and *U. granulatus* to St. Joseph Atoll and determining whether individuals dispersed from this ecosystem
- Determining the spatial extent of the atoll utilised by individuals and whether this varied seasonally

3.2 Methods and materials

Data collected from the passive acoustic monitoring of dasyatids, as described in Chapter 2, were used in this chapter. Analyses were conducted on the data collected between December 2014 - November 2017. For *U. granulatus*, data collected before this period were also analysed separately because detections for the majority of individuals ceased before December 2014. Detections from receivers that were installed during the period (see Chapter 2 for details) were excluded. Analyses on detections of *U. granulatus* prior and subsequent to December 2014 were referred to as *U. granulatus* I and *U. granulatus* II, respectively, throughout the results.

3.2.1 Residency and dispersal

An individual was considered present in St. Joseph Atoll if it was detected two or more times on a receiver in or on the fringe reef of the atoll. A residency index (RI) was calculated for each individual, where:

RI = no. of days detected / no. of days monitored

The number of days monitored was taken as the period from when an individual was tagged to the last date of detection in the atoll. Values ranged from 0 - 1, with lower values representing lower levels of residency and vice-versa. A Mann-Whitney U test was used to determine whether residency significantly differed between sexes and a Pearson's correlation coefficient was used to determine if residency was significantly correlated with size of individuals.

Dispersal was investigated by determining if residency, averaged for all individuals, decreased with time by correlating monthly RI values (no. of days detected in each month / total no. of days in each month) against time at liberty. Detections on receivers on the Amirantes Bank were also investigated to determine if individuals were detected outside of the atoll once detections in the atoll ceased. To determine whether dispersal was related to the size of an individual, a correlation between disc-width (DW) and the number of days until an individual's final detection in the atoll (which was assumed to be the time of dispersal) was performed.

3.2.2 Space use and the effect of seasonality

Individuals displayed very restricted movements and were only detected on a mean of two receivers per day. As such, traditional estimates of space use, such as kernel utilisation distributions and home range sizes, could not be utilised. Therefore, to provide an understanding of the extent of horizontal movements, the distance between an individual's tagging location and each receiver it was detected on, was calculated. These distances were then grouped into 1km intervals, and the percentage of detections recorded in each distance interval (for all individuals) was calculated. This was done separately for the north-west (NW) and south-east (SE) monsoon seasons.

The extent of the atoll utilised was further investigated using network analyses (Ledee *et al.*, 2015). Treating acoustic arrays as networks is becoming increasingly popular in literature as it not only provides an understanding of which receivers are utilised in an array, but it also highlights the movements and connections between the receivers (Jacoby & Freeman, 2016). This understanding of how individuals move throughout the array allows improved insight into the movement patterns of individuals. In this approach, receivers are treated as nodes and pairs of subsequent detections at different receivers are treated as edges. Various metrics can then be calculated to describe the networks (Jacoby *et al.*, 2012a). Four network metrics were used in this chapter to provide insights into the extent of the array utilised by individuals: i) node density, which is the proportion of available nodes in the network that were utilised, ii) edge density, which is the proportion of available edges in the network utilised, iii) node centrality, which is the number of edges a node has, and iv) mean path length, which is the number of consecutive nodes connected by edges.

Networks were constructed and network metrics calculated using the R package iGraph (Csardi & Nepusz, 2006). Networks were first constructed for each individual for the entire period in which they were detected in the atoll to determine overall space use. Networks were then constructed on a seasonal basis to determine whether season had an effect on spatial utilisation of the atoll. Only edges that occurred within 24 hours of each other were considered. Generalised linear mixed models (GLMMs) were used to determine if edge and node densities significantly differed between seasons. Covariates in the models included season, time (a numeric vector indicating the number of seasons passed since time of tagging), and size and sex of the individual, given that these factors may also influence spatial use of the atoll. A binomial distribution with a logit link was chosen as both edge and node densities reflect proportions of the network utilised. Models were run separately for each species. Further details on the modelling approach can be found in Chapter 2.

3.3 Results

3.3.1 Residency and dispersal

The *U. granulatus* I group of individuals were monitored for relatively short periods (mean = 157 days, range = 11 - 264 days), given that data was only collected for 8 months and that this group excluded individuals that were detected subsequent to December 2014. Detections were sporadic, particularly for the smaller individuals (<60cm DW), and two individuals were not detected at all (Table 3.1). With the exception of one outlier (#21), juvenile RI values were generally low with a median RI of 0.22 (range = 0.02 - 0.95). The only adult in this group had an RI of 0.57. The *U. granulatus* II group of individuals and all *P. ater* individuals were monitored for longer periods, with a mean of 658 days (range = 213 - 1326) and 389 days (range = 162 - 890), respectively. Residency indices were highly variable across individuals (range = 0.04 - 1) however, most individuals displayed high levels of residency (Table 3.1). *P. ater* juveniles had a median RI of 0.75 (range = 0.12 - 1) and sub-adults/adults a median of 0.71 (range = 0.04 - 1). *U. granulatus* II juveniles had a median RI of 0.57 (range = 0.04 - 0.97) while the only adult in this group had an RI of 0.8. Residency was not significantly different between sexes for both species and tended to increase with DW, but a significant correlation was only found for *P. ater* (r = 0.47, df = 17, p = 0.04) (Figure 3.1).

There was evidence to suggest dispersal from the atoll as only four individuals (two *P. ater* and two *U. granulatus*) were still detected in the atoll at the end of the four year monitoring period, of which three were sub-adults or adults at the time of tagging. Detections for all other individuals in the atoll ceased at different times between May 2014 and February 2017 (Table 3.1). In addition, mean monthly RI significantly decreased with time for both species (r = -0.91, df = 27, $p < 0.01$ for *P. ater*; r = -0.94, df = 27, $p < 0.01$ for *U. granulatus* II) (Figure 3.2). There appeared to be no effect of size on dispersal as DW was not significantly correlated with the number of days between tagging and final detection date.

Three *P. ater* and four *U. granulatus* individuals were detected on the Amirantes bank subsequent to their last detections in the atoll (Figure 3.3). The *P. ater* individuals had multiple detections on the Amirantes Bank months apart, although only at singular locations. Detections occurred at a sand cay ~25kms south of the atoll, at Poivre atoll ~32kms south, and on the Amirantes Bak drop-off ~9kms south for each individual. One *U. granulatus* individual (#33) appeared to take up residence around D'Arros Island (~ 1km west of St. Joseph Atoll) as it was detected on multiple receivers located on the shallow reefs surrounding D'Arros for many months. The remaining *U. granulatus* individuals were each only detected for one day outside of the atoll at various locations on the Amirantes Banks (but no further than 17kms away). Interestingly, a *P. ater* individual (#15) made a long distance trip to Desnouefs, the southernmost landfall on the Amirantes Bank ~90kms away, in only four days. After being detected here for one day, the individual moved back to the St Joseph Atoll (being recorded at Poivre Atoll along the way about 30kms south of St Joseph), where it took up residency for another year (Figure 3.3).

There appeared to be a seasonal effect on dispersal as the majority of dates of last detections occurred in the warm NW-monsoon season. Eight *P. ater* individuals dispersed in the first warm season subsequent to tagging and two individuals dispersed in the second warm season subsequent to tagging. One individual (#9) fell outside of this pattern and its last detection in the atoll was in May. Similarly, for *U. granulatus* II, all three individuals that dispersed did so in the warm season. For *U. granulatus* I, a few individuals fell outside of this pattern, but given the sporadic nature of their detections, it is difficult to draw any conclusions from their data.

Table 3.1: Details of *P. ater* and *U. granulatus* individuals acoustically tagged in St. Joseph Atoll, where DW = disc width (cm), DT = date tagged, DL = date of last detection in atoll, DM = number of days monitored, DD = number of days detected, RI = residency index. * denotes individuals that were considered sub-adults/adults at the time of tagging.

Individual #	Species	DW (cm)	Sex	DT	DL	DM	DD	RI
1	*P. ater*	49	f	2015-05-13	2016-04-08	331	48	0.15
2	*P. ater*	54	m	2015-05-20	2016-04-14	330	281	0.85
3	*P. ater*	55	f	2015-05-13	2015-12-12	213	73	0.34
4	*P. ater*	56	m	2015-05-14	2017-01-27	624	73	0.12
5	*P. ater*	58	m	2015-05-15	2016-01-24	254	254	1
6	*P. ater*	60	m	2015-05-19	2016-10-30	530	320	0.6
7	*P. ater*	61	m	2015-05-12	2016-02-04	268	238	0.89
8	*P. ater*	61	m	2015-05-14	2015-12-30	230	183	0.8
9	*P. ater*	62	f	2015-05-14	2016-05-06	358	270	0.75
10	*P. ater*	66	f	2015-05-13	2016-04-15	338	117	0.35
11	*P. ater*	73	m	2015-05-13	2015-12-08	209	201	0.96
12	*P. ater*	73	m	2015-05-19	2015-12-11	206	143	0.69
13	*P. ater*	74	m	2015-05-12	2016-01-08	241	228	0.95
14	*P. ater*	76	f	2015-05-12	2016-10-19	526	253	0.48
15	*P. ater*	77	f	2015-05-18	2016-12-03	565	427	0.76
16*	*P. ater*	81	f	2015-05-18	2017-10-24	890	605	0.68
17*	*P. ater*	84	f	2015-05-20	2015-12-30	224	213	0.95
18*	*P. ater*	89	f	2015-05-19	2016-06-21	399	282	0.71
19*	*P. ater*	103	f	2015-05-19	2015-10-28	162	6	0.04
20*	*P. ater*	104	m	2015-05-21	2017-10-27	890	891	1
21	*U. granulatus*	30	m	2014-03-15	2014-07-02	109	82	0.75
23	*U. granulatus*	38	m	2014-03-16	2014-03-27	11	1	0.09
24	*U. granulatus*	46	m	2014-03-16	2014-05-11	56	15	0.27
25	*U. granulatus*	47	m	2014-03-10	2014-08-13	156	38	0.24
26	*U. granulatus*	47	m	2014-03-16	2014-04-27	42	1	0.02
27	*U. granulatus*	55	f	2014-03-10	2014-05-31	82	13	0.16
28	*U. granulatus*	58	m	2014-03-10	2014-06-12	94	4	0.04
29	*U. granulatus*	63	m	2014-03-11	2017-10-22	1321	755	0.57
30	*U. granulatus*	67	f	2014-03-14	2014-08-26	165	77	0.47
31	*U. granulatus*	67	m	2014-03-16	2014-05-05	50	1	0.02
32	*U. granulatus*	68	m	2015-05-19	2015-12-18	213	49	0.23
33	*U. granulatus*	70	f	2015-05-19	2016-10-20	520	428	0.82
34	*U. granulatus*	71	m	2014-03-14	2014-11-23	254	48	0.19
35	*U. granulatus*	73	f	2014-03-11	2014-11-30	264	208	0.79
36	*U. granulatus*	73	m	2015-05-18	2016-03-01	288	278	0.97
37	*U. granulatus*	74	f	2014-03-14	2014-11-14	245	232	0.95
38	*U. granulatus*	77	m	2014-03-14	2014-11-03	234	105	0.45
39*	*U. granulatus*	80	m	2014-03-11	2017-10-27	1326	1059	0.8
40*	*U. granulatus*	81	m	2014-03-14	2014-10-21	221	126	0.57

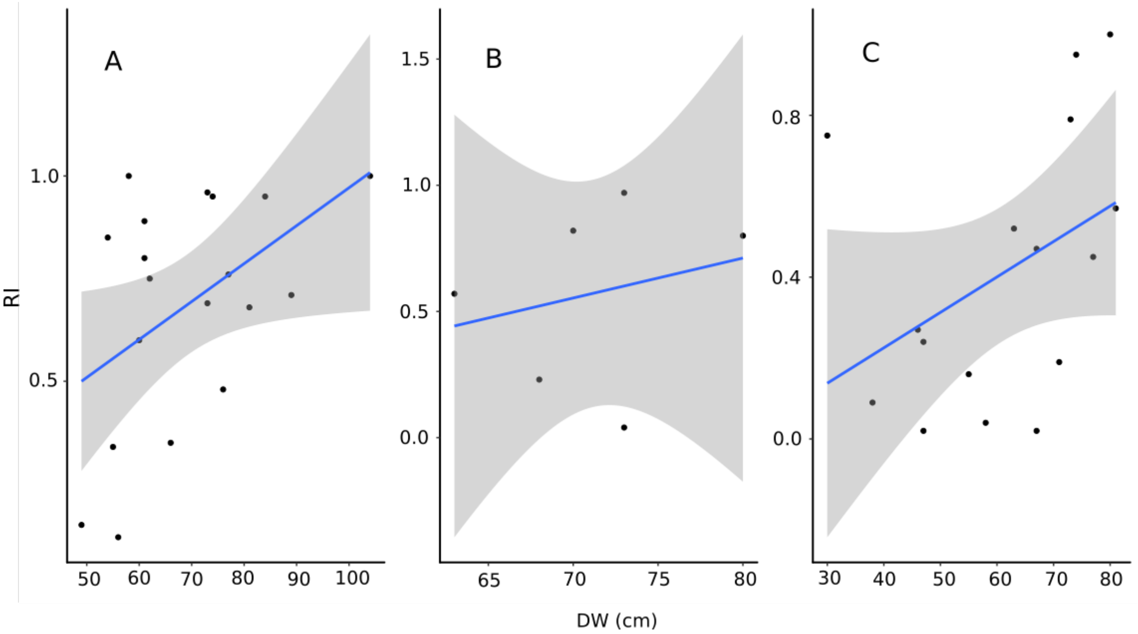

Figure 3.1: Scatterplot of residency indices (RI) against disc-width (DW) (cm) for each tagged *P. ater* (A), *U. granulatus* II (B), and *U. granulatus* I (C) individual. Grey bar represents 95% confidence intervals of regression line.

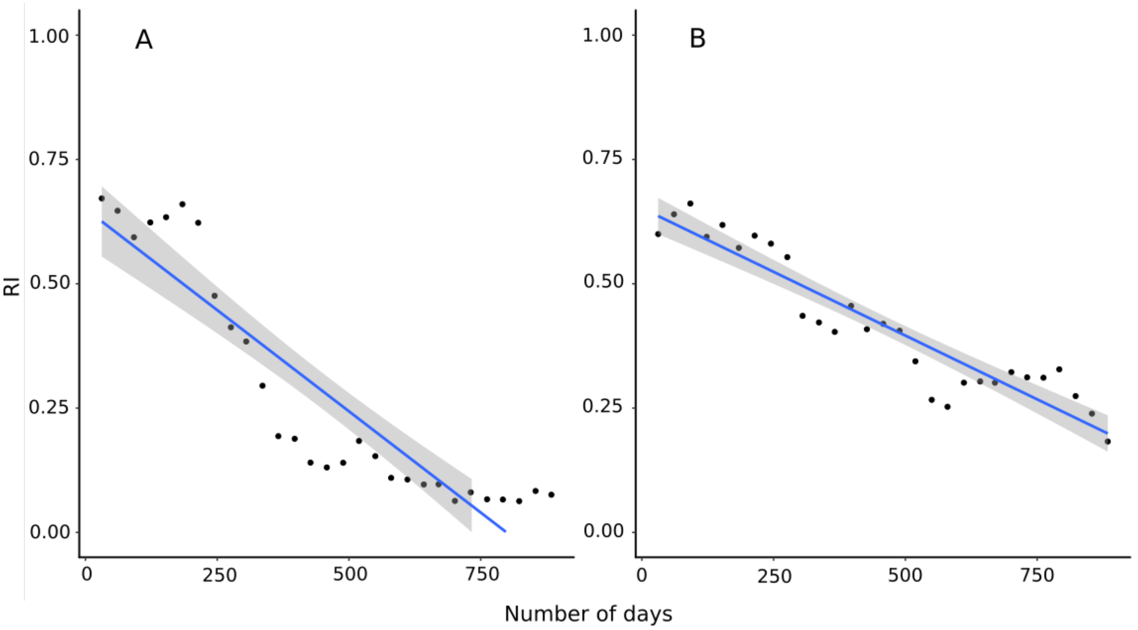

Figure 3.2: Scatterplot of monthly residency indices (RI) averaged across individuals against the number of days between acoustic tagging and the date of last detection in St. Joseph Atoll for *P. ater* (A) and *U. granulatus* II (B). Grey bar represents 95% confidence intervals of regression line.

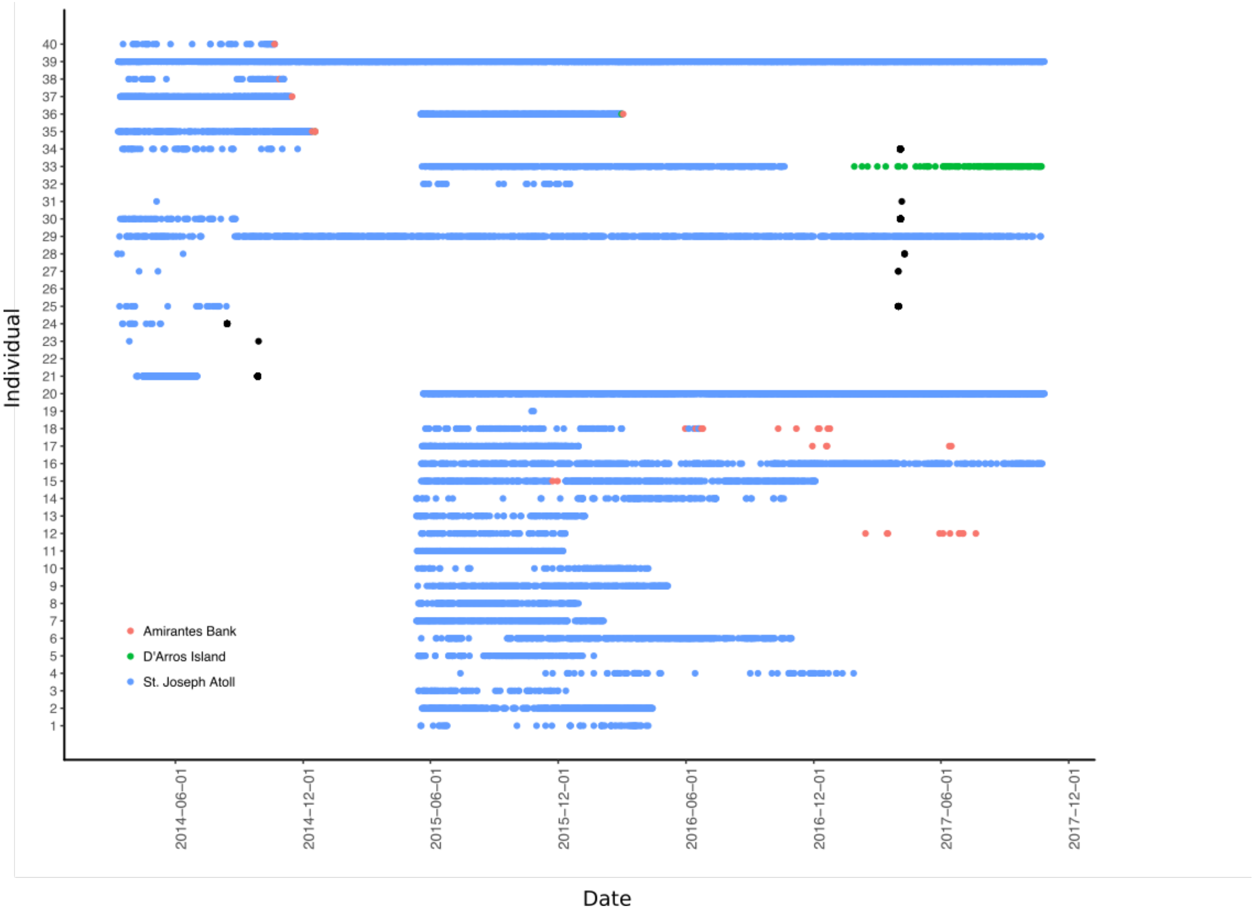

Figure 3.3: Daily presence of acoustically tagged dasyatids within St. Joseph Atoll and outside of the atoll on the Amirantes Bank and around D'Arros Island specifically. Individuals #1 - 20 are *P. ater* and #21 - 40 are *U. granulatus*. Black dots represent the estimated battery death of acoustic transmitters.

3.3.2 Space use and the effect of seasonality

While detected in St. Joseph Atoll, all tagged juveniles utilised small areas and showed fidelity to their tagging locations. The majority of detections occurred within 1km of the tagging location, particularly in the SE-monsoon season (Figure 3.4). These highly restricted movements resulted in fragmented networks (Figure 3.5) and low network metric values. Overall, *P. ater* and *U. granulatus* II utilized only 1% of available edges on average, while *U. granulatus* I utilised a mean of 0.04%. *P. ater* and *U. granulatus* II only utilised 20% of available nodes on average, *U. granulatus* I a mean of 10%. There was only a mean of 1 edge per node for *P. ater* and *U. granulatus* II, and a mean of 0.3 edges per node for *U. granulatus* I. There was a mean path length of 2 nodes for *P. ater* and *U. granulatus* II, and a mean of 1 node for U. granulatus I (Figure 3.6).

Individuals were detected in St. Joseph Atoll year round but there were significant differences in the extent of the atoll utilised between the NW- and SE-monsoon seasons. When considering the linear distances traversed by individuals, a higher proportion of detections occurred at larger distances from tagging locations in the NW- compared to the SE-monsoon season (Figure 3.4). The best fit GLMMs for network metrics included season as a significant predictor of both edge and node densities for *P. ater* (Table 3.2). Both metrics were significantly higher in the warmer NW-monsoon season. Best fit models also included the number of seasons passed and disc-width as significant predictors of network metrics, and these were both positively correlated network metrics. Models could not be run for *U. granulatus* as there was not enough data, but trends were similar.

Network maps with edges summed for individuals provided a visualisation of this increased space use in the atoll during the NW-monsoon season (Figure 3.7). There was an increased utilisation of the fringe reef and lagoon habitats during this warmer season however, season only effected the extent of movement and not the main receivers utilised by individuals. Individuals were still found to have the majority of their detections on the same nodes between seasons. There was one exception; a large majority of detections for *P. ater* #16 were in the south of the atoll around Benjamin Island in the NW-monsoon season, but most detections during the SE-monsoon were recorded in the north of the atoll around Resource Island.

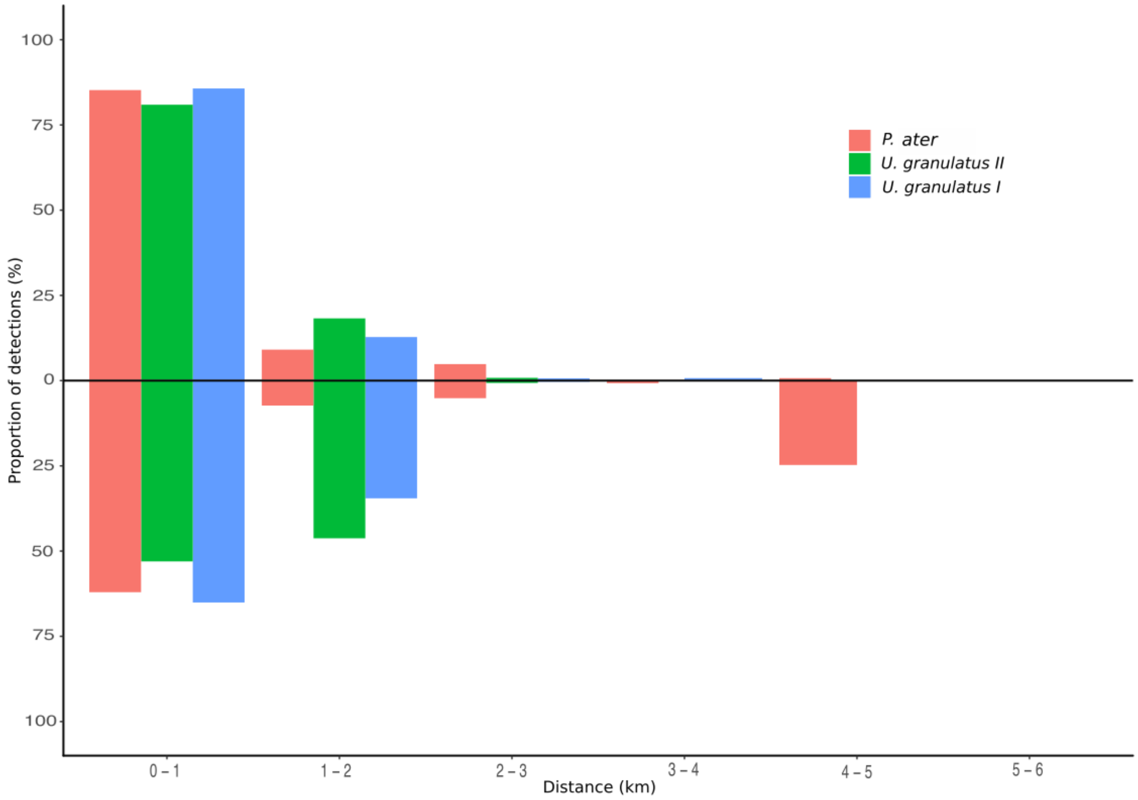

Figure 3.4: The percentage of acoustic detections recorded in each distance category from the tagging locations of dasyatids. Bars above the zero line were detections recorded in the south-east monsoon season and those below the zero line were in the north-west monsoon season. Red represents *P. ater* detections, green and blue represents detections from *U. granulatus* pre- and post- December 2014, respectively.

