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Refining the Ecological Role of Stingrays in Coral Reef Ecosystems

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Abstract

Coral reefs are some of the most diverse and complex ecosystems in the world. They are known for their ability to support a high diversity of marine life. Unfortunately, coral reefs and their associated marine life are under intense anthropogenic pressures; climate change, habitat loss and overfishing are some of the main threats. These pressures also have negative effects on stingrays inhabiting coral reef areas. Management and conservation of stingrays is currently hindered by a lack of knowledge on essential aspects of their ecology. Therefore, this thesis aimed to refine the roles juvenile stingrays play in coral reef habitats and determine the importance of coral reef habitat to stingray populations in a nursery area in the Great Barrier Reef. To do so, this thesis addressed five specific aims: (1) review the current knowledge on batoid nursery areas; (2) identify movement patterns and determine habitat use of juvenile cowtail stingrays *Pastinachus ater*; (3) evaluate the accuracy of towed-float GPS tags to assess movement patterns and habitat use of stingrays; (4) identify diel movement patterns and habitat use of juvenile mangrove whiprays *Urogymnus granulatus*; and (5) investigate the relative trophic relationships of four juvenile elasmobranch species within a communal nursery area, with an emphasis on stingrays.

Nursery areas are crucial for many elasmobranch species, providing advantages such as increased access to prey and reduced mortality. To date, batoid nurseries have been poorly studied in comparison with shark nurseries. The current decline of batoid populations worldwide, resulting in serious extinction threats, highlights the importance of better understanding these critical habitats. This dissertation presented a synthesis of the available knowledge on batoid nurseries and suggests the use of a combination of well-established criteria to standardize batoid nursery definition.

Two telemetry approaches were used to examine movement patterns and habitat use of two stingray species commonly found in coral reef habitats: cowtail stingrays *Pastinachus ater* and mangrove whiprays *Urogymnus granulatus*. First, active acoustic telemetry was used to investigate cowtail stingray movements. Active acoustic telemetry provided fine-scale results, but had some limitations (e.g. potential human disturbance and difficulties in performing night tracks). Therefore, a new method was developed – towed-float GPS telemetry –- to investigate mangrove whipray activity patterns and habitat use. Lastly, stable

isotope analysis was used to define the contribution of potential carbon sources in the food web and the trophic position and relationship of four juvenile elasmobranch species (mangrove whipray, cowtail stingray, blacktip reef shark *Carcharhinus melanopterus* and giant shovelnose ray *Glaucostegus typus*).

Active acoustic telemetry results generated a total of 14 active tracks of cowtail stingrays ranging from 4.91 to 9 hours. Cowtail stingrays moved at an average speed of 2.44 m.min^{-1} + 0.87 SE, with minimum distances travelled ranging from 546 to 1446 meters. Tracking data showed that juvenile cowtail stingrays move in response to tidal cycles, moving faster and with straighter pathways during incoming and outgoing tides, compared to low and high tides. Juvenile cowtail stingrays also showed a strong affinity to sand flat areas and mangrove edge areas, but were infrequently detected in mangrove root habitats. These areas provide food resources and potential refuges for juvenile rays to avoid potential predators. Reef crest habitats were identified as secondary refuge for juveniles during the lowest tides.

Towed-float GPS tags were tested on juvenile stingrays with active tracking performed simultaneously for comparison. Individuals travelled 1332.15 ± 269.58 m SE across Pioneer Bay at an average speed of 6.87 m.min^{-1} and average tracking time of 3.7 hours. Stationary tests demonstrated that the quality of the data obtained by towed-float GPS tags could not be matched by active, acoustic or ARGOS telemetry – on average reaching 99% of successful location recording and <15 meters accuracy. Location Error varied significantly based on the number of satellites detected, with error decreasing as satellite number increased.

Towed-float GPS telemetry showed juvenile mangrove whiprays travelled distances from 394 to 2189 meters during tracks, moving at a mean rate of movement of 4.51 m.min^{-1} ± 3.1 SE with track durations ranging from 1.5 to 9.0 hours. Juvenile mangrove whipray movements were strongly influenced by tidal cycles and rate of movement was significantly different between day and night. Individuals moved faster and chose more direct paths during the outgoing and incoming tide, and were significantly faster during the day than at night. Juvenile mangrove whiprays showed preference for mangrove root habitats during high tides. These areas are thought to reduce juvenile stingray predation risk.

Stable isotope analysis showed all juvenile elasmobranchs in Pioneer Bay are at a trophic level of ~4. Isotopic niche size of blacktip reef sharks was smaller than both stingrays, and cowtail stingrays showed the largest niche size. Results showed strong evidence of niche partitioning between mangrove whiprays and cowtail stingrays with differences in feeding strategy. Nearshore pelagic and benthic prey items (e.g. crabs, annelid worms and small baitfishes) contributed most to juvenile elasmobranch diets, while mangrove or offshore prey and carbon sources appear to not have significant input.

Results of telemetry and stable isotope analysis revealed juvenile stingrays were fully dependent on the Pioneer Bay system. This dissertation confirms juvenile stingrays play important roles as mesopredators and energetic links within the Pioneer Bay nursery area. By looking at fine scale movements and trophic relationships, this PhD provides important information to better understanding juvenile stingray's ecology, but also to support management and conservation policies.

Table of Contents

List of Tables

List of Figures

Chapter 1: General Introduction

Coral reefs are some of the most diverse and complex ecosystems in the world. They are known for their ability to support a high diversity of marine life (Hughes *et al.*, 2003). Unfortunately, coral reefs and their associated ecosystems have experienced substantial declines in abundance, diversity, and habitat structure in recent decades (Chin *et al.*, 2011; Hughes *et al.*, 2003). These changes have occurred for numerous reasons, including nutrient and sediment pollution (Bellwood *et al.*, 2004; Bruno & Selig, 2007), ocean acidification (Burke *et al.*, 2011), rising ocean temperatures (Wilson *et al.*, 2002), diseases, and coral bleaching (Knowlton, 2001). Furthermore, the growth of human populations has caused high demand for fish as a source of protein, with coral reefs supplying fish to almost one billion people in tropical regions. As human populations have grown, the pressure on coral reef fish populations has increased resulting in the depletion of fish stocks and degradation of coral reef systems (MacNeil *et al.*, 2015). According to Wilkinson (2006), approximately 30% of coral reefs worldwide are already badly damaged and an estimated 60% may be totally lost by 2030. Even the Great Barrier Reef (GBR), recognized as one of the largest and least deteriorated coral reefs in the world, has experienced significant changes in coral cover, coral health and fish populations (De'ath *et al.*, 2012; GBRMPA, 2019; Hughes *et al.*, 2018).

Elasmobranch species (sharks and rays) associated with coral reefs have also been affected by anthropogenic pressures (Dulvy *et al.*, 2014; Heupel *et al.*, 2019; Robbins *et al.*, 2006). The situation may be similar for stingrays (family Dasyatidae), some of the most common representatives of rays on coral reefs, but data are more limited than for sharks. Overall, coral reefs support both small and large benthic stingrays . Small benthic stingrays (e.g. juvenile rays, maskrays, bluespotted ribbontail rays *Taeniura lymma*) can be found in sandy or mangrove habitats near coral reefs and are often restricted to shallow waters. Large benthic batoids (e.g. mangrove whiprays *Urogymnus granulatus*, cowtail stingray *Pastinachus ater*, porcupine rays *Urogymnus asperrimus*, pink whiprays *Pateobatis fai*) also have bottom-dwelling habits in sandy areas associated with coral reefs, but have the ability to move longer distances and explore deeper areas (Last *et al.*, 2016). Despite their different habit use and characteristics, all stingray types are negatively affected by anthropogenic stresses in coral reef habitats.

A recent global analysis identified Dasyatidae as one of the most endangered families of elasmobranchs (Dulvy *et al.*, 2014). The major threat to stingray species is unsustainable levels of by-catch, although they are also targeted in some fisheries (D'alberto *et al.*, 2019). Human population growth (Cohen, 2005) and changes in climate (Chin *et al.*, 2010; Rahmstorf *et al.*, 2007) also represent rising threats for stingrays around the world. Due to their life history characteristics (e.g. slow growth, large body size, late sexual maturity, low fecundity, and high longevity), stingray populations can be rapidly depleted when exposed to one or more of these anthropogenic pressures (Fowler & Cavanagh, 2005). Furthermore, stingrays have a close relationship between stock size and recruitment, which results in the need for long recovery periods after over-exploitation (Stevens *et al.*, 2000). Unfortunately, the ability to better understand population declines and effectively manage stingrays is hindered by the absence of knowledge on essential aspects of their biology and ecology (Cartamil *et al.*, 2003; Cerutti-Pereyra *et al.*, 2014; Last *et al.*, 2016). For example, species-specific data on stingray scale and timing of movements (Le Port *et al.*, 2012), level of site fidelity (Vaudo & Lowe, 2006), location of foraging and nursery grounds (Le Port *et al.*, 2012) and trophic ecology (Kanno *et al.*, 2019; Shipley *et al.*, 2018) are lacking for most species.

To date, stingrays are thought to perform important ecological roles in coral reef environments. Firstly, they are thought to function as key mesopredators. Their dorso-ventrally flattened shape and ventral mouth permit capture of small prey in benthic habitats (Matern *et al.*, 2000), but they are also at risk of being a food source for larger predators (Navia *et al.*, 2017). As such, stingrays are thought to occupy an intermediate position in marine food chains, providing a connection between higher level predators and lower trophic level organisms (Vaudo & Heithaus, 2011). Secondly, stingrays function as bioturbators in benthic habitats. Suctorial feeding movements to access infauna and meiofauna (i.e. jetting water, moving pectoral fins) suspend soft sediments and form feeding pits (Lynn-Myrick & Flessa, 1996; O'shea *et al.*, 2012; Takeuchi & Tamaki, 2014). This process, a form of bioturbation, has significant impacts on physical and biological habitat properties, such as benthic species abundance, oxygen dissemination into sediments and nitrogen cycling (Gilbert *et al.*, 1995; Laverock *et al.*, 2011; O'shea *et al.*, 2012). Thirdly, stingrays serve as

energetic links. Limited data indicate rays may remain within restricted home ranges, but also have capacity to move long distances between different habitats (Collins *et al.*, 2007). Thus, stingray movements can connect widely separated ecosystems at short time scales (diel or tidal movements) (Cartamil *et al.*, 2003; Davy *et al.*, 2015; Matern *et al.*, 2000) or over longer periods (seasonal or ontogenetic migrations) (Aguiar *et al.*, 2009; Ajemian & Powers, 2014; Ebert & Cowley, 2003). These patterns demonstrate that stingray movements on coral reefs can be important as energetic linkages between reefs, coastal, pelagic, and deep-water habitats (Heupel *et al.*, 2015). Despite the ecological significance of stingrays in coral reef systems, the extent of influence of these roles is generally poorly understood and almost completely unknown for juveniles that use these ecosystems as nursery grounds.

Nursery areas can be critical for populations with low fecundity and slow growth (Heupel *et al.*, 2019; Martins *et al.*, 2018). These areas are known to provide both biotic and abiotic resources for the development and survival of juveniles (Castro, 1993; Heupel & Simpfendorfer, 2002; Heupel & Simpfendorfer, 2011; Yokota & Lessa, 2006). Survival during early life stages is essential for the stability of populations as a whole (Cerutti-Pereyra *et al.*, 2014; Heupel & Simpfendorfer, 2002). Several studies to date have focused on the importance of nursery areas for shark species (e.g. Conrath & Musick, 2010; Curtis *et al.*, 2013; Froeschke *et al.*, 2010; Kinney & Simpfendorfer, 2009; Simpfendorfer & Milward, 1993; Wetherbee *et al.*, 2007), but little attention has been given to rays – with existing studies covering only 6% of all described species (Martins *et al.*, 2018). Therefore, better understanding the dynamics of newborn and young of the year stingrays within established nursery areas is important for developing effective management and conservation practices (Dulvy *et al.*, 2014; Fowler & Cavanagh, 2005).

Given increasing anthropogenic pressure in coastal areas, coral reefs and the biodiversity they support need better management and protection (Pandolfi *et al.*, 2003). Understanding juvenile stingray ecology and the roles they play in coral reefs is crucial to assessing their ecosystem function and connectivity (Espinoza *et al.*, 2015; Munroe *et al.*, 2015), their vulnerability to anthropogenic threats and environmental changes (Schlaff *et al.*, 2014), and also for the development of efficient management and conservation strategies (Knip *et al.*, 2012) –- not only to manage stingray species as a fishery resource, but also to manage coral

reef ecosystems and ensure that the important and unique roles that stingrays play are maintained (Tilley & Strindberg, 2013). Thus, this study aimed to understand the roles stingrays play in coral reef habitats and determine the importance of coral reef habitat to stingray populations using a protected bay on Orpheus Island in the Great Barrier Reef as a case study.

Orpheus Island is a volcanic island located within the Palm Island Group – central portion of the Great Barrier Reef (GBR). The island is known for its numerous marine and estuarine habitats that support high biodiversity. Pioneer Bay – the chosen study site – is situated on the western side of Orpheus Island. This bay is 400 m wide with approximately 0.8 km^2 of open water area. Its terrain is mostly flat, which results in robust semi-diurnal (Parnell, 1986) and meso-tidal (Hopley *et al.*, 1983) influence (~ 3.5 m). Pioneer Bay is composed of a small mangrove area (Red mangrove, *Rhizophora mangle*; white mangrove, *Avicennia marina*; and myrtle mangrove, *Osbornia octodonta*), sand flats, an inner reef that includes coral rubble and dead micro atolls, a reef crest area with living corals along the seaward edge and, finally, the reef slope about 100 m from cemented beach deposits (Hopley *et al.*, 1983; Parnell, 1986). Here two species of stingrays occur in high abundance – mangrove whiprays and cowtail stingrays (Davy *et al.*, 2015; Kanno *et al.*, 2019). Several other ray species also occur in the area in lower abundance (e.g. blue-spotted maskray *Neotrygon kuhlii*, blue-spotted ribbontail ray *Taeniura lymma*, pink whipray *Pateobatis fai* and reticulate whipray *Himantura australis*), making this an ideal study system.

In the face of increasing threats to both Dasyatidae rays and coral reef habitats, the major goal of this thesis was accomplished by addressing five specific research aims: review the current knowledge on oviparous and viviparous batoid nursery areas (chapter 2); identify movement patterns and determine habitat use of juvenile *Pastinachus ater* using active acoustic telemetry (chapter 3); evaluate the accuracy of towed-float GPS tags to assess movement patterns and habitat use of stingrays (chapter 4); identify diel movement patterns and habitat use of juvenile *Urogymnus granulatus* using GPS telemetry (chapter 5); and investigate the relative trophic relationships of four juvenile elasmobranch species, with an emphasis on stingrays, using stable isotope analysis (chapter 6). Finally, chapter 7 summarized all data collected throughout this PhD and discussed these findings, comparing with studies

developed in the same study area and/or across the world, to provide a robust picture of juvenile stingray ecological roles in coral reef ecosystems.

Chapter 2: Batoid nurseries: definition, use and importance

2.1 Introduction

Nursery areas are crucial for the health of many elasmobranch populations (e.g. Heupel *et al.*, 2007). These areas provide biotic and abiotic features that benefit the development and survival of juveniles (Castro, 1993; Heupel & Simpfendorfer, 2011). In the past 30 yr, studies of shark nursery areas have significantly progressed our understanding of their function and importance (e.g. Grubbs & Musick, 2007; Heupel & Simpfendorfer, 2005; Heupel & Simpfendorfer, 2011; Keeney *et al.*, 2005; Mccandless *et al.*, 2007; Simpfendorfer & Milward, 1993; Stevens & West, 1997). However, the use of nurseries by batoid species remains poorly understood.

Batoids are the most diverse group of cartilaginous fishes (Aschliman *et al.*, 2012). However, batoids are the most endangered group of elasmobranchs, with 19.9% of species listed in a Threatened category by the IUCN Red List (Dulvy *et al.*, 2014; Last *et al.*, 2016). Batoids are increasingly taken by artisanal and industrial fisheries around the world, which has contributed to population declines (Cailliet *et al.*, 2005; Cavanagh *et al.*, 2003; Dulvy *et al.*, 2014; Dulvy *et al.*, 2003; Stevens, 2000; Stevens *et al.*, 2005; White *et al.*, 2006). Habitat loss (Stevens *et al.*, 2005) and changes in climate (Chin *et al.*, 2010; Rahmstorf, 2007) also represent increasing threats to many species. Due to their relatively unproductive life history characteristics (e.g. slow growth, large body size, late sexual maturity, low fecundity and high longevity), batoid populations can rapidly be depleted when exposed to one or more of these anthropogenic pressures (Fowler & Cavanagh, 2005; Stevens, 2000). Collapses of populations of the common skate *Dipturus batis* (Brander, 1981; Dulvy & Reynolds, 2002), the purple eagle ray *Myliobatis hamlyni* (White & Kyne, 2010) and all sawfish species (Pristidae) (Dulvy *et al.*, 2016), for example, have all been reported as a result of intense human pressures. Furthermore, stock size and recruitment are closely related in batoids, resulting in long recovery periods after over-exploitation (Holden, 1974; Stevens, 2000). Therefore, intensive management and conservation effort is required to sustainably fish these populations or aid their recovery (Simpfendorfer & Dulvy, 2017).

The ability to effectively manage and conserve batoid populations is affected by a lack of knowledge on their biology and ecology (Cerutti-Pereyra *et al.*, 2014; Last *et al.*, 2016). The ecology and life history of batoids are poorly understood (256 Data Deficient species on the IUCN Red List) and long-term species-specific data are scarce, such as the scale and timing of movements (Bonfil, 1999), level of philopatry (Vaudo & Lowe, 2006) and location or presence of foraging, mating and nursery areas (Le Port & Lavery, 2012). The identification of elasmobranch nurseries is complicated by their different reproductive modes. Many batoids exhibit aplacental viviparous reproduction, but skates (4 families: Rajidae, Arhynchobatidae, Gurgesiellidae, Anacanthobatidae; 38 genera; at least 288 described species) are a strictly oviparous group (Conrath & Musick, 2012). Historically, elasmobranch nursery theories have largely been developed based on viviparous species models (e.g. Heupel *et al.*, 2007). Hoff (2016) did consider nursery areas for oviparous skates, but there is a need to develop a unified definition of nursery areas that are suitable for all elasmobranch reproductive modes. Hence, the aim of this review was to provide a synthesis of the current knowledge on oviparous and viviparous batoid nursery areas. In addition, I aimed to contribute to a better understanding of ecological roles of batoids within these areas, which is crucial for developing effective management strategies for batoids and their nursery habitats worldwide.

2.2 Elasmobranch nursery area concepts

Historically, nursery areas were defined as places where mature females give birth and juveniles reside until they reach maturity (Bass, 1978; Castro, 1993; Meek, 1916; Springer, 1967). According to Springer (1967), Bass (1978) and Branstetter (1990), these places should offer abundant food resources and lower predation risk for neonates. Thus, most estuarine and shallow marine ecosystems were automatically identified as nurseries based on the presence of juveniles and assumptions of high productivity and protection against predation (Beck *et al.*, 2001). This concept was widely accepted and applied, although a clear definition of what constituted a nursery was not developed. Lack of a clear definition of nursery habitats inhibited conservation efforts because the identification of vast areas as nurseries meant that protection was expensive and difficult to implement (Heupel *et al.*, 2007). Thus, the need for a refined definition of nursery areas increased over time, with the first step to resolve the definition proposed by Beck *et al.* (2001).

Beck *et al.* (2001) specifically noted that nursery areas for marine animals were not just places where juveniles occur, but regions where juveniles occur at higher densities, avoid predation more successfully and grow at a faster rate than the average for that species. Beck *et al.* (2001) also stated that nurseries contribute more individuals per unit area to adult stocks than other habitats where juveniles occur. Consequently, not all areas where juveniles are found are nurseries. This approach to defining nursery areas was more precise and reduced part of the risk of diluting management and conservation efforts. However, the definition outlined by Beck *et al.* (2001) had gaps, especially regarding inter-annual variability in nursery use and recognition of the difficulty in defining the contribution of a single area to an adult population. While Beck *et al.* (2001) suggested that some habitats are more likely to be nursery areas than others, testable approaches to identify these areas were not proposed. To provide practical means to identify nursery areas for elasmobranchs, Heupel *et al.* (2007) proposed a set of criteria specific for the group based on Beck *et al.* (2001) concept, but incorporating aspects such as higher than average abundance and philopatry as metrics. Using this approach, an elasmobranch nursery area should be defined based on 3 criteria where newborn or young-of-the-year individuals (1) are more commonly encountered in the area than in other areas, (2) have a tendency to remain or return for extended periods and (3) repeatedly use the area or habitat across years.

This definition allowed researchers to test the existence of nursery areas for sharks in a straightforward manner. Moreover, Heupel *et al.* (2007) proposed new terms to the literature to define areas used by juvenile elasmobranchs that are not nurseries, such as pupping, birthing and egg-laying or hatching grounds. Heupel *et al.* (2007) criteria have been successfully applied by elasmobranch researchers (e.g. Espinoza *et al.*, 2011; Francis, 2013; Froeschke *et al.*, 2010; Henderson *et al.*, 2010; Hussey *et al.*, 2009), but in general have been limited to viviparous species.

Identifying nursery areas for skates and other oviparous species has proven more challenging because of their reproductive mode. Until recently, authors commonly identified skate nursery areas as those where high densities of eggs occur (egg density criterion), with little consideration of the abundance of neonates and juveniles (Amsler *et al.*, 2015; Hoff, 2008; Hunt *et al.*, 2011; Treude *et al.*, 2011). Hoff (2016) emphasized the difficulty in defining

nurseries for oviparous batoids and developed a set of criteria to identify their nursery areas. He suggested separating the areas used for eggs and juveniles, with the addition of two terms to the literature: 'egg case nursery' and 'juvenile nursery'. To be defined as an egg case nursery, an area should have high densities of eggs and egg cases in contact with the benthos or permanent structures. In addition, the area must be used as an egg-laying area over multiple years, and newborns should leave the area promptly after hatching. The egg case nursery definition uses criteria similar to those of Heupel *et al.* (2007), making it easily testable. Hoff's (2016) juvenile nursery was defined as an area that should have a high abundance of neonate and juvenile skates, be distinct from the egg case nursery and strongly contribute to population recruitment. This definition is also similar to that of Heupel *et al.* (2007) for shark nursery areas, and is functionally equivalent.

However, some confusing points in the nomenclature and concepts of oviparous batoids have been observed, especially regarding the definition of egg-laying and hatching sites as nurseries. According to Heupel *et al.* (2007), egg-laying and hatching sites could be nurseries, but only if the post-hatching young remain in the same area, a criterion which contradicts Hoff (2016) description. Generally, elasmobranch nursery areas are defined as those where many of the young live after birth (Bass, 1978; Beck *et al.*, 2001; Branstetter, 1990; Springer, 1967), but eggs are a developmental stage that contain unborn individuals. The requirements for optimal development of eggs and juveniles may also differ. In other words, optimal conditions for egg development are not always advantageous for juvenile growth. Hoff (2008, 2010), Love *et al.* (2008) and Hunt *et al.* (2011), for example, observed no or very few neonates and juvenile skates in areas identified using the egg density criteria, reinforcing that newborn skates are likely to leave these areas soon after hatching. Thus, the egg case nursery as defined by Hoff (2016) is different than an egg-laying ground since not all areas where eggs are found would meet the egg case nursery criteria. Under such usage, almost all of the areas previously identified as skate nursery areas are in fact egg case nursery areas and not juvenile nurseries. This is not to say that these species do not have juvenile nurseries just that the areas identified to date are egg case nurseries and juvenile nursery areas remain to be determined.

Importantly, for a single species, egg case nursery and juvenile nursery areas could overlap and therefore result in a single nursery area serving both functions. Although there are no known examples, overlaps in egg case and juvenile nursery grounds might occur in batoid species with small home ranges or restricted distributions. Nevertheless, if egg and juvenile nurseries overlap in a delineated area, but are not mostly segregated from the adult population, this area cannot be a nursery (Knip *et al.*, 2010). This area might provide benefits for a population (e.g. food abundance, optimal temperature) but does not provide specific advantages for egg development or juvenile growth and survival separate from the needs of adults. Difficulty in distinguishing egg case and juvenile nurseries is also an issue for oviparous sharks. For example, Cau *et al.* (2014) and Cau *et al.* (2017) identified a nursery area for the small spotted catshark *Scyliorhinus canicula* in the central-western Mediterranean Sea using the egg density criteria, although several specimens of *S. canicula* of different life stages were also observed in the same area.

In fact, both egg case nursery and juvenile nursery areas are essential fish habitats, and their importance to populations must be recognized. For this reason, these definitions should not be aggregated, as has been common in the literature. Confusion and inconsistency in the literature highlights the need for understanding and defining these essential habitats for early life stage batoids. However, the use of multiple and sometimes confusing terms could impede conservation and management efforts by under- or overestimating the importance of specific areas, diluting resources and delaying effective protection. I suggest that Hoff's (2016) second term, 'juvenile nursery area', be simplified to 'nursery', since it is consistent with Heupel *et al.* (2007), which defines nursery areas as associated with the presence and occurrence of juveniles. Thus, I recommend the use of the Heupel *et al.* (2007) criteria for nursery areas and the Hoff (2016) criteria for egg case nursery as they provide clear, simple, testable and widely applied methods to standardize the definitions for all elasmobranchs.

Therefore, for the purposes of viviparous and oviparous elasmobranch populations, the criteria for an area to be considered a nursery would be (1) new-born or young-of-the-year individuals are more commonly encountered there than in other areas, (2) newborn or young-of-the-year individuals have a tendency to remain or return for extended periods and (3) newborn or young-of-the-year individuals repeatedly use the area or habitat across years. For oviparous

species, an egg nursery area can be identified using the following criteria: (1) high densities of eggs and egg cases in contact with benthic or stationary materials, (2) adults use the area or habitat to lay eggs repeatedly over multiple years and (3) newborn or young-of-the-year individuals leave the area promptly after hatching.

2.3 Prevalence of elasmobranch nursery area use

Many elasmobranch species use nursery areas (e.g. Castro, 1993; Cerutti-Pereyra *et al.*, 2014; Deangelis *et al.*, 2008; Freitas *et al.*, 2009; Heupel *et al.*, 2007; Speed *et al.*, 2010; Yokota & Lessa, 2006). These areas can be critical to early life stages of large-bodied species with low fecundity and slow growth (Hussey *et al.*, 2017; Yokota & Lessa, 2006). These species generally have relatively small litters and longer periods between reproductive events. Hence, survival during early life is crucial for population persistence (Cerutti-Pereyra *et al.*, 2014; Heupel *et al.*, 2007). For this reason, slow-growing and low-fecundity species are more likely to benefit from delineated nursery areas that increase survival rates of the young-of-the-year age class (Heupel & Simpfendorfer, 2011).

Nevertheless, exceptions may occur. As emphasized by Springer (1967), McElroy *et al.* (2006) and Heupel *et al.* (2007), some elasmobranch species do not use distinct nursery grounds. Small elasmobranch species with productive life history characteristics and fast reproductive cycles often lack nurseries (Knip *et al.*, 2010). For those species, the absence of a nursery, which could result in high mortality rates, is overcome by their relatively rapid growth, early sexual maturity and high reproduction. Knip *et al.* (2010) also hypothesised that the level of protection against predation in a nursery could be small or even irrelevant for small-bodied elasmobranch species because some small-bodied sharks are likely to be preyed upon throughout their life, even in nursery areas, as juveniles of co-occurring larger species could be potential predators. Thus, the use of nurseries is determined by the life history components of each species and as such may be traded off for other advantages (Branstetter, 1990).

2.4 Batoid use of nursery areas

Nursery areas have been reported in a range of batoids, including sawfishes, stingrays, skates, guitarfishes and numbfishes (Table 2.1). Here, I examine some of the studies that have described batoid nursery areas in a variety of habitats and consider application of nursery theory to these species.

Identification of batoid nursery areas has historically used differing criteria (Table 2.1). Few studies occurred prior to 2007, but those that did used the occurrence of neonates and pregnant females as indicators (e.g. Yokota & Lessa, 2006). Since 2007, most studies on viviparous batoids have used the Heupel *et al.* (2007) criteria (11 studies), while the egg density criterion has been used for the majority of studies on oviparous batoids (8 studies).

Whether all batoid species rely on nursery areas is unclear due to the limited number of studies on these species. Existing studies cover less than 6% of the currently described species — only 38 of 663 species and 12 of 26 living families. In addition, the existing studies are restricted to a few families — mainly Arhynchobatidae (softnose skates), Dasyatidae (whiptail stingrays) and Pristidae (sawfishes). Sawfishes in particular have been increasingly studied due to their imminent threat of extinction (Dulvy *et al.*, 2014). The concentration of research effort to a select few families shows a strong bias in batoid nursery research. As a consequence, knowledge about nurseries of several ecologically and economically important batoid species, and/or threatened families remain scarce or non-existent.

Research bias is also evident when habitat types for batoid nursery areas are evaluated (Table 2.1). The majority of Dasyatidae and Pristidae species inhabit shallow, sheltered coastal and tropical waters. On the other hand, Arhynchobatidae species tend to be found in cold and deep waters, such as the eastern Bering Sea and western Antarctic Peninsula. This tendency shows that very little attention has been given to pelagic batoids inhabiting open water/off-shore systems. The reason for focusing on particular batoid families and habitats is not clear, but might reflect the limited number of researchers dedicating time to better understand batoids and current limitations faced by these researchers to meet funding requirements to access remote locations.

Table 2.1. Identified batoid nursery areas to date

FAMILY	SPECIES	LOCATION	HABITAT	CRITERIA	REFERENCES
AETOBATIDAE	*Aetobatus narinari*	Northeastern Brazil	Coastal sandbanks and rocky reef	Occurrence of neonates, small juveniles and pregnant females	Yokota & Lessa (2006)
ARHYNCHOBATIDAE	*Bathyraja aleutica*	Eastern Bering Sea	Flat sandy mud	Egg density and distribution	Hoff (2008)
	Bathyraja interrupta	Eastern Bering Sea	Flat sandy mud	Egg density and distribution	Hoff (2008)
	Bathyraja parmifera	Eastern Bering Sea	Flat sandy mud	Egg density and distribution	Hoff (2008)
	Raja rhina	Southern California Bight	Rocky outcrop sitting on the edge of a submarine canyon	Egg density and distribution	Love *et al.* (2008)
	Bathyraja parmifera	Eastern Bering Sea	-	Egg density and distribution	Hoff (2009)
	Bathyraja aleutica	Eastern Bering Sea	Flat sandy to muddy bottom	Egg density and distribution	Hoff (2010)
	Bathyraja interrupta	Eastern Bering Sea	Flat sandy to muddy bottom	Egg density and distribution	Hoff (2010)

Bathyraja parmifera	Eastern Bering Sea	Flat sandy to muddy bottom	Egg density and distribution	Hoff (2010)
Bathyraja smirnovi	Shiribeshi Seamount Sea of Japan	Rocky area	Egg density and distribution	Hunt *et al.* (2011)
Bathyraja spp.	Eastern Mediterranean Sea	Cold-seep carbonates	Egg density and distribution	Treude *et al.* (2011)
Bathyraja spp.	Western Antarctic Peninsula	Sandy and rock seabed	Egg density and distribution	Amsler *et al.* (2015)
Bathyraja aleutica	Eastern Bering Sea	Flat sandy to muddy bottom	Hoff *et al.*(2016)	Hoff *et al.*(2016)
Bathyraja parmifera	Eastern Bering Sea	Flat sandy to muddy bottom	Hoff *et al.*(2016)	Hoff *et al.*(2016)
Bathyraja brachyurops	Southwest Atlantic Ocean, northern part of the Argentine Continental Shelf	Sands, shells, gravels, muds, consolidated sediments and rocks	Egg density and distribution	Vazquez *et al.* (2016)
Bathyraja macloviana	Southwest Atlantic Ocean, northern part of the Argentine Continental Shelf	Sands, shells, gravels, muds, consolidated sediments and rocks	Egg density and distribution	Vazquez *et al.* (2016)

	Bathyraja sp.	Southwest Atlantic Ocean, northern part of the Argentine Continental Shelf	Sands, shells, gravels, muds, consolidated sediments and rocks	Egg density and distribution	Vazquez *et al.* (2016)
	Bathyraja albomaculata	Southwest Atlantic Ocean, northern part of the Argentine Continental Shelf	Sands, shells, gravels, muds, consolidated sediments and rocks	Egg density and distribution	Vazquez *et al.* (2016)
	Psammobatis lentiginosa	Southwest Atlantic Ocean, northern part of the Argentine Continental Shelf	Sands, shells, gravels, muds, consolidated sediments and rocks	Egg density and distribution	Vazquez *et al.* (2016)
	Psammobatis normani	Southwest Atlantic Ocean, northern part of the Argentine Continental Shelf	Sands, shells, gravels, muds, consolidated sediments and rocks	Egg density and distribution	Vazquez *et al.* (2016)
DASYATIDAE	*Hypanus americanus*	Northeastern Brazil	Coastal sandbanks and rocky reef	Occurrence of neonates, small juveniles and pregnant females	Yokota & Lessa (2006)

	Hypanus guttatus	Northeastern Brazil	Coastal sandbanks and rocky reef	Occurrence of neonates, small juveniles and pregnant females	Yokota & Lessa (2006)
	Hypanus marianae	Northeastern Brazil	Coastal sandbanks and rocky reef	Occurrence of neonates, small juveniles and pregnant females	Yokota & Lessa (2006)
	Hypanus americanus	United States Virgin Islands	Shallow seagrass flat	Heupel *et al.* (2007)	DeAngelis *et al.* (2008)
	<u>*Bathytoshia lata*</u>	Kaneʻohe Bay, Hawaii, USA	Mud habitats	Heupel *et al.* (2007)	Dale *et al.* (2011)
	Himantura uarnak	Ningaloo Reef, Western Australia	Reef lagoon	Heupel *et al.* (2007)	Cerutti-Pereyra *et al.* (2014)
	Pastinachus ater	Ningaloo Reef, Western Australia	Reef lagoon	Heupel *et al.* (2007)	Cerutti-Pereyra *et al.* (2014)
	Urogymnus asperrimus	Ningaloo Reef, Western Australia	Reef lagoon	Heupel *et al.* (2007)	Cerutti-Pereyra *et al.* (2014)
	Urogymnus granulatus	Orpheus Island, Australia	Reef flat and mangroves	Heupel *et al.* (2007)	Davy *et al.* (2015)
GLAUCOSTEGIDAE	<u>*Glaucostegus cemiculus*</u>	The Gulf of Gabes, south-eastern Tunisian, central	Sandy bottoms	Occurrence of females with encapsulated	Bradai *et al.* (2005)

		Mediterranean Sea		eggs, neonates and juveniles	
	Glaucostegus typus	Ningaloo Reef, Western Australia	Reef lagoon	Heupel *et al.* (2007)	Cerutti-Pereyra *et al.* (2014)
	Glaucostegus cemiculus	The Gulf of Gabes, south-eastern Tunisian, central Mediterranean Sea	Sandy-muddy bottoms	Occurrence of gravid females, neonates with visible umbilical scars and the permanence of juveniles	Enajjar *et al.* (2015)
NARCINIDAE	*Narcine brasiliensis*	Northeastern Brazil	Coastal sandbanks and rocky reef	Occurrence of neonates, small juveniles and pregnant females	Yokota & Lessa (2006)
	Narcine brasiliensis	Paraná, Brazil	Mangrove shorelines and sandy-muddy bottoms	Heupel *et al.* (2007)	Martins *et al.* (2009)
PRISTIDAE	*Pristis pectinata*	South Florida, USA	Shallow, sheltered and mangrove shorelines	Heupel *et al.* (2007)	Simpfendorfer *et al.* (2010)

	Pristis pectinata	South-western Florida, USA	Shoreline habitats with overhanging vegetation	Heupel *et al.* (2007)	Poulakis *et al.* (2011)
	Pristis pectinata	Florida, USA	Mangrove shorelines and shallow euryhaline habitats	Heupel *et al.* (2007)	Norton *et al.* (2012)
	Pristis zijsron	Western Australia	Tidal mangrove creeks	Heupel *et al.* (2007)	Morgan *et al.* (2015)
RAJIDAE	*Raja asterias*	South Ligurian and north Tyrrhenian Sea	Muddy bottoms	High abundance of juveniles	Serena & Relini (2005)
	Raja. Clavata	Ionian Sea	-	High abundance of juveniles	Serena and Relini (2005)
	Raja spp.	Outer Thames estuary, Isle of Wight, Bristol Channel and Lleyn Peninsula	-	High abundance of juveniles	Ellis *et al.* (2004)
	Raja brachyuran	Portugal Coast	Sandy and rock seabed	Occurrence of juveniles and adults	Serra-Pereira *et al.* (2014)
	Raja clavata	Portugal Coast	Sandy and rock seabed	Occurrence of juveniles and adults	Serra-Pereira *et al.* (2014)