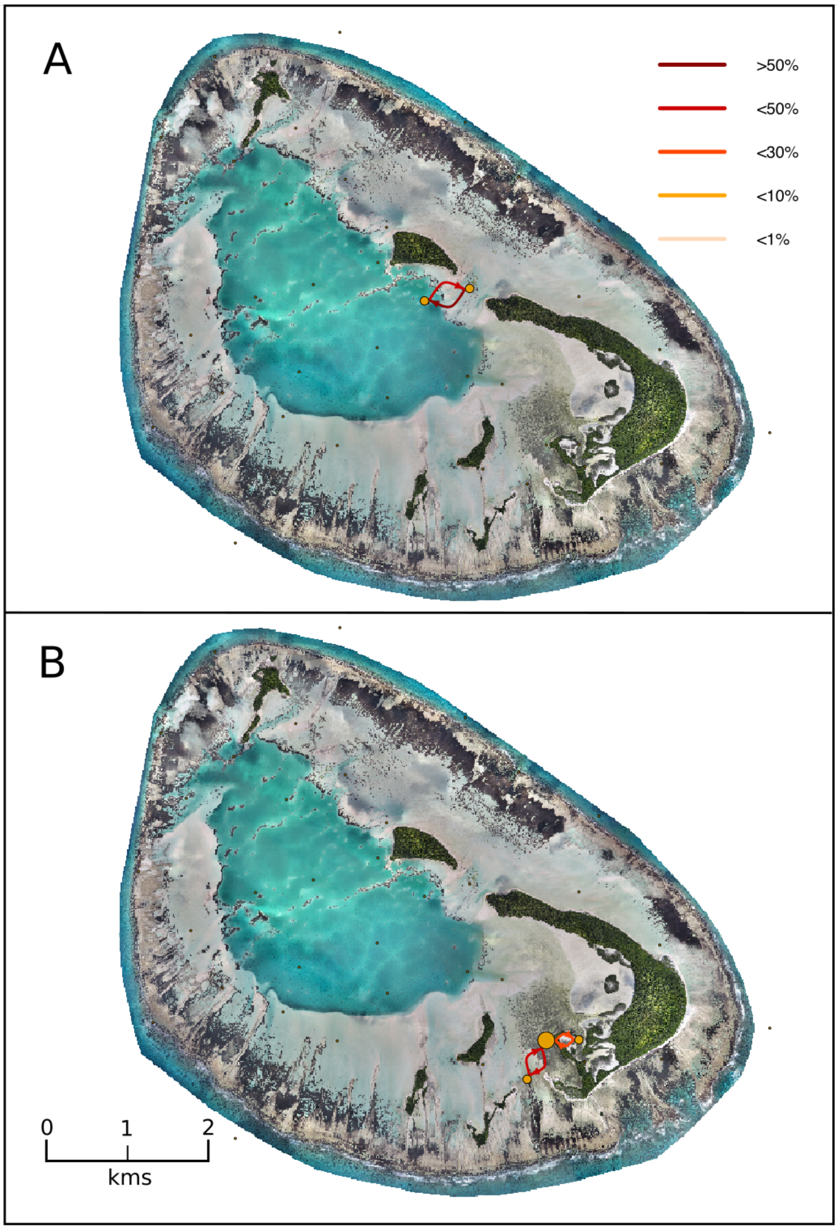

Figure 3.5: Examples of restricted receiver array usage by dasyatids, determined through network analysis. A = map of network constructed for *P. ater* individual #9 and B = map of network constructed for *U. granulatus* individual #33. Yellow dots represent nodes/acoustic receivers. The size of the node is proportional to the number of detections recorded by the receiver. Lines between nodes represent edges/movements between receivers. The colour of the edge represents the proportions of movements between receivers.

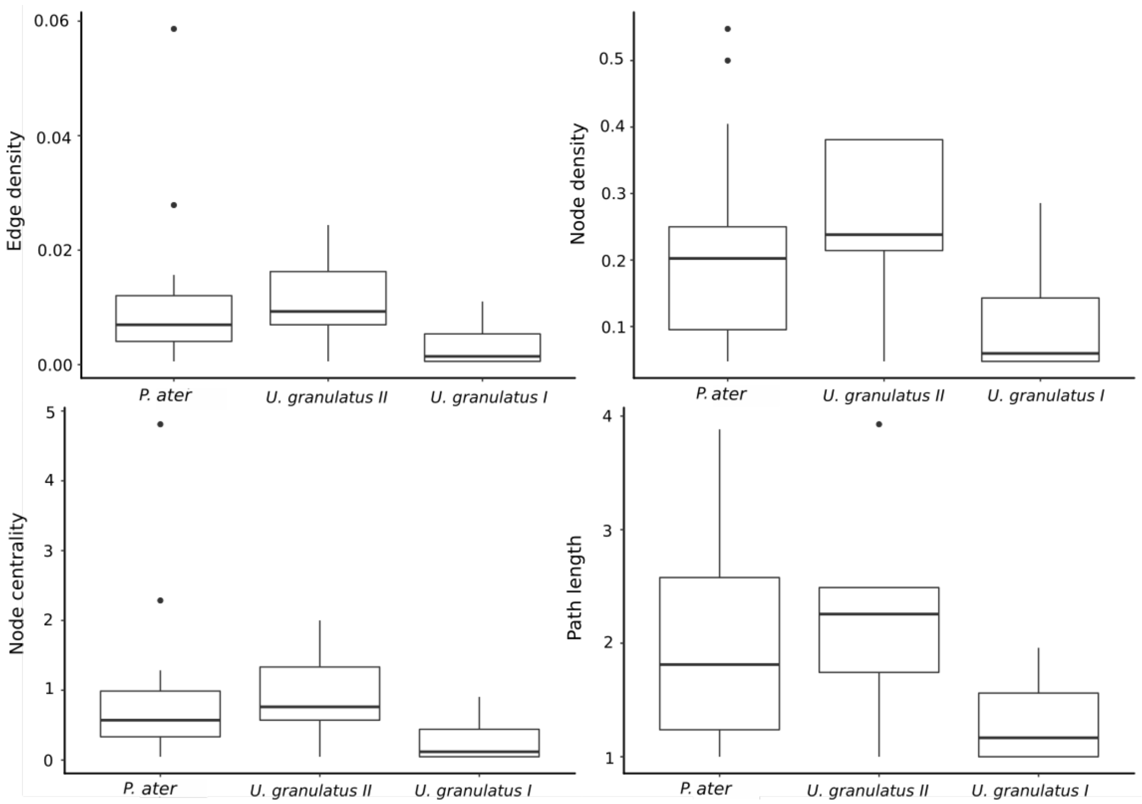

Figure 3.6: Boxplots of the calculated network metrics for each dasyatid species. Networks were constructed on acoustic detections for each tagged dasyatid for the time they were detected within St. Joseph Atoll. *U. granulatus* I and *U. granulatus* II represent detections pre- and post- December 2014. Dots represent outliers in the data.

Table 3.2: Best fit (ΔQAICc < 2) ranked generalised linear mixed models of network metrics (edge and node densities) for *P. ater*. Covariates included disc-width (DW), season, number of seasons passed since tagging, sex, and the random effect of individual dasyatid (id). Degrees of freedom (df), quasi-Akaike's information criterion corrected for small samples (QAICc), changes in in QAICc relative to the top ranked model (ΔQAICc), and the QAICc weights.

Edge density					Node density				
Model	df	QAICc	Δ QAICc	weight	Model	df	Δ QAICc	Δ QAICc	weight
~ DW + Season + (1|id)	4	131.31	0	0.72	~ DW + Season + (1|id)	4	151.68	0	0.43
~ DW + Season + No. seasons + (1|id)	5	133.17	1.86	0.28	~ DW + Season + No. seasons + (1|id)	5	151.88	0.2	0.39
					~ DW + No. seasons + Season + (1|id)	4	153.47	1.79	0.18

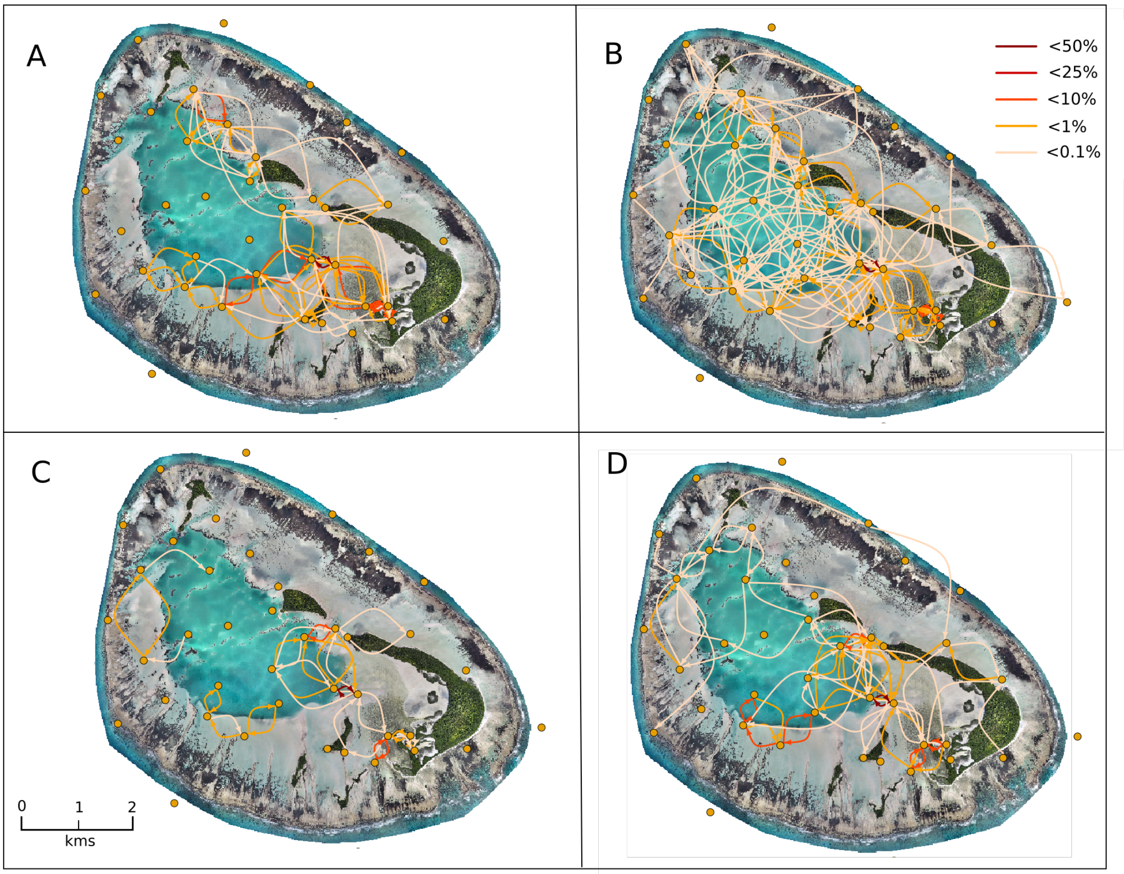

Figure 3.7: Seasonal networks constructed for acoustically tagged dasyatids (networks summed for all tagged individuals). A = network for *P. ater* in the south-east monsoon season, B = network for *P. ater* in the north-west monsoon season, C = network for *U granulatus* in the south-east monsoon season, and D = network for *U granulatus* in the north-west monsoon season. Yellow dots represent nodes/acoustic receivers. Lines between nodes represent edges/movements between receivers. The colour of the edge represents the proportions of movements between receivers.

3.4 Discussion

Nursery identification

According to Martins *et al.* (2018), to delineate a batoid nursery, newborn individuals (<1 year old) i) need to be more commonly encountered than in other areas, ii) need to remain or return to the area for extended periods, and iii) need to repeatedly use the area across multiple years. This chapter provides data to support all three criteria. By having an acoustic array with receivers both inside and outside the atoll, tagged dasyatids could be shown to be largely reliant on St. Joseph Atoll, with the number of detections outside of this area negligible. Long-term site affinity was also evident as the majority (78%) of *P. ater* and *U. granulatus* individuals were detected in the atoll for periods longer than 6 months, and approximately half (55%) were detected for periods longer than one year. During these periods of detection, residency was variable across individuals, but generally high, and most were detected for more than 50% of days monitored.

Similar levels of site affinity were found for *U. asperrimus* juveniles in St. Joseph Atoll (Elston 2016) however, it was difficult to classify these juveniles as newborn because there are no size-at-birth estimates for this species. Conversely, these estimates exist for *U. granulatus* and, the smallest tagged individuals in this study were similar in size to the estimated size at birth (14-28cm DW). Movements of larger individuals were also tracked (>50cm DW) and results suggest that newborns and older juveniles for this species were all dependent on St. Joseph Atoll. *P. ater* is estimated to be born at approximately 18cm DW. The smallest tagged juveniles in this study were larger in size (∼50cm DW), but they may still be less than 1 year old. By inference, St. Joseph Atoll can be classified as a nursery habitat for one of the species (*U. granulatus*), and it may be a nursery habitat for the other two species.

A prior study identified a nursery for *U. granulatus* in Pioneer Bay, Australia (Davy *et al.*, 2015), where juveniles were found to have relatively higher levels of residency than what was reported in this chapter (mean RI of 0.81 and 0.57 respectively). However, this was presumably due to the receiver array set-up in St. Joseph Atoll. Even though there was a high receiver coverage, they were not placed close enough to have overlapping detection radii, creating ‘listening’ gaps in the atoll. It is plausible to suggest that when individuals were not detected, it was not due to a lack of presence, but rather they were simply not in range of a receiver. This was also potentially why residency levels of *U. granulatus* prior December 2014 were the lowest. Individuals detected in this time period were the smallest tagged (some < 50cm DW) and these small individuals were often seen in very shallow waters (depth < 20cm) (personal observations). Acoustic receivers cannot be placed in these areas, thus residency indices were likely artificially low.

Sharks have been shown to utilise atolls as nurseries (Wetherbee *et al.*, 2007; Papastamatiou *et al.*, 2010) however, this is the first study to identify an atoll as a dasyatid nursery (all others have been coastal bays). Atolls have the capacity to provide sufficient food and refuge to juveniles and St. Joseph Atoll, in particular, appears to be a highly suitable nursery habitat. It does not have a dominant channel like most other atolls and the subsequent absence of strong currents has resulted in an abundance of soft sediment habitat with seagrass beds. Even though juvenile dasyatids may not directly feed in the seagrasses themselves, these productive beds provide a reliable source of carbon and nutrients that juveniles are still reliant upon (Vaudo & Heithaus 2011, see Chapter 4). In addition, the shallow reef flats provide protection as they cannot often be traversed by larger sharks (Lea *et al.*, 2016). Therefore, even though offshore atolls are generally more difficult to reach compared to coastal bays, future studies should take these ecosystems into account when searching for batoid nurseries.

Dispersal

When considering habitat use throughout ontogeny, there should be dispersal of older juveniles from nurseries to other habitats (Beck *et al.*, 2001; Heupel *et al.*, 2018a). Dispersal has only previously been inferred in dasyatid nurseries, for example, only juveniles of *U. granulatus* were present in Pioneer Bay, Australia, and individuals were hypothesised to disperse upon reaching a certain size threshold (Davy *et al.*, 2015). The data presented in this chapter provided convincing evidence of juvenile dispersal from St. Joseph Atoll. Many *P. ater* and *U. granulatus* juveniles had relatively consistent detections in the atoll that suddenly ceased. Furthermore, a subset of these juveniles were subsequently detected on receivers outside of the atoll. This indicated that juveniles left the atoll to exploit other areas on the Amirantes Bank, a phenomenon also seen in *U. asperrimus* juveniles (Elston, 2016). It was possible that the observed changes in detection patterns were a result of predation by larger sharks however, this scenario was not applicable to all individuals as some were detected on the Amirantes Bank months apart.

Interestingly, there was a lack of correlation between the size of the individual and time until dispersal. Juveniles of *P. ater* ranging in size from 49cm - 84cm DW all dispersed in the same season and individuals falling in the same size range only dispersed one year later in the following warm season. This indicated that there was not a sudden, mass dispersal event from the nursery area when dasyatids reached a specific size threshold, as has previously been hypothesised for *U. granulatus* juveniles (Davy *et al.*, 2015). Additionally, *P. ater* individuals were found to increase their spatial utilisation of the atoll with time. This might be linked to individual's expanding their home ranges and making exploratory movements before dispersing from the atoll. Further evidence for this hypothesis comes from the fact that larger resident individuals were also found to roam more widely in the atoll than smaller individuals. This gradual increase in home range size as individuals grow, and the subsequent gradual dispersal from nursery areas, has been seen before in immature lemon sharks *Negaprion brevirostris* in Bimini, Bahamas (Franks, 2007; Chapman *et al.*, 2009) and in immature sicklefin lemon sharks *N. acutidens* in Mangrove Bay, Australia (Oh *et al.*, 2017). Reasons for this gradual dispersal may be linked to the allometric changes in mouth gape with increasing size and the subsequent changes in prey through ontogeny (Powter *et al.*, 2010).

Finally, it is worth noting that residency indices were found to be positively correlated with size. This could be due to the fact that the wider ranging movements by larger individuals increased their chances of being detected on a receiver. Additionally, larger individuals were implanted were larger transmitters that have higher outputs which could also be influencing this trend.

The dispersal of individuals from St. Joseph Atoll likely has ecological consequences for the entire Amirantes Bank because juvenile dispersal is known to effect the population dynamics and stock structure of a species (Avice, 2004). Individuals were detected at various locations on the bank, suggesting that they dispersed to different areas. This movement across the Amirantes Bank would spread genetic material, potentially mediating gene flow between the small and isolated habitats on the bank, while also linking separate ecosystems through nutrient flow (Sheaves, 2009). Movement across the bank is possible given the generally shallow depths of mostly less than 40m however, it was unlikely that individuals left the bank as it is surrounded by open ocean with depths in excess of 2000m (Stoddart *et al.*, 1979).

It is also worthwhile mentioning the individual (*P. ater* #15) that left the atoll, being detected on a receiver at the southernmost landfall of the Amirantes Bank (∼90km away), and then subsequently returned to the atoll, where it remained for a further year before detections ceased. Since this migration was restricted to a single individual it is difficult to reconcile, however, it does reveal that benthic batoids are capable of traveling long distances at speed. This is not often thought possible for this faunal group given their limited locomatory efficiency (Di Santo & Kenaley, 2016). The individual travelled 90kms in 4 days (an average of 22.5km per day), with speed estimates that are similar to what larger pelagic manta rays *Manta alfredi* can accomplish (Jaine *et al.*, 2014). It also raises interesting questions about benthic batoid navigational skills. While it is possible that this individual rediscovered St. Joseph Atoll by chance, it is more likely that it navigated it's way back. These long distance movement events may be more common in dasyatids than previously thought, and could be relevant to the scale of receiver arrays when attempting to investigate the movements of these animals.

Adult residency

Despite the evidence for dispersal, there were a small number of individuals that were detected in the atoll up until the end of the monitoring period, some showing consistent daily detections for periods up to 3.5 years. Most of these individuals were sub-adults or adults at the time of tagging. There are two possible explanations for this. Firstly, it may be that while most juveniles disperse, some remain resident in the atoll into adulthood. Alternatively, these adults may have come from other potential nursery areas on the Amirantes Bank, before dispersing to and taking up residence in St. Joseph Atoll. Although no other dasyatid nurseries have been identified on this bank, the lack of research in this area means that this remains a possibility. Further telemetry studies at different areas on the bank, coupled with a genetic investigation, would be needed to investigate this further.

The co-occurrence of *P. ater* and *U. granulatus* juveniles and adults in St. Joseph Atoll was different to what was found for *U. asperrimus.* Adults of this latter species were encountered in the atoll only on very rare occasions, and the only tagged adult was not resident to the atoll (Elston, 2016). However, sympatry of juvenile and adult batoids has been found in previous studies (Vaudo & Heithaus, 2009; Cerutti-Pereyra, 2012) and might be linked the lack cannibalism in batoids. For certain shark species, it is beneficial that individuals of different ontogenetic stages occur in spatially distinct locations, as larger sharks will often prey upon smaller sharks. However, this is not the case with batoids (only one cannibalism incident has been recorded for this group (Branco-Nunes *et al.*, 2016)), potentially facilitating this co-occurrence. It also suggests that the atoll provides suitable habitat types to adults in addition to juveniles, but raises questions as to how resources are partitioned so as to minimize competition between these ontogenetic stages. Other elasmobranchs that share the same habitat among size classes do so by targeting different prey, for example, adult leopard sharks *Triakis semifasciata* feed in nursery areas but shift from feeding on fish eggs to crustacean as they age (Ebert & Ebert, 2005). Furthermore, Heupel *et al.* (2018a) argues that if juveniles and adults co-occur, they need to use different habitat types within the ecosystem for it to be considered a nursery. Therefore, investigating differences in diet and habitat use in St. Joseph Atoll between juvenile and adult dasyatids warrants investigation.

Seasonal changes

Network analysis was found to be a useful tool when investigating the extent to which individuals utilised the atoll and how this varied over time. Overall, resident juveniles displayed limited movements and only utilised small, restricted portions of the atoll. However, individuals were found to increase the extent of their movements in the warmer NW-monsoon season, similarly to *U. asperrimus* juveniles (Elston 2016). This trend was partially driven by the increased utilisation of the lagoon and fringe reef; habitats that are deeper and cooler compared to the shallow sand flats, and thus this movement pattern may be linked to behavioural thermoregulation. A similar finding was reported for juvenile batoids that were found to roam less widely in cooler months (Cerutti-Pereyra *et al.*, 2014), however, this behaviour was not linked to behavioural thermoregulation as hypothesised in this study. Regardless, the movement to actively exploit optimal thermal environments has been observed in batoids before (Fangue & Bennett, 2003; Vaudo & Lowe, 2006; Matern *et al.*, 2000; Dabruzzi *et al.*, 2013) and the effect of the environment should be explored to determine if dasyatids in St. Joseph Atoll are indeed changing movement patterns to behaviourally thermoregulate.

It is important to note the potential effect that environmental factors may play on the detection efficiency of receivers and it's subsequent role in this observed pattern. Higher wind speeds reduce the detection efficiency of receivers in the reef flat habitat of St. Joseph Atoll (Chapter 2, Elston (2016)). Wind speeds are much lower in the NW-monsoon season, which could be driving the observed increase in the rate of detection in this season. However, a large portion of the detections in the NW-monsoon season were in the deeper lagoon and fringe-reef habitats. The detection efficiency of receivers in these deeper habitats are likely not as susceptible to change with increasing wind speeds which, in part, suggests that the observed trend was not solely related to this phenomenon.

Conclusions

Knowledge of the extent of movements and the degree of site fidelity of animals are essential for appropriate management and conservation strategies (Speed *et al.*, 2010; Field *et al.*, 2011; Oh *et al.*, 2017; Henderson *et al.*, 2018). The identification of the high levels of site affinity displayed by all three dasyatid species to St. Joseph Atoll could provide distinct conservation benefits. Two of the three species are considered threatened by the IUCN Red List of Threatened Species. St. Joseph Atoll is small, manageable area, and thus suitable for the designation as a Marine Protected Area (MPA). Ensuring that anthropogenic impacts at this atoll remains low through the designation of an MPA could ensure the continued success of this dasyatid population on the entire Amirantes Bank.

Chapter 4

Dietary habits of dasyatids informed through stomach content and stable isotope analyses

4.1 Introduction

Information regarding the trophic interactions and position of a species is required to understand their ecological role in a food web (Heithaus *et al.*, 2013; Bornatowski *et al.*, 2014). Understanding the trophic ecology of mesopredators is particularly relevant as they occupy an intermediate trophic position, linking apex predators to lower levels in a food web (Ritchie & Johnson, 2009).

In marine environments, small benthic batoids occupy mesopredatory positions as both predators and prey (Navia *et al.*, 2017). Given this mesopredatory nature, in combination with the potential that large sharks have to mediate communities through top-down control, batoids are hypothesised to be able to transmit effects of declining shark abundances down the food chain through a phenomenon called trophic cascades (Myers *et al.*, 2007; Heithaus *et al.*, 2008; Baum & Worm, 2009). However, the roles of benthic batoids in these purported trophic cascades have recently been called into question (Grubbs *et al.*, 2016). There are high levels of trophic complexity and redundancy (multiple species feeding on the same resources) in many marine food webs and batoids display conservative life history traits, all of which render 'mesopredator release' in response to declining shark populations unlikely in certain ecosystems (Navia *et al.*, 2010, 2012, 2017). Nevertheless, benthic batoids have the potential to play important predatory roles (VanBlaricom, 1982; Thrush *et al.*, 1994; Peterson *et al.*, 2001; Blanco-Parra *et al.*, 2012) and could exert influence over prey communities. Unfortunately, a comprehensive understanding into the trophic habits and thus ecological roles of benthic batoids is hindered by a lack of detailed dietary assessments for many species.