	Raja montagui	Portugal Coast	Sandy and rock seabed	Occurrence of juveniles and adults	Serra-Pereira *et al.* (2014)
	Amblyraja doellojuradoi	Southwest Atlantic Ocean, northern part of the Argentine Continental Shelf	Sands, shells, gravels, muds, consolidated sediments and rocks	Egg density and distribution	Vazquez *et al.* (2016)
	Zearaja chilensis	Southwest Atlantic Ocean, northern part of the Argentine Continental Shelf	Sands, shells, gravels, muds, consolidated sediments and rocks	Egg density and distribution	Vazquez *et al.* (2016)
MYLIOBATIDAE	*Myliobatis goodei*	Southern Brazil	Flat sandy mud	Heupel *et al.* (2007)	Araujo *et al.* (2016)
	Myliobatis ridens	Southern Brazil	Flat sandy mud	Heupel *et al.* (2007)	Araújo *et al.* (2016)
GYMNURIDAE	*Gymnura micrura*	Northeastern Brazil	Coastal sandbanks and rocky reef	Occurrence of neonates, small juveniles and pregnant females	Yokota & Lessa (2006)
GURGESIELLIDAE	*Fenestraja plutonia*	Cape Lookout	Coral banks	Occurrence of females with encapsulated eggs and neonates	Quattrini *et al.* (2009)
RHINOBATIDAE	*Pseudobatos percellens*	Northeastern Brazil	Coastal sandbanks and rocky reef	Occurrence of neonates, small	Yokota & Lessa (2006)

				juveniles and pregnant females	
	Pseudobatos productus	Southern California, USA	Estuary	Heupel *et al.* (2007)	Farrugia *et al.* (2011)
	Rhinobatos rhinobatos	The Gulf of Gabes, south-eastern Tunisian, central Mediterranean Sea	Sandy-muddy bottoms	Occurrence of gravid females, neonates with visible umbilical scars and the permanence of juveniles	Enajjar *et al.* (2015)
RHINOPTERIDAE	*Rhinoptera bonasus*	Northeastern Brazil	Coastal sandbanks and rocky reef	Occurrence of neonates, small juveniles and pregnant females	Yokota & Lessa (2006)

2.5 Key factors affecting batoid use of nurseries

Batoids that demonstrably use nurseries are attracted to these locations for a combination of biotic and abiotic features. Differences in these features can directly affect abundance and distribution (Sguotti *et al.*, 2016). Even small variations can lead to spatial and temporal variability (Felley & Felley, 1986; Heupel & Hueter, 2002). Shallow coastal ecosystems, for example, are some of the most variable in the marine world. Given the common (but not exclusive) use of coastal habitats as nurseries by batoids and their variable nature, juveniles that rely on these habitats must be able to cope with significant environmental changes over relatively short time periods (e.g. Knip *et al.*, 2010; Schlaff *et al.*, 2014). Thus, the features determining batoid abundance, residency and fidelity in nursery areas will depend on the species and its geographical location. A better understanding of these features will be crucial to long-term assessments of batoid spatial ecology and development of site-specific management. Here, I discuss some common features affecting batoid use of nurseries.

2.5.1 Energy partitioning into food acquisition

Obviously, food is an essential nursery feature. If there is not enough food, survival will be too low. However, energy requirements and resulting behaviour may differ greatly between species. In theory, abundant food enhances juvenile development and survival. However, in practice, this is not always true. Davy *et al.* (2015) observed that food was not a major driver of mangrove whipray *Urogymnus granulatus* movements within a nursery area. Thus, while the availability of food resources is an important feature, it is not the only essential nursery feature for some batoids.

2.5.2 Predator avoidance

Several authors have hypothesised that, even by delaying maturity and recruitment, the permanence of juvenile batoids in an area protected from large predators would be an important strategy for population health — a trade-off between survival and maturity (Branstetter, 1990; Dale & Holland, 2012; Dale *et al.*, 2013; Dale *et al.*, 2011). However, nursery areas are not always predator-free. Heupel *et al.* (2007) highlighted that nurseries can have high levels of predators, but individuals may employ behavioural strategies to avoid them. According to Costa *et al.* (2015), juvenile Brazilian large-eyed stingrays *Hypanus marianae* spend more time in turbid, shallow waters with low prey abundance to minimize

predation risk. Hoff (2007) hypothesised that neonate Alaska skate *Bathyraja parmifera* move out of egg case nurseries shortly after emergence to avoid large predators. Davy *et al.* (2015) also cited predator avoidance as a major driver of habitat use patterns of juvenile mangrove whiprays *U. granulatus* that stayed in shallow areas and in mangrove root habitats of the nursery to avoid encounters with large blacktip reef *Carcharhinus melanopterus* and lemon *Negaprion acutidens* sharks. Even larger batoids could present risks to juveniles. Branco Nunes *et al.* (2016) reported the first evidence of predation between dasyatid species remains of *H. marianae* were found in the stomach contents of larger southern stingray *Hypanus americanus*. Thus, given the small size and limited swimming ability of many juvenile batoids (Blake, 2004; Dale *et al.*, 2011), it is likely that predation rates can be high even inside nursery grounds.

The potential for cannibalism or intra-specific predation might be the major difference between shark and batoid nurseries. Cannibalism is common among shark groups (Compagno, 2001) and thus can be an important factor in the need for nursery areas where the juveniles of a species are separated from the adults. Morrissey & Gruber (1993) and Guttridge *et al.* (2012), for example, reported intra-specific predator–prey interactions between juvenile, large juvenile and adult lemon sharks *Negaprion brevirostris*. On the other hand, the occurrence of cannibalism within batoid taxa has never been reported, and thus there are no known effects relative to nursery use.

2.5.3 Temperature

Temperature is a factor that affects the distribution and movement of a large number of species including elasmobranchs (e.g. Schlaff *et al.*, 2014). Variations in water temperature have been shown to influence the biology and ecology of batoids (e.g. Cerutti-Pereyra *et al.*, 2014; Fangue & Bennett, 2003; Hopkins & Cech, 2003). For example, Amsler *et al.* (2015) reported that temperature played an important role in the embryonic development and hatching of skate eggs off the western Antarctic Peninsula. Le Port *et al.* (2012) reported temperature was a major factor in short-tail stingray *Bathytoshia brevicaudata* movement patterns, while Dabruzzi *et al.* (2012) observed that ribbontail rays *Taeniura lymma* could identify optimal zones along a thermal gradient with a high level of accuracy. The ability to remain in a desired thermal range is important because juvenile ribbontail rays are often exposed to rapid and extreme temperature fluctuations in shallow coastal waters. Still, according to Dabruzzi *et al.* (2012), juvenile ribbontail rays must remodel biochemical pathways to improve

physiological functions before recruitment and migration to the cooler, more stable habitats occupied by adults.

Batoids may also exploit variations in temperature to enhance oxygen consumption and digestive efficiency (Di Santo & Bennett, 2011; Sims *et al.*, 2006). Matern *et al.* (2000) and Wallman & Bennett (2006) observed batoids foraging at high temperatures and moving to cooler areas after foraging to optimize digestive processes. In contrast, Tenzing (2014), through physiological tests, observed no significant variation between feeding and resting temperatures of *U. granulatus* at Orpheus Island, Australia. According to Tenzing (2014), the use of high temperature habitats might provide an ecological advantage for the species, accelerating digestive rates and, in the long term, juvenile development. Thus, higher temperatures might increase growth rates of juveniles (Jirik & Lowe, 2012; Wearmouth & Sims, 2008), increase reproductive success, improve feeding efficiency and increase survival through predator avoidance (Wallman & Bennett, 2006). Therefore, the benefits of using nurseries with high temperatures may outweigh the potential physiological costs.

2.5.4 Salinity

Batoid movements and habitat use can also be influenced by salinity variations (Poulakis *et al.*, 2012; Simpfendorfer *et al.*, 2011). Juvenile sawfish seek out specific salinity levels to optimize their development or survival (Norton *et al.*, 2012; Simpfendorfer *et al.*, 2011). Similarly, Collins *et al.* (2008) and Heupel and Simpfendorfer (2008) suggested that some elasmobranch species actively move to remain within a specific salinity range, minimizing energetic costs of osmoregulation and freeing up energy for other processes (e.g. growth, sexual maturation). On the other hand, Poulakis *et al.* (2011) reported that smalltooth sawfish *Pristis pectinata* can remain in a nursery area under a wide range of salinities and continue to grow rapidly, suggesting that osmoregulation may have little influence on habitat selection for this species. Effects of environmental parameters such as salinity need to be explored in more detail to more fully define any relationships with batoid habitat use and selection, and how they influence nursery area use.

2.5.5 Oxygen levels

Dabruzzi and Bennett (2014) observed that the Atlantic stingray *Hypanus sabinus* commonly used shallow waters with reduced levels of dissolved oxygen. They hypothesised that by

spending time in hypoxic areas, *H. sabinus* excludes direct competition with less hypoxia-tolerant organisms (Di Santo & Bennett, 2011) and reduces their vulnerability to predators. Thus, the ability to tolerate low oxygen concentrations might be an important advantage to some species of juvenile batoids and is likely a determining factor for use of some nurseries in shallow coastal areas.

2.6 Ecological and trophic role of batoids in nursery habitats

The existing information on batoid ecological roles is based on limited research on a small number of species and locations, restricting our understanding largely to shallow water areas (e.g. Jacobsen & Bennett, 2013). However, due to their high diversity and abundance, batoids are thought to play a number of key ecological roles that are integral to the functioning of several ecosystems, including nursery habitats (Costa *et al.*, 2015). Here, I discuss three of these roles.

2.6.1 Energetic links

The limited data available indicate that juvenile batoids often remain within a restricted area over the short and medium term (weeks to months) (Davy *et al.*, 2015; Vaudo & Lowe, 2006), but some species have the capacity to move longer distances between habitats (Collins *et al.*, 2007; 2008). These movements may have a variety of ecological effects, such as linking trophic webs and enhancing or redirecting nutrient and energy flows (Sheaves, 2009). Thus, batoid movements can connect separated ecosystems at short time scales (Cartamil *et al.*, 2003; Davy *et al.*, 2015; Matern *et al.*, 2000; Silliman & Gruber, 1999) or over long time periods if they undertake ontogenetic or long-range migrations (Aguiar *et al.*, 2009; Ajemian & Powers, 2014; Ebert & Cowley, 2003). These movements also enhance nursery area ecological roles by transporting energy and nutrients through biologically mediated pathways (Sheaves, 2009). Based on current knowledge (Table 2.1), these connections would mostly be from very shallow coastal and estuarine habitats to deeper habitats. Further studies on deep-water species are needed to understand their role in linking energy between habitats.

2.6.2 Bioturbation

Many batoid species feed on infauna and meiofauna within soft sediments. To access these prey resources, batoids employ a number of behaviours (e.g. beating pectoral fins and jetting water) that suspend soft sediments and often form feeding pits — excavated depressions in

the sediment (Lynn-Myrick & Flessa, 1996; O'shea *et al.*, 2012; Takeuchi & Tamaki, 2014). This process, known as bioturbation, is very common in shallow coastal and estuarine nurseries with soft substrates and has a significant impact on the physical and biological habitat properties of intertidal and subtidal areas (O'shea *et al.*, 2012), such as density and distribution of benthic fauna (Dabruzzi *et al.*, 2012). At a fine scale, the formation of feeding pits facilitates oxygen penetration into sediments, extending the zone of oxygenation (Gilbert *et al.*, 1995) and affecting the nitrogen cycle (Kogure & Wada, 2005). Bioturbation may also enable other species to benefit from prey items that are disturbed or excavated during foraging activities (Heithaus *et al.*, 2010; Vanblaricom, 1982). Kiszka *et al.* (2014) detected the association of southern stingrays *Hypanus americanus* and bar jacks *Caranx ruber*, where stingray bioturbation allowed *C. ruber* to access resources otherwise unavailable. Similarly, Kajiura *et al.* (2009) observed double-crested cormorants *Phalacrocorax auritus floridanus* taking advantage of *H. americanus* bioturbation to feed on teleosts. Thus, batoid bioturbation may fulfil a number of roles in soft sediment habitats within and beyond nursery grounds.

2.6.3 Trophic roles

Batoids can consume a wide range of prey items and have different feeding mechanisms and behaviours. They have highly variable dentition and jaw morphology among species, sex and/or life stage (Dean *et al.*, 2007; Pardo *et al.*, 2015). Batoid diets can also be influenced by aspects such as location, or predator and prey distribution (Ebert & Cowley, 2003). Feeding strategy is another important indicator of batoid trophic relationships. Their approaches can be categorized as continuous feeders, ambush predators or filter feeders (Wetherbee & Cortés, 2004).

On average, batoid trophic levels vary between 3.4 and 3.9, but can reach higher levels, such as a 4.2 for rasptail skate *Rostroraja velezi* (Navia *et al.*, 2017). Navarro-González *et al.* (2012), for example, observed Mediterranean starry rays *Raja asterias* sharing trophic position with sea birds, and large demersal and pelagic fish. Batoids also play a role as food sources for larger predators in the food web (Chapman & Gruber, 2002; Dean *et al.*, 2017; Visser, 1999). Therefore, most batoids function as mesopredators, providing the connection between top predators and lower trophic level organisms (Vaudo & Heithaus, 2011). Navia *et al.* (2017) emphasized that, as a highly diverse group of mesopredators, batoids influence the stability and robustness of ecosystems (Dunne *et al.*, 2004). Therefore, batoids likely play numerous and crucial roles in the structure and functioning of food webs. Unfortunately, due to the lack

of knowledge of batoid ontogenetic changes and life cycles, the specific roles played by juvenile batoids in nursery habitats is poorly known.

2.7 Ontogenetic shifts and partitioning of resources in nurseries

Ontogenetic shifts can be a major driver of changes in batoid diet composition (Colloca *et al.*, 2010; Gray *et al.*, 1997; Jacobsen & Bennett, 2012). These shifts could be linked to morphological, behavioural and physiological features (Scharf *et al.*, 2000). Dale *et al.* (2011), for example, observed that diet composition of brown stingrays *Bathytoshia lata* was closely related to body size. Juvenile brown stingrays tended to prey on small and abundant items due to their limited mouth gape, swimming speed and foraging abilities. On the other hand, larger brown stingrays tended to ingest less abundant but more energetically valuable items due to their greater foraging capabilities. Heithaus (2007) also emphasized the possible difficulty of larger juveniles in capturing small-sized prey. Thus, at some point, large juveniles need to switch habitats, moving away from nurseries to attain their specific dietary needs and meet their higher energetic demands. This strategy could drastically reduce time and energy used during foraging activities (Scharf *et al.*, 2000) and competition for food resources with smaller conspecifics, producing a recognized evolutionary benefit (Carrier *et al.*, 2012; Dale *et al.*, 2011).

Partitioning of resources also allows the coexistence of competing or closely related marine predators (Mcpeek, 2014). Several examples of partitioning of food resources between batoid species have been reported (Bizzarro *et al.*, 2017; Bornatowski *et al.*, 2014; Kemper *et al.*, 2017; Mabragana & Giberto, 2007; Platell *et al.*, 1998; Treloar *et al.*, 2007). Pardo *et al.* (2015), for example, detected localised dietary partitioning between sympatric batoids in Australia, where several species foraged at the same spatiotemporal scale, but each species exhibited different prey preferences. Partitioning may also reduce competition for food resources among newborn batoids, decreasing mortality rates during early life stages. The existence of partitioning suggests that batoids may also use communal nurseries as this is a common feature in these areas (Kinney *et al.*, 2011). The use of communal nurseries is known to provide benefits in reducing predation for sharks (Simpfendorfer & Milward, 1993) and may have had great importance in their life histories. However, limited data have been presented suggesting this for batoids. Davy *et al.* (2015) briefly discussed the coexistence of two stingray species in the same bay at Orpheus Island, Australia, and there is evidence that communal groupings of batoids provide increased predator protection (Semeniuk & Dill,

2006). Vazquez *et al.* (2016) also indicated the presence of communal egg case nurseries for several skate species in the Argentine shelf-break front. However, much more research is needed to clarify the use of communal nurseries and their benefits for batoids.

2.8 Conservation of batoid nursery areas

The low fecundity and slow growth rates of some batoids suggest that juvenile survivorship is one of the most crucial features for sustaining stocks (Cortés, 2002; Frisk, 2010; Goldman *et al.*, 2012). Unfortunately, nursery areas, especially those in shallow coastal areas, are susceptible to the influence of anthropogenic pressures (Dale *et al.*, 2013; Lotze *et al.*, 2006) (Lotze et al. 2006, Dale et al. 2013). For example, coastal nurseries can be directly affected by nutrient and sediment pollution due to their close proximity to human communities. Furthermore, coastal nursery areas support not only batoids, but often other fishery resources of major economic significance. As such, juvenile batoids in these areas may be threatened through bycatch in coastal fisheries (Heithaus, 2007). The high level of philopatry seen in many of the batoid species studied (Braun *et al.*, 2014; Flowers *et al.*, 2016; Hunter *et al.*, 2006; White *et al.*, 2013) means that they may not respond well when exposed to high anthropogenic pressures. According to Heupel *et al.* (2007), some elasmobranch species tend to remain in their habitats, even when highly altered by human activities. This situation can lead to a catastrophic scenario for batoids, resulting in high rates of juvenile mortality and low rates of recruitment.

Thus, understanding the dynamics of batoid populations in nursery areas is crucial to improving conservation outcomes for some species (Camhi *et al.*, 2009; Dulvy *et al.*, 2014; Fowler & Cavanagh, 2005). However, designating wide swaths of the coastline as protected nursery areas is probably not an efficient use of resources, or even politically possible. Obtaining better data to specifically target manageable areas for protection is much more likely to be successful. Thus, precise identification of areas that support important life stages (Le Port *et al.*, 2012; Yokota & Lessa, 2006), and improved understanding of batoid life histories are crucial to assessing their ecosystem function and connectivity (Espinoza *et al.*, 2015; Munroe *et al.*, 2015), their vulnerability to anthropogenic threats and environmental changes (Schlaff *et al.*, 2014) and the development of efficient management and conservation strategies (Knip *et al.*, 2012) — not only to manage batoid species as a fishery resource, but also to manage habitats (Tilley & Strindberg, 2013).

2.9 Conclusions

Knowledge of batoid nursery areas is limited compared to that of sharks, although both groups appear to use nurseries in very similar ways. I recommend the use of the Heupel *et al.* (2007) criteria for identifying nurseries as a simple and effective way to define a nursery area for juvenile sharks and batoids. I also support the specific concept of egg case nurseries as proposed by Hoff (2016) that separates areas important for egg development in oviparous species from those important for juvenile and adult life stages. Adopting these definitions will promote the use of standardized criteria and terminology, which will assist conservation and management efforts while reducing under- or overestimations of nursery importance, dividing resources for conservation purposes and hindering effective protection.