To fully assess the roles of benthic batoids, not only is it necessary to quantify these vertical interactions in a food web (i.e. interactions between predator and prey), but an understanding of horizontal interactions is also required. Dasyatids often occur sympatrically and in abundance in tropical shallow sandy ecosystems (Vaudo & Heithaus, 2009; O'Shea *et al.*, 2013; Cerutti-Pereyra *et al.*, 2014; Last *et al.*, 2016a) which could lead to high levels of competition if resources are limiting. The partitioning of resources along some ecological axis (trophic, spatial, and/or temporal) is a straightforward response to competitive effects (Hardin, 1960; Scheffer & van Nes, 2006) but species can also coexist if they are sufficiently similar (Scheffer & van Nes, 2006; Vergnon *et al.*, 2009, 2012). Nevertheless, competition is a fundamental component of the environment that has the potential to influence community structure and the realised niche of individual organisms (Papastamatiou *et al.*, 2006).

Little is understood about the effects of competition on elasmobranch communities (Papastamatiou *et al.*, 2006). This is particularly pertinent for batoids but, the few studies that have investigated competitive effects do so by focussing on the partitioning of dietary resources. These studies have yielded varying results with some revealing significant dietary differences between sympatric species (Platell *et al.*, 1998; Yick *et al.*, 2011; Marshall *et al.*, 2008; Bornatowski *et al.*, 2014; Yemken *et al.*, 2017), whereas others reveal significant dietary overlap (Vaudo & Heithaus, 2011; Jacobsen & Bennett, 2012; O'Shea *et al.*, 2013). High dietary overlap in these latter studies are attributed to either abundant prey resources (limited competitive effects), or batoids are partitioning resources along some other gradient, for example, micro-habitats. However, evolutionary processes could have lead to the stable coexistence of these similar species regardless of competitive effects (Scheffer & van Nes, 2006). Further investigations into these patterns of similar or dissimilar resource use in batoid communities may provide further insights into the potential structuring effect of competition on communities.

Investigating resource partitioning may be particularly relevant in nursery areas. Nurseries have been thought to provide substantial food resources in a low predation environment (Branstetter, 1990; Simpfendorfer & Milward, 1993), but if several abundant species utilise the same nursery, competition for food and habitat may be high (Bethea *et al.*, 2004). Indeed, there have been examples where new-born sharks in nurseries were highly susceptible to mortality (Heupel & Hueter, 2002; Duncan & Holland, 2006). In another case, sympatric juvenile sharks have been found to consume similar prey items when they do not co-occur in nurseries, but partition resources when they do co-occur, probably in efforts to reduce competitive effects (Matich *et al.*, 2017). Levels of resource partitioning and competition have not yet been investigated in dasyatid nurseries, but are important aspects to consider when trying to understand the ecology of species in these systems.

Studies have also investigated intra-specific dietary preferences and have found that batoids often partition trophic resources between size classes or ontogenetic stages (Platell *et al.*, 1998; White *et al.*, 2004; Marshall *et al.*, 2008; Valls *et al.*, 2011; Yick *et al.*, 2011; Jacobsen & Bennett, 2012; O'Shea *et al.*, 2013). These ontogenetic dietary changes may even cause the same species to occupy different trophic levels and perform different trophic roles throughout its life (Navia *et al.*, 2017). It is important to consider these potential ontogenetic dietary changes when characterising the dietary habits of a species.

The dietary composition of *U. asperrimus* juveniles in St. Joseph Atoll has been quantified (Elston *et al.*, 2017) and individuals were found to specialise on infaunal prey, most notably the polychaete family Capitellidae. However, detailed dietary assessments of the other two species, *P. ater* and *U. granulatus*, have yet to be conducted, both within this ecosystem and across their distributions. Determining the dietary compositions of these species will not only provide insights into their roles as predators, but also information as to whether there is potential resource partitioning in this ecosystem and what trophic level these species occupy. An understanding into the nutrient flow within the atoll can also be obtained through determining which basal carbon sources dasyatids were most reliant upon. This chapter aimed to utilise both stomach content and stable isotope analyses to assess the dietary habits of dasyatids that occur in the atoll. Specific objectives were to determine:

- The dietary compositions of *P. ater* and *U. granulatus* and the basal carbon sources that they were reliant upon
- Whether diet differed significantly inter- or intra-specifically for all three dasyatid species
- The trophic level of each species and ontogenetic stage (i.e. juvenile and adult)

4.2 Methods and materials

4.2.1 Sample collection and processing

Stomach contents

Captured *P. ater* and *U. granulatus* individuals (procedure detailed in Chapter 2) were subjected to non-lethal gastric lavage for the collection of stomach contents. The procedure is detailed in Elston *et al.* (2015) but, briefly, individuals were placed ventral side up on a water-filled kayak and a plastic hose that was connected to a bilge pump was eased into the stomach of the dasyatid via the mouth. Seawater was pumped into the stomach to facilitate the regurgitation of stomach contents. The hose was removed once there was visual expansion of the abdomen. If individuals did not regurgitate stomach contents on the first lavage attempt, a second and sometimes third attempt was conducted. Flushed stomach contents were then collected by a mesh basket placed under the individual and transferred to a vial containing 70% ethanol. In the lab, prey items were identified to the lowest possible taxonomic level, blotted dry and items of a given taxon were weighed collectively (0.001g). Stomach content that was too digested for identification was excluded from analyses.

Stable isotope samples

Muscle samples were collected from all three dasyatid species for stable isotope analyses. A biopsy punch was used to remove a small ($\sim$1cm^3) section of muscle from the ventral surface of the pectoral fin of dasyatids. Primary producers and benthic invertebrates were collected from the reef flats of St. Joseph Atoll to determine food web dynamics in the atoll. Seagrasses and algae were collected by hand and epiphytes were removed. Sediment samples were collected using shovels and prawn pumps, from which infaunal invertebrates (e.g. prawns, polychaetes, bivalves) were removed. Epifaunal gastropods were collected by hand. All collected fauna were kept alive for 12 hours so that their digestive tracts could be evacuated (Bergamino *et al.*, 2014). All collected samples were then kept on ice until they could be frozen in the lab.

Samples were subsequently thawed and rinsed with distilled water. For hard-shelled invertebrates, muscle tissue was extracted for processing. Samples were then dried in an oven at 60°C for at least 48 hours and homogenised using a mortar and pestle. Samples that contained calcium carbonate (for e.g. small soft-shelled invertebrates whose shells had not been removed prior to processing) were decalcified so that only organic carbon could be analysed (Nieuwenhuize *et al.*, 1994). To accomplish this, samples were weighed into silver cups and 40μL of 1M HCl were pipetted onto samples. Effervescence was observed through a dissecting microscope, samples were dried and the process repeated until effervescence stopped. As this treatment is known to effect δ^{15}N (Bunn *et al.*, 1995), a bulk untreated sample was also kept.

Lipid extraction is generally not required for batoids as their muscle tissue has a low lipid content (Carlisle *et al.*, 2017). However, urea can potentially artificially lower δ^{15}N values as it is thought to be depleted in ^{15}N, which is of particular concern for elasmobranchs as they produce urea as a waste product of metabolism (Fisk *et al.*, 2002; Logan & Lutcavage, 2010). The magnitude of this effect still remains unclear though; one study reported no change in δ^{15}N values following urea extraction (Logan & Lutcavage, 2010), two reported a significant increase in δ^{15}N values (Kim *et al.*, 2012; Burgess & Bennett, 2017), while one reported variable outcomes for different species (Carlisle *et al.*, 2017). To determine if lipid and/or urea extraction was required for this study, a sub-sample of muscle tissue from nine randomly chosen individuals across the size range were lipid extracted. Several studies have noted an increase in δ^{15}N following lipid extraction in a manner consistent with the removal of urea, suggesting lipid extraction removes urea as well as lipids (Hussey *et al.*, 2010, 2012; Kim *et al.*, 2012; Li *et al.*, 2016; Papiol *et al.*, 2017). A bulk untreated sub-sample was used for comparison. Lipid extraction followed a modified Bligh & Dyer (1959) method: tissue was agitated twice in a 2:1 chloroform:methanol solution at 30°C for 24 hours, the solvent decanted and the sample air dried. A Wilcoxon signed rank test revealed that δ^{13}C and δ^{15}N did not change significantly after lipid extraction ($V = 28$, $p = 0.57$ and $V = 7$, $p = 0.14$ for δ^{13}C and δ^{15}N values respectively). In addition, δ^{15}N values decreased slightly, instead of the expected increase that urea removal would effect (mean δ^{15}N = 10.45 and 10.07 for bulk and lipid extracted samples respectively). These results indicated that neither lipid nor urea extraction was necessary in this study.

4.2.2 Stable isotope analysis

Between 400-600μg of invertebrate and dasyatid muscle tissue and between 1300-1500μg of flora were weighed into tin capsules using a microbalance. These samples were analysed for stable carbon and nitrogen isotope ratios and total percent carbon and nitrogen using a Delta V Advantage Mass spectrometer (Thermo) coupled to a Costech 4010 Elemental Combustion system and a ConFlo IV gas interface at the Great Lakes Institute for Environmental Research, University of Windsor. Analytical precision, based on the standard deviation of four standards (NIST1577c, internal lab standard (tilapia muscle), USGS40 and urea, n=33 for all) measured <0.19% for δ^{15}N and <0.18% for δ^{13}C.

Standard delta notation (δ) was used to express δ^{15}N and δ^{13}C as parts per thousand deviations from the international standards as follows:

δ^{15}N or δ^{13}C = [(Rsample/Rstandard - 1)] * 1000

where R = ^{13}C/^{12}C or ^{15}N/^{14}N. PeeDee Belemnite for carbon and atmospheric nitrogen were used as standard reference materials.

4.2.3 Statistical analyses

Dietary composition

Stomach contents

Cumulative prey curves were constructed to determine whether a sufficient number of stomach contents had been sampled to accurately describe the diet of *P. ater* and *U. granulatus* (Ferry *et al.*, 1997). A species accumulation curve was plotted using the R vegan package (Oksanen *et al.*, n.d.), and the order in which samples were analysed was randomised 100 times. If the curve reached an asymptote, it is assumed that a new prey type will only rarely be introduced into the diet and the sample number is considered sufficient. To quantitatively determine if the slopes reached an asymptote, a linear regression was conducted on the mean cumulative number of prey taxa in the final four stomach content samples. The slope of the linear regression was statistically compared to a slope of 0 using a Student's t-test (t = (b - 0) / Sb where b is the slope of the linear regression and Sb is the standard error of the slope) (Bizzarro *et al.*, 2007).

Prey items identified from stomach contents were grouped to family level or higher and three indices were used to describe dietary composition: percent frequency of occurrence (%F) i.e. the percentage of stomachs which contained a particular prey group, percent weight (%W) i.e. the weight of each prey group divided by the total weight of stomach contents (Hyslop, 1980), and a percent Index of Importance (%IoI) defined as:

$$\%IoI_a = \frac{100 \times (\%F_a + \%W_a)}{\sum_{a=1}^{n} \%F + \%W}$$

Values range from 0 - 100; lower values indicate a prey item is not an important component in the diet and high values indicate the opposite. The more popular percent Index of Relative Importance (%IRI) was not calculated as prey items could not be accurately enumerated.

A feeding strategy plot was created by plotting %F against the prey-specific weight (%WP), calculated as the percentage weight of a prey item for all stomachs containing that prey item (i.e. this excludes samples not containing that prey item) (Amundsen *et al.*, 1996). These plots demonstrate the importance of each prey item as a component of the diet, the feeding strategy (i.e. generalist or specialist feeder) and inter-individual and intra-individual niche width. If a prey item falls into the upper left of the diagram, there is specialisation by individual predators; if a prey item falls into the upper right, there is specialisation by the population; and if prey items fall into the lower quadrants, the population is thought to have a generalist strategy as prey items are eaten in low quantities (Amundsen *et al.*, 1996).

To further quantify trophic breadth, the Levin's standardised niche breadth (Krebs, 1989) was calculated for each species using the R spaa package (Zhang, 2016). Values range from 0-1 and low values indicate a specialist predator with a diet dominated by few taxa while high values indicate a generalist diet (Krebs, 1989).

Stable isotopes

The dietary compositions of juvenile dasyatids were modelled using a Bayesian stable isotope mixing model (SIAR package, Parnell & Jackson 2013). This model allows for the incorporation of uncertainty associated with multiple prey sources of a consumer through the addition of error around consumer and prey stable isotope values, as well as error associated with trophic enrichment factors (TEFs) (Inger *et al.*, 2010). The model produces a probability distribution for proportions of prey items consumed based on their relationship with consumer tissue values. TEF values, i.e. the change in isotopic values between prey and consumer, are required for these models. To date, no accurate experimental TEF values have been calculated for dasyatids, however, Galván *et al.* (2016) estimated TEF values for the smallnose fanskate *Sympterygia bonapartii* based on a controlled feeding experiment, and Tilley & Strindberg (2013) suggested TEF values for dasyatids based on stomach content information available in literature. Thus, the models were run using these two different TEF values; δ^{15}N = 2.7±0.22% and δ^{13}C = 0.9±0.33% (Tilley & Strindberg, 2013), and δ^{15}N = 1.5±0.2% and δ^{13}C = 1.3±0.25% (Galván *et al.*, 2016). C:N ratios were >3.5 for collected invertebrates suggesting a high concentration of lipids and the equation from Caut *et al.* (2013) was used to model lipid extracted values for these samples. Invertebrates were then plotted in 2D isotopic space to visually assess which species were sufficiently isotopically different from each other for the inclusion into the mixing model.

Further Bayesian mixing models were run to provide estimates of the proportion of carbon derived from different primary producers in the muscle tissue of juvenile dasyatids. Three potential resource pools were assumed: benthic macroalgae, seagrass and phytoplankton in the atoll (estimated from filter-feeding bivalves as a proxy).

Each resource pool was assigned a trophic level (1 for primary producers and 2.1 for filter-feeders (Ebert & Bizzarro, 2007)), and the mean trophic level for stingrays was calculated (see next section). Isotopic fractionation was scaled to the number of trophic transfers between resource pools and stingrays: TEF values of 3.4±1% for d15N and 0.4±1.3% for δ^{13}C were used for transfers between primary producers and primary consumers, TEF values of 2.7±0.22% for δ^{15}N and 0.9±0.33% for δ^{13}C were used for transfers between primary consumers and dasyatids (secondary consumers).

Inter- and intra-specific differences in trophic niche

Stomach contents

Multivariate analyses were conducted on the weight of prey items in stomach content samples to determine if dietary compositions differed significantly between species, sexes or with size. A permutational multivariate analysis of variance (PERMANOVA) was chosen as it is not as sensitive as a one-way analysis of similarities (ANOSIM) to the presence of zero values and it considers both categorical and continuous dependent variables (Anderson, 2001). A Euclidean dissimilarity matrix was developed, based on the weight of prey items for each individual, and the adonis function in the vegan package was used to perform the PERMANOVA (Anderson, 2001). If the PERMANOVA highlighted significant differences, a similarity percentages analysis (SIMPER) was calculated to determine which prey categories contributed most to the dissimilarity.

The Schoener and Pianka indices of overlap were also calculated using the spaa package to determine dietary overlap between *P. ater* and *U. granulatus*. Values range from 0 (no overlap) - 1 (complete overlap). Schoener values of >0.6 are considered biologically relevant (Mabragana & Giberto, 2007).

Stable isotopes

In order to investigate differences in isotopic niche, a hierarchical cluster analysis was performed. This was based on the resemblance matrix of δ^{15}N and δ^{13}C mean values for all three species (standardised data) while using the Ward's minimum variance method of linking of Euclidean distances. To determine whether differences in isotopic values between the identified clusters were significant, ANOVA and Kruskal-Wallis tests (if residuals were not normally distributed) were employed. If significant differences were found ($p<0.05$), post hoc Tukey or Dunn tests were employed to determine which groups were different from each other. Mann-Whitney U tests were used to determine if δ^{15}N values and δ^{13}C values were significantly different between males and females (for each species).

Standard corrected ellipse areas (SEAc) (**?**) were calculated for each species and size class as a measure of trophic niche breadth. This metric was chosen because it uses multivariate ellipse-based Bayesian metrics and Bayesian inference techniques allow for robust statistical comparisons between samples of different sizes. The SEAc represents a measure of the total amount of isotopic niche exploited by a species and high values of SEAc represent a high trophic niche breadth. SEAcs were calculated using the SIBER package (Jackson *et al.*, 2011). The percentage overlap of the SEAcs was also calculated to quantify the level of isotopic niche space overlap between species and size classes.

Trophic level

The trophic level (TL) for each species and ontogenetic stage was calculated using stomach content ($\mathrm{TL}_{\mathrm{sc}}$) and stable isotope ($\mathrm{TL}_{\mathrm{si}}$) data. $\mathrm{TL}_{\mathrm{sc}}$ was determined as follows:

$$TL_{\mathrm{sc}} = 1 + (\sum_{j=1}^{n} P_{\mathrm{j}} \times TL_{\mathrm{j}})$$

where n is the number of prey categories, j, TL is the trophic level and P is the proportion of each prey category (Cortés, 1999). The TL of each prey category was taken from Ebert & Bizzarro (2007).

$\mathrm{TL}_{\mathrm{si}}$ was calculated as follows:

$$TL_{\mathrm{si}} = \frac{\lambda + (\delta^{15}N_{\text{secondary consumer}} - \delta^{15}N_{\text{base}})}{\Delta_{\mathrm{n}}}$$

where λ is the trophic level of the base organism used to estimate $\delta^{15}\mathrm{N}_{\mathrm{base}}$, $\delta^{15}\mathrm{N}_{\mathrm{consumer}}$ is measured directly, Δ is the TEF value per trophic level. The base species chosen was the Strombidae gastropod sampled from the sand flats of the atoll and its corresponding TL was 2.1 (Ebert & Bizzarro, 2007). The two different TEF values used in the mixing models used were used for the calculation (Tilley & Strindberg, 2013; Galván *et al.*, 2016).

4.3 Results

A total of 70 *P. ater*, 50 *U. granulatus*, and 30 *U. asperrimus* individuals were caught for the collection of stomach content and muscle samples, details of which can be found in Table 4.1. Gastric lavage resulted in the successful collection of stomach contents from 60% of *P. ater* and 74% of *U. granulatus* individuals. The average lavage duration was 34 secs and ranged from 5 secs to 2 min 23 secs. Short-term post-release survival was 100% with no observed shark predation/encounters and no obvious signs of distress. Three different primary producer species, two filter-feeding invertebrates and three detritivorous invertebrates were collected for stable isotope analyses and were included in mixing models (Table 4.2).

Table 4.1: Details of dasyatids captured for the collection of stomach contents and muscle samples (for stable isotope analyses). n = number of individuals

Species	**Disc width (cm)**	**n (total, male, female)**
	Stomach content collection	
P. ater	28 - 140	50, 24, 26
U. granulatus	28 - 100	39, 23, 16
	Muscle collection	
P. ater	28 - 140	39, 19, 20
U. granulatus	27 - 100	34, 20, 14
U. asperrimus	43 - 110	30, 16, 14

Table 4.2: Details of the primary producers and invertebrates collected for stable isotope analyses. n = number of individuals

Species	**n**	**Section sampled**
Primary producers		
Chlorophyta	25	whole
Thalassia hemprichii	9	leaves
Thalassendron ciliatum	12	leaves
Filter-feeders		
Mactricidae	28	foot
Pinna muricata	16	adductor muscle
Consumers		
Strombidae	19	foot
Capitellidae	16	whole
Callianiassidae	21	whole

4.3.1 Dietary composition

Stomach contents

The majority of stomach content collections came from juvenile dasyatids, while adult sample size was low (n = 4 and 1 for *U. granulatus* and *P. ater*, respectively), therefore, adult stomach contents were removed from analyses. Cumulative prey curves did not reach an asymptote for either species as the slope of the regression line was statistically different from 0 (t = 30.93 and 18.75; p = 0.001 and 0.003 for *U. granulatus* and *P. ater*, respectively). The number of stomach content samples was thus not yet sufficient to provide a complete insight into the dietary composition of these two species. Regardless, curves appeared to be nearing an asymptote and overall trends in the prey types consumed can still be evaluated (Figure 4.1).

A variety of infaunal and epifaunal benthic organisms as well as small quantities of fish were identified in the stomach contents of both dasyatid species. Most prey items could only be identified to family level or higher and a total of 7 phyla and 13 families were identified. The majority of prey items were consumed whole and were not mechanically handled, apart from the molluscs and some brachyurans which had been crushed. Decapod crustaceans were cumulatively the most important prey component in the diet of *U. granulatus* juveniles (%W = 70.27 and %IoI =

78.53). Specifically, ghost shrimps Callianassidae and swimming crabs Portunidae were consumed by roughly half of the individuals sampled (albeit at low quantities) (Table 4.3 and Figure 4.2). The feeding strategy plot also revealed a small number of individuals to consume large proportions of annelids and fish (Actinopterygii), but remaining prey items were consumed infrequently and in low proportions. Conversely for *P. ater*, molluscs were cumulatively the most important prey category (%W = 55.17 and %IoI = 43.87). Bivalves, specifically, was the only prey group to be eaten in high quantities by a large proportion of the individuals sampled. Cumulatively, crustaceans and annelids were also important prey categories (%IoI = 22.96 and 22.23 respectively), but as with *U. granulatus*, individual families were consumed infrequently by a small number of individuals (Table 4.3 and Figure 4.2). Levin's standardized niche breadth values for juveniles of both species were small and indicative of specialist predators (B = 0.2 and 0.05 for the *U. granulatus* and *P. ater*, respectively).

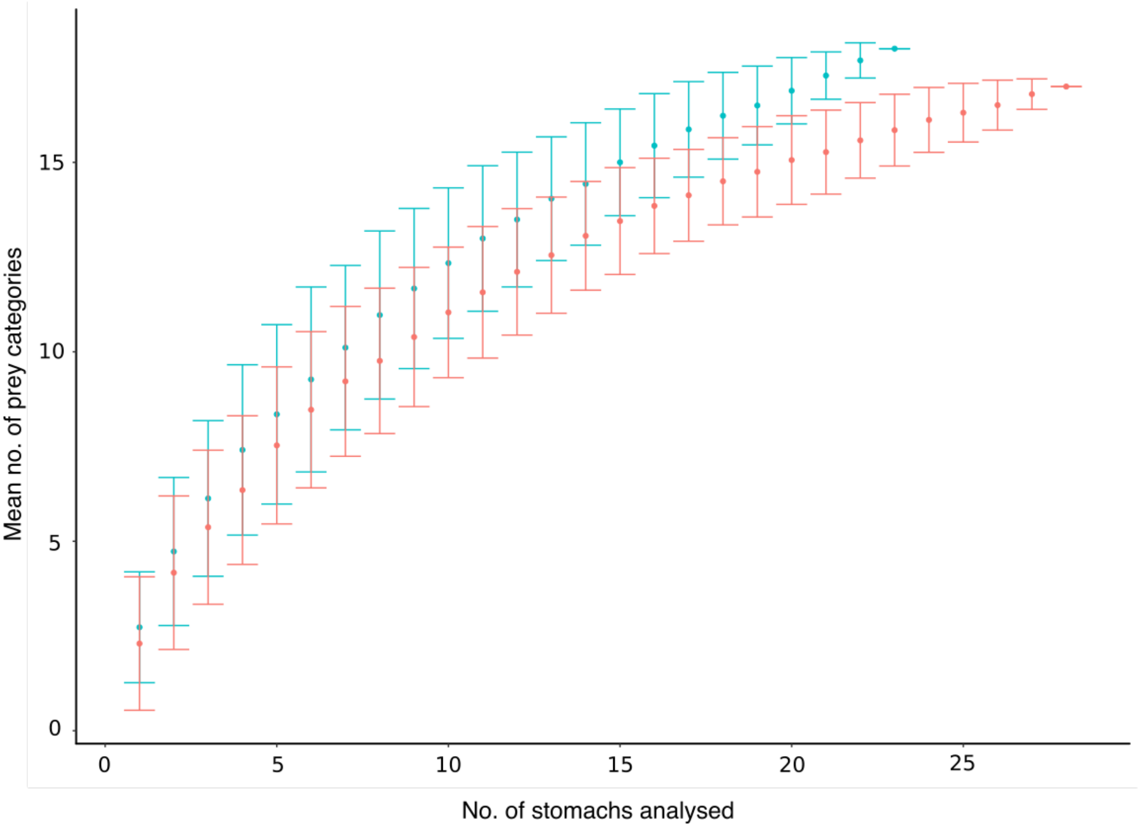

Figure 4.1: Cumulative prey curves for *P. ater* (blue) and *U. granulatus* (pink). Error bars denote standard deviation surrounding the mean number of cumulative prey categories for the number of stomach content samples analysed (after 100 randomisations).

Table 4.3: Percentage weight (%W), percentage frequency (%F) and percentage index of importance (%IoI) of prey items collected from stomach contents of *U. granulatus* and *P. ater*.

		U. granulatus			*P. ater*		
Prey		%W	%F	%IoI	%W	%F	%IoI
Crustacea		**70.27**		**78.53**	**6.60**		**22.96**
Caridea		0.73	10.34	2.92	0.08	4.17	1.08
Axiidea	Callianassidae	18.99	55.17	19.55	1.94	12.50	3.69
Brachyura							
	Calappidae	1.99	13.79	4.16	-	-	-
	Ocypodidae	0.01	3.45	0.91	1.26	25.00	6.70
	Portunidae	22.63	41.38	16.87	-	-	-
	Majidae	1.60	10.34	3.15	-	-	-
	Xanthidae	0.34	3.45	1.00	-	-	-
	Unidentifiable brachyura	6.06	27.59	8.87	1.21	8.33	2.44
Dendrobranchiata							
	Penaeidae	4.70	17.24	5.78	0.14	4.17	1.10
Anomura							
	Diogenidae	-	-	-	0.03	4.17	1.07
	Hippidae	1.31	3.45	1.26	-	-	-
Amphipoda		-	-	-	0.08	4.17	1.08
Stomatopoda		2.51	10.34	3.39	0.03	4.17	1.07
Unidentifiable crustacea		9.42	31.03	10.66	1.83	16.67	4.72
Mollusca		**0.03**		**0.92**	**55.17**		**43.87**
Bivalvia		-	-	-	52.68	75.00	32.60
Gastropoda							
	Nassariidae	-	-	-	0.10	4.17	1.09
	Muricidae	-	-	-	0.44	4.17	1.18
	Unidentifiable gastropoda	0.03	3.45	0.92	1.29	29.17	7.78
Unidentifiable mollusca		-	-	-	0.66	4.17	1.23
Annelida		**20.10**		**13.48**	**20.39**		**22.23**
Polychaeta							
	Capitellidae	2.53	20.69	6.12	14.65	41.67	14.38
	Glyceridae	0.28	6.90	1.89	0.62	8.33	2.29
	Unidentifiable polychaeta	-	-	-	2.15	8.33	2.68
Unidentifiable annelida		17.30	3.45	5.47	2.97	8.33	2.89
Nemertea		0.26	3.45	0.98	0.09	4.17	1.09
Sipuncula		-	-	-	17.59	12.50	7.68
Chordata							
Actinopterygii		9.27	10.34	5.17	0.16	8.33	2.17
Echinodermata							
Echinoidea		0.07	3.45	0.93	-	-	-

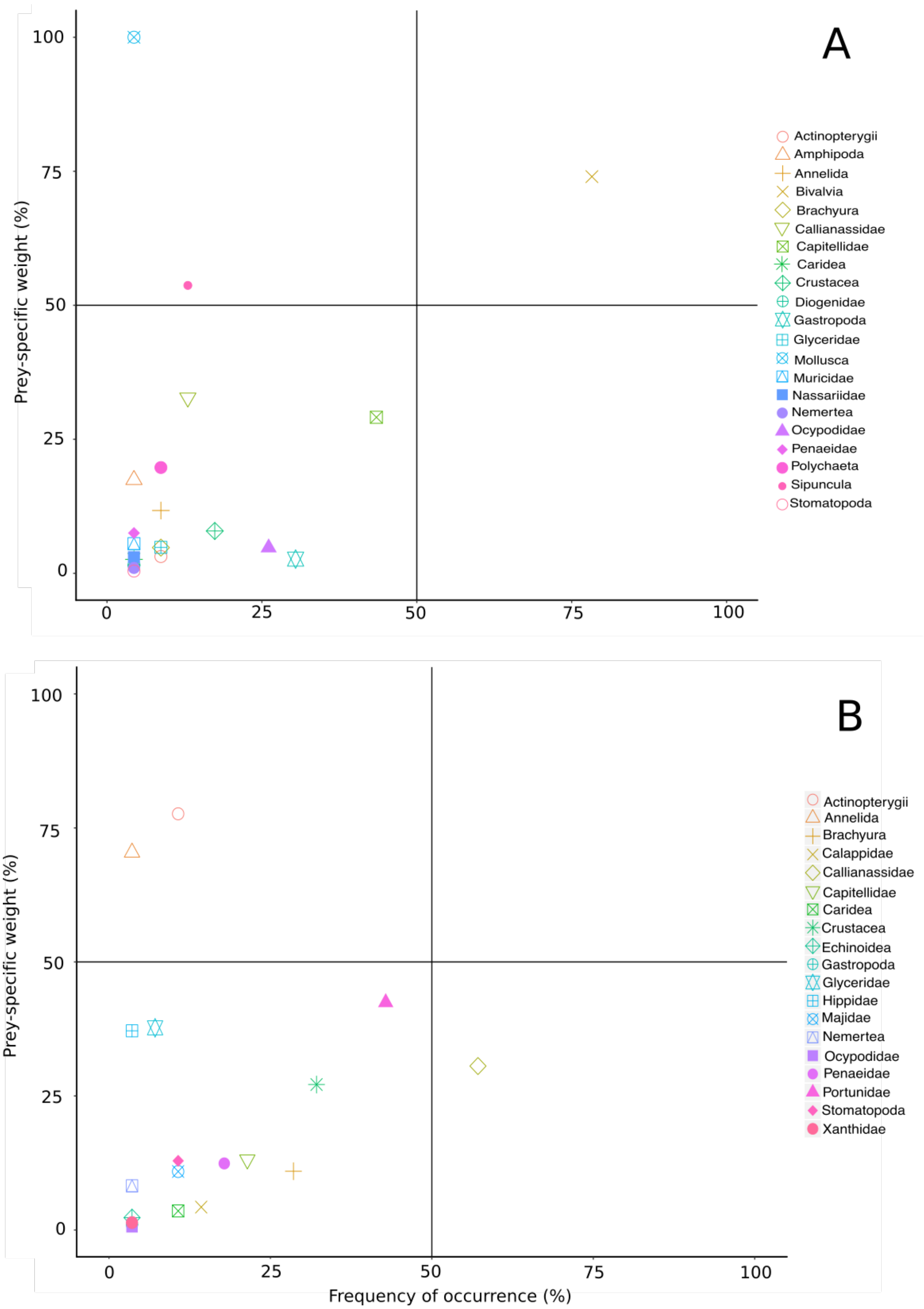

Figure 4.2: Feeding strategy plot for *P. ater* (A) and *U. granulatus* (B). Upper quadrants represent prey items that are eaten in large quantities, while those in the lower quadrants are eaten in small amounts. The right quadrants represent prey items that are eaten by a large portion of the population, while those in the left are eaten by few individuals in the population.

Stable isotopes

Trying to estimate dietary compositions using Bayesian mixing models proved relatively uninformative in this study. All collected invertebrates were relatively isotopically similar (with the exception of Capitellidae) (Figure 4.3) and not all prey items identified through stomach contents were collected for stable isotope analyses (e.g. brachyurans, sipunculids, nematodes and fish were not sampled). As such, some individuals fell outside of the prey polygon and model results were inconclusive.

Mixing models to determine which primary producers contributed the largest proportions of carbon to dasyatid diets were more successful. Sampled primary producers had largely different δ^{13}C values; seagrass had the highest values, benthic algae had the lowest and phytoplankton values estimated from filter feeders fell in between the two (Figure 4.4). Both *P. ater* and *U. granulatus* juveniles were most reliant on carbon obtained from seagrass sources as the largest proportional contribution of carbon was obtained from seagrass, but there were also small proportions of carbon obtained from phytoplankton sources. There were negligible contributions of carbon from chlorophyta algae (Figure 4.4).

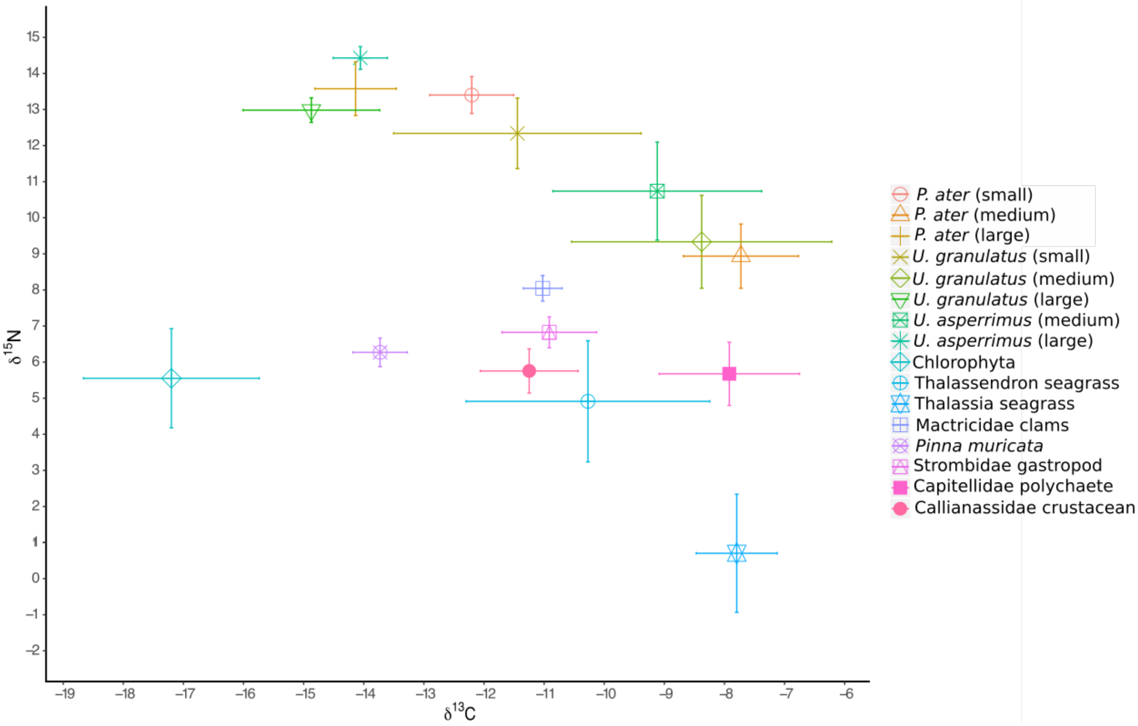

Figure 4.3: Biplot of isotope ratios, δ^{15}N and δ^{13}C, for collected dasyatid muscle samples and flora and invertebrate fauna of the reef flats of St. Joseph Atoll. Error bars denote standard deviation around the mean of each group. Dasyatids were divided into separate size classes based on cluster analysis results.

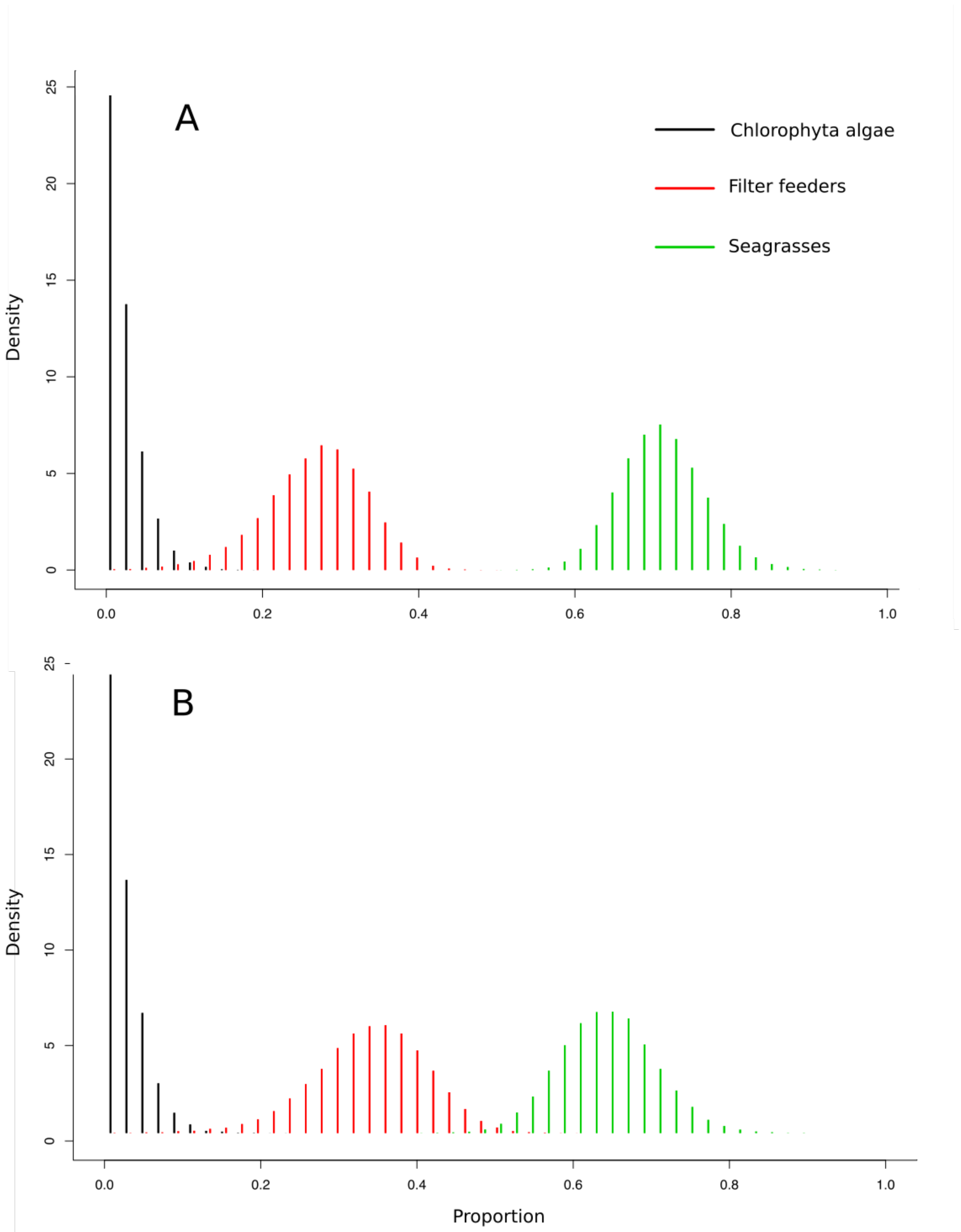

Figure 4.4: The probability distribution for proportional contributions of primary producers to the diet of dasyatids determined through Bayesian mixing models. Primary producers included in models were chlorophyta algae, filter feeding *P. muricata* and Mactricidae clams and Thalassendron and Thalassia seagrasses.

4.3.2 Inter- and intra-specific differences in trophic niche

Stomach contents

There were significant differences in the stomach content compositions between juveniles of *P. ater* and *U. granulatus* (Table 4.4). The SIMPER highlighted that three prey groups contributed most to this dissimilarity. A visual representation of these prey groups revealed that *U. granulatus* consumed larger proportions of Callianiassidae and Portunidae whereas *P. ater* consumed larger proportions of bivalves (Figure 4.5). There was very little dietary overlap between the two species (Schoener index = 0.16 and Pianka index = 0.09). Furthermore, there were significant dietary differences between size classes but not between sexes (Table 4.4).

Table 4.4: Results of the PERMANOVA performed on stomach content data collected for *P. ater* and *U. granulatus* to determine if dietary composition varied between species, sexes and with size. DF = degrees of freedom.

	DF	F statistic	R^2	p-value
Species	1	6.5582	0.13803	0.001
Size	1	2.9862	0.06285	0.001
Sex	1	0.9678	0.02037	0.453

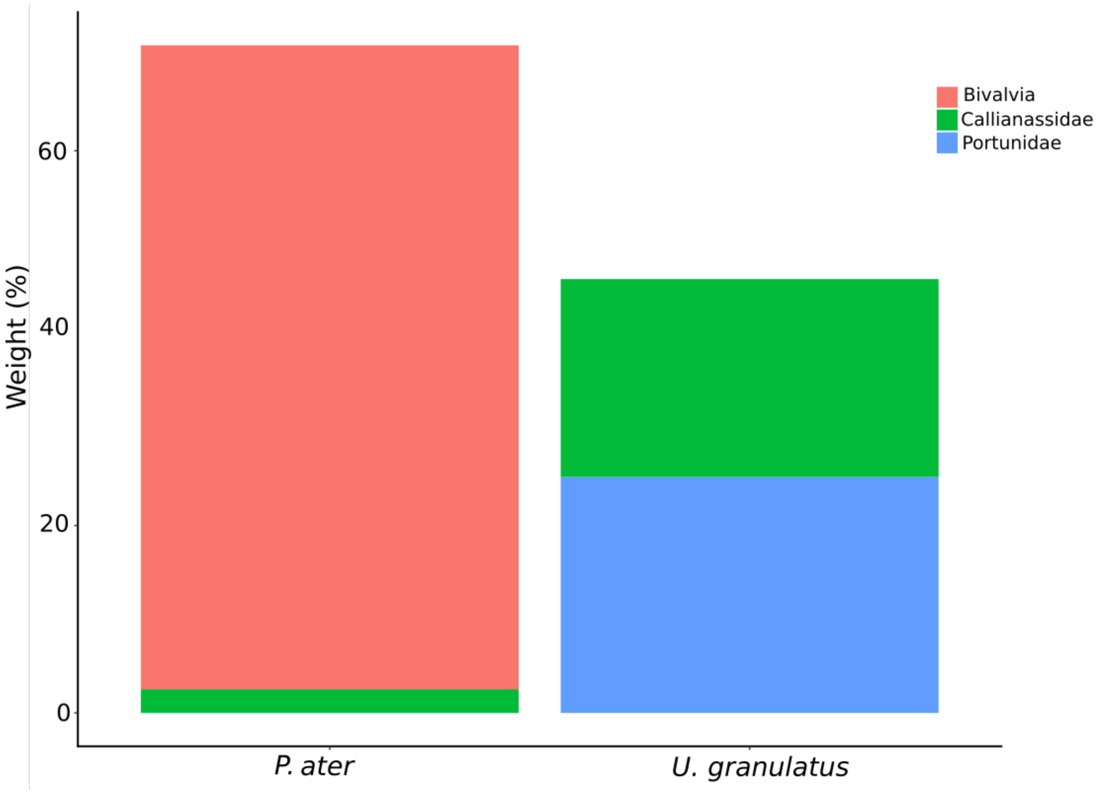

Figure 4.5: Percentage weight contributions of prey items that contributed most to the dissimilarity of the stomach contents between *P. ater* and *U. granulatus* juveniles.

Stable isotopes

Hierarchical clustering performed on δ^{13}C and δ^{15}N values revealed two distinct groups, and individuals of similar sizes rather than of the same species clustered together. One group contained both the smallest and largest individuals for *P. ater* and *U. graunulatus*, barring one exception, while the other group contained all the medium sized individuals for both species (Figure 4.6). As such, *P. ater* and *U. granulatus* were divided into three size classes; small, medium and large (Table 4.5). Conversely, *U. asperrimus* did not appear to cluster by size and data were split into two size classes based on known maturity determined at capture; the medium size class contained all juveniles while the large size class contained adults (maturity confirmed at capture) (Table 4.5).

There were significant differences between these identified size class groups for both δ^{15}N and δ^{13}C ($F = 36.83$, $DF = 7$, $p < 0.01$ for δ^{13}C and $F = 32.12$, $DF = 7$, $p < 0.01$

for δ^{15}N). Contrary to stomach content data, post-hoc tests showed no significant differences in inter-specific comparisons, with the exception that the medium *U. asperrimus* size class had significantly different δ^{15}N and δ^{13}C values compared to the medium size classes of the other two species (Table 4.6). Conversely, ontogenetic shifts in isotopic niche were evident as all medium size classes had significantly lower and higher δ^{13}C and δ^{15}N values respectively compared to the small and large size classes, the latter of which were not found to be significantly different from each other (Table 4.6). There were no significant differences in δ^{13}C and δ^{15}N values between sexes for *P. ater*, *U. granulatus* and *U. asperrimus*.

Two *U. granulatus* juveniles were outliers in the data, displaying both lower δ^{13}C and δ^{15}N values. This could artificially enlarge SEAc values so these individuals were removed when calculating SEAcs. SEAcs could not be calculated for the the large *P. ater* and *U. asperrimus* size classes due to small sample sizes. SEAc estimates revealed the small *U. granulatus* and the medium *U. asperrimus* size classes to occupy the largest isotopic niche spaces and to display the largest levels of overlap, particularly with each other. Medium *U. granulatus* and *P. ater* size classes also had large degrees of overlap with each other, but lay completely separate to the somewhat overlapping isotopic niches of small *P. ater* and large *U. granulatus* size classes (Figure 4.7).

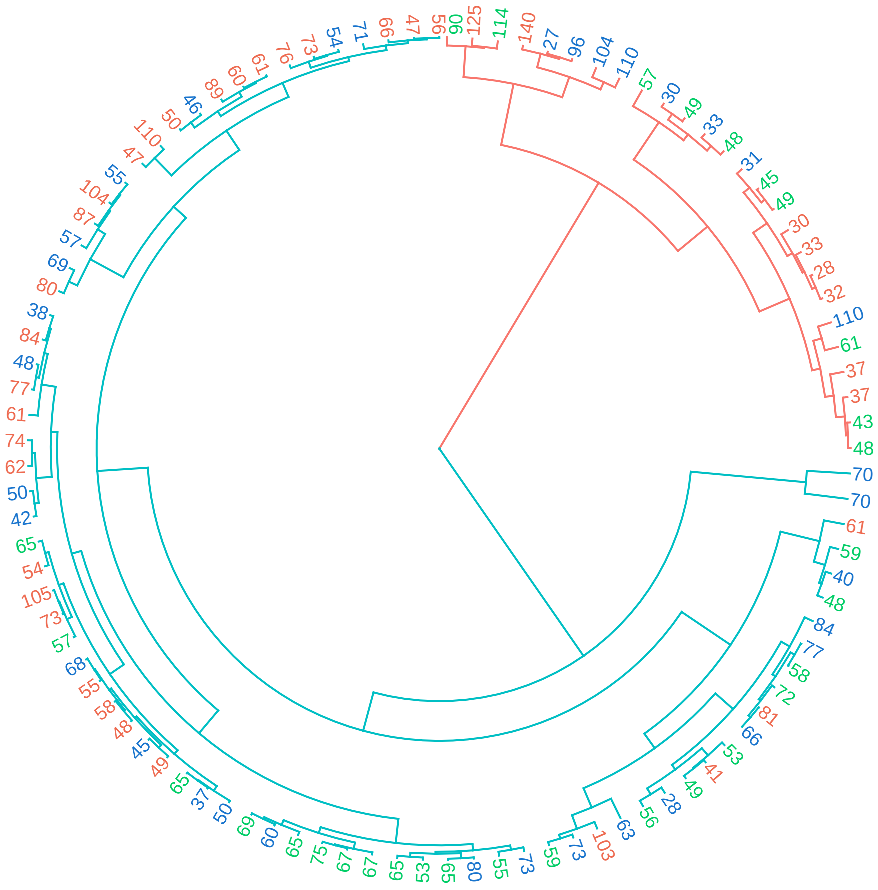

Figure 4.6: Output of hierarchical cluster analysis performed on δ^{13}C and δ^{15}N values for *P. ater*, *U. granulatus* and *U. asperrimus*. Branch colours represents the two major clusters identified. Numbers at the end of branches represent disc-widths (cm) of *P. ater* (red), *U. granulatus* (blue), and *U. asperrimus* (green).

Table 4.5: Details of size class groups defined for *P. ater*, *U. granulatus*, and *U. asperrimus* based on heirarchical cluster grouping. Included are the size ranges of the groups (DW = disc-width), number of individuals in each group (n), and the mean ± standard deviations of isotopic ratios for each group.

	n	DW (cm)	δ^{15}N	δ^{13}C
P. ater - small	6	28 - 37	13.40 ± 0.51	-12.20 ± 0.70
P. ater - medium	30	41 - 110	8.94 ± 0.89	-7.73 ± 0.96
P. ater - large	2	125 - 140	13.58 ± 0.74	-14.14 ± 0.68
U. granulatus - small	5	27 - 33	12.34 ± 0.98	-11.44 ± 2.06
U. granulatus - medium	25	37 - 84	9.33 ± 1.29	-8.38 ± 2.16
U. granulatus - big	4	96 - 110	12.98 ± 0.34	-14.88 ± 1.14
U. asperrimus - medium	28	43 - 75	10.74 ± 1.36	-9.12 ± 1.74
U. asperrimus - big	2	90 - 114	14.42 ± 0.31	-14.06 ± 0.45

Table 4.6: Results of post-hoc tests based on the ANOVA to determine differences in δ^{15}N values (results above diagonal line) and δ^{13}C values (results below diagonal line) between the different size classes of *P. ater*, *U. granulatus*, and *U. asperrimus*. S = small, m = medium, l = large size classes. Stars denote significant differences.