At least in the coastal nurseries that have been studied to date, juvenile batoids play important ecological roles in nursery areas, functioning as mesopredators, vectors for energy transfer and bioturbators. However, little data are available to enable a full evaluation of batoid ecological roles in nursery areas. In addition, the concentration of identified nurseries in coastal areas highlights a research bias. A broader approach is required to better evaluate the presence of batoid nurseries in deeper and offshore ecosystems. Batoid species are at risk of extinction due to increasing anthropogenic threats and environmental changes; identification of nursery areas and a better understanding of batoid ecology are important for improving management of batoid stocks and sensitive areas, such as nurseries. By compiling what is known about the use of nursery areas by batoid species, this review provides a foundation to move towards broader and practical approaches to identify and conserve batoid populations.

Chapter 3: Activity patterns and habitat use of juvenile *Pastinachus ater* in a coral reef flat environment

3.1 Introduction

Stingrays (family Dasyatidae) are a diverse and widespread group of elasmobranchs. They can be found in a variety of habitats across the globe (Last *et al.* 2016), including coral reefs. In these ecosystems, stingrays are thought to play important ecological roles, such as connecting trophic webs across habitats, enhancing nutrient recycling and energy flows, and controlling prey populations via predation and/or physical disturbances of soft-bottom microhabitats (Dabruzzi *et al.*, 2012; Martins *et al.*, 2018; O'shea *et al.*, 2012; Sheaves, 2009; Thrush, 1991). Stingrays are also an important fishing resource in many parts of the world (Dulvy *et al.*, 2008; Dulvy *et al.*, 2014; Jabado *et al.*, 2018), especially in developing countries where fish represent an significant portion of local food intake (Dulvy *et al.*, 2017). Due to increasing anthropogenic pressures over recent decades, stingray populations are facing elevated risks of extinction based on population declines caused by fishing and habitat loss (Dulvy *et al.*, 2014).

Currently, some aspects of stingray biology are reasonably understood, such as reproduction, diet, age and growth (Ebert & Cowley, 2008; Hayne *et al.*, 2018; Jacobsen & Bennett, 2012; Pierce *et al.*, 2011; Rastgoo *et al.*, 2018; Saadaoui *et al.*, 2015; Veras *et al.*, 2014). Nevertheless, little is known about the movement patterns and habitat use of stingrays inhabiting coral reefs (Cerutti-Pereyra *et al.*, 2014; Simpfendorfer & Heupel, 2004) – even less about juvenile dasyatid rays. This lack of knowledge is a concern because, generally, survival during early life stages is essential for the health and persistence of slow growing, late-maturing and low fecundity species with a strong relationship between recruitment and population size, such as stingrays (Heupel *et al.*, 2007; Kinney & Simpfendorfer, 2009). Understanding the range and regularity of juvenile stingray movements and their direct relationships with the use of reef ecosystems may be beneficial in determining their dependence on reef habitats, their ecological role within these areas (Cartamil *et al.*, 2003), and their response to anthropogenic threats (Collins *et al.*, 2007).

Juvenile stingrays are often found in shallow soft bottom microhabitats associated with coral reefs (Cerutti-Pereyra *et al.*, 2014; Vaudo & Heithaus, 2009; Yokota & Lessa, 2007). While adults can feed in a wider variety of benthic habitats and move over deeper areas in search for

food, juveniles distribution is often limited to these shallow soft bottom microhabitats where encounters with larger predators are reduced (Heupel *et al.*, 2018; Kanno *et al.*, 2019) and feeding opportunities are increased (Marshall *et al.*, 2008).

Acoustic telemetry has been shown to be a successful tool to assess the activity patterns of both sharks and rays in coral reef habitats (Cartamil *et al.*, 2003; Dewar *et al.*, 2008). However, the use of passive acoustic telemetry in areas juvenile stingrays often inhabit — shallow and turbid waters, and intertidal zones — is compromised by limitations such as shallow depth, habitat complexity, and water flow that affect detection ranges (Davy *et al.*, 2015; Heupel *et al.*, 2006). Active telemetry is an effective alternative in such situations (in some cases the only feasible option) that can provide detailed movement information in habitats where physical attributes hinder the use of most telemetry methods (Brownscombe *et al.*, 2019; Cartamil *et al.*, 2003). Thus, the broad aim of this work was to use active acoustic telemetry to determine fine-scale diel movement patterns and habitat use of juveniles of a stingray species known to commonly use coral reef systems – the cowtail stingray, *Pastinachus ater.*

3.2 Methods and Materials

3.2.1 Study area

Pioneer Bay is located on the western side of Orpheus Island, in the Central Region of the Great Barrier Reef, Australia (Fig. 3.1). This 400-metre wide bay has a reef flat and live coral areas influenced by semi-diurnal tidal variation. Mangrove patches (red mangrove, *Rhizophora mangle*; white mangrove, *Avicennia marina*; and myrtle mangrove, *Osbornia octodonta*) occur along the cemented beach deposits, especially in the southern flat area.
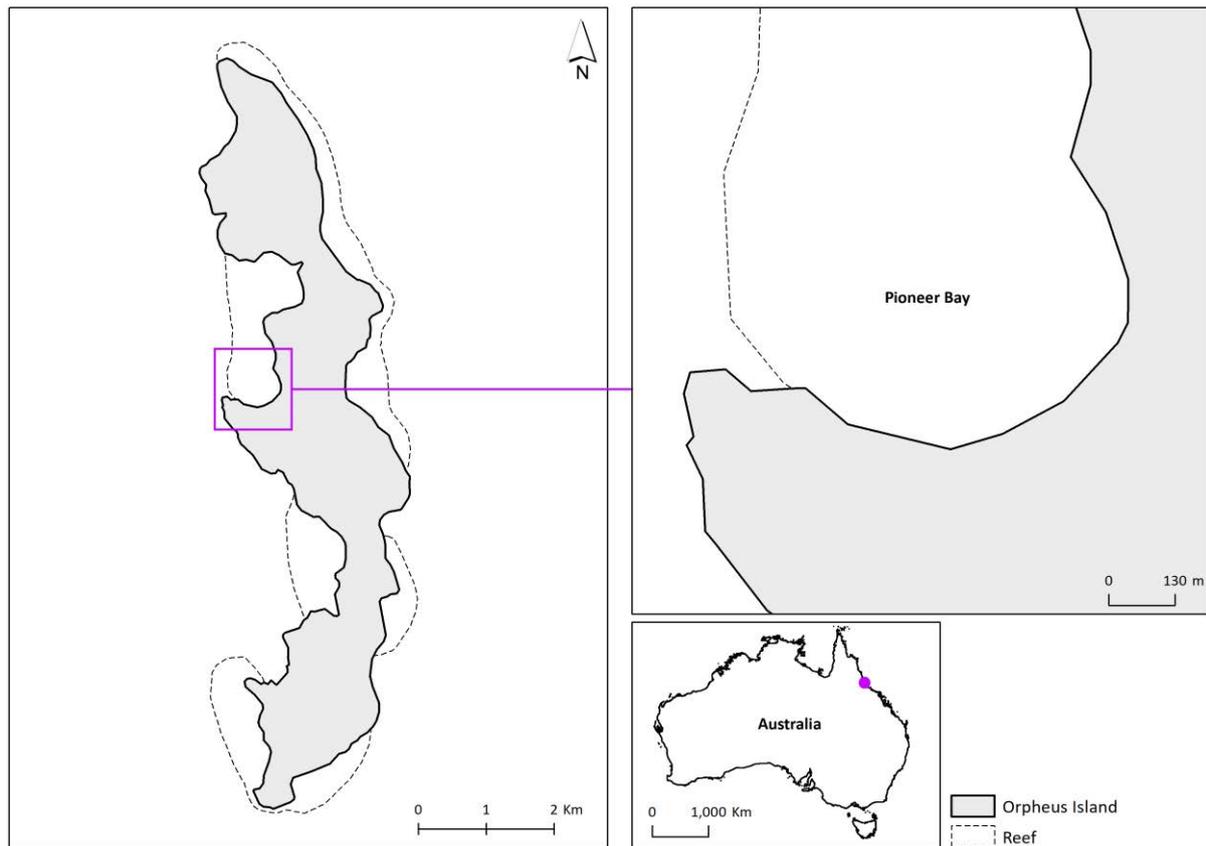
Fig. 3.1. Map of Orpheus Island, Queensland, Australia

3.2.2 Study species and capture methods

The cowtail stingray, *Pastinachus ater,* is a widespread species in the Indo–West Pacific. It inhabits intertidal lagoons, reef flats, reef slopes, bays and estuarine habitats. Individuals are born at approximately 18 cm disc width (DW) and can reach ~200 cm DW in full maturity (Last *et al.*, 2016). Detailed maturity features for *P. ater*, such as length at first maturity (Lm), are unknown. Thus, only an estimate of male maturity can be determined by examining the level of calcification of claspers.

Cowtail stingrays were captured in the shallow waters of Pioneer Bay during outgoing tides using seine and dip nets between April and December 2016. Each animal was manually immobilized for measurements (sex and disc width) and attachment of individually numbered spiracle tags. Individuals were fitted with a Vemco V9 acoustic transmitter (21 mm in length, 1.6 g in water). Date, time and GPS coordinates of the capture and release sites were also recorded. Handling procedures took less than 5 minutes.

Each Vemco V9 acoustic transmitter operated at a specific frequency (81, 78 and 75 KHz). Due to the short duration of the tracks, transmitters were set to emit one acoustic signal per second, resulting in an estimated battery life of five to seven days. Stingrays were released at their site of capture and actively tracked on foot both visually and with a hand held directional hydrophone (Vemco VH110) connected to an ultrasonic acoustic receiver (Vemco VR100). The location of the animal was recorded with a hand-held GPS every 5 minutes. Observers maintained a pre-established distance of 10 meters from the tagged animal - minimising any potential impact on stingray natural movements yet remaining within the location error associated with the hand-held GPS. Depth, habitat type and behaviour of tagged stingrays were also recorded for analysis. Each stingray was continually tracked for 4.91 to 9 hours per day (average 6.83 hours) for two days and always during daylight hours. All tracks started at the outgoing tide and were completed during the incoming tide, when the water reached the mangrove patches or when natural light was no longer available. After two tracks, each animal was recaptured and the acoustic tag quickly removed.

Tidal stages were define as: High tide – 1 hour before to 1 hour after the highest tide height of a tidal cycle; Low tide – 1 hour before to 1 hour after the lowest tide height of a tidal cycle; Incoming tide – period of increasing tide height between low and high tides; Outgoing tide – period of decreasing tide height between high and low tides.

3.2.3 Statistical Analysis

Tracking data were analysed using the *adehabitatLT* package in R. Initially, rate of movement (ROM) – defined as the distance travelled by a specimen within 5 minutes - was calculated in m.min^{-1} for all tracks. To identify the importance of the different habitats within Pioneer Bay for juvenile cowtail stingrays, 95% and 50% kernel utilisation distributions (KUD) were estimated using the package *adehabitatHR*. The method of Lavielle (Calenge, 2015), a function used to estimate the number of segments building a track, was applied to fragment each track into intervals of specific movement patterns. Segments were plotted against tide heights to graphically show possible variations in movement patterns throughout different tidal heights. . Tortuosity of movements – the degree of straightness of each track (ranging from 0 to 1, where zero indicates random movement and one indicates linearity) – was evaluated through an index of linearity. The index was calculated iteratively for every 5

adjacent location points. To understand the effects of tide levels and sex on ROM and tortuosity, second order polynomial natural splines were applied using the R package *glmmTMB* (Brooks *et al.*, 2017) with individual as a random factor. ROM values were square-root transformed before analysis to achieve normality.

3.3 Results

Seven cowtail stingrays (4 males and 3 females) were manually tracked between April and December 2016 (Table 3.1). Disc width of individuals varied from 29 cm to 50 cm, with a mean of 39.5 cm. All male specimens were sexually immature. Due to the lack of external features to assess maturity levels, females were also considered sexually immature based on their similar DW to the males and the known use of Pioneer Bay as a nursery ground (Davy *et al.*, 2015). Each individual was tracked over 2 days, generating a total of 14 active tracks ranging from 4.91 to 9 hours. Specimens moved at an average speed of 2.44 m.min^{-1} + 0.87 SE, with minimum distances travelled ranging from 546 to 1446 meters (Fig. 3.2).

Table 3.1. Details of manual tracking events for juvenile cowtail stingrays at Orpheus Island. DW, disc width.

Stingray	Sex	DW	Track	Date	Duration of track (h)	Min. dist. Travelled (m)	Speed (m.min^{-1})
1	M	37	1	1/04/2016	8	903.57	1.94
			2	2/04/2016	9	1147.53	2.17
2	F	42	1	2/04/2016	7.83	1174.69	2.55
			2	4/04/2016	5.33	585.20	1.77
3	F	33	1	5/04/2016	4.91	602.21	2.40
			2	6/04/2016	6.91	1013.25	3.44
4	M	50	1	9/04/2016	5.58	546.28	1.65
			2	10/04/2016	6.75	594.90	1.65
5	M	34	1	11/04/2016	8	672.27	1.49
			2	12/04/2016	8.16	788.02	1.65
6	M	50	1	9/12/2016	5.75	1423.08	4.24
			2	11/12/2016	5.08	884.64	2.90

7	F	29	1	13/12/2016	5.75	834.15	2.41
			2	14/12/2016	7	1446.35	3.85
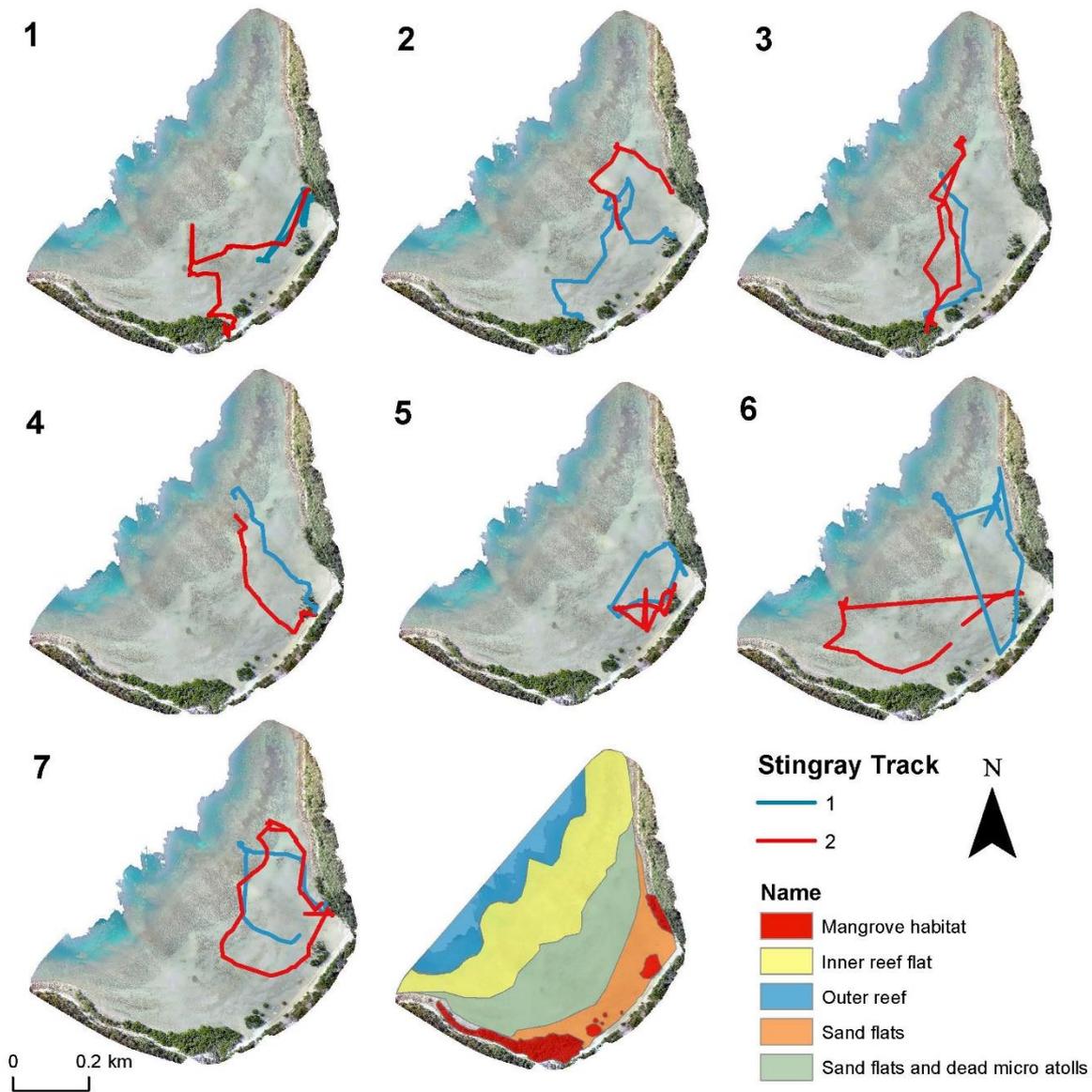
Fig. 3.2. Tracks of juvenile cowtail stingrays and habitat types in Pioneer Bay. Each map represents two tracks of the same individual. Different styles of movement: (1) across the bay; (3) returning to the same area; (4) direct movements towards the reef flat; (6) use of northwest portion of the bay by one individual.

All tracked individuals remained in Pioneer Bay during their tracks – suggesting that juveniles do not, or only on rare occasion, leave the bay. In fact, in four tracks, individuals

34

returned to within less than 100 meters of their capture point on the next incoming tide. Despite the range of tidal heights in which tracks took place (57 – 363 cm), none of the individuals were observed in depths > 1 meter. Both tortuosity (p < 0.0001) and ROM (p < 0.0001) were found to be influenced by tidal height (Fig. 3.3).
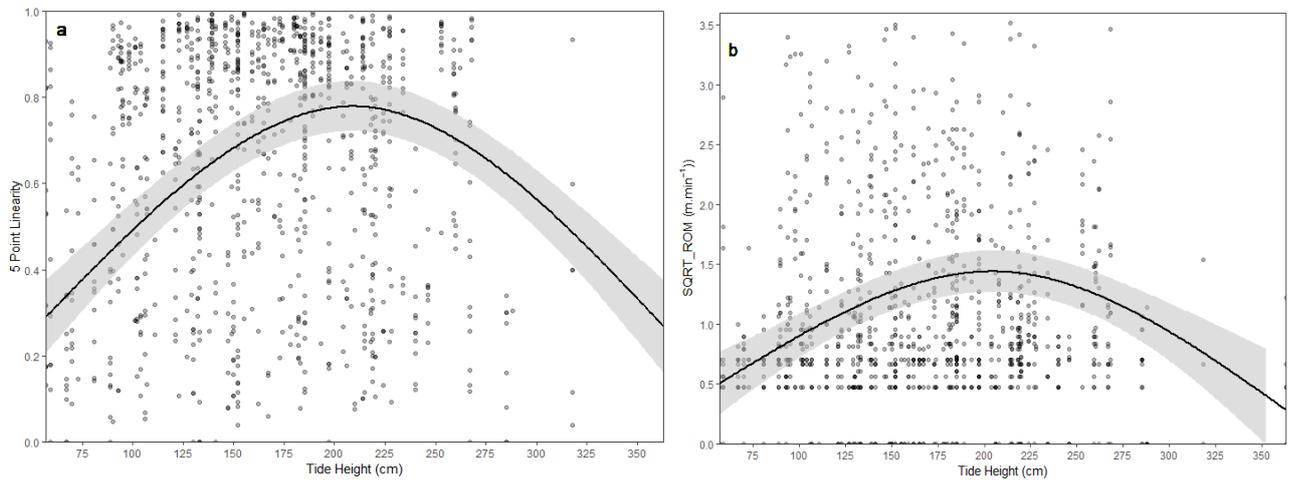
Fig. 3.3. Effects of tide height on (a) linearity and (b) ROM of juvenile cowtail stingrays. Points represent individual locations and 95% confidence intervals are represented by the grey area.