	P. ater (s)	*P. ater* (m)	*P. ater* (l)	*U. granulatus* (s)	*U. granulatus* (m)	*U. granulatus* (l)	*U. asperrimus* (m)	*U. asperrimus* (l)
P. ater (s)	-	0.00*	1.00	0.77	0.00*	1.00	0.00*	0.95
P. ater (m)	0.00*	-	0.00*	0.00*	0.89	0.00*	0.00*	0.00*
P. ater (l)	0.82	0.00*	-	0.89	0.00*	1.00	0.02*	0.99
U. granulatus (s)	0.99	0.00*	0.49	-	0.00*	0.99	0.08	0.35
U. granulatus (m)	0.00*	0.81	0.00*	0.00*	-	0.00*	0.00*	0.00*
U. granulatus (l)	0.18	0.00*	1.00	0.04*	0.00*	-	0.01*	0.81
U. asperrimus (m)	0.00*	0.03*	0.00*	0.07	0.71	0.00*	-	0.00*
U. asperrimus (l)	0.85	0.00*	1.00	0.53	0.00*	1.00	0.00*	-

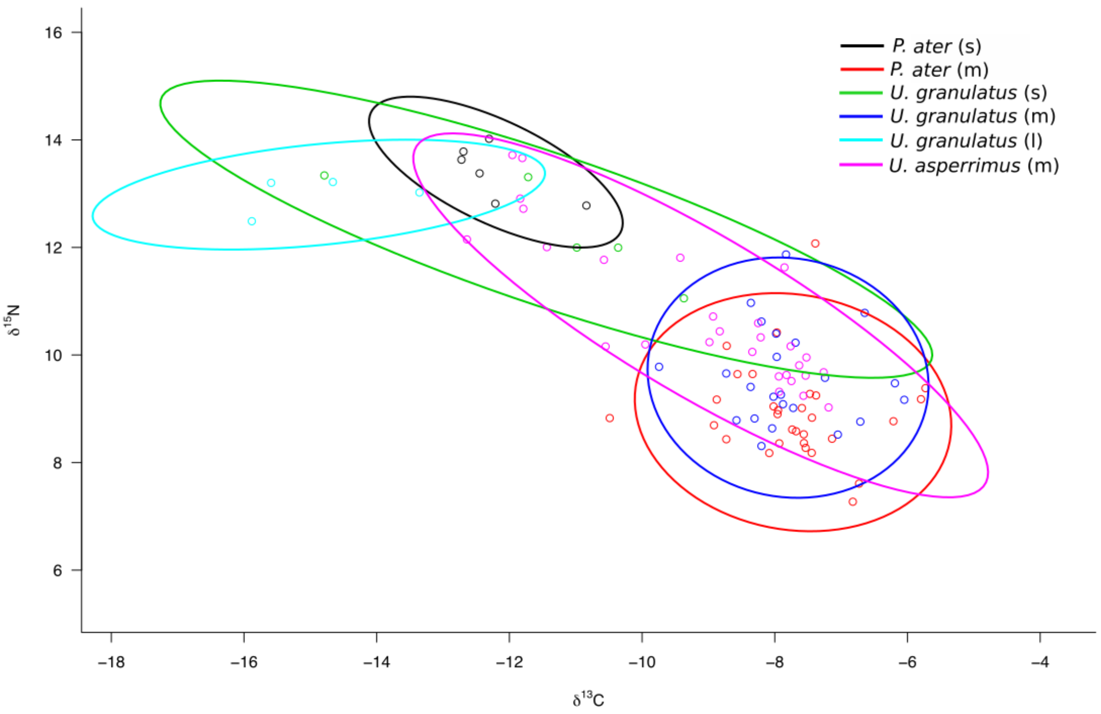

Figure 4.7: Standard corrected ellipse areas (SEAc) calculated from δ^{13}C and δ^{15}N values for the different size classes of *P. ater*, *U. granulatus* and *U. asperrimus*. S = small, m = medium, l = large size classes.

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4.3.3 Trophic level

The trophic level (TL) estimates for juvenile dasyatids were similar when calculated with stable isotope and stomach content data (mean TL = 3.2 and 3.4 respectively). Adult dasyatids were feeding at higher trophic levels compared to juveniles (mean TL = 4.6) but TL estimates were similar between species. TEF values from Galván *et al.* (2016) resulted in unusually high TL estimates for adults (ranging from 5.97 - 7.17) and were considered inaccurate for this study (Table 4.7).

Table 4.7: Trophic level (TL) estimates calculated from stable isotope ($\mathrm{TL}_{\mathrm{si}}$) and stomach content ($\mathrm{TL}_{\mathrm{sc}}$) for juvenile and adult *P. ater*, *U. granulatus*, and *U. asperrimus*. The use of two different TEF values, from Tilley & Strindberg (2013) and Galván *et al.* (2016), resulted in two different $\mathrm{TL}_{\mathrm{si}}$ estimates.

Group	$\mathrm{TL}_{\mathrm{si}}$ (Galvan *et al.*, 2016)	$\mathrm{Tl}_{\mathrm{si}}$ (Tilley *et al.*, 2013)	$\mathrm{TL}_{\mathrm{sc}}$
P. ater adult	6.51	4.55	
P. ater juvenile	3.51	2.88	3.20
U. granulatus adult	5.97	4.25	
U. granulatus juvenile	3.77	3.03	3.50
U. asperrimus adult	7.17	4.92	
U. asperrimus juvenile	4.71	3.55	3.60

4.4 Discussion

The combination of stable isotope and stomach content data provided detailed insights into the trophic ecology of dasyatids in St. Joseph Atoll and their inter- and intra-specific dietary differences. Similar to other dasyatid species (Jacobsen & Bennett, 2013), *P. ater* and *U. granulatus* juveniles were found to be continuous foragers as there was a high number of prey items in the stomachs at varying stages of digestion and there was a low occurrence of empty stomachs. The vast majority of prey consisted of infaunal and epifaunal organisms that reside on/in sand flats, suggesting these individuals forage on the surface and sub-surface layers of the sand flats.

Inter-specific dietary differences

Stomach content analysis revealed significant inter-specific differences in diet, even though species possess similar body forms, benthic lifestyles and size. Juveniles of *U. granulatus* and *P. ater* each occupied a narrow trophic niche breadth and both were specialist predators on different infaunal prey (decapod crustaceans and bivalves, respectively). Juveniles of *U. asperrimus* were also found to be specialist predators at St. Joseph Atoll , feeding largely on polychaetes (Elston, 2016), suggesting that each species exploits a different trophic niche in the atoll. Despite these differences, juveniles had similar TL estimates highlighting that these species feed at the same trophic levels in the food web.

Theoretical models show that the partitioning of resources is one (but not the only) expected outcome when competitive effects are present (Scheffer & van Nes, 2006). However, it is also important to note that resource partitioning may not necessarily only be a result of competition, but other factors too (Ross, 1986). Regardless, while one cannot infer direct causality from the observed patterns of resource partitioning, it may be indicative of competitive effects and that food may be a limiting resource in St. Joseph Atoll. This is potentially due to the fact that the atoll is a nursery area supporting high abundances of juvenile dasyatids (Chapters 2). Furthermore, food has been found to be a limiting resource in other elasmobranch nursery areas (Duncan & Holland, 2006). Alternatively, these species may have previously evolved morphological or behavioural differences (as discussed below) which can facilitate dietary specialisation in the absence of competition.

Dietary partitioning can be facilitated by a variety of factors including morphological features such as mouth morphology and dentition, foraging behavior and depth, and temporal/spatial differences in foraging (Platell *et al.*, 1998; Platell & Potter, 2001; Marshall *et al.*, 2008; Vaudo & Heithaus, 2011). Although detailed dentition information for these species is absent, it has been noted that *P. ater* has unique hexagonal, high crowned rough teeth (Compagno *et al.*, 1989) that may allow them to crush harder prey than most other dasyatids. Indeed, molluscs consumed by *P. ater* in this study were mechanically crushed while *U. granulatus* and *U. asperrimus* largely consumed prey whole. Conversely, other studies have shown that *P. ater* consumes large proportions of annelids in Western Australia (Vaudo & Heithaus, 2011; O'Shea *et al.*, 2013), suggesting that this species may specialise on different prey items throughout it's range. There appears to be subtle differences in foraging depth between dasyatid species in St. Joseph Atoll. Previously conducted sediment sampling (Elston *et al.*, 2017) revealed that bivalves, which were consumed by *P. ater*, were located near the surface of the substratum, while polychaetes and decapods, which were consumed by *U. granulatus* and *U. asperrimus*, occurred deeper in the sediment (pers. obs.). The distribution and composition of benthic fauna can vary across microhabitats (Darnell, 1990), thus fine-scale habitat partitioning may be facilitating the differences in diet, but this still needs to be investigated.

Inter-specific dietary differences between juveniles of *P. ater* and *U. granulatus* were not reflected in the stable isotope data. This might be due to the differences in time-scales between methods as stomach contents provide a 'snapshot' view of the diet whereas stable isotopes integrate dietary compositions over longer time periods (MacNeil *et al.*, 2006). However, it is probably an artefact of similar isotopic values between the main prey items (decapods and molluscs). *U. asperrimus* juveniles were found to be isotopically distinct because their main prey item (Capitellidae) was isotopically different to other prey items (Elston *et al.*, 2017).

Results from mixing models to estimate dietary compositions were equivocal. Not all prey species identified in stomach contents were collected for the inclusion into mixing models and many stingray samples subsequently fell outside of the prey polygon. In addition, the accuracy of TEF values utilised remains in question as no TEF values for dasyatids have been estimated through controlled feeding studies. This highlights the need for accurate TEF values for stingrays and the importance of a priori information related to diet so that all potential prey can be sampled. However, it must also be noted that mixing models might not be feasible in such systems where potential prey share similar isotopic values due to the reliance on similar carbon pathways.

Intra-specific dietary differences

Stable isotope data revealed distinct ontogenetic shifts in diet, with adults having significantly different isotope values compared to juveniles. Multiple studies have found that batoids change diet through ontogeny (Smale & Cowley, 1992; Vaudo & Heithaus, 2011; Yick *et al.*, 2011; O'Shea *et al.*, 2013; Tilley & Strindberg, 2013) and these changes may lead to species occupying different trophic levels and thus performing different trophic roles through ontogeny (Navia *et al.*, 2017). Indeed, adults were found to feed at higher trophic levels compared to juveniles, given their higher δ^{15}N values. Juveniles had TL estimates consistent with mesopredators (2.9 - 3.6) and may play important roles in structuring the benthic community of the St. Joseph Atoll. Conversely adults had trophic levels corresponding to top predators (TL>4). Given the small sample size of adults, details into their dietary preferences remain in question, but it may be that they shift to a diet based more on fish than on benthic organisms, which would effect the increase in δ^{15}N values. Indeed, studies have shown that larger rays have greater foraging capabilities and can ingest larger, more mobile species (Kyne & Bennett, 2002; Collins *et al.*, 2007; Marshall *et al.*, 2008; Jacobsen & Bennett, 2012).

Despite this significant change in diet with ontogeny, few studies take size into account when estimating TL for dasyatids, a trend highlighted by a review of dietary studies that reported TL estimates for 25 dasyatid species (Jacobsen & Bennett, 2013). TL estimates in this review ranged from 3.16 - 4.08 (mean = 3.62), which largely concur with the TL estimates of juveniles in this study (2.9 - 3.6). Specifically, the mean TL for *P. ater* juveniles in this study (mean TL = 3.05) was similar to the TL obtained in other studies (TL = 3.2 (Jacobsen & Bennett, 2013) and 3.5 (Vaudo & Heithaus, 2011)). López-García *et al.* (2012) was the only study in the review that took ontogenetic stage into account when calculating TL and found that adults of *Dasyatis longa* had a TL of 4.2, which is similar but still lower than the TL estimates for adults in this study (4.3 - 4.9). It is important to note the low sample size of adults as well as the potential inaccuracy of TEF values, which may be effecting the accuracy of TL estimates. However, similarity in TL_{si} and TL_{sc} values for juveniles suggest TEF values may be sufficient to accurately estimate

TL. Nevertheless, this study highlights the importance of calculating TL for each ontogenetic stage, especially in dasyatids, where shifts from a mesopredatory to top predatory lifestyle may be common.

Adult rays also had significantly lower δ^{13}C values compared to juveniles. Seagrasses contributed the highest proportions of carbon to the diet of juveniles, confirming they feed in the atoll. A similar result was obtained by Vaudo & Heithaus (2011), who found that an elasmobranch mesopredatory community was highly dependent on a seagrass-based food web. This suggests that even though mesopredatory elasmobranchs feed on prey from the sand flats, there are important nutritional flows from adjacent seagrass beds (potentially via detrital pathways) that sustain communities. This further emphasises the importance of seagrass beds, particularly to species that are not typically associated with seagrass (Vaudo & Heithaus, 2011). Conversely, adult carbon isotope values were smaller than those of seagrasses, characteristic of a more offshore system with phytoplanktonic primary producers (Michener & Kaufman, 2007). This is unsurprising given the dispersal of juveniles found in the previous chapter and suggests that ontogenetic changes in diet are likely facilitated by a habitat shift.

Maternal meddling

It was also found that the smallest individuals and the largest individuals (which were adults) had isotopic values that were not significantly different. However, rather than this being a function of similar dietary preferences, it is a function of maternal meddling, a trend observed before in sharks (Olin *et al.*, 2011). There is a transfer of maternal resources to individuals during gestation which ceases after birth, but elasmobranch muscle tissue has a long turn-over rate ($\approx$ 1 year, MacNeil *et al.* 2006). Thus, stable isotopes of individuals less than a year old are influenced by their mother's diets and foraging location and do not reflect what the individuals themselves are consuming (Olin *et al.*, 2011). This was also reflected in the large isotopic niche space occupied by the small *U. granulatus* size class. These individuals were not in isotopic equilibrium and stable isotope values become less and less influenced by maternal signatures as they grow, leading to greater variability in isotopic values between individuals.

Importance of stingrays and stability of ecosystem

Dasyatids have the potential to effect the structure and function of the food webs in which they occur because they participate in many of its trophic roles (Navia *et al.*, 2017). Given that each species in St. Joseph Atoll specialises on a different prey group, they may have strong predator-prey interactions and may play influential roles in structuring these communities (Bascompte *et al.*, 2005). In addition, given this specialisation, it suggests relatively low levels of trophic redundancy and the ecosystem might be sensitive to the removal of one of these species (Navia *et al.*, 2012). However, species responses to change are difficult to predict and dasyatids may increase their dietary niche if one species is removed and competition is reduced, as has been seen in juvenile sharks (Matich *et al.*, 2017). To investigate this, determining the diet of these species under variable levels of competition would be needed. In addition, it is important to remember that dasyatids are not the only mesopredators present in the atoll. Bonefish *Albula glossodonta*, mullet (Mugilidae), and juvenile blacktip *Carcharhinus melanopterus* and sickle-fin lemon *Negaprion acutidens* sharks all inhabit the atoll, some of which may feed on the same prey, thereby increasing the trophic redundancy and stability of the system. Finally, given that these species are trophic omnivores (ie a species feeding at different trophic levels (Novak, 2013)), it increases the functional redundancy within trophic groups which is important in maintaining structure and robustness of food webs (Bascompte *et al.*, 2005; Navia *et al.*, 2017).

Chapter 5

Habitat use patterns

5.1 Introduction

Identifying the mechanisms that facilitate the coexistence of similar species has long been an important question in community ecology (Schoener, 1974). One such evolutionary mechanism is that similar species will often use limiting resources in different ways (Mcpeek, 2014). Competing species may partition food, space, and/or time and among marine fishes in particular, resource partitioning commonly involves dietary divergences, whereby functionally similar species evolve dissimilar diets (Ross, 1986). This is also often the case within sympatric batoid communities and studies have revealed that dietary differentiation can facilitate coexistence among this abundant faunal group (Yick *et al.*, 2011; Bornatowski *et al.*, 2014; Pardo *et al.*, 2015).

Despite the evidence for dietary differentiation in batoid communities, the potential for differences in habitat utilisation remain unclear. Habitat partitioning is an important aspect to consider because while differences in dietary resource use are often due to morphological adaptations (Jacobsen & Bennett, 2012; Pardo *et al.*, 2015), studies on various elasmobranchs show that differences in diet can also be linked to differences in habitat (White *et al.*, 2004; Heupel *et al.*, 2018b). However, most dietary studies on sympatric batoids have been conducted over broad spatial scales, which may have obscured the effects of habitat partitioning (for example Jacobsen & Bennett 2012; Bornatowski *et al.* 2014).

Indeed, species may utilise different habitats over very small spatial scales, for example, O'Shea *et al.* (2013) suggested that sympatric dasyatids may partition habitat at the scale of metres, such as between the reef lagoon and the outer reef. In addition, different species may utilise the same habitat but at different temporal scales (Speed *et al.*, 2011). Recent acoustic telemetry studies on dasyatids revealed distinct diel peaks in detections (Campbell *et al.*, 2012; Brinton & Curran, 2017), something that may facilitate temporal habitat partitioning. The partitioning of habitat has been identified in sympatric shark communities, particularly in nursery areas (White *et al.*, 2004; Pikitch *et al.*, 2005; DeAngelis *et al.*, 2008). However, the limited evidence for habitat partitioning in batoids have found high levels of spatial and temporal overlap (Vaudo & Heithaus, 2009, 2012).

Resources can be partitioned through a variety of mechanisms, including different species occupying different habitats, but also by the movement from one habitat to another as individuals of a species increase in body size (Ross, 1986). For sharks specifically, there is increasing evidence that different size classes coexist through this intra-specific habitat partitioning as juveniles and adults occupy habitats with different depths, temperatures, salinity levels, or benthos types (Pikitch *et al.*, 2005; Simpfendorfer *et al.*, 2005; McAuley *et al.*, 2007; Speed *et al.*, 2010). Conversely, even though sympatric batoid communities can consist of both juveniles and adults (Vaudo & Heithaus, 2009; Cerutti-Pereyra, 2012), it is unclear whether these ontogenetic stages segregate in their habitat use, spatially and temporally.

Three dasyatid species are resident to St. Joseph Atoll, and both juveniles and adults are present (Chapter 3). There is both inter- and intra-specific (ontogenetic) prey partitioning (Chapter 4). In addition, juveniles were found to restrict their movements to small portions of the atoll (Chapter 3). Therefore, whether inter- and intra-specific dietary differences were related to differences in habitat utilisation remains unknown. The reef flats of St. Joseph Atoll are relatively homogenous given the lack of a tidal channel however, there may be subtle distributional differences in the benthic invertebrate fauna that these dasyatids prey upon. Thus, the aim of this chapter was to determine how three dasyatids (*P. ater*, *U. granulatus*, and *U. asperrimus*) spatially and temporally utilised habitats in the atoll, and whether this varied intra- and inter-specifically. Specific objectives were:

- To characterise the habitat preferences of three dasyatid species and ontogenetic stages in St. Joseph Atoll
- To describe fine-scale spatial and temporal habitat utilisation by juveniles and whether this varied between species, sexes and with size

5.2 Methods and materials

Acoustic telemetry data, as described in Chapter 2, were used in the data analyses for this chapter. As inter-specific differences in habitat use were being investigated, acoustic data collected from all three dasyatid species (*P. ater*, *U. granulatus* and *U. asperrimus*) were included in the analyses.

5.2.1 Habitat preference

Habitat selection indices were calculated to determine whether dasyatid species and ontogenetic stages (i.e. juvenile and adult) preferentially utilised different habitats in St. Joseph Atoll. Three broad habitat types were delineated, namely reef flats, lagoon and fringe reef (Figure 5.1). Each acoustic receiver in the array was assigned to a habitat type and the proportions of detections on each receiver for each species-ontogenetic group was calculated. A chi-square goodness of fit test was used to test the null hypothesis that individuals utilised a habitat type in proportion to its availability. The utilisation of a habitat type (u) was defined as the sum of detections on receivers within a habitat type / the total number of detections on receivers within the atoll, expressed as a percentage. The availability of a habitat type (a) was defined as the number of receivers within a habitat type / the total number of receivers within the atoll, expressed as a percentage. If the null hypothesis was rejected, a modified Strauss linear index of selection ($L = u_i - a_i$) was used to quantify habitat type selection where L is the selection value, u is the percent use of habitat type i, and a is the percent availability of habitat type i. If L is positive it indicates selection for a particular habitat type, negative L values indicate avoidance, and zero values indicate random use of a habitat type (Munroe *et al.*, 2015). Selection indices were calculated for each species and ontogenetic stage.

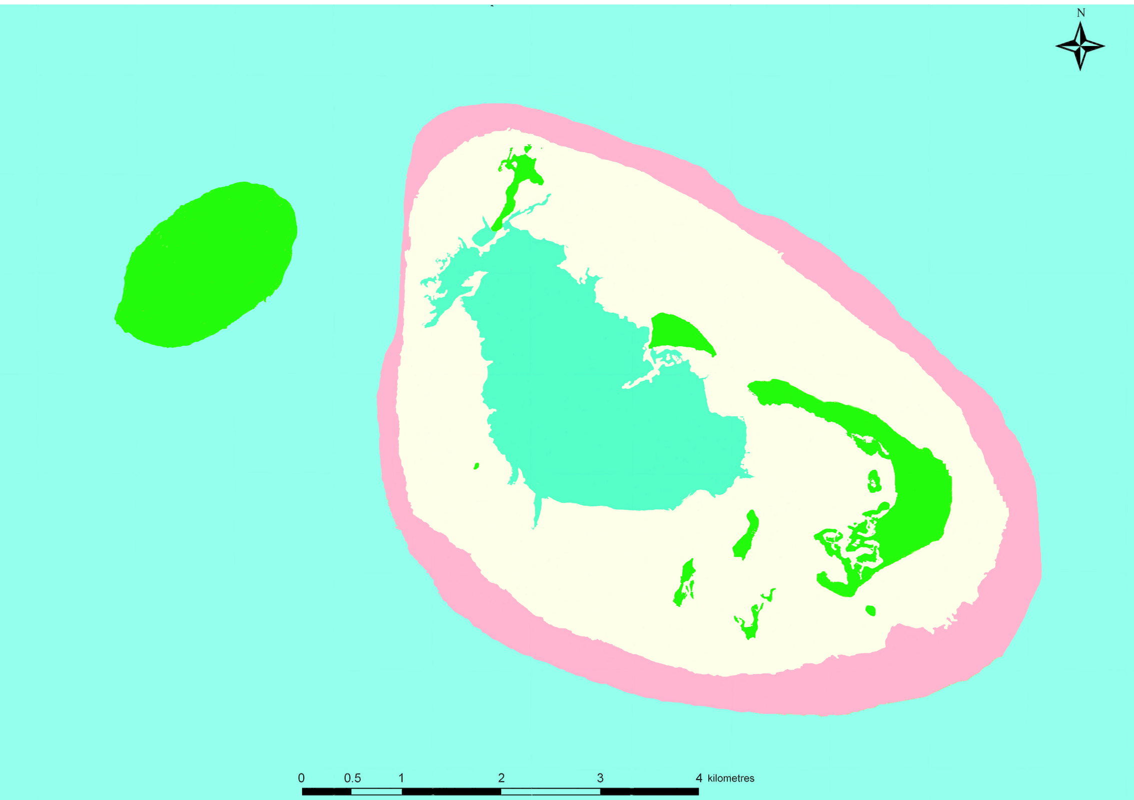


Figure 5.1: Map of St. Joseph Atoll showing delineated habitat types. Pink is fringe reef, beige is the reef flat, turquoise blue is the lagoon of the atoll, green are the islands present.

As juveniles were the most abundant ontogenetic stage in St. Joseph Atoll (Chapter 2), and represented the majority of tagged individuals, the following analyses of spatial and temporal habitat use and overlap relating to the second objective were restricted to this ontogenetic stage.

5.2.2 Spatial patterns of habitat use

Kernel density estimates providing the size and location of an animal's space use, as well as the overlap between these, have often been used to assess habitat partitioning (e.g. Speed *et al.* 2011). However, given that these estimates were not possible in this study (Chapter 3), the fine-scale spatial differences in habitat use by juvenile dasyatids were described by the proportion of detections for each individual at each receiver in the array. Firstly, the primary receiver (i.e. the receiver that recorded the most detections) was identified for each individual, and the proportion of detections recorded by that receiver was calculated. The location of each individual's primary receiver was plotted on a habitat map to visually identify habitat use patterns. A permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001), which considers both categorical and continuous independent variables, was then used to determine whether the dasyatid species, sexes, or sizes segregated spatially by receiver use. A Euclidean dissimilarity matrix was developed, based on the proportion of detections at each of the receivers for each individual over their period of detection in the atoll. The adonis function in the vegan package in R was used to perform the PERMANOVA (Anderson, 2001).