During outgoing tides, when water levels drop and most of the reef flat becomes exposed, individuals moved toward the reef crest or outer crest margin. During this period, rays exhibited more direct and active movements (ROM = 3.27 m.min^{-1} + 2.44 SE; Tortuosity = 0.725 + 0.06 SE) and were often observed in intense searching mode and/or feeding in shallow sand flat areas (11 searching and 23 feeding events recorded). Searching mode was characterized by fast and circular movements, whereas foraging mode included attempts to capture preys on the sandy bottom – with suspension of sediments and formation of feeding pits. During incoming tides, rays used shallow areas, cruising at the limit of the water line and moving into areas as soon as there was sufficient water depth (tide height > 160 cm). During these incoming tides, stingrays interspersed resting behaviour (waiting for more areas to be made available by the rising water level) with more directed movements toward the shoreline (ROM = 2.61 m.min^{-1} + 2.12 SE; Linearity = 0.689 + 0.10 SE). Occasional searching behaviours were observed during the incoming tide. However, individuals were not observed feeding either at the high or low tides during tracks.

Juvenile cowtail stingrays moved in less linear paths and showed lower rates of movements during the highest (ROM = 0.678 m.min^{-1} + 0.28 SE; linearity = 0.339 + 0.13 SE) and lowest parts of the tides (ROM = 1.49 m.min^{-1} + 0.96 SE; linearity = 0.527 + 0.14 SE). At the lowest tidal phase, individuals were observed using sand patches on the edge/within the reef flat as resting points. Individuals covered themselves with sand and remained immobile until the tide started to move back in (Fig. 3.4). The average depth in these areas was approximately 20 cm. At the peak of the high tide, cowtail stingrays were more commonly encountered in a resting mode at mangrove patch edges and sand flats. They were rarely observed penetrating deep into mangrove habitats. Both ROM and tortuosity values did not differ significantly between sexes (p = 0.09; p = 0.65).

Fig. 3.4. Immobile juvenile cowtail stingray at the edge of the reef flat at the lowest tide. The yellow ring is the individually numbered spiracle tag where the Vemco V9 acoustic transmitter was attached to and the pink tape was used to facilitate visual monitoring.

The observed movement patterns of juvenile cowtail stingrays described above were reinforced by the Lavielle method. The number of segments of each track varied from three to 11. The results suggest that long periods of reduced activity (approximately 2 – 3 hours) were interspersed by short sections of variable behaviour (< 60 minutes). Long segments in most tracks occurred during low and high tides, while short periods of variable behaviours

mostly occurred during incoming or outgoing tides (Fig. 3.5). The maximum distance from shore recorded for each individual directly related to the height of the tide ($p < 0.0001$; Fig. 3.6). A wider tide variation throughout the day required individuals to move further toward the reef flat to remain in shallow water. KUD results showed shallow sand flat areas located in the southeast portion of Pioneer Bay were intensively used by juvenile cowtail stingrays, constituting their primary habitat (Fig. 3.7). Mangroves in the southern part of the bay when water depth was not greater than one meter, with both feeding and resting behaviours were observed in these areas in three instances (Fig. 3.8). Rocky bottom areas of the western portion of the bay and outer reef were rarely utilized.
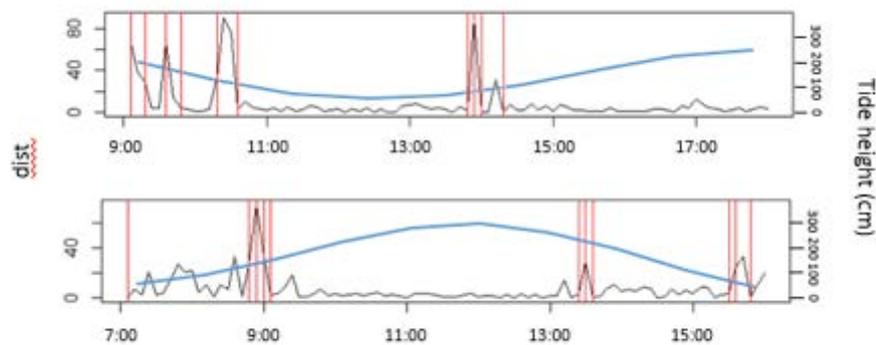
Fig. 3.5. Lavielle method segmentation for two juvenile cowtail stingray active tracks in different tide heights. Black lines represent dist (distance between successive relocations), red lines indicate the boundaries of segments and the blue lines show the tide level.
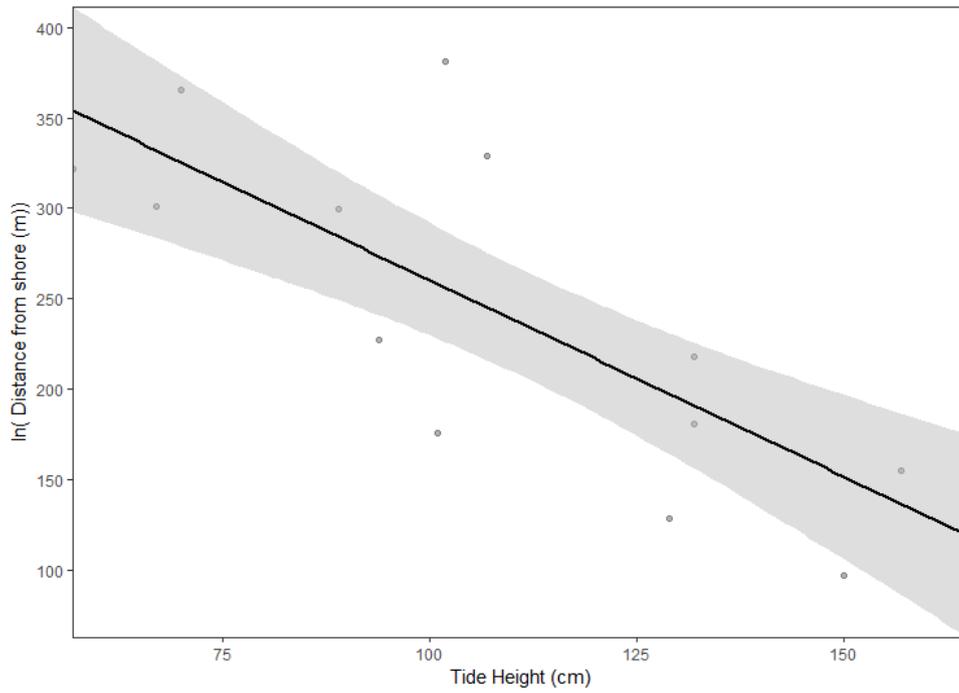
Fig. 3.6. Relationship between the maximum recorded distance from shore and tide height at low tide. 95% confidence intervals are represented by the grey area.
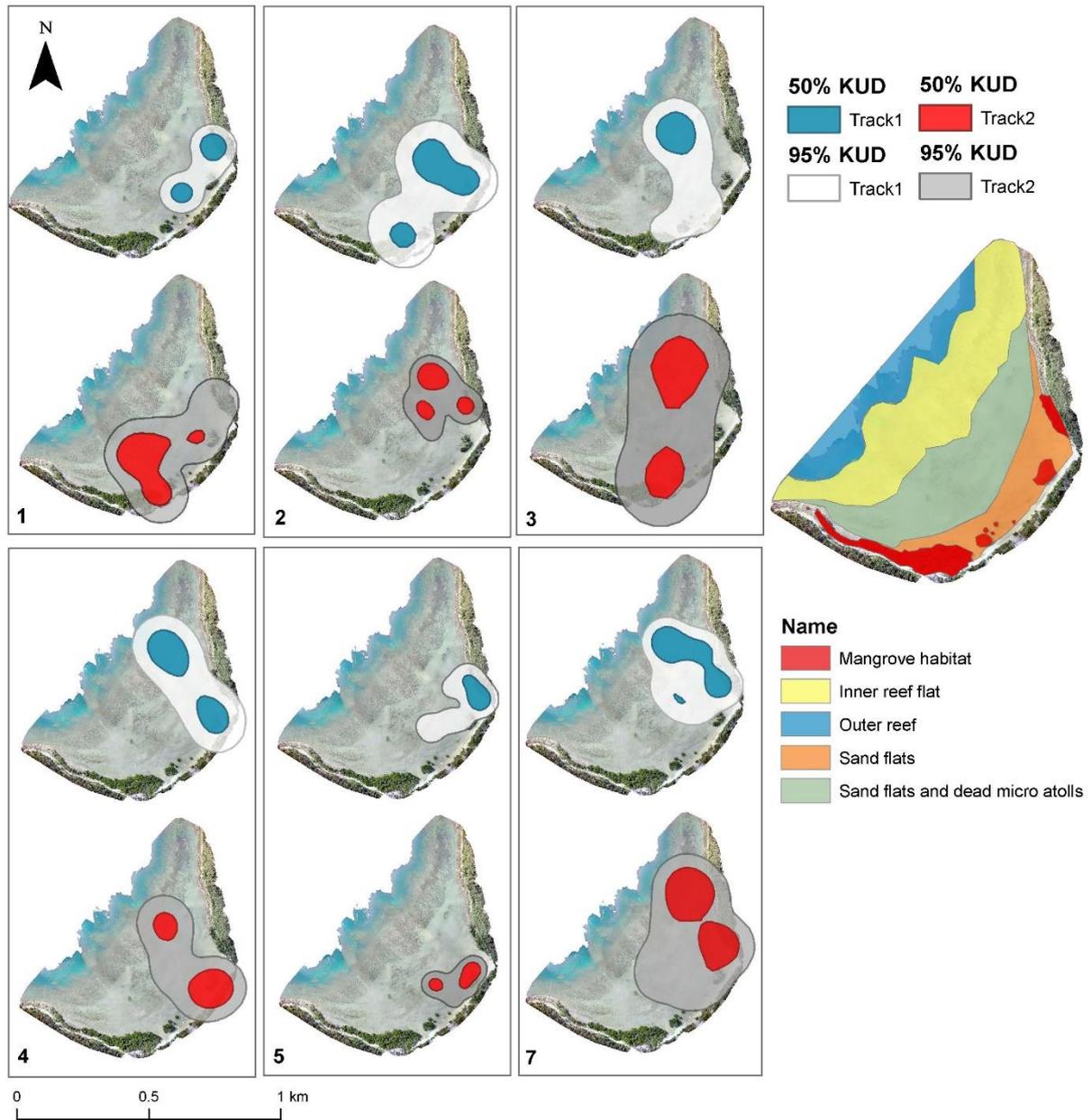
Fig. 3.7. 95% and 50% kernel utilisation distributions (KUD).
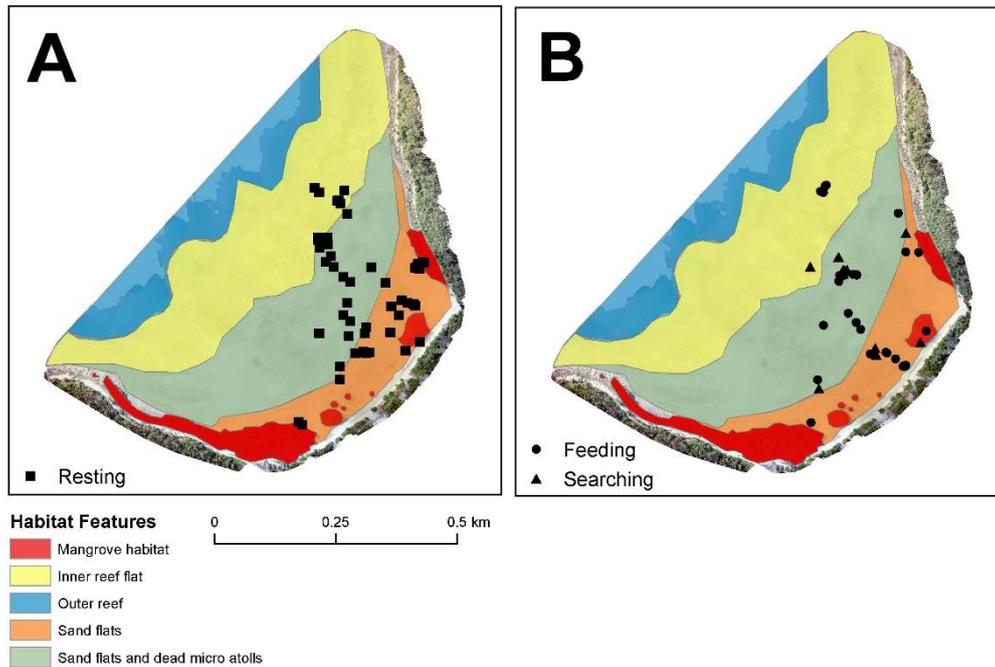
Fig. 3.8. Location of (a) resting and (b) feeding/searching areas used by juvenile cowtail stingrays at Pioneer Bay.

3.4 Discussion

The use of active acoustic telemetry showed that juvenile cowtail stingrays move in response to tide cycles on an inter-tidal reef flat. Our results suggest that juveniles use shallow waters and/or camouflage techniques to avoid potential predators, and to access food resources on sand flats. Juvenile cowtail stingrays spent the entire monitoring period within Pioneer Bay, with individuals not leaving the reef flat or reef crest for deeper waters. Although considered a major driver of stingray movement and habitat use, tide variation did not appear to be as influential to juvenile cowtail stingray habitat use as they were for mangrove whiprays (*Urogymnus granulatus*) which were also tracked in Pioneer Bay (Davy et al 2015). According to Davy *et al.* (2015), mangrove whiprays appear to reduce risk of predation by entering into mangrove habitats when the tide allows, residing within the complexity structures of mangrove roots for as long as these structures were available. Juvenile mangrove whiprays only used sand flats when transiting to the fringing reef area – a secondary refuge during the lowest tides. In contrast, this study showed that juvenile cowtail stingrays were observed using mangrove habitats much less frequently even at higher tides, showing a stronger affinity to sand flat areas. This result is supported by video monitoring of mangrove areas by Kanno *et al.* (2019).

Dasyatid species are known to commonly use sandy-bottom intertidal areas (Pardo *et al.*, 2015; Pierce *et al.*, 2011; Takeuchi & Tamaki, 2014). These areas can offer higher prey availability or easiest access to food resources (Ebert & Cowley, 2003; Elston *et al.*, 2017; O'shea *et al.*, 2013). In addition, juvenile cowtail stingray preference for shallow and soft sediment areas could reduce exposure to predators that cannot access these shallow waters (Cartamil *et al.*, 2003; Collins *et al.*, 2007), and facilitate anti-predator behaviours, such as camouflage by burying and reduced movement rates (Aguiar *et al.*, 2009). Vaudo and Heithaus (2009) also demonstrated that the activity space of four batoid species (*Glaucostegus typus*, *Himantura australis*, *Pateobatis fai* and *Pastinachus ater*) was limited to nearshore and shallow waters of Shark Bay (Australia) likely as a result of predator avoidance.

Furthermore, as demonstrated by Papastamatiou *et al.* (2009) and Espinoza *et al.* (2015) for reef sharks, larger individuals often require a broad activity space in order to fulfil their energetic requirements. Stingray activity space in early life stages therefore might also be limited by low energy intake requirements. Thus, the limited movement and habitat use of juvenile cowtail stingrays within Pioneer Bay could be a trade-off between foraging opportunities, low energetic requirements and predator avoidance - while also reducing the direct competition for resources with co-occurring mangrove whiprays (see Chapter 6). Other strategies to avoid predation, such as aggregation behaviour, were not observed during the study period, but have been reported in other areas for cowtail stingrays (Semeniuk & Dill, 2004).

ROM and Linearity were higher during the incoming and outgoing tides, with juvenile cowtail stingrays moving at faster rates and choosing more direct paths toward the reef flats or mangrove edge areas. Similar results were found by (George *et al.*, 2019) for blacktip reef sharks at Pioneer Bay suggesting this may be a common strategy for small, potentially vulnerable elasmobranchs in this area. Results suggest that, during tide changes, juvenile cowtail stingrays moved with a purpose and often changed their behaviour. By moving faster and using the shortest pathways, juvenile cowtail stingrays might reduce both their time in open/exposed areas during incoming tides and also the chance of getting trapped in shallow pools during outgoing tides. On the other hand, juvenile cowtail stingrays moved at a slower pace and chose random paths during high and low tides. During these tide phases, long periods of inactivity were observed, with individuals often adopting camouflage as a predator avoidance strategy.

Although variations between sexes have been documented in shark and ray movement pattern studies (Schlaff *et al.*, 2014), both ROM and tortuosity values did not differ significantly between sexes in this study. Similar results have been found by Dale *et al.* (2011) and Cerutti-Pereyra *et al.* (2013), indicating that differences in movement patterns and habitat use between sexes do not occur for some batoid species at early life stages. The restricted activity space could also be a factor hindering sexual segregation or sex-specific behaviours. These differences may develop once individuals become sexually mature and have larger areas of available habitat. However, this study only involved a small number of individuals, so further studies are required to fully understand the potential for ontogenetic shifts in cowtail stingray behaviour.

Different from the results found by Davy *et al.* (2015) for mangrove whiprays, juvenile cowtail stingrays were observed feeding more often during the outgoing tide. In addition, juvenile cowtail stingrays were not detected feeding either at highest or lowest tides. Results also showed individual feeding activities were mostly restricted to the soft-bottom area located on the southeast portion of Pioneer Bay. According to Ajemian *et al.* (2012), the use of specific patches for foraging purposes is often related to the availability of resources. Unfortunately, information on cowtail stingray diet and prey availability in Pioneer Bay are not available. However, several studies on bottom dwelling batoid species have shown their preferences for benthic invertebrates, such as annelids, crustaceans, molluscs, bivalves and gastropods (Elston *et al.*, 2017; O'shea *et al.*, 2013) – all of which are commonly found across the sand flats of Pioneer Bay (A Martins pers. obs.). Furthermore, longer-term studies and larger sample sizes would be needed to confidently proclaim that juvenile cowtail stingrays are permanent residents of Pioneer Bay and that observed movement patterns and habitat use are consistent over time.