5.2.3 Temporal patterns of habitat use

The proportions of hourly detections (over the 24h diel cycle) were used to investigate whether there were temporal peaks in detections on the reef flats and lagoon habitats (the fringe reef was excluded because of the small number of detections in this habitat type). Detections were filtered so that only the first detection for every hour for each juvenile was kept (to avoid the influence of large numbers of detections if individuals were detected at the same receiver for a long period of time and to reduce possible effects of receiver detection efficiency in analyses). Each acoustic detection was assigned to an hourly bin (0-23), and the total number of detections for each hourly bin was summed (for each individual). Chi-squared tests were used to determine whether hourly detections differed from an even distribution. A PERMANOVA was then used (following a similar approach as above) to determine whether dasyatid species, sexes, or sizes utilised the receiver array differently over the diel cycle. A Euclidean dissimilarity matrix was developed, based on the proportion of detections in each hourly bin for each individual. Analyses were conducted separately for the reef flats and lagoon habitats.

5.2.4 Spatiotemporal patterns of habitat use

Social network analysis may be a useful tool in determining whether individuals have overlapping habitat usage. When constructing social networks, individuals are treated as nodes and edges occur when they associate or interact with one another (Farine & Whitehead, 2015). It therefore combines both spatial and temporal components of co-occurrence, as opposed to analysing these separately as done above. Combining social network analysis with acoustic array data is still in its infancy and has its limitations, but it can still provide useful insights. In this approach, the 'gambit of the group' is used, which defines all individuals within a group at a point in time as being associated (Farine & Whitehead, 2015) (i.e. if individuals co-occur within range of an acoustic receiver in a specified time frame, they are considered to be associating). It is difficult to infer actual sociality from traditional acoustic array data given the wide reception range of acoustic receivers (Mourier *et al.*, 2017) (although it may be accomplished using newer techniques such as VPS arrays (Armansin *et al.*, 2016)). In traditional receiver arrays individuals may be located 500m apart, but in this approach they are still considered to be associating, which is not biologically relevant when investigating strict social interactions. Despite this, the technique can be useful in determining the co-occurrences of individuals, whether individuals of the same phenotypic traits co-occur more than expected from random, and thus whether species, sexes, or size classes may be partitioning habitat on a spatiotemporal scale.

An approach described by Farine *et al.* (2015) was adapted and used for this chapter. The R package asnipe was used to build the social networks, and only individuals that occurred at the same receiver within 30 minutes of each other were assumed to be co-occurring. As strict social interactions were not being investigated (as discussed above), this time frame was deemed appropriate. Edges in the network were weighted by the simple-ratio association index that ranges from 0 (two individuals never co-occurred in the same group) to 1 (individuals always co-occurred in the same group). The assortativity coefficient based on weighted edges was then calculated using the R package assortnet (Farine *et al.*, 2014). This index is a measure of the tendency of nodes in a network to be connected to other nodes that have the same/similar phenotypic trait. The trait can be a categorical or continuous variable and the assortative coefficient was used to explore whether dasyatids co-occur more often with individuals of the same species, sex, and/or size. Telemetry data collected between July - September 2015 was used to construct the network as this is was the period when all individuals were tagged, and the majority of individuals were still being detected in the atoll.

Two permutation methods were then used to calculate the significance of the assortative coefficient (following Farine *et al.* 2015). Permutations randomise the original data to create a posterior distribution against which the observed value can be assessed. This randomisation is important as the observations of individuals interacting are non-independent, violating the assumptions of most parametric statistical tests. The first method was the node permutation method which represents the phenotypic null model. This consisted of randomly re-allocating the phenotype of the nodes while maintaining the same edge structure of the network. The second method was the data stream permutation method which represents the spatiotemporal null model. This consists of swapping the observations of two individuals observed in different groups, with swaps being restricted to control for space and time. The swaps were restricted to occur only between groups that occurred at the same receiver on the same day. Therefore, the time and location of group remains constant, but the details of group membership changes, and it tests whether structure exists at each location. By comparing both these null models, it allows for the partial differentiation between potential patterns arising from spatial and social effects. The randomisation was repeated 1000 times for each model and a p-value was obtained by comparing the coefficient expected values with the observed one.

5.3 Results

5.3.1 Habitat preference

The reef flats habitat contained the largest number of receivers (50%), followed by the fringe reef (29%) and lagoon (21%) habitats. Differences in the utilisation of these habitats were found between ontogenetic stages but not between species. For *P. ater*, *U. granulatus* and *U. asperrimus* juveniles, the vast majority of detections were recorded on the reef flats, a small amount in the lagoon and very few on the fringe reef (Table 5.1). As such, the chi-square goodness of fit test indicated that habitat type was not utilised in proportion to availability (p<0.001 for all groups). Habitat selection indices revealed a preference to the reef flats, while the lagoon and fringe reef habitats were avoided by all juveniles (Table 5.1).

Resident adults appeared to be more reliant on the lagoon habitat. *P. ater* adults had the majority of their detections in this habitat and habitat selection indices revealed a preference for the lagoon habitat, whereas the reef flats and fringe reef habitats were avoided (Table 5.1). Conversely, *U. granulatus* adults had most detections on the reef flats, as with the juveniles, but they had a higher proportion of detections in the lagoon compared to juveniles and selection indices revealed a neutral use of the lagoon by adults (i.e. it was neither preferred or avoided) (Table 5.1). It is interesting to note that only juveniles for both species had detections on the fringe reef and no adults were detected in this habitat. The only tagged *U. asperrimus* adult was not resident to the atoll.

Table 5.1: The percentage of acoustic detections and habitat selection values (L) for each habitat type in St. Joseph Atoll, for each dasyatid species and ontogenetic stage.

Habitat	**No. of detections (%)**		**L**	
	P. ater juvenile	*P. ater* adult	*P. ater* juvenile	*P. ater* adult
Reef flats	90.57	24.18	40.57	-25.82
Lagoon	9.37	75.82	-12.06	54.39
Fringe reef	0.06	0.00	-28.51	-28.57
	U. granulatus juvenile	*U. granulatus* adult	*U. granulatus* juvenile	*U. granulatus* adult
Reef flats	97.19	81.52	47.19	31.52
Lagoon	2.72	18.48	-18.70	-2.95
Fringe reef	0.08	0.00	-28.49	-28.57
	U. asperrimus juvenile	*U. asperrimus* adult	*U. asperrimus* juvenile	*U. asperrimus* adult
Reef flats	89.05	-	39.05	-
Lagoon	10.82	-	-10.61	-
Fringe reef	0.13	-	-28.44	-

5.3.2 Spatial patterns of habitat use

A high proportion of detections was recorded by primary receivers, particularly for *P. ater* (mean ± SD = 72 ± 23%) and *U. granulatus* juveniles (mean ± SD = 76 ± 16%), but less so for *U. asperrimus* juveniles (mean ± SD = 54 ± 15%). The primary receivers were located throughout the reef flats of the atoll, with most being situated at the south-east corner (Figure 5.2). Spatial receiver use did not appear to be grouped by species, sex or size (Table 5.2).

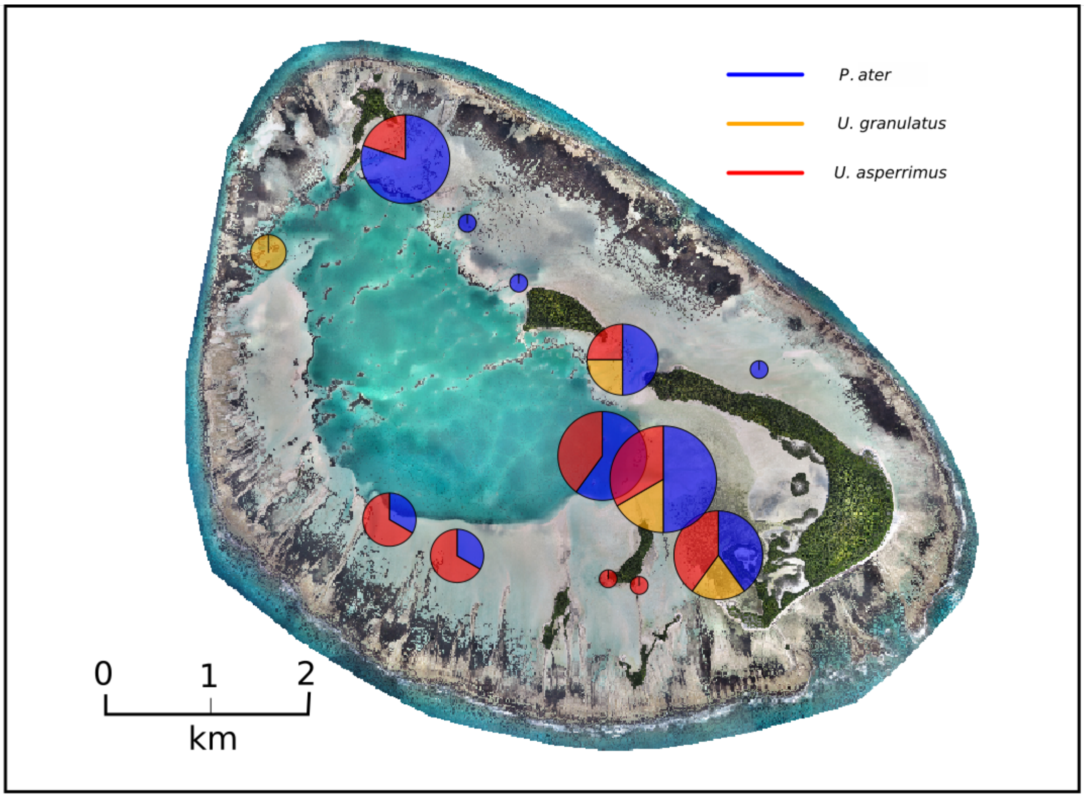


Figure 5.2: Map of St. Joseph Atoll showing the location of the primary receiver for each acoustically tagged dasyatid. The size of the pie chart is proportional to the number of individuals whose primary receiver is located there. Blue represents *P. ater* juveniles, yellow *U. granulatus* juveniles, and red *U. asperrimus* juveniles.

Table 5.2: Results of the PERMANOVA performed on the proportion of detections at each acoustic receiver for each tagged dasyatid. Independent variables included species, size (disc-width cm) and sex.

	DF	F statistic	R^2	p-value
Species	2	1.11	0.05	0.26
Size	1	1.48	0.04	0.06
Sex	1	0.84	0.02	0.71
Residuals	36		0.89	

5.3.3 Temporal patterns of habitat use

Chi-squared tests showed that hourly detections of *P.ater* in the reef flat habitat differed significantly ($p<0.01$) from an even distribution for all individuals except one (#8). When examining plots of these peaks, individual variation was evident. Seven individuals had peaks in their detection frequencies around dawn and/or dusk, four had peaks during the day (mostly between 12:00 and 15:00), three had peaks during the night, and 3 displayed no dominant peaks (Figure 5.3). For the lagoon habitat, chi-squared tests were significant for only 10 individuals (some individuals were absent from this habitat or had a very low numbers of detections). Most individuals had peaks during the night, two had peaks during the day and one had a peak in both the night and day (Figure 5.3).

Detections for *U. granulatus* displayed more consistent trends. In the reef flat habitat, chi-squared tests were significant for all individuals and detections peaked around dawn and dusk, with very few detections during the day. Conversely, in the lagoon habitat, chi-squared tests were significant for only two individuals, but both had detections that peaked during the day (Figure 5.3).

Even though chi-squared tests revealed that detections for *U. asperrimus* in the reef flat habitat significantly differed from an even distribution for all individuals, trends were not consistent. Detection peaks occurred at all times throughout the diel cycle for different individuals, but most individuals had peaks during the day ($n = 7$), a few at dawn/dusk ($n = 3$) and a few during the night ($n = 2$). In the lagoon habitat, chi-squared tests were significant for only seven individuals, most had detections peaks during the day, one had during the night, and two had peaks in both the day and night (Figure 5.3).

The different species, sexes and sizes did not differ significantly in their hourly proportions of detections for the reef flat habitat, but a significant difference was found between species in the lagoon habitat (Table 5.3).

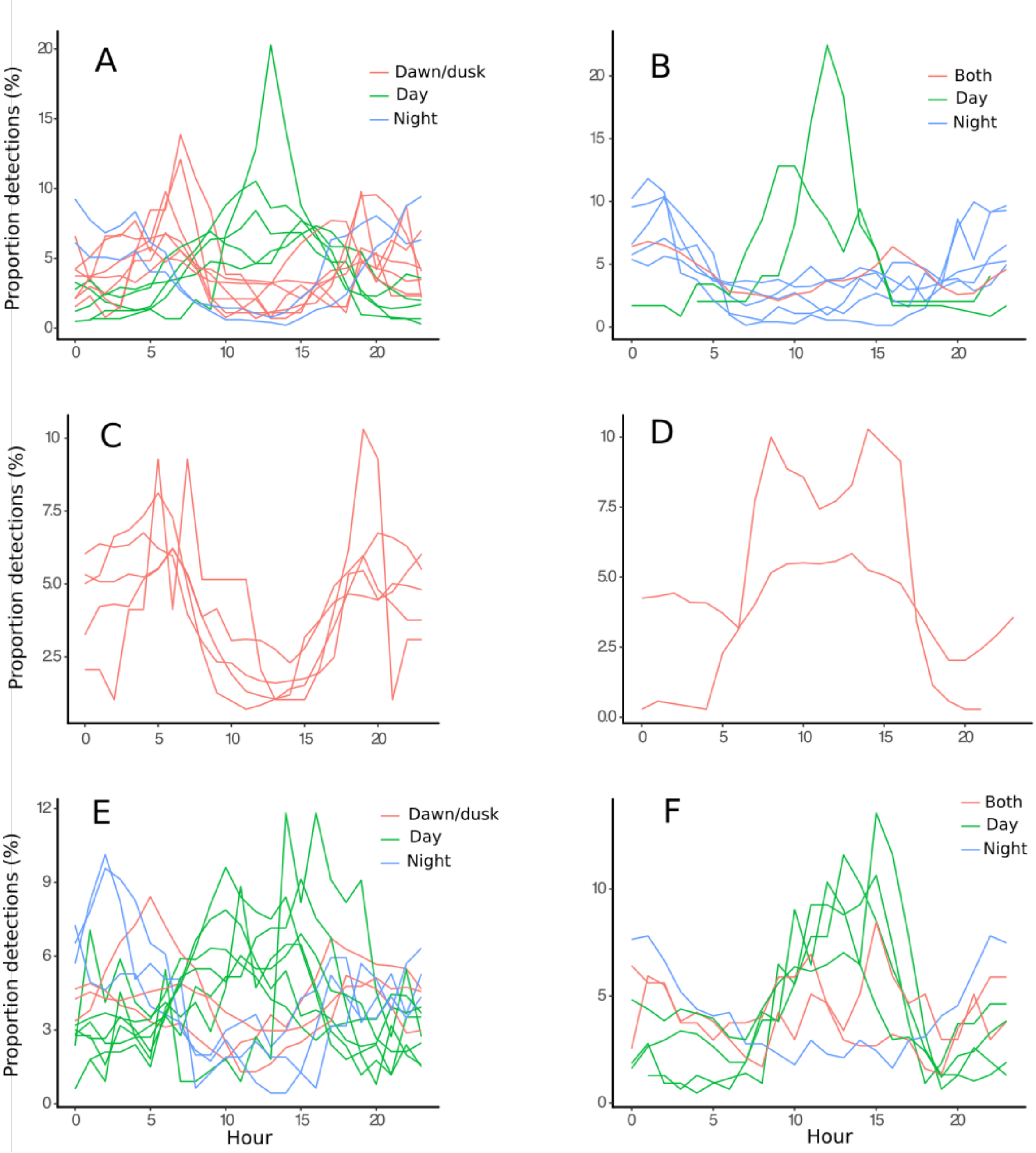
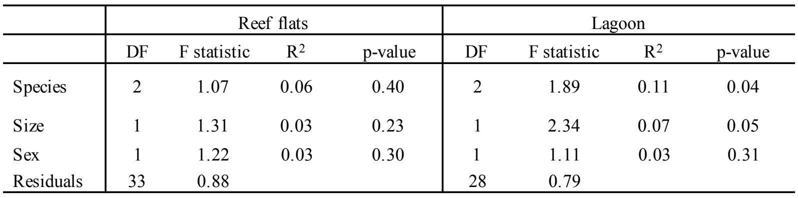


Figure 5.3: Individual proportions of hourly detections in St. Joseph Atoll for for acoustically tagged *P. ater* (A, B), *U. granulatus* (C, D), and *U. asperrimus* (E, F). Figures on the left represent detections in the reef flat habitat (A, C, E) and figures on the right represent detections in the lagoon habitat (B, D, F). Colours represent when diel peaks occur.

Table 5.3: Results of the PERMANOVA performed on the proportion of detections in each hour (over the 24h diel cycle) for each tagged dasyatid. Independent variables included species, size (disc-width cm) and sex.



	Reef flats				Lagoon			
	DF	F statistic	R^2	p-value	DF	F statistic	R^2	p-value
Species	2	1.07	0.06	0.40	2	1.89	0.11	0.04
Size	1	1.31	0.03	0.23	1	2.34	0.07	0.05
Sex	1	1.22	0.03	0.30	1	1.11	0.03	0.31
Residuals	33	0.88			28	0.79		

5.3.4 Spatiotemporal patterns of habitat use

Social network analysis revealed that most individuals co-occurred with others at a receiver at the same time, with only two individuals (#41 and #47) displaying no edges. Individuals also displayed variable edge weights, some were only connected to others of the same species (e.g. #1, #3 and #4), but most were connected to hetero- and con-specific individuals (Figure 5.4). Individuals appeared to associate more with heterospecifics, with the other sex and with individuals of similar sizes however, assortativity coefficients were not significantly different from the phenotypic null models ($p > 0.05$ for all models), suggesting that these levels of assortment were not different from random. Coefficients were significantly different from the spatiotemporal null models ($p < 0.05$ for all models), which indicates that phenotypes were randomly distributed in space (given that the spatial variation in the number of each phenotype was not maintained in these spatiotemporally controlled null models).

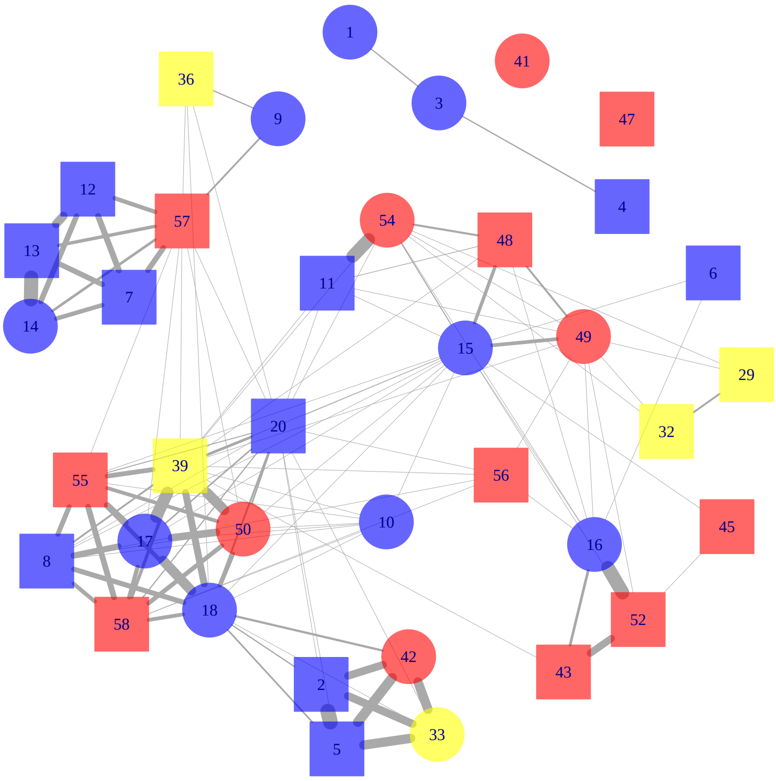


Figure 5.4: Social network for acoustically tagged dasyatid juveniles. Each node colour represents a different species: *P. ater* = blue, *U. granulatus* = yellow, *U. asperrimus* = red. Each node shape represents sex: male = square and female = circle. The edge width represents the simple ratio index (i.e. the proportion of times that individuals co-occurred in a group).

5.4 Discussion

Preferential habitat selection by juveniles

P. ater, *U. granulatus*, and *U. asperrimus* juveniles exhibited distinct habitat preferences in St. Joseph Atoll, occurring more frequently in the reef flat habitat, while avoiding the deeper lagoonal and fringe reef habitats. A similar habitat preference was found for juvenile sicklefin lemon sharks *Negaprion acutidens* in an intertidal coastal bay (Oh *et al.*, 2017). Animals are known to select habitats to satisfy their requirements for resources and refuge (Nathan *et al.*, 2008). The preference for the reef flats in St. Joseph Atoll is likely, in part, due to the refuge opportunities they provide. Three species of sharks are resident to St. Joseph Atoll (blacktip reef *Carcharhinus melanopterus*, sicklefin lemon *Negaprion acutidens*, and tawny nurse *Nebrius ferrugineus* sharks) (Lea *et al.*, 2016) and juvenile dasyatids were sometimes found with shark bite wounds on their pectoral fins (Elston pers. obs.), indicating that predation is a risk in St. Joseph Atoll. The shallow reef flats are largely inaccessible to these sharks; they occur more frequently in the deeper lagoon and only move onto the reef flats at high tides (Filmalter *et al.*, 2013; Lea *et al.*, 2016) and juvenile dasyatids may be utilising the shallow reef flats to avoid these predators. Another line of evidence to support this predator avoidance hypothesis is that resident adult dasyatids were found to be more reliant upon the deeper lagoon habitat. With the increase in body size, predation risk is lowered, and these larger individuals can then exploit deeper waters in relative safety (Vaudo, 2011; Oh *et al.*, 2017). Predation risk has been used to explain habitat selection by juvenile dasyatids in previous studies. For example, juvenile southern stingrays *Dasyatis americana* were abundant on the depauperate shallow sand flats of the Glovers Reef Atoll, trading energy for safety (Tilley, 2011). In addition, juveniles of *U. granulatus* were found to remain in shallow waters to avoid encounters with large blacktip reef and lemon sharks in an intertidal bay (Davy *et al.*, 2015).

However, there may also be other reasons behind this habitat choice, for example, juveniles may be utilising the reef flats for the availability of prey. Dasyatids were often observed feeding on the shallow sand flat areas and feeding pits were numerous in this habitat (Elston pers. obs.). Given both the presence of large seagrass beds on the flats as well as the pristine nature of the atoll, it might be a productive habitat hosting large benthic invertebrate communities that these dasyatids prey upon. However, a previous study has found the sand flats of a similarly pristine atoll in the Caribbean to be relatively depauperate compared to deeper habitats (Tilley, 2011). Therefore, the composition and abundance of the invertebrate community would need to be investigated to determine if this habitat provides the most abundant prey resources.

Finally, juveniles may prefer the reef flats based on environmentally beneficial conditions, for example warmer temperatures. Batoids are known to select habitats based on their thermal advantages (Matern *et al.*, 2000; Vaudo & Lowe, 2006), and these dasyatids may be seeking out warmer waters to increase metabolic and growth rates. However, the high individual variability in diel detection peaks may suggest that temperature does not have a significant influence on movement patterns. Further investigation is needed to determine if thermoregulatory behaviours are present.

Inter- and intra-specific habitat partitioning

Sympatric shark communities have been found to partition habitat on a small-scale, occupying different areas within coral reefs and bays (DeAngelis *et al.*, 2008; Heupel *et al.*, 2018b). Conversely, a sympatric batoid community was found to spatially overlap in a small bay (Vaudo & Heithaus, 2009, 2012). This chapter had similar results to the latter study, with juvenile dasyatids exhibiting no clear evidence for habitat partitioning on the reef flats of St. Joseph Atoll. There was individual variability in the spatial and temporal utilisation of the reef flats. Spatially, the primary receivers of individuals were spread across the reef flats (although the largest cluster occurred in the south-east corner of the atoll). Temporally, individual diel peaks in detection frequencies occurred throughout the 24 hour cycle (although the largest proportion of individuals had peaks at dawn/dusk). However, despite this individual variability, there were no significant inter-specific differences in spatial or temporal habitat utilisation on the reef flats.

Social network analysis further confirmed this as there were random levels of co-occurence between con- and hetero-specifics. The same was found when comparing different sexes or sizes of individuals. These results suggest that this sympatric dasyatid community are ‘indifferent neighbours’, coexisting randomly with different and alike phenotypes. *P. ater* has been known to form facultative resting groups with con- and hetero-specifics in Shark Bay, Australia, in response to an increase in the perceived risk of predation. While it is difficult to infer direct behaviour from telemetry data, it may be that juvenile dasyatids in St. Joseph Atoll are co-occurring for similar reasons. Alternatively, environmental conditions may necessitate the use of certain habitats at different times, causing these observed patterns of random co-occurrence.