3.5 Conclusion

In this study, the use of active acoustic telemetry has helped to provide the first detailed examination of the spatial ecology of cowtail stingrays in a coral reef flat environment and the first to focus on juvenile movements. Results have shown juvenile cowtail stingray preferences for sand flat and mangrove edge habitats within Pioneer Bay. These areas provide food resources and the ability to more successfully avoid potential predators. Coral reef flats were identified as secondary refuge for juvenile cowtail stingrays during the lowest tides. In

addition, juvenile cowtail stingray movements were shown to be strongly guided by tidal cycles. These findings highlight the need to better comprehend the movement patterns and habitat use of juvenile cowtail stingrays at different spatial and temporal scales. Future research is necessary to fully unveil the major drivers involved in juvenile cowtail stingray seasonal and ontogenetic movement patterns and habitat use within coral reef flat environments. This information is important to establish a full understanding of juvenile cowtail stingray ecology, but also could support management and conservation policies.

Chapter 4: Towed-float GPS telemetry: a tool to assess movement patterns and habitat use of juvenile stingrays

4.1 Introduction

Biotelemetry devices have become increasingly useful in assessing behaviour, energetics, physiology and ecological aspects of free-swimming marine animals (Cerutti-Pereyra *et al.*, 2014; Ogburn *et al.*, 2017). Recent improvements to tracking devices and systems have supported studies over broader spatial and temporal scales (Braun *et al.*, 2014; Bullock *et al.*, 2015; Heupel *et al.*, 2015).Modern devices have also been developed to go beyond simple animal movement, collecting data such as acceleration, magnetic fields, pH, water depth, temperature and salinity (Browning *et al.*, 2018; Cooke *et al.*, 2004; Hart & Hyrenbach, 2009). Telemetry capabilities are rapidly improving, and size and price of this technology are reducing, hence, diversifying ecological research. Advances in telemetry not only improve our understanding of the biology of species, but can also help improve management (Hussey *et al.*, 2015).

A variety of biotelemetry methods have been used to track marine animals (Ajemian & Powers, 2014). These include acoustic telemetry, where information is transmitted to moored or mobile receivers (Heupel *et al.*, 2006), satellite telemetry where locations of tags are estimated by satellite-based systems, and logger-based telemetry where data are stored for post-recapture processing (Hussey *et al.*, 2015). Some telemetry approaches combine more than one of these methods, especially to overcome the need to recapture animals to obtain stored data (Cooke *et al.*, 2016). The choice of telemetry approach for a particular study requires consideration of the aims, species, location and budget (Riding *et al.*, 2009). Species that occur in environments that challenge conventional telemetry equipment make study more difficult and may require innovative approaches. For example, species that occur in shallow structurally complex habitats make acoustic approaches difficult because of limited signal-transmission distances (Costa *et al.*, 2015; Royer & Lutcavage, 2008), or those that live in the deep sea where tagging animals is difficult and the extreme pressures can damage equipment (Cooke, 2013).

GPS telemetry is a biotelemetry approach that has been broadly and successfully employed to assess movement patterns of terrestrial and aerial animals (Sims *et al.*, 2009). This technology has reduced many of the bias and precision issues often reported in other

telemetry methods, such as acoustic and ARGOS-based satellite telemetry (Hebblewhite & Haydon, 2010). However, GPS-telemetry devices for marine animals have historically been large and heavy, drastically reducing their use. In addition, tracking marine animals with GPS loggers has proved challenging, because of irregular-surfacing behaviour or bottom-dwelling habits that limit data acquisition (Schofield *et al.*, 2007). Recently, terrestrial ecologists developed very small data-logging GPS tags for use on birds and small mammals (Ryan *et al.*, 2004). This advancement also supported the use of GPS telemetry for smaller marine species because of sophisticated systems that allow prompt recording of GPS locations when individuals surface or move close to the surface (Sims *et al.*, 2009). Despite significant improvements in size, weight, accuracy and precision, the need to recover GPS loggers to obtain data remains an issue. The need to recover loggers has meant that this technology has not been widely adopted to assess movements of organisms with low recapture rates, such as stingrays.

A recent global analysis identified stingrays (superorder Batoidea, order Myliobatiformes) as one of the most endangered families of elasmobranch (Dulvy *et al.*, 2014). Unsustainable by-catch, habitat destruction and changes in climate are rising threats for stingrays around the world (Chin *et al.*, 2010). Worryingly, stingrays are highly susceptible to these human pressures mainly because of their life-history features, such as low fecundity, late sexual maturity and slow growth (Stevens, 2000). Therefore, a better understanding of stingrays' activity patterns through the use of non-lethal biotelemetry techniques is essential for effective management and conservation of the group (Papastamatiou & Lowe, 2012).

Stingray anatomy and behaviour are a consideration in decisions about which telemetry techniques to use. This is particularly true for juveniles. In some species, their small size and dorso-ventrally flattened body (Last *et al.*, 2016) hamper the attachment of large loggers or telemetry devices (Grusha & Patterson, 2005). In addition, juvenile stingrays often inhabit shallow and muddy waters, which hinders the use of acoustic telemetry (Heupel *et al.*, 2015). Blaylock (1990), Le Port *et al.* (2008), Riding *et al.* (2009), Ajemian and Powers (2014) and Branco Nunes *et al.* (2016), for example, used satellite telemetry to assess movement patterns of batoids. However, these studies used large stingray (short-tailed stingray, *Bathytoshia brevicaudata*; southern stingray, *Dasyatis americana*) and pelagic myliobatid ray (cownose ray, *Rhinoptera bonusus*; New Zealand eagle ray, *Myliobatis tenuicaudatus*; spotted eagle ray, *Aetobatus narinari*) species that were capable of carrying large telemetry packages. Such an approach is not appropriate for juvenile stingrays. Nevertheless, the occurrence of juvenile

stingrays in shallow water, and the shrinking size of GPS logger tags, means that they may be able to tow a small tag in a float to provide position estimates; much like eagle rays did in the study of Riding *et al.* (2009).

No study has attempted to use GPS logger telemetry for juvenile stingrays. If suitable, this methodology would enable longer tracks, collect greater amounts of high-accuracy location data, reduce labour costs and reduce observer-induced behavioural biases. In some situations, it may also allow data collection beyond the ability of human observers, such as, in difficult-to-access areas, limiting weather conditions, and over long distances. Thus, the present study aimed to evaluate the performance of GPS loggers attached to floats (towed-float GPS tags) as an effective, accurate, minimally invasive and less labour-intensive tool to assess fine-scale movement patterns and habitat use of juvenile mangrove whiprays *Urogymnus granulatus*

4.2 Methods and materials

4.2.1 Study area

Orpheus Island is located in the central region of the Great Barrier Reef, within the Palm Island Group. The island stretches for 12 km and comprises 1368 ha. Pioneer Bay is one of several bays on the western side of Orpheus Island. This bay has an open water area strongly influenced by tidal variation. Tides in Pioneer Bay are semi-diurnal (Parnell, 1986) and mesotidal, reaching a maximum of 3.5 m at high tides. The 400-m-wide Pioneer Bay (0.8 km^2 of open water area) is composed of a reef flat (Parnell, 1986) and living corals along the seaward edge (Hopley, 1983). The inner reef flat consists of sand, coral rubble and abundant dead micro atolls. Living corals can be observed on the outer reef, ~100 m from cemented beach deposits. A small area of mangrove (red mangrove *Rhizophora mangle*; white mangrove *Avicennia marina*; and myrtle mangrove *Osbornia octodonta*) is located in the southern inner reef flat.

4.2.2 Study species and catching methods

The mangrove whipray, *Urogymnus granulatus* (family Dasyatidae), is a large-bodied stingray (up to 141-cm disc width) widely distributed in the tropical waters of the Indo-West Pacific region. Juveniles are found in shallow, turbid coastal waters, especially in mangroves

and estuaries. Unfortunately, there is little information in the scientific literature pertaining to its life history, spatial ecology and population dynamics.

Juvenile mangrove whiprays are common benthic inhabitants of sandy and mangrove habitats of Pioneer Bay (Davy *et al.*, 2015). Individuals were captured under mangrove roots or on shallow sandy and reef flat areas of Pioneer Bay by using seine or dip nets, between 25 and 27 April 2016. Once captured, stingrays were measured (disc width, DW), sexed and tagged with a uniquely numbered spiracle tag (Fig. 4.1a). Date, location, and time of capture and release were recorded. None of the procedures took longer than 5 min.
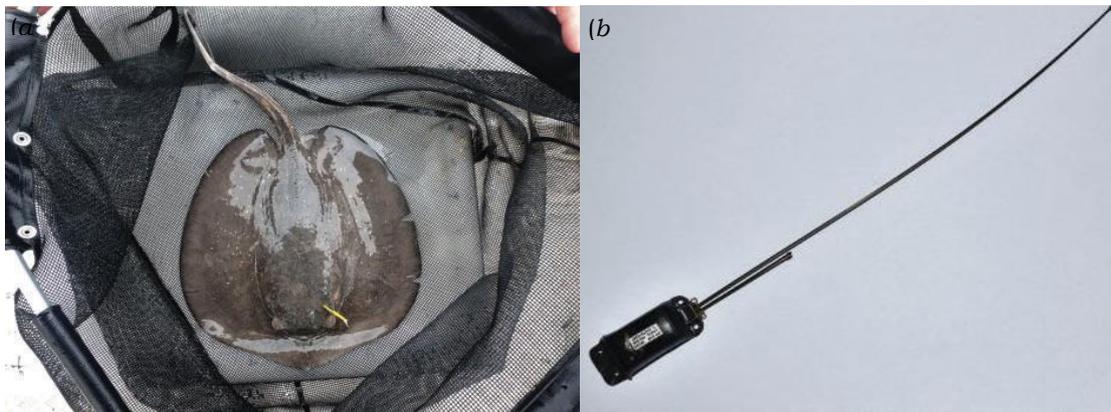
Fig. 4.1. GPS logger tagging of juvenile stingrays. (a) Tagged juvenile mangrove whipray. (b) Lotek Wireless PinPoint Beacon 120 GPS logger tag.

4.2.3 GPS device

GPS logger tags (Lotek, Wireless PinPoint Beacon 120) were used for this research. Loggers were customised to suit the project goals, i.e. they were waterproofed, weighed 20g (5% of estimated juvenile stingray of 400 g) and measured 40 X 16 X 10 mm (L X W X H). A lightweight and flexible antenna was attached to each tag to allow detection of GPS satellite signals (Fig. 4.1b). Tags could record up to 1500 location attempts and their rechargeable nature allows their long-term re-use when recaptured. Each tag had a programmable schedule that defined the interval of location recording. An embedded Lotek radio beacon enabled tag relocation after a programmed period. Recorded data were downloaded from recovered tags by using Lotek Wireless PinPoint Host software.

4.2.4 Accuracy and precision of the GPS device

Stationary trials were performed to test the accuracy and precision of tags in determining locations across Pioneer Bay. Several locations were chosen as test areas and divided into the following three categories: (1) fixed points above water with a clear view of the sky (Uncovered), (2) fixed points in mangrove trees to simulate when stingrays moved into or adjacent to mangrove habitats (Covered) and (3) on tethered float-mounted GPS device to simulate animal tracks (Float). It is important to point out that, during Float tests, the accuracy was expected to be less certain than for fixed-station tests, because tethered floats could move 1–2 m from a central point of attachment, depending on the tide and wind. Devices were set to record one location every 5 min and left in place for 12 h, i.e. one full GPS satellite constellation cycle. The true location of each fixed station was taken with a hand-held GPS Garmin GPSMAP78sc (accuracy to <10 m from the true location).

To determine the performance of each device at each location, the following factors of positional accuracy and precision were measured: (1) the fix success rate (FSR), i.e. attempts that successfully acquired a location (proportion of the total amount of fixes, $n = 145$); and (2) location error (LE), i.e. the linear distance between fix position recorded by the loggers and the true location. Each of these metrics depends on the number of satellites and their geometric configuration at the time of computing a GPS point. A minimum of four satellites was needed to record a three-dimensional (3-D) fix and the adoption of dilution of precision (DOP) filters, which is a metric that expresses the precision of a successful location fix, was necessary for an indication of good satellite geometry. In this study, locations were validated if they were based on at least four satellites (Sea Mammal Research Unit SMRU, http://smub.st-and.ac.uk, accessed 25 November 2018; Schofield *et al.*, 2007) and had DOP values of <10 (Adrados *et al.*, 2002). To assess the impact of these factors on the FSR and LE, data were assessed in both raw and filtered form (detailed below). By quantifying the FSR and LE in stationary trials, a baseline reference was established to determine the relative accuracy of the towed-float GPS tags.

4.2.5 Accuracy of float-mounted GPS devices

Stability, buoyancy, relative hydrodynamic drag and ability to avoid entanglement of different-shaped floats were tested in a salt-water tank before design finalisation. Small cone-shaped foam floats with a short lead keel showed the best results in these trials. The size and

weight of the float were also considered to achieve minimum drag levels (~10 cm long and 40 g, 10% of the average body mass in air). The towed-float GPS device was attached to the spiracle tag of mangrove whiprays with 2-kg test monofilament fishing line, ~1.5 m in length. Once attached, each device was towed by the stingray throughout shallow reef flat habitats (Fig. 4.2). Previous research by Davy *et al.* (2015) demonstrated that juvenile mangrove whiprays rarely entered water >0.5 m deep; so, they were not expected to pull the float below the surface. Tags were set to record locations once every 5 min. Tags were fitted during the falling tide and retrieved at high tide. Devices were recovered by cutting the fishing line when tracks were terminated. The ability of mangrove whiprays to remove the tag by themselves in the case of entanglement was tested during the second track, by letting the individual move into mangrove roots where they take refuge at high tides (A. P. B. Martins, unpubl. data). Active acoustic telemetry was performed simultaneously to the towed-float GPS telemetry for comparison purposes.

Vemco V9 acoustic transmitters, measuring 21 mm in length, weighing 1.6 g in water and emitting signals every second at the frequency of 81 KHz, were also attached to the spiracle tags of each individual. A Vemco VR100 acoustic receiver connected to a directional hydrophone was placed in a recreational kayak and towed by foot by an observer. The movements of each tagged individual were recorded by the observer every 5 min by using a hand-held GPS. The distance between the observer and the tagged animal was only a few metres and, so, smaller than was the error associated with the hand-held GPS.

Fig. 4.2. Towed-float GPS device attached to a juvenile mangrove whipray.

4.2.6 Data processing, screening and analysis

Date, time, latitude and longitude were recorded every 5 min with the GPS logger. Raw data from the GPS tags provided a time-series of successful and unsuccessful fix attempts, while additionally reporting the number of satellites used in computation and the corresponding DOP values. Only fixes taken at the same time by active and GPS tracking tags were included in the analysis, reducing uncertainty in the distance between the real and estimated points. To estimate the LE, latitude and longitude values were projected onto UTM coordinates (Zone 55). Data points were screened to remove significant outliers (i.e. LE > 250 m, $n = 1$). Furthermore, positional fixes were analysed in both a raw (unfiltered) and filtered (number of satellites of ≥ 4 and DOP of <10) form to explore the effects of satellite number and constellation geometry on the performance of the tags.

Stationary tests: The effects of applying filters to the data were tested by calculating differences in the mean LE for each treatment by using a Welch's t-test to account for the unevenness of sample sizes. A one-way ANOVA was performed to look at the differences in LE among treatments. Values of LE were log transformed to meet assumptions of normality. To evaluate differences in the number of satellites among treatments and how LE varied with the number of satellites, Kruskal–Wallis non-parametric tests were utilised because of violations in the assumptions of normality. A Tukey's honest significant difference (HSD) test was also utilised to provide multiple pairwise comparisons among the means of the treatments.

Tracks: The distance and speed of each individual were calculated by, first, estimating the linear distance between each positional fix and then dividing by the time interval between each fix (5 min). Location errors between hand-held GPS units and GPS tags were estimated to assess the accuracy and precision of float mounted GPS devices. Each successful position fix was also categorised into two different habitat types (mangrove and reef flat) by overlaying each GPS track with a satellite image of Pioneer Bay; successful fixes were considered to be in mangroves only if they were contained within mangrove areas. Welch's t-tests for unequal variances were performed to evaluate differences in the mean LE among habitat types.

This study was conducted under Great Barrier Reef Marine Park Authority Permit G15/37987.1 and James Cook University Animal Ethics Permit A2310.

4.3 Results

4.3.1 Accuracy and precision of the GPS loggers

Unfiltered v. filtered datasets: In total, 24 stationary tests were performed between 25 and 27 April 2016 (Uncovered = 10, Covered = 11, Float = 3). Accuracy and precision of GPS loggers varied significantly between unfiltered and filtered datasets (Table 4.1). For example, LE and DOP values decreased by 22 and 125% respectively, from unfiltered to filtered datasets. The FSR also decreased from unfiltered to filtered datasets. The average number of connected satellites increased by 16% when filters were applied. However, when the different treatments were considered, only Covered tests showed significant differences between unfiltered and filtered datasets. This result suggests an advantage of applying filters when tracking animals through covered habitats, such as mangroves. Thus, all further analysis was completed using the filtered dataset.

Table 4.1. Summary of fix success rate (FSR), mean location error (LE) both with and without dilution of precision (DOP) filter for each treatment. *t*-values are significant at *, $P < 0.001$. s.e., standard error.