Sympatric elasmobranchs can either partition both prey resources and habitat (White *et al.*, 2004), or they can partition one or the other (Papastamatiou *et al.*, 2006). Results for this study are in agreement with the latter suggestion, as dietary differences between dasyatid species (Chapter 4) do not appear to be driven by differences in habitat utilisation on the reef flats. Instead, dietary divergence may be facilitated by species-specific morphological adaptations or differences in foraging behaviour.

There was some evidence for the temporal partitioning of the lagoon habitat, with the majority of *P. ater* juveniles occurring in this habitat more during the night, while the majority of *U. granulatus* and *U. asperrimus* juveniles occurred in this habitat more during the day. Although detections in the lagoon were rare for juveniles in comparison to detections on the reef flats, the use of this habitat might be related to physical phenomena, such as the tidal cycle, and should be investigated further. However, the reasons behind these inter-specific diel differences in the utilisation of the lagoon remain unclear.

There was also evidence for ontogenetic habitat segregation, as resident adults were more reliant on the deeper lagoonal habitat. The ontogenetic segregation of individuals is common in elasmobranchs (Speed *et al.*, 2010; Grubbs, 2010; Ajemian & Powers, 2016), and larger sharks have also been found to move from shallow seagrass beds to a deeper atoll lagoon (Pikitch *et al.*, 2005). This ontogenetic segregation probably facilitates the intra-specific dietary partitioning found in Chapter 4 and could have evolved as a consequence of competition for resources. Alternatively, larger individuals may not be as susceptible to predation and can exploit deeper waters (Sims, 2003).

Caveats

There are three caveats in this chapter that need to be mentioned. The first is that all individuals were caught and tagged on the reef flats of the atoll. Even though it is unlikely that juveniles occur in the deeper lagoonal habitats (given the evidence that juvenile rays are known to avoid areas with high predation risk (Tilley, 2011; Cerutti-Pereyra *et al.*, 2014; Davy *et al.*, 2015)), as no fishing effort was directed toward this habitat, it cannot be ruled out that there may be juveniles that occur in this habitat.

The second caveat relates to the findings of the high inter-specific habitat overlap. This hypothesis was tested at the level of receiver utilisation. Given that receivers are located in close proximity (between ∼200m to ∼600m apart), habitat partitioning was tested at a relatively fine spatial scale. However, individuals may still be partitioning different habitats that these analyses could not identify. For example, some individuals may occur more in or adjacent to seagrass bed or island fringes, whereas others may occur over bare mud-flats (where benthic faunal compositions may differ) (O'Shea *et al.*, 2013). To resolve this, even finer-scale spatial information would be required, for example through active tracking or through a Vemco Positioning System (VPS) receiver array (where receiver detection ranges are overlapping to provide a more exact estimate of individual locations) (as done by Armansin *et al.* (2016)).

The third caveat relates to the potential differences in receiver detection efficiency between the shallow reef flats and the deeper lagoon (where physical disturbance will have a greater and lesser negative impact on detection efficiency respectively) and how this might effect habitat selection indices. However, the habitat that recorded most detections was the reef flat habitat, which suggests that receiver detection efficiency was not the reason behind the large number of detections in this habitat type.

Conclusions

Tracking multiple species simultaneously is useful when elucidating how species interact with each other within a habitat, thereby providing insights into resource partitioning (Heupel *et al.*, 2018b). This is one of the few studies to examine habitat partitioning in a sympatric batoid community using acoustic telemetry. The fine-scale data that acoustic telemetry can provide is critical given the small-scales over which benthic dasyatids are hypothesised to partition habitat (O'Shea *et al.*, 2013). However, this study, as with others (Vaudo & Heithaus, 2009, 2012), have yet to identify habitat partitioning in batoid communities.

Social network analysis was found to be a useful tool when evaluating habitat partitioning intra- and inter-specifically as it combines both spatial and temporal data to determine when individuals are co-occurring. This can then be used to test hypotheses of co-occurrence due to both spatial or social effects (Farine *et al.*, 2015). While drawing inferences related to social interactions is limited given the large spatial range of receiver detections (Mourier *et al.*, 2017), the associations of individuals can be used to test co-occurrence rates between like phenotypes, which feeds directly into whether similar phenotypes are sharing or partitioning habitat. This type of analysis has the potential to unlock further insights into animal movement patterns as determined through acoustic telemetry, and its future use should be advocated.

Understanding the spatial dynamics and habitat preferences of coexisting species is necessary to determine species-specific habitat requirements, and thus it provides valuable information on how to manage areas effectively for conservation. Specifically, knowledge of habitat partitioning by multiple species can be used to influence the design of marine protected areas to take into account species complexes rather than single taxa (for e.g. Lea *et al.* 2016).

Chapter 6

The influence of physical environmental factors on juvenile dasyatid movement patterns

6.1 Introduction

Elucidating animal ecology necessitates understanding both habitat selection and the reasons behind this selection (Morris, 2003). This knowledge of the 'why' of habitat selection assists in predicting animal movement and further informs how animals will respond to anthropogenic influences such as climate change or habitat degradation (Sakabe & Lyle, 2010; Speed *et al.*, 2012; Vaudo & Heithaus, 2013). However, recent reviews show that many elasmobranch tracking studies have not identified the drivers behind movement patterns, and they simply report when and where animals occur (Sims, 2010; Hammerschlag *et al.*, 2011; Flowers *et al.*, 2016).

Shallow nearshore environments are dynamic in nature and the selection of appropriate habitats within these environments will depend on a variety of physical factors (for e.g. temperature, tidal flow, salinity) and biological processes (for e.g. predation risk and prey availability) (Sims, 2003; Speed *et al.*, 2010; Carlisle & Starr, 2010). The dynamism of these factors is often manifested over a variety of temporal scales and movement patterns often change over large-scale seasonal cycles to smaller-scale diel and tidal cycles. As there are multiple drivers behind habitat selection, trade-offs will occur. For example, batoids were shown to select shallow nearshore habitats at low tide, which may serve as a refuge from predation but, this was likely a metabolically expensive choice due to high temperatures (Vaudo & Heithaus, 2013).

There is also a link between an animal's biology and it's external environment which will effect habitat selection. For example, the physiological state of an animal will be an optimised under a subset of environmental conditions and animals may avoid habitats where such environmental conditions are not optimal (Brown *et al.*, 2004). These environmental thresholds may be species- or population- specific, allowing each to exploit different ecosystems (Fangue & Bennett, 2003; Dabruzzi *et al.*, 2013).

Elasmobranchs are ectothermic and therefore temperature, in particular, can have a significant effect on physiological and biological aspects such as growth and metabolic rates, embryonic development, parturition, and the rate of digestion (Fangue & Bennett, 2003; Hight & Lowe, 2007; DiGirolamo *et al.*, 2012; Dale *et al.*, 2013). Consequently, temperature effects elasmobranch movement and certain species exhibit behavioural thermoregulation (whereby individuals will select habitats in a thermally heterogenous environment to maintain body temperatures within an optimal range) in both field and lab settings (Matern *et al.*, 2000; Hopkins & Cech, 2003; Sims *et al.*, 2006; Vaudo & Lowe, 2006; DiGirolamo *et al.*, 2012; Speed *et al.*, 2012). Salinity and oxygen levels may also affect habitat selection in batoids, for example, the Atlantic stingray *Hypanus sabinus* commonly used shallow areas with reduced dissolved oxygen, likely in an effort to reduce levels of competition and predation risk (Dabruzzi & Bennett, 2014). However, these effects are not as well studied and understood as temperature effects.

Tides and the associated changes in water levels, particularly in shallow nearshore areas, will potentially have one of the strongest impacts on elasmobranch movement as it influences all other physical aspects of the water column (such as temperature and oxygen concentration) (for example Carlisle & Starr (2010); Brinton & Curran (2017); Smith & Curran (2017)). In addition, tides also change environmental biotic factors such as predation risk (as larger predators cannot access very shallow waters) and prey availability (as tides will alter the availability of intertidal flats). Indeed, tides were a significant driver of the movement of *U. granulatus* juveniles in Orpheus Bay, Australia and it was hypothesised individuals were utilising the tides to remain in shallow waters to avoid encounters with larger sharks present in the bay (Davy *et al.*, 2015).

St. Joseph Atoll represents a heterogenous environment: the reef flats are a very shallow habitat that is constantly in flux (for e.g. tides significantly change the availability of these flats and temperature is highly variable), whereas the lagoon habitat is a more stable environment. The previous chapter revealed that juvenile dasyatids in St. Joseph Atoll preferentially selected for the shallow flats, but also occurred in the deeper lagoon. Given the environmental heterogeneity of the atoll, this chapter aimed to determine if:

- the daily presence of juveniles to the atoll varies with the environmental factors of temperature, precipitation and wind speed

- the habitat utilisation within the atoll (i.e. flats vs. lagoon habitats) is effected by the environmental factors of temperature, precipitation, tidal and diel stage

6.2 Materials and methods

Juvenile dasyatids were passively monitored with an acoustic receiver array and acoustic detections were related to the local physical environmental data that was collected (as described in chapter 2). Generalised linear mixed models GLMMs were used to model the influence of physical variables on dasyatid presence and all GLMMs were run with a binomial distribution and a 'logit' link function as response variables were binary (presence/absence). For further details on the modelling approach, see chapter 2.

Given the variability in daily residency found in chapter 3, it was first investigated whether physical factors influenced daily presence of *P. ater* and *U. granulatus* juveniles to St. Joseph Atoll. Data from *U. asperrimus* were excluded as this question has already been addressed before for this species (Elston, 2016). A GLMM was used to determine if daily presence (coded as 0 for absent and 1 for present for each individual) was effected by daily mean air temperatures, precipitation, and wind speeds. Two GLMMs were conducted: GLMM1 included data from both *P. ater* and *U. granulatus* that spanned a one-year period (1 June 2015 - 31 May 2016) while GLMM2 included only data from *U. granulatus* in the prior year (1 April 2014 - 31 November 2014). This latter dataset was included in a separate model because most *U. granulatus* detections occurred during this period, but data needed to be filtered to exclude any detections recorded by receivers that were installed during 2014 (see Chapter 2 for details).

Secondly, it was investigated whether physical factors influenced the habitat utilisation of juvenile dasyatids in St. Joseph Atoll. Every detection was coded as either a reef flat or lagoon detection (see map in chapter 5) and was matched to the temporally closest environmental variables. A GLMM was then used to determine if habitat utilisation (coded as 0 for lagoon or 1 for reef flats presence) was influenced by water temperature on the flats, tidal height, and precipitation (the latter of which probably effects salinity in the shallow waters). The diel stage was also included as a factor (coded as dawn/day/dusk/night). Because of this GLMM set-up, if the probability of being detected on the flats increased, it automatically followed that the probability of detection in the lagoon decreased (and vice-versa). Data from each species was modelled separately and data from *U. asperrimus* were included because prior investigations into environmental influences on movement did not take into account the effect of environment on flats vs. lagoon presence (Elston, 2016). In addition, models were run separately for each season, given the evidence that season effected movement patterns (Chapter 3). Four months of telemetry data in each season were analysed for both *P. ater* and *U. asperrimus* (SE-monsoon: 1 June 2015 - 31 September 2015, NW-monsoon: 1 December 2015 - 31 March 2016). Telemetry data collected in the prior year was analysed for *U. granulatus* and only spanned one season (1 June 2014 - 30 September 2014) as most individuals were no longer detected in the NW-monsoon season subsequent to tagging. The detections recorded by receivers that were installed in 2014 were excluded for *U. granulatus*.

To further investigate the role of tides on habitat utilisation by juvenile dasyatids, telemetry detections were binned into 10cm tidal height bins based on the tidal height that was matched to each detection. However, due to the uneven distribution of tidal heights across the range (0-2m) of the tidal cycle (see results), the frequency of detections in each tidal bin needed to be corrected for bias. To do this, every 10-minute tidal height record calculated by the tidal model (see details in chapter 2) were also binned into 10cm tidal height categories and the proportion of tidal height occurrences in each bin was calculated. The frequency of telemetry detections in each tidal bin was then divided by the proportion of tidal height occurrences in each tidal bin. These corrected frequencies were then used to calculate the proportions of telemetry detections in each 10cm tidal bin. Furthermore, a chi-squared test was used to determine if the distribution of telemetry detections across the tidal range was significantly different from the distribution of the occurrence of each tidal height across the tidal range. These analyses were conducted separately for flats and lagoon habitats.

6.3 Results

6.3.1 Daily presence to the atoll

Results from GLMM1 performed on *P. ater* and *U. granulatus* daily presence to the atoll showed that the probability of daily detection was significantly influenced by only wind speeds and air temperature. The model predicted that the probability of daily atoll detection decreased by 17% with increasing wind speeds and increased by 6% with increasing temperatures. The GLMM2 included all three variables as significant influences on *U. granulatus* daily presence (i.e. temperature, wind speed and precipitation). This model predicted larger effects by variables and the probability of daily detection in the atoll increased by 55% and 40% with increasing precipitation and temperature respectively, while detection probability decreased by 40% with increasing wind.

6.3.2 Habitat utilisation

Water temperature on the reef flats varied significantly across the diel cycle, with peaks during the late afternoon and the lowest values at dawn, whereas temperatures in the lagoon were relatively stable over the diel cycle. Temperatures in both the reef flats and lagoon habitats varied with season, with higher temperatures recorded in the NW-monsoon season (Figure 6.1).

The best-fit GLMMs revealed that the diel stage (i.e. dawn/day/dusk/night) had a significant effect on the habitat utilisation of all three dasyatid species and that temperature on the reef flats was a further significant effect for *P. ater* and *U. asperrimus* (but not *U. granulatus*). These effects were significant for both seasons but in different ways (Table 6.1). For both *P. ater* and *U. asperrimus*, the probability of lagoon detections was highest during the night in the SE-monsoon season, but highest during the day in the NW-monsoon season (Figure 6.2). Additionally, there was a higher probability of detections on the reef flats with increasing temperatures in the SE-monsoon seasons, while there was a higher probability of detections in the lagoon with increasing temperatures in the NW-monsoon season. Precipitation was found to have a minor and insignificant influence on habitat presence.

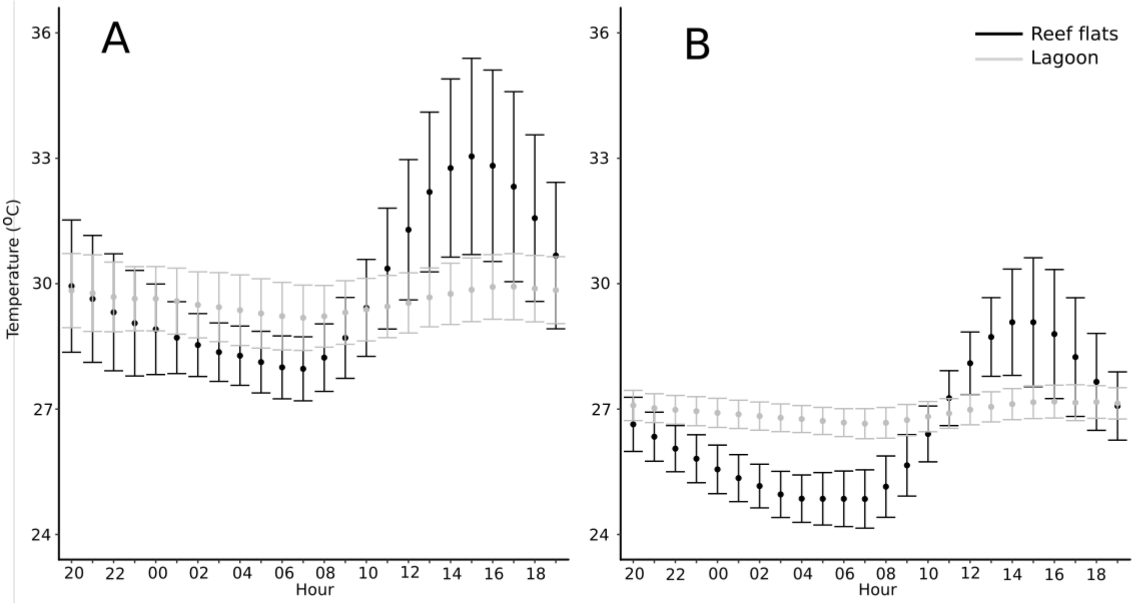


Figure 6.1: Mean hourly temperatures for the reef flats and lagoon habitats of St. Joseph Atoll for the NW-monsoon season (A) and the SE-monsoon season (B). Error bars denote standard deviation.

Table 6.1: Predictions by General Linear Mixed Models of environmental effects on dasyatid habitat utilisation for each season in St. Joseph Atoll. Significant effects included reef flats temperature (°C), tidal height, and diel stage. Outputs include the predicted magnitude that the effect has on the probability of detection, and the relationship of the effect to the probability of detection on the reef flats.

		SE-monsoon season		NW-monsoon season	
Species	Effect	Magnitude of effect (%)	Relationship to probability of detection	Magnitude of effect (%)	Relationship to probability of detection
P. ater	Temperature	0.2	Positive	2	Negative
	Tidal height	0.3	Positive	4	Positive
	Diel stage	0.2	-	2	-
U. asperrimus	Temperature	0.3	Positive	19	Negative
	Tidal height	2	Positive	24	Positive
	Diel stage	0.8	-	3	-
U. granulatus	Temperature	-	-	-	-
	Tidal height	5	Positive	-	-
	Diel stage	0.7	-	-	-

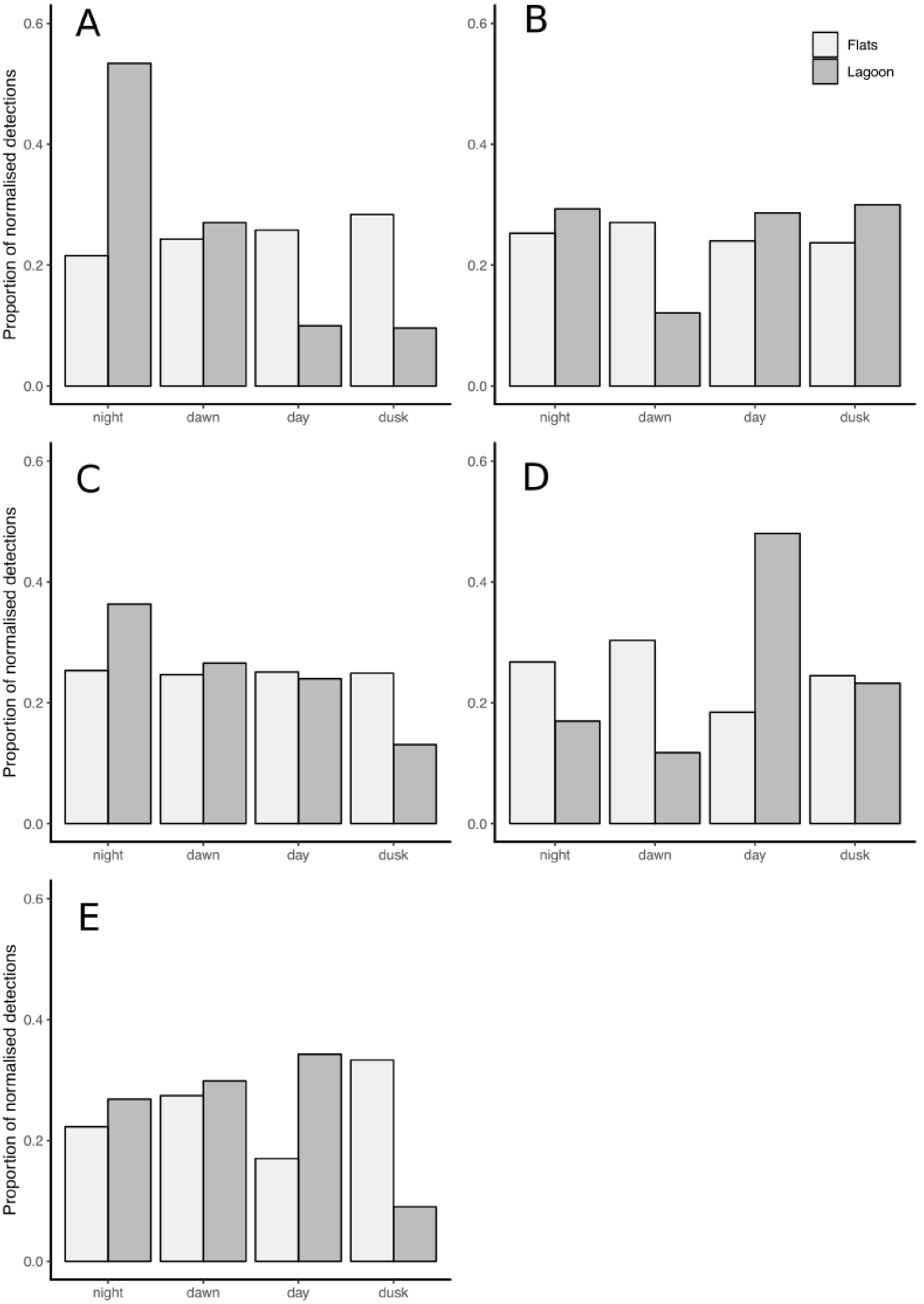
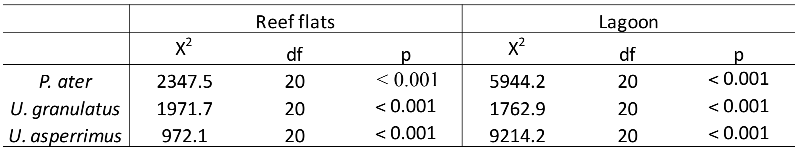


Figure 6.2: Proportion of acoustic detections by dasyatids in each diel stage (normalised by the number of hours in each diel stage) for *P. ater* (A and B), *U. asperrimus* (C and D), and *U. granulatus* (E). Figures on the left (A, C, E) are for the SE-monsoon season and figures on the right (B, D) are for the NW-monsoon season.

GLMMs also showed that tidal height had a constant significant effect on habitat utilisation for all three species in both seasons, with the probability of detections on the reef flats positively correlated to tidal height (Table 6.1). These results were further supported by the chi-squared tests which found that the distribution of telemetry detections in each tidal height bin was significantly different to the distribution of tidal height occurrences (for each habitat and for each species) (Table 6.2). Detections in the lagoon habitat peaked at low tides (<0.5m) while detections on the reef flats peaked at the highest tides (>1.7m) (Figure 6.3).

Table 6.2: Results of chi-squared (X^2) tests to determine if the tidal heights corresponding to dasyatid acoustic detections had a significantly different distribution to the occurrence of tidal heights in St. Joseph Atoll.



	Reef flats			Lagoon		
	X^2	df	p	X^2	df	p
P. ater	2347.5	20	< 0.001	5944.2	20	< 0.001
U. granulatus	1971.7	20	< 0.001	1762.9	20	< 0.001
U. asperrimus	972.1	20	< 0.001	9214.2	20	< 0.001

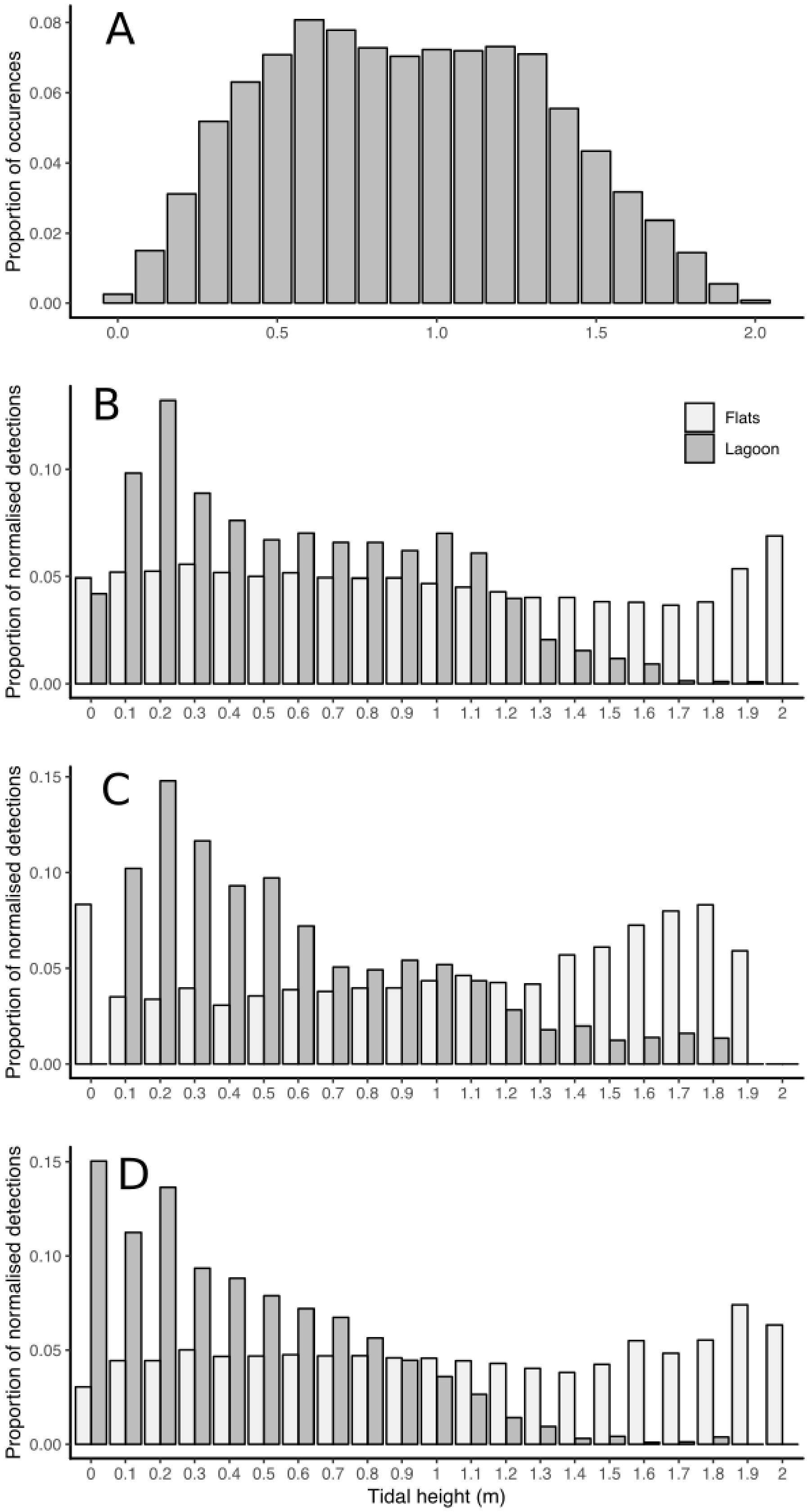


Figure 6.3: Histogram of tidal height bin occurrences for June 2015 - May 2016 in St. Joseph Atoll (A). The proportions of acoustic detections (normalised with the proportion of occurrences each tidal height bin) in each tidal height bin for each habitat type (reef flats or lagoon) recorded by *P. ater* (B), *U. asperrimus* (C), and *U. granulatus* (D).

The magnitude of the effect of each factor on habitat utilisation was highest during the NW-monsoon season. For example, models predicted that the probability of *P. ater* juveniles being detected on the flats vs. lagoon varied only 0.2% with increasing flats temperatures in the SE-monsoon season, while this value increased to 2% in the NW-monsoon season. In addition, models predicted abiotic variables to have the largest influence on *U. asperrimus* habitat presence when compared to other species. For example, in the NW-monsoon season, the increasing flats temperatures influenced the probability of habitat presence by 2% in *P. ater* and by 19% in *U. asperrimus* (Table 6.1).

6.4 Discussion

Daily presence

Juvenile dasyatids had highly variable levels of daily presence in St. Joseph Atoll (Chapter 3) and the GLMMs presented here suggest that this variability, at least in part, was caused by physical environmental factors. Firstly, GLMMs illustrated that the probability of daily detection was significantly negatively correlated with wind speeds. It is difficult to reconcile whether this is due to receiver detection efficiency or actual seasonal variation in movement patterns of dasyatids. Wind speed is known to reduce detection efficiency of receivers, both in this (Chapter 2) and other arrays (Heupel *et al.*, 2006). The SE-monsoon season is characterised by persistent strong winds and detections in this season were restricted when compared to the detections in the NW-monsoon season, which could be due to the influence of strong wind speeds on detection efficiency. However, the increase in detections recorded in the NW-monsoon season were largely in the deeper lagoon habitat, where wind is unlikely to have as large an effect on detection efficiency as on the shallow flats. This discrepancy suggests that these seasonal patterns in detections may not only be due to the changes in receiver detection efficiency but may also be driven by actual changes in movement patterns. However, there is still the likelihood that daily detection was, at least in part, compromised by increased wind speeds and the subsequent reduction in receiver detection efficiency.

Secondly, the GLMM highlighted that, for *U. granulatus*, the probability of daily detection increased with increasing precipitation. This trend is unlikely related to receiver detection efficiency, because precipitation has been found to negatively effect detection efficiency at shallow depths (Huveneers *et al.*, 2016) but, it is probably linked to the differences in seasonal utilisation of the atoll by juvenile dasyatids. The positive correlation between the probability of daily detections and precipitation and temperature further suggests individuals utilised larger proportions of the receiver array in the warmer and wetter NW-monsoon season, as found in Chapter 3.

Habitat utilisation

The habitat utilisation by juvenile dasyatids in St. Joseph Atoll significantly varied over the diel cycle. Diel patterns in behaviour have often been seen before in elasmobranchs and have been attributed to foraging (Cartamil *et al.*, 2003; Carlisle & Starr, 2010; Hammerschlag *et al.*, 2017a), predator avoidance (Holland *et al.*, 1993; Cartamil *et al.*, 2003), or as a strategy to optimise physiological processes through behavioural thermoregulation (Matern *et al.*, 2000; Hight & Lowe, 2007). If foraging or predator avoidance were the drivers behind diel movement patterns, one would expect a consistent pattern through time. However, in this study, the diel pattern of habitat usage changed between the two seasons. Additionally, temperature was a significant influence on habitat utilisation. Together, this suggests that behavioural thermoregulation may be the reason behind the observed diel patterns in this study.

More specifically, dasyatids appeared to avoid temperature extremes, seeking out the thermal stability of the lagoon when temperatures on the flats became too high or low. For both *P. ater* and *U. asperrimus*, when temperatures on the flats reached their lowest during the nights of the cooler SE-monsoon season, individuals occurred more frequently at these times in the contextually warmer lagoon. Similarly, when temperatures on the flats reached their highest during the days of the warmer NW-monsoon season, juveniles occurred more frequently at these times in the contextually cooler lagoon. The avoidance of warm temperatures appeared to be more important than the avoidance of cool temperatures, as models predicted physical factors to have larger influences on habitat use in the warmer NW-monsoon season.

The inconsistency of habitat utilisation over the diel cycle between seasons has been noted before, where Atlantic stingrays *Dasyatis sabina* were hypothesised to avoid colder waters in the winter (Brinton & Curran, 2017). For other species that behaviourally thermoregulate, some employ the 'hunt-warm rest-cool' strategy while others restrict their movement during warmer periods over the diel cycle (Matern *et al.*, 2000; Cerutti-Pereyra *et al.*, 2014). Seasonally, some species are known to seek out warmer waters at certain times of the year (Vaudo & Lowe, 2006; Jirik & Lowe, 2012). In this study, dasyatids selected for a thermal niche and avoided temperature extremes, which may ensure that physiological processes are continually optimised. High temperatures may increase metabolic rates to the point where they are unsustainable, while low temperatures may slow down growth rates too much (Dale *et al.*, 2013). The preference to remain in a narrow thermal niche has been witnessed in a laboratory setting where tropical juvenile ribbontail stingrays *Taeniura lymna* were found to have small thermal niches and individuals actively moved in tanks to remain in this narrow thermal niche (Dabruzzi *et al.*, 2013).

Even though data for *U. granulatus* showed an opposite trend, with more day-time (instead of night-time) detections in the lagoon during the SE-monsoon season, the GLMM revealed that neither diel cycle nor temperature significantly influenced habitat presence. These results must be treated with caution as there was only limited data for one season (SE-monsoon) for this species. Regardless, it raises questions as to whether this species may be more thermally tolerant in comparison to the other two. Laboratory studies show that different species can have different thermal tolerances (Fangue & Bennett, 2003; Dabruzzi *et al.*, 2013). However, species-specific responses to temperature changes in the wild remain an enigma as the majority of studies that investigate temperature influence on batoid movement only consider one species (Cartamil *et al.*, 2003; Vaudo & Lowe, 2006; Jirik & Lowe, 2012). More multi-specific research is needed to determine how different species respond to the same thermal environment.

Dasyatid habitat utilisation was also influenced by the tidal cycle. Detections in the lagoon occurred at a significantly higher proportion at low tides while detections on the flats occurred more during the high tides for all three species. Movements of elasmobranchs in shallow nearshore areas are more often associated with tides than diel patterns (Smith & Merriner, 1985; Wetherbee *et al.*, 2007; Papastamatiou *et al.*, 2009; Carlisle & Starr, 2010; Filmalter *et al.*, 2013; Brinton & Curran, 2017). Tidally-influenced movement patterns are often attributed to foraging because, in shallow environments, the amount of foraging habitat available to elasmobranchs can change over the tidal cycle, resulting in movements into prey-rich intertidal habitats with the high tide to forage. In this study, tidally-linked movement patterns were probably related to physical habitat availability. At low tides, the flats represented a very shallow habitat with some regions completely exposed, presenting a high risk of stranding and restricting movement in this habitat. Conversely, at high tides there was a sufficient water depth on the flats to allow dasyatids to move into and around this habitat more freely. Dasyatids are known to feed on the flats (Chapter 4) and the utilisation of this habitat may be linked to foraging opportunities.

However, for juvenile dasyatids, tidally-linked movement patterns are also likely driven by predator-avoidance strategies and not only foraging opportunities (Davy *et al.*, 2015). At St. Joseph Atoll, the utilisation of the flats increased at high tides for both dasyatids and for shark species that are resident to the atoll (Lea, 2017). This suggests that both dasyatids and sharks were constrained to utilise the deeper lagoonal waters at low tides, but at high tides, dasyatids move onto the flats, followed by the sharks. These sharks may utilise the high tides to hunt on the flats (Filmalter *et al.*, 2013; Lea, 2017), suggesting that predation risk for juvenile dasyatids may be significant in this habitat. Additionally, juveniles were occasionally seen with shark bites on their pectoral fins, further confirming this predation risk (personal observations). However, the flats still provide refuge opportunities, as dorsoventrally-flattened dasyatids were often seen to seek out shallow island fringes or sand banks that were more inaccessible to sharks (Elston, pers. obs.).

Conclusions

In conclusion, numerous environmental variables appeared to affect dasyatid spatial dynamics at St. Joseph Atoll. Increasing wind speeds may have compromised the ability of the receiver array to detect individuals on a daily basis, but seasonal patterns in movement were still probable as individuals were more likely to be detected in warmer weather with higher levels of precipitation (i.e. the north-west monsoon season). Both temperature and its effect on habitat utilisation varied over diel and seasonal cycles, while tides consistently influenced habitat utilisation for all three dasyatid species between seasons.

Together, results suggest that the preference for the shallow reef flat habitat as found in Chapter 5 might be related to biological phenomenon such as prey availability and predator-avoidance opportunities. Conversely, the utilisation of the lagoon habitat appears to be driven by physical phenomenon as individuals move into this habitat when temperatures on the flats become too high or too low, and when tidal changes restrict the usage of the flats.

U. asperrimus has, in particular, been hypothesised to be highly susceptible to the effects of climate change (Chin *et al.*, 2010). The significant influence by temperature on movement patterns in this chapter supports this notion, and may suggest that these species will be significantly effected by the changing temperatures that climate change will bring. With increasing temperatures, these individuals may need to increase their utilisation of the lagoon habitat to keep metabolic costs sustainable.

Chapter 7

General discussion

Understanding the ecology of a species requires a broad contextual knowledge, something difficult to achieve with single-species research. Simultaneously collecting data from multiple species provides insights into how they interact with each other and their environment (Heupel *et al.*, 2018b). Furthermore, through the use of multiple complementary technologies, ecological insights on a variety of spatial and temporal scales can be collected, something that is needed to elucidate what ecological role a species can play. For example, species that move large distances can connect and transfer energy between ecosystems (Lundberg & Moberg, 2003), whereas resident individuals will effect local resource use and energy transfer with in an ecosystem (Heupel *et al.*, 2018b).

This thesis examined a sympatric dasyatid community in an isolated, near pristine ecosystem and determined levels of residency and dispersal, dietary behaviours, habitat use patterns, and the physical environmental factors that influenced these patterns. Insights were gained over varying spatial and temporal scales. Spatially, inter-specific horizontal overlap at the scale of hundreds of meters was identified while large-scale insights were gained by looking at dispersal across the Amirantes Bank. Temporally, there was evidence of how movement patterns changed over hourly to seasonal scales. Trophically, the identification of recently ingested dietary items in comparison to long-term dietary trends determined through stable isotopes revealed inter- and intra-specific prey partitioning. Collectively, this evidence provides a relatively comprehensive understanding into the dynamics of the sympatric dasyatid community at St. Joseph Atoll but, there are still questions remaining.

7.1 The significance of a communal nursery area

Juveniles of three dasyatid species displayed high, long-term levels of site fidelity to St. Joseph Atoll (Chapter 3, Elston 2016), meeting the criteria suggested to identify a batoid nursery (Martins *et al.*, 2018). The presence of young-of-year individuals was also confirmed through stable isotope data. There were distinct maternal isotope signatures in the muscle tissue of the smallest sampled juveniles (Chapter 4), which suggests that these individuals were less than one year old (MacNeil *et al.*, 2006). There was ontogenetic segregation as, after periods of juvenile residency, individuals dispersed from the atoll. Dispersed individuals were detected at various locations on the Amirantes Bank, suggesting that this atoll contributes to the adult dasyatid population across large portions of the bank (Chapter 3). Although a small number of adults were also resident to the atoll, which thereby constitutes as an overlap in spatial distributions with juveniles, these ontogenetic stages segregated by habitat type and thus by depth. The reef flats of the atoll were shallow in nature (<2m depth), while the lagoon was deeper (>3m depth). Resident adults tended to use the deeper lagoon more, whereas juveniles used the shallow reef flats of the atoll more (Chapter 5). Additionally, carbon isotope values suggested ontogenetic prey partitioning. Juveniles were reliant upon a seagrass-based food chain, whereas adults had significantly lower carbon values, characteristic of a more offshore system with phytoplanktonic primary producers. Juveniles and adults also fed at different trophic levels (Chapter 4). Both the habitat and trophic segregation of ontogenetic stages further clarifies this ecosystem as a nursery area.

St. Joseph Atoll can also be classified as a communal nursery area because juveniles of multiple species were present (Simpfendorfer & Milward, 1993). Although nursery use may increases a species' fitness as predation risk is lowered (Heupel *et al.*, 2018a), there is potentially a disadvantageous increase in inter-specific competition for food. Furthermore, other benthivorous fish were also resident in the atoll (for example permit *Trachinotus falcatus*) (Cowley, pers. comm.), which may further increase competition for food. Prior work in communal shark nursery areas have identified that species may reduce these competitive effects by the partitioning of prey resources (Kinney *et al.*, 2011). Chapter 4 identified similar trends with each dasyatid species consuming significantly different proportions of benthic invertebrate prey. This is the first study to identify trophic resource partitioning in a dasyatid nursery and the partitioning may be due morphological variability, differences in foraging behaviour or foraging depth in the substrate. Regardless, it probably facilitates the co-occurrence of these three species (Ross, 1986).

Movement and habitat use has also been known to play a role in resource partitioning (White *et al.*, 2004; Heupel *et al.*, 2018b) however, this did not seem to be the case in this study. All three species displayed overlapping areas of high use and co-occurred randomly with con- and hetero-specifics (Chapter 5). Juveniles could be sharing habitat to derive social benefits and learn from each other, a phenomenon that has received very little research attention in elasmobranch studies but may be relevant for future studies (Jacoby *et al.*, 2012b). Alternatively, another reason for habitat overlap could be increased safety benefits, something that has been hypothesised for the heterospecific grouping seen in other *P. ater* in Australia (Semeniuk & Dill, 2005, 2006).

7.2 Temporal patterns of habitat use and the influence of environment

There appeared to be a significant influence of a variety of environmental factors on the habitat use of dasyatids. Overall, juveniles preferentially occurred on the shallow reef flats and avoided the deeper lagoon habitat (Chapter 5). However, the rare usage of this latter habitat type was necessitated by temperature and tides.

A recent review has highlighted that elasmobranch movement is often influenced by the diel cycle, a consistent and predictable environmental cue but, that elasmobranchs do not appear to alter their habitat use across the diel cycle in a consistent manner (Hammerschlag *et al.*, 2017a). This is unsurprising given the variable foraging and reproductive patterns of this faunal group. Even within this study, habitat use across the diel cycle was inconsistent, varying significantly between individuals and seasons (Chapter 5, Chapter 6). Despite this variability, temperature appeared to be the consistent driver behind the observed diel patterns, as both habitat use and temperature varied accordingly with season. Specifically, in the SE- and NW-monsoon seasons, both *P. ater* and *U. granulatus* juveniles avoided the coldest and warmest temperatures on the reef flat habitat respectively. The tropical location of the atoll means temperatures do not fluctuate as much as they do in temperate regions. However, the shallow nature of the reef flats means that temperatures in this habitat fluctuate the most. Avoidance of warm temperatures appeared to be more influential on movement patterns than the avoidance of cold temperatures though. Dasyatids increased the extent of their movements in the warmer NW-monsoon season and utilised the lagoon habitat more in this season (Chapter 3) and models predicted temperature to have a larger effect on habitat use in the NW-monsoon season (Chapter 6). Temperature is known to effect the habitat use of many ectothermic marine species, given the effect it has on the physiology of these species, and warm temperatures, in particular, may increase the metabolic rates above sustainable levels (Fangue & Bennett, 2003; Brown *et al.*, 2004).

The tidal cycle is another environmental cue that is known to effect elasmobranch habitat use in shallow nearshore areas (Carlisle & Starr, 2010; Brinton & Curran, 2017; Smith & Curran, 2017). In this study, there was indeed a consistent effect of the tidal cycle and individuals were detected more frequently in the lagoon at low tide and on the reef flats at high tide (Chapter 6). This was probably driven in part by physical habitat availability, with parts of the reef flats becoming inaccessible at low tides. This may also relate to the avoidance of temperature extremes, as shallow waters at low tides on the reef flats may become very warm (up to 40°C), which may be approaching the upper thermal maximum for these dasyatids (Dabruzzi *et al.*, 2013).

The interplay between temperature, tides, and diel cycle, and how it effects habitat use in dasyatids, was complicated. It required relatively detailed and long-term movement data, something possible with passive acoustic telemetry. Additionally, habitat use patterns were difficult to discern when not taking season into account (for e.g. determining overall diel patterns in Chapter 5 failed to discern seasonal patterns in diel habitat use as discovered in Chapter 6). Only analysing data in one season, or averaging annual data, would have missed these nuances in behaviour. Future telemetry studies should focus on acquiring this inter-seasonal data in combination with environmental data, even in relatively seasonally stable tropical locations, to further elucidate the cues behind batoid movement.

7.3 Ecological significance

Juvenile dasyatids spent multiple months being largely reliant on the reef flat of St. Joseph Atoll (Chapters 3 and 5), suggesting they may play significant ecological roles in this confined habitat. Furthermore, each species was found to specialise on different benthic invertebrate prey (Chapter 4). This suggests strong predator-prey links between dasyatids and the invertebrate community, which could be ecologically significant. However, batoids are known to be highly plastic in their dietary habits and change their prey in response to differing prey availability and levels of competition (Navia *et al.*, 2011; Tilley *et al.*, 2013; Matich *et al.*, 2017). Indeed, if one dasyatid species was removed, the remaining species might change their dietary habits and increase their trophic niche as has been observed before in elasmobranch juveniles (Matich *et al.*, 2017). Regardless, given the high abundance of dasyatids at St. Joseph Atoll, they may have significant influences in structuring prey communities on the reef flats where they feed, as hypothesised by Tilley & Strindberg (2013) in Glover's Reef Atoll, Belize. The bioturbative effect that this community has is also likely large, where their foraging behaviour in the sandy substrates over which they occur is potentially important to the resuspension of organic nutrients into the water column (Yahel *et al.*, 2008).

It is also interesting to note the high trophic level (TL) calculated for adults (mean TL of 4.6), suggesting a shift to an apex-predatory diet with an increase in size, further highlighting that batoids may play different ecological roles throughout ontogeny (Navia *et al.*, 2017). The species present in St. Joseph Atoll attain large sizes as adults (DW $>$ 130cm) and they may become prolific hunters. Larger dasyatids are indeed known to feed on larger quantities of fish (Jacobsen & Bennett, 2013). Unfortunately, the gastric lavage performed on adults failed to provide a significant number of prey items so their detailed trophic habits could not be confirmed. Further sampling of adults in nearby reefs might provide further insights into their dietary habits and potential ecological roles.

7.4 Contribution to species-specific knowledge

Given the paucity of information available for dasyatids, this study contributed significant novel species-specific information. For *P. ater*, there are no estimates for size-at-maturity, but a male caught at 74cm DW was not yet considered mature (based on clasper calcification), while a male caught at 104cm DW was mature, suggesting maturity occurs in this range. This is also the first study to provide information on the movement patterns for this species. While residency to small areas was the most common behaviour, one individual displayed a unique behaviour whereby it travelled ∼90km in four days, suggesting that this species is capable of traversing large distances at relatively high speeds. Dietary insights were also novel and specialisation for benthic molluscs was found.

For *U. granulatus* there is novel information regarding their distribution as the currently assumed range does not extend as far south as the Seychelles in the western Indian Ocean (Last *et al.*, 2016a). The estimated size-at-maturity for males is 55-65cm DW (Last *et al.*, 2016a) however, males in this area appeared to mature at larger sizes as a male caught at 73cm was still considered immature, while a male caught at 77cm was mature. One previous study has monitored the movements of *U. granulatus* juveniles in a shallow bay (Davy *et al.*, 2015) and similar movement behaviours (residency to a confined area and the influence of the tidal cycle on movements) were identified in this study. Detailed dietary insights were lacking for this species, and this study revealed a benthic crustacean specialisation in the atoll.

7.5 Conservation implications

The use of nursery habitats by species appears to often be driven by predator-avoidance strategies and these areas often play a significant role in the overall abundance of the species (Kinney & Simpfendorfer, 2009). As such, the management and protection of nursery areas provides distinct conservation benefits. Despite this, the identification of a nursery area often fails to convert into actual conservation policy or action (Heupel *et al.*, 2018a). St. Joseph Atoll was indeed a rare case because the movement patterns of sharks and turtles were communicated to the Seychelles government, and were then used to delineate the boundaries of the St. Joseph Special Marine Reserve, a designated no-take MPA (Lea *et al.*, 2016). However, this designation was subsequently repealed and has yet to be reinstated (Payet, 2014).

Small-scale fishing pressure is high in the south-west Indian ocean, including Seychelles, and interactions between elasmobranchs and these fisheries are common (Temple *et al.*, 2018). Although St. Joseph Atoll remains relatively untouched by these fisheries given its remoteness, this may not remain so in the future. The increased levels of marine resource exploitation means that fishing pressures have been placed on remote elasmobranch populations, leading to their declines (Graham *et al.*, 2010). Additionally, the neighbouring D'Arros Island (only 1km away) is one of the privately-owned Seychelles islands. The island may be bought and developed, which could degradate the habitats of St. Joseph Atoll and pave the way for significant recreational fishing activity, as present on the nearby Desroches island (∼45kms away). Fishing activity may have a notable negative impact on this community, given their high reliance to this small and confined atoll (Filmalter *et al.*, 2013).

As St. Joseph Atoll and D'Arros Island are small and easily definable areas, they are appropriate for spatial management tools. Specifically, the reinstatement of the MPA will likely ensure the continued success, not only of the dasyatid population reliant on this ecosystem, but the many other threatened species that occur here (Lea *et al.*, 2016). Furthermore, Seychelles has signed a debt-for-conservation deal designed by The Nature Conservancy, in which it has pledged to increase it's marine protection from 0.04% to 30% of it's EEZ by 2020 (Sey, 2018). St. Joseph Atoll is ideal for the designation of an MPA because it one of the very few remote locations in the Seychelles where research has been conducted to confirm the dependency of vulnerable wildlife to the habitat (Filmalter *et al.* 2013; Lea *et al.* 2016, Chapter3).

7.6 Conclusions

Dasyatids were encountered in high abundances within St. Joseph Atoll, a relatively pristine ecosystem, and this thesis represents the first ecological assessment of this faunal group, both locally and nationally throughout Seychelles. Although this is a local assessment and applied results could be locally beneficially (i.e. for MPA designation), results can be applied on a much wider scale to understand batoid communities and how their behaviour changes under anthropogenic stress. These findings, in particular, indicate complex interactions in a sympatric mesopredator community, where success is a balancing act between predators, prey and competitors. Results suggest these mesopredators may be regulated by food supply in pristine conditions, given the inter-specific partitioning of prey that was evident. Additionally, predators also likely influence their habitat use patterns in a top-down manner, given the reliance to shallow reef flat habitat, despite the evidence that this is a thermally stressful environment. Overall, the differences in habitat use and variations in diet appear to be mechanisms which allow these mesopredators of different species and ontogenetic stages to co-occur within the confines of a small ecosystem. Using multiple technologies to study multiple species was vital to reveal the subtleties in their spatial and trophic behaviour. The understanding of batoid ecology remains severely limited, and this thesis contributes significant knowledge in this area. Future research should focus on different habitat types to determine if behaviours are similar when communities are not constrained by such isolated habitats. The definition of these behaviours across varying ecosystems is crucial to understanding and preserving ecosystem function (Heupel *et al.*, 2018b).

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