Treatment	N	FSR ± SE (%)		LE (m; mean ± SE)		t
		Filtered	Unfiltered	Filtered	Unfiltered	
Uncovered	10	90.4 ± 0. 03	99.5 ± 0. 002	12.23 ± 14.66	12.18 ± 14.85	0.078
Covered	11	66.2 ± 0.04	86.5 ± 0.02	13.43 ± 16.70	21.12 ± 47.84	-5.5512*
Float	3	81.5 ± 0.06	99.5 ± 0.001	7.75 ± 8.28	9.18 ± 23.29	-1.1921

Stationary GPS logger tests: The FSR of GPS loggers followed expected trends across all treatment locations. Open areas, such as Uncovered and Float treatments, had a 13% increase in successful fix rates when compared with the Covered treatment (Float *v.* Covered and Covered *v.* Uncovered, Tukey's HSD, $P < 0.001$) (Table 4.1). The number of satellites used ranged from four, the minimum necessary for computation, to 11, which is the maximum acquired throughout this study. The average number of satellites varied significantly among treatment locations (Kruskal– Wallis: $x^2 = 86.94$, d.f. = 2, $P < 0.001$; Fig. 4.3a). Both Uncovered and Float tests had significantly higher FSRs than did the Covered treatment.
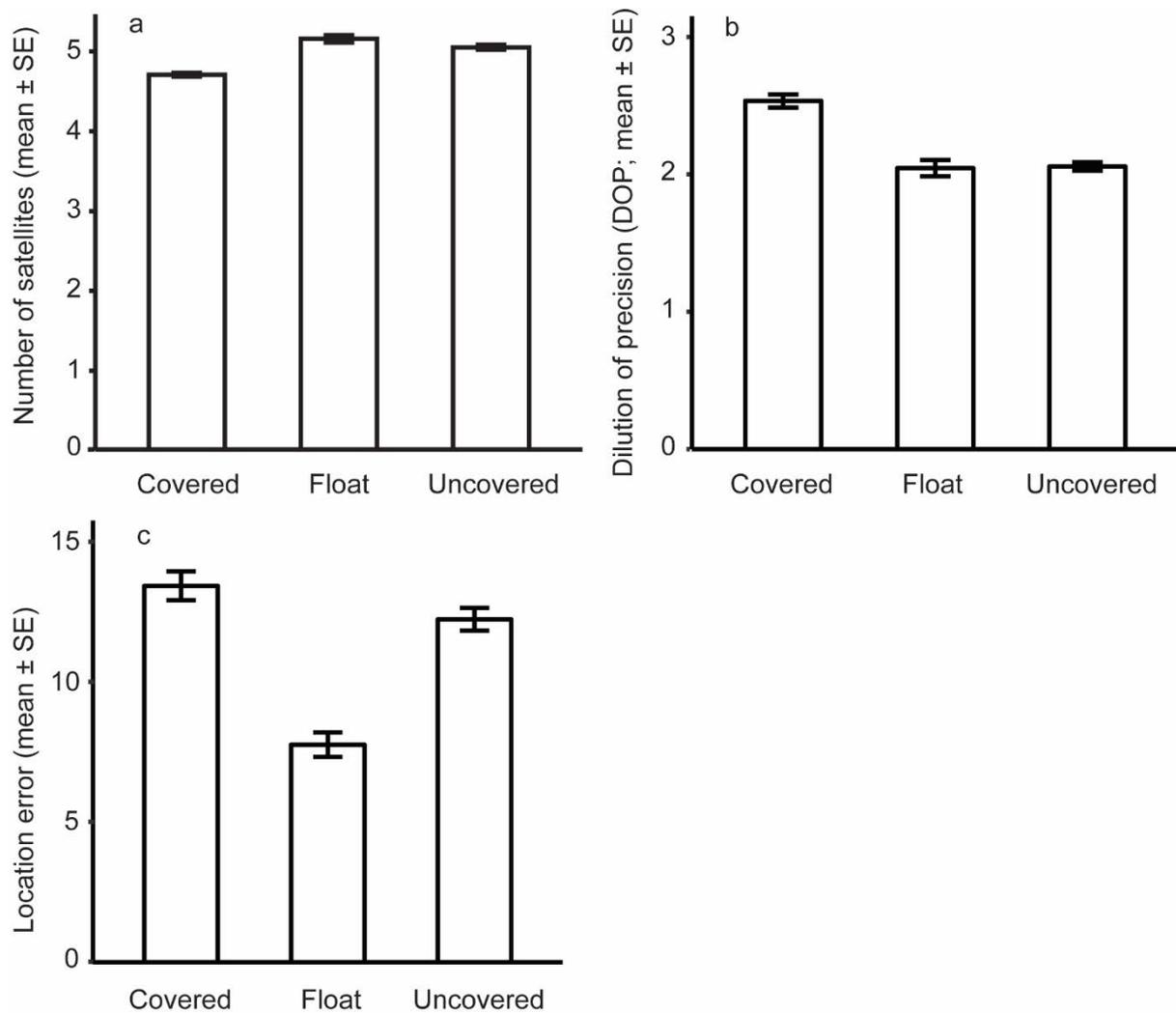
Fig. 4.3. Performance of GPS tags during stationary tests (using a dilution of precision (DOP) filter) among different location types (covered, uncovered and float). Mean (a) number of connected satellites, (b) DOP values and (c) location error (m). Error bars show ± standard error.

Values of DOP ranged from 0.6 to 9.4 (with 10 being the allowed maximum value) and, similar to previous trends, both Uncovered and Float tests recorded lower average DOP values (Float *v*. Covered and Covered *v*. Uncovered, Tukey's HSD, $P < 0.001$; Fig. 4.3b). Although LE varied significantly among Covered, Uncovered and Float tests (ANOVA: $F = 35.49$, d.f. = 2, $P < 0.001$), the Float treatment recorded the lowest average LEs (7.75 ± 0.4 m) when compared with both Uncovered (12.2 ± 0.4 m) and Covered (13.4 ± 0.5 m) treatments (Float *v*. Covered and Float *v*. Uncovered, Tukey's HSD, $P < 0.001$; Fig. 4.3c). Thus, both open locations (Uncovered and Float) had the highest accuracy and precision from the GPS tags.

The LE also varied significantly on the basis of the number of satellites, generally decreasing as the satellite number increased, except at the highest numbers of satellites (Kruskal–Wallis, $x^2 = 206.87$, d.f. = 7, $P < 0.001$, Fig. 4.4). Location error decreased by 78% when fix locations decreased from nine to four satellites. In contrast, LE tended to increase when fix locations were acquired from more than nine satellites, most likely owing to the low number of recorded values (10–11 satellites: $n = 2$).
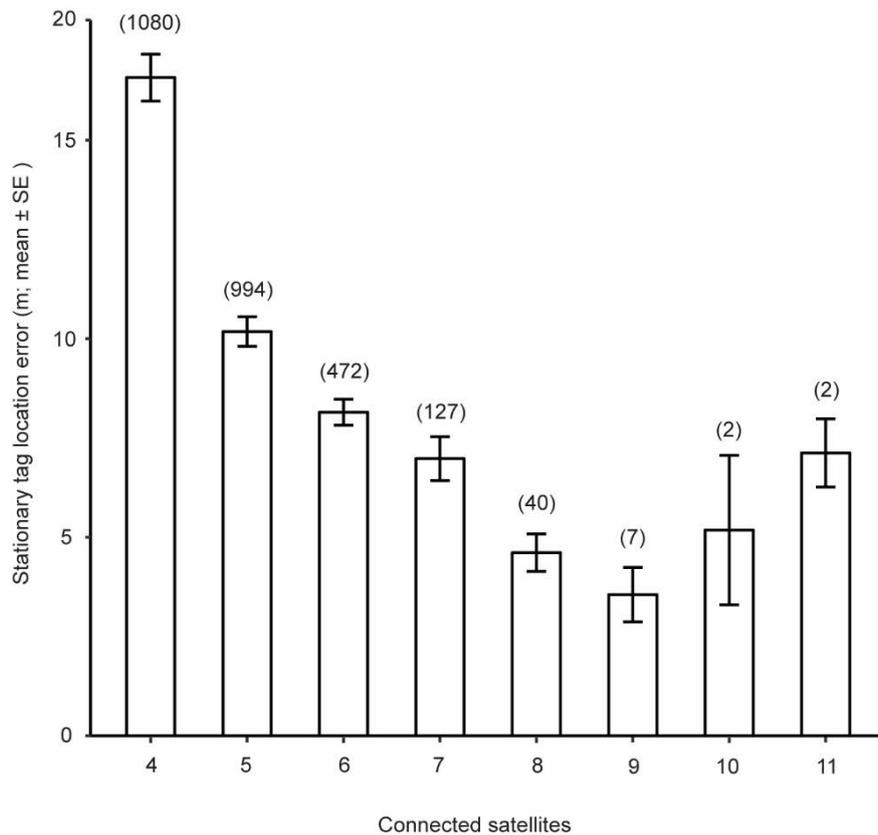
Fig. 4.4. Location error (mean ± s.d. and maximum, m) of GPS fixes grouped by number of acquired satellites fixes.

4.3.2 Accuracy of float-mounted GPS device during stingray tracks

Four juvenile mangrove whiprays (2 female and 2 males; average 32.2 cm DW) were equipped with towed-float GPS devices and tracked between 25 and 27 April 2016. All tracks were performed during the day. On average, each individual travelled 1332.15 ± 269.58 m (mean ± s.e.) across reef flat and mangrove habitats at an average speed of 6.87 m.min^{-1}. The average duration of each track was 223.9 ± 36.4 min (mean ± s.e.; Table 4.2).

53

Table 4.2. General information of each track.

Track	Sex	DW	Date of track	Duration of track (h)	Distance of track (m)	Speed (m.min^{-1})
A	F	32	25/04/2016	5:41	1817.7	6.99
B	M	35	26/04/2016	4:03	1783.9	8.49
C	F	30	26/04/2016	2:50	486.88	3.04
D	M	32	27/04/2016	2:30	1240.1	8.85

During tracks, the number of recorded satellites ranged from three to eight, with mean values for each track just over four satellites (Fig. 4.5a). As seen in stationary testing, LE tended to decrease with an increasing number of satellites. Mean DOP values differed among tracks, but were always low (Fig. 4.5b). Location error was significantly different among tracks (Fig. 4.5c), possibly being related to differences in DOP values. Overall, the LE of individual locations ranged from 1 to 87 m during tracks (Fig. 4.6), yet, on average, remained consistent and did not vary significantly between mangrove and reef-flat habitats ($t = 0.416$, $P = 0.678$; Fig. 4.7).
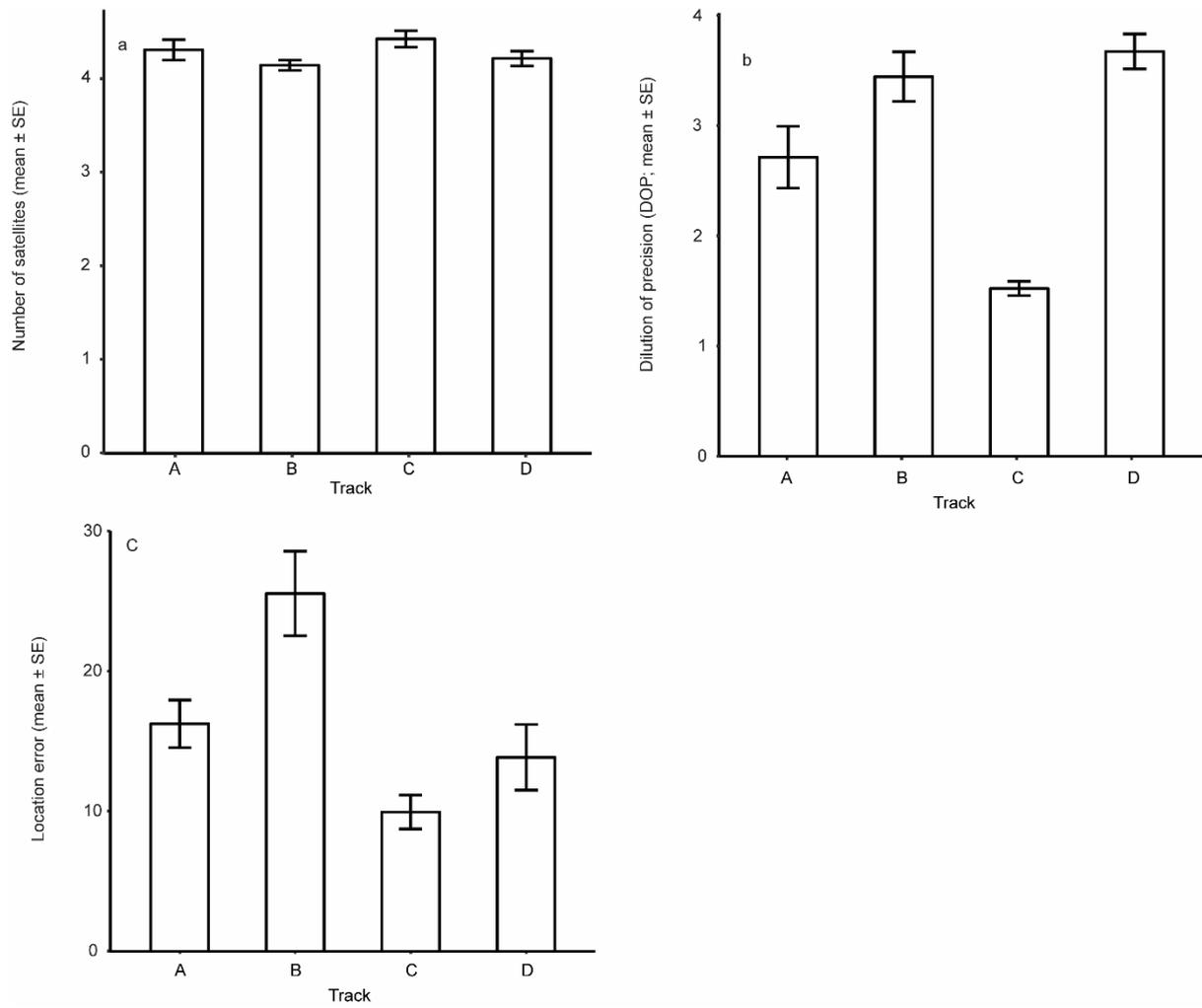
Fig. 4.5. Performance of GPS tags during tracking of juvenile mangrove whiprays (Tracks A, B, C, D). Mean (a) number of connected satellites, (b) dilution of precision (DOP) values and (c) location error (m). Error bars show ± standard error.
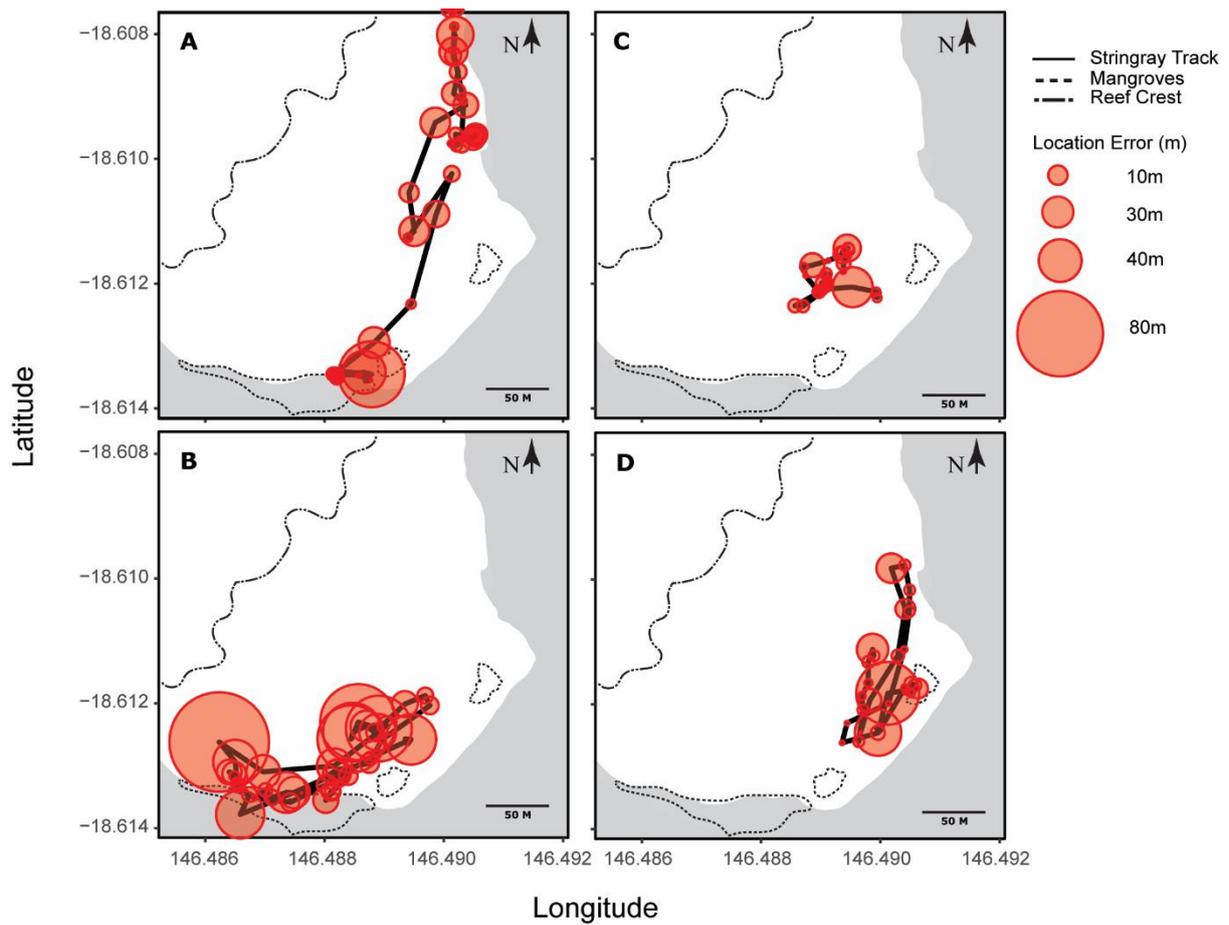
Fig. 4.6. Towed-float GPS-logger tracks from four individual juvenile mangrove whiprays across the reef flat and mangrove habitats in Pioneer Bay, Orpheus Island. Circles indicate size of location error at each positional fix taken every 5 min and are sized relative to map scale.
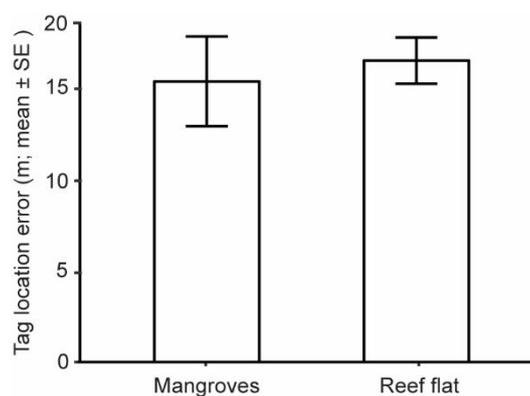
Fig. 4.7. Mean location errors of towed-float GPS loggers between different habitats: mangrove (covered) and reef flat (uncovered). Error bars show ± standard error.

4.4 Discussion

The results of the study have demonstrated that towed-float GPS tagging is a useful tool in studying the movements of juvenile stingrays, such as mangrove whiprays, that live in shallow water. Float-mounted devices allowed GPS tags to maintain the connection to satellites during the entire period of tracking and reached 99% of successful fixes, which is higher than the 90.5% recorded by Riding *et al.* (2009) for tracks of eagle rays. When compared with active acoustic telemetry, GPS tags provided very similar location estimates. The average difference in location per fix was < 15 m, fitting well within the average of modern GPS loggers (10–28 m; Hansen & Riggs, 2008).

Stationary tests helped evaluate the performance of GPS tags before attachment to the study species (Frair *et al.*, 2010). Tests showed that reef-flat areas generally acquired a higher number of satellites and more successful fixes, and were, therefore, the areas that were likely to produce the highest accuracy and precision for the GPS tags in Pioneer Bay. Negative effects of mangrove canopy cover on the GPS-logger performances, such as reduced location precision and fix rates, were observed. Frair *et al.* (2010), Webb *et al.* (2013) and Forin-Wiart *et al.* (2015) found similar decreases in location data quality because of the interference of thick canopy cover over GPS devices. To minimise this aspect during tracks, mangrove whiprays were tagged during the falling tide, when they move out of mangrove habitats, and retrieved at high tide, when they return to mangrove patches and tags tangled on mangrove roots. In addition, results showed that the application of filters had no significant influence on data recording for loggers located in the reef flat; however, these filters were essential to ensure accurate results for areas with canopy cover. Thus, filters were demonstrated to be effective tools to improve location accuracy and essential when assessing movement patterns of species such as mangrove whiprays that use covered habitats.

During tracks with filters applied, LE values were consistent and did not vary significantly between reef-flat and mangrove habitats. Although canopy cover influenced accuracy and precision of data recorded during the stationary tests, during tracks, the largest LEs were surprisingly found in open areas. The number of satellites per recorded fix turned out to be the major negative factor in data recording. Additional research is required to fully understand this aspect.

The developed float device showed adequate stability, buoyancy and retention of the GPS logger. The chosen length of the monofilament, first adopted by Sims *et al.* (2009), facilitated

continuous communication between GPS loggers and satellites, and reduced the drag forces on the attachment point that could possibly have affected mangrove whipray movements. Thus, the attachment of a towed-float device proved effective for slow-moving mangrove whiprays in Pioneer Bay, corroborating results found by Riding *et al.* (2009) and Sims *et al.* (2009), who also obtained high-quality results for low-speed fish species. Our results reinforced those of previous studies because data showed that juvenile mangrove whiprays usually swim at a low speed unless disturbed.

The use of towed-float GPS tags on mangrove whiprays has three potential disadvantages. The first disadvantage is the stress in response to the attachment of tags (Weimerskirch *et al.*, 2002). The towed-float GPS device was developed to cause minimal damage and stress. Individuals showed a short-term reaction to capture and tagging procedures, with some moving away from the capture point and others resting immobile at the site of release (A. P. B. Martins, unpubl. data). However, all tagged individuals maintained speeds (mean 6.7 m.min^{-1}) similar to those observed by Davy *et al.* (2015; mean 5–6 m.min^{-1}, depending on tide), suggesting that the towed float had little effect on their regular movement. So, it appears that tagging stress was minimal and did not have lasting effects. The second disadvantage is the possibility of entanglement on mangrove roots, rocks and coral reefs (Gifford *et al.*, 2007). This problem was solved through the use of a 2-kg monofilament as the tether connecting the towed-float device to the stingrays. In case of entanglement, the line broke easily, causing minimal damage to the animal and reducing the stress of recapture for tag removal. However, this also meant that movements could not be fully investigated by this methodology at high tide (e.g. how far do they move on high tides). A combination of methodologies is needed to address this issue. Finally, the method may not provide sufficiently accurate results to answer research questions. Estimation of LEs is essential for evaluation of any telemetry method (Royer & Lutcavage, 2008). In the present study, the average distance between the true and predicted locations was 12.1 ± 0.28 m, which is within the average precision of modern GPS loggers (10–28 m) established by Hansen and Riggs (2008). This result provided a sufficient level of precision to estimate fine-scale habitat-use patterns and swimming speeds of tagged juvenile mangrove whiprays.

Despite the above-cited potential issues, the quality of the spatial data obtained with GPS tags in Pioneer Bay could not have been replicated with such accuracy by other conventional telemetry methods. Human resources and bias, tidal cycles and night periods, for example, limit active tracking. Meso-scale tides and the shallow and sandy characteristics of Pioneer

Bay hamper the use of passive acoustic telemetry. This was confirmed by Davy *et al.* (2015), who used passive acoustic telemetry to track mangrove whiprays at Orpheus Island and obtained low spatial accuracy because of environmental conditions, and Welsh *et al.* (2012), who identified the detection range for 9-mm transmitters as being low within Pioneer Bay, namely, 60 m, which is only a fraction of the reported range in deeper, less complex habitats. Mangrove whipray benthic habits could negatively affect the capability of ARGOS-based systems in recording high-accuracy data (tens of metres) and could never achieve similar temporal resolution because of the limited number of satellite overpasses in the tropics (Riding *et al.*, 2009). Archival geolocation tags and pop-up satellite archival tags (PSAT) would not be useful to assess movement of stingrays in small areas because of the large size of loggers and positional errors up to hundreds of metres for light-based geolocation (Elston *et al.*, 2015; Hazel, 2009; Svedäng *et al.*, 2007). Thus, towed-float GPS tags provide an excellent option for tracking the movements of small, limited-range animals in very shallow water for short periods.

Despite the small number of tracked animals, towed-float GPS tags showed potential to provide insight into juvenile mangrove whipray movements, which could be applied to similar species in other locations. In addition to the quality of the data obtained, this method was advantageous by allowing deployment of multiple towed-float GPS tags simultaneously, because no further monitoring is required after tag deployment (Riding *et al.*, 2009). When well employed, the use of GPS loggers will enable the description of poorly known movement patterns, ontogenetic shifts, habitat preferences and essential habitats. As a result, the finer-scale data that GPS loggers provide in open habitats could have important application in studies that inform fisheries management and conservation, helping address interdisciplinary ecological issues and aid management decisions for essential habitats and threatened species (Hart & Hyrenbach, 2009; Schofield *et al.*, 2007).

4.5 Conclusions

This study has demonstrated the potential of towed-float GPS telemetry for assessing geographical extent, movement patterns, site fidelity, spatial dynamics, habitat preferences and behaviour data of juvenile stingrays over short periods. Use of this methodology must be carefully designed according to the study species, its life stages and study areas. If well applied, GPS loggers can provide more accurate data on juvenile stingray locations and movements than do other telemetry methods, especially when used in inter-tidal habitats. Our

study was the first to use of towed-float GPS telemetry to document fine-scale movements of mangrove whiprays. Broad-scale use of this technique could enhance our understanding of habitat use and conservation, movement patterns and ecology of juvenile stingray populations and their essential habitats.

Chapter 5: Tidal-diel patterns of movement, activity and habitat use by juvenile mangrove whiprays using towed-float GPS telemetry

5.1 Introduction

Understanding movement and habitat use is an essential step to reveal species interactions and their relationship with ecosystem dynamics (Morales *et al.* 2010). Animal tracking has been widely used for understanding these processes in marine animals (e.g. Brinton & Curran, 2017; Mendonca *et al.*, 2018). Detailed information on how and why a species accesses particular sites also makes it possible to develop more effective conservation strategies (e.g. Buchholz, 2007; Shipley *et al.*, 2017) and, as a result, reduce ecological consequences of anthropogenic disturbances (Van Cleave *et al.*, 2018).

Stingrays (Dasyatidae) are particularly susceptible to anthropogenic disturbances due to their life history characteristics (Dulvy *et al.*, 2014) and use of heavily fished coastal habitats. Habitat destruction, intense coastal development and especially overfishing are the main causes of declines in stingray populations (Jabado *et al.*, 2018). Stingrays are often caught as bycatch, but are also important food resources, particularly in developing countries, and so retained when captured (Dulvy *et al.*, 2017). Unfortunately, effective management and conservation of stingray population is hindered by limited data on their movement patterns and habitat use (Cartamil *et al.*, 2003; Collins *et al.*, 2007) – specifically their fine-scale spatial use.

Tag and recapture techniques (Lewis, 1983; Schmid, 1988), acoustic telemetry (Cerutti-Pereyra *et al.*, 2013; Collins *et al.*, 2007; Speed *et al.*, 2013), satellite telemetry (Le Port *et al.*, 2008) and accelerometer packages (Otaki *et al.*, 2015; Ward *et al.*, 2019) have provided some insights into stingray movement over the past few decades. Recent research has also demonstrated that GPS logger tags can be used with juvenile stingrays when they occur in shallow water (Martins *et al.*, 2019). However, very few studies have looked at fine-scale movements of stingrays, even less have looked at juvenile fine-scale movements within nursery areas (e.g. Dale *et al.*, 2011; Davy *et al.*, 2015; Elston, 2016).

Thus, key space-use patterns for juvenile stingrays, such as how activity varies with diel period and tidal cycles, remain poorly understood. This study therefore aimed to examine diel movement patterns in a common stingray, the mangrove whipray (*Urogymnus granulatus*), that occurs within a reef flat habitat of the Great Barrier Reef using a novel

tracking method – towed-float GPS telemetry (Martins *et al.*, 2019). More specifically I: (1) recorded fine-scale movements, (2) examined possible influences of tidal height, time of day and sex on movements, and (3) analysed habitat use, identifying important microhabitats for juvenile mangrove whiprays.

5.2 Methods and Materials

5.2.1 Study area and species

Mangrove whiprays (*Urogymnus granulatus*) are common inhabitants of Indo-West Pacific coastal areas (Last *et al.*, 2016). Juveniles are typically found in proximity to mangrove and estuarine habitats, using predominantly shallow and turbid areas. Due to the increasing pressure on coastal environments, especially overfishing and habitat loss, mangrove whipray populations in much of their range are in decline (Last *et al.*, 2016). Currently, the mangrove whipray is assessed by the International Union for Conservation of Nature (IUCN) Red List as Vulnerable globally (Manjaji Matsumoto *et al.*, 2016), but in Australia faces less threat and is assessed as Least Concern (Simpfendorfer *et al.*, 2019) (Shark Report Card https://www.sharkreportcard.org/).

This study was conducted in Pioneer Bay, one of the west-facing bays of Orpheus Island in the central region of the Great Barrier Reef, Australia (Fig. 3.1). Pioneer Bay is known for sheltering juvenile mangrove whiprays all year around, but with varying abundance between seasons (Davy *et al.*, 2015; Kanno *et al.*, 2019). For this study, individuals were captured with seine and dip nets in shallow waters, mainly under mangrove roots. Individuals were captured throughout both wet and dry seasons, from April 2016 to August 2017. Specimens captured during the outgoing tide were measured (disc width – DW), fitted with a spiracle tag for identification, and released at the same location as capture. Spiracle tags had a unique number ID for animal identification and were also used as attachment point for towed-float GPS tags. GPS tags were attached to the spiracle tags with 2-kg test monofilament fishing line, approximately 1.5 m in length, as described in (Martins *et al.*, 2019). Date, time of capture and GPS location were recorded. Individuals captured during low, incoming or high tide were transferred to the Orpheus Island Research Station and kept in circular tanks (2-m diameter) until the next outgoing tide. Individuals were then taken back to the location of capture for measurements, tag attachment and release. As demonstrated in Chapter 4, individuals exhibited normal behaviour soon after capture and handling. Towed-float GPS

tags were equipped with Lotek Wireless PinPoint Beacon 120 transmitters (25 x 14 x 10 mm) set to record a GPS position every 5 minutes to describe short-term movements of juvenile mangrove whiprays throughout the bay. Tags were expected to become entangled in mangrove roots once the stingray entered mangrove areas during high tides and, as a result, detach from the animal as the monofilament line breaks. An integrated Lotek radio beacon facilitated tag recovery in mangrove patches after the programmed period of one tidal cycle. GPS loggers had rechargeable batteries and were reused.

5.2.2 Data analysis

Data were downloaded from recovered tags using Lotek Wireless PinPoint Host software. Following Martins *et al.* (2019), a Dilution of Precision (DOP) filter was applied to the data to improve geolocation quality – removing GPS points recorded with poor satellite geometry. The first five GPS points of each track were removed to reduce the effects of capture and handling on animal behaviour. Analyses of filtered tracking data were carried out with R (R Core Team, 2017) using the *adehabitatLT* package (Calenge *et al.* 2015). Rate of movement (ROM), the distance travelled by a specimen per minute ($m.min^{-1}$), was calculated per 5-min interval and for the whole track for all tracks. ROM was calculated separately for four tidal stages (low – one hour on either side of low tide; high – one hour on either side of high tide; outgoing – decreasing tide between high and low, and incoming tide – increasing tide between low and high), day and night, and between sexes. Hourly tide heights were extracted from records from the Bureau of Meteorology website (http://www.bom.gov.au/, 22/04/2019). Tides were classified as either "spring" (3 days either side of the full or new moon) or "neap" (all others) to examine differences in movements based on tidal extent. A Linearity Index (L) – the Euclidian distance between the beginning and ending point of a track divided by total track length – was calculated for every group of five consecutive GPS positions (5 point linearity) to identify how straight stingray movements were during tracks (Batschelet, 1981). Linearity Index values range from 0 to 1, with values close to zero showing non-linear movement and values close to one demonstrating direct movement.

Second order polynomial natural splines with individual as a random factor were applied using the R package *glmmTMB* to determine the effects of tide level, sex and time of the day on ROM and Linearity. In addition, general linear mixed models were performed to evaluate possible relationships between ROM and Linearity and tide types (spring and neap), and also

between activity space and duration of the tracks. ROM values were log transformed before analysis to normalise the data.

Finally, using the R package *adehabitatHR* (Calenge *et al.* 2015), Kernel utilisation distributions (KUD) were used to estimate space utilization during day and night periods. To do so, detections were pooled into day (between 0600 and 1759 hours) or night (between 1800 and 0559 hours). Both KUD and tracks were then overlaid on a photo-mosaic image of Pioneer Bay using ArcMap software to provide a full overview of juvenile mangrove whipray habitat utilisation in Pioneer Bay.

5.3 Results

A total of 11 young-of-the-year and juvenile (4 males and 7 females) mangrove whiprays were tracked in Pioneer Bay using towed-float GPS telemetry. One individual was recaptured and tracked twice, first in March and then in August 2017. One individual did not enter the mangrove area during high tide, therefore its tag was removed only during the second tidal cycle, resulting in one long track separated into two (day and night) for further analysis. Thus, 13 tracks were analysed, six during the day and seven at night. Specimens varied in DW from 28 to 54 cm and track length ranged from 1.5 to 9.0 hours. Juvenile mangrove whiprays did not leave Pioneer Bay during tracking and travelled distances from 394 to 2189 meters, with an overall mean ROM of 4.51 m.min^{-1} ± 3.1 SE and L of 0.69 ± 0.16 (Table 5.1; Fig. 5.1).

Table 5.1. Details of juvenile mangrove whiprays tracked at Orpheus Island using towed-float GPS telemetry.

Tracks	Sex	DW (cm)	Time of the day	Date	Duration of track (hr)	Min. dist. travelled (m)	Activity space (km²)	ROM (m.min⁻¹)	L
1	M	28	Day	27/04/2016	1.58	1064	1.1	11.20	0.69
2	F	38	Day	10/11/2016	3.50	2189	4.7	10.68	0.91
3	F	54	Day	13/11/2016	5.25	1983	3.9	6.84	0.61
4	F	34	night	20/08/2017	7.25	1132	1.2	2.60	0.73
5	M	35	Day	16/08/2017	6.50	1525	2.3	3.91	0.63
6	M	31	night	20/08/2017	5.0	692	0.4	2.30	0.66
7	M	30	Day	2/03/2017	1.58	394	0.1	4.14	0.31
8	M	31	night	21/08/2017	5.66	846	0.7	2.49	0.50
9	F	42	night	14/08/2017	4.83	789	0.6	2.72	0.48
10	F	30	night	25/08/2017	3.66	836	0.6	3.80	0.48
11	F	30	Day	26/08/2017	9.0	1626	2.6	3.53	0.61
12	F	32	night	17/08/2017	3.0	425	0.1	2.36	0.31
13	F	30	night	25/08/2017	5.0	697	0.4	2.32	0.60
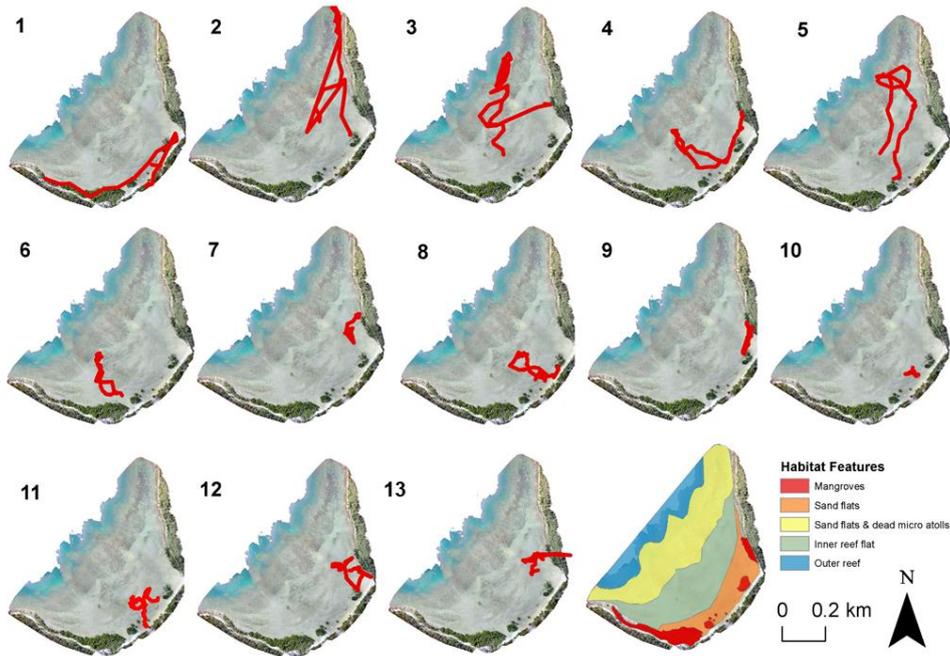
Fig. 5.1. Tracks of juvenile mangrove whiprays using towed-float GPS telemetry and habitat types in Pioneer Bay. Day tracks: 1, 2, 3, 5, 7 and 11; Night tracks: 4, 6, 8, 9, 10, 12 and 13.

Both ROM and Linearity varied significantly at different tide phase (ROM $p < 0.001$; L $p < 0.001$) (Fig. 5.2). Individuals moved faster and with straighter paths during outgoing (ROM of 6.10 m.min^{-1} ± 3.2 SE and L of 0.68 ± 0.1 SE) and incoming (ROM of 5.26 m.min^{-1} ± 7.46 SE and L of 0.68 + 0.2 SE) tides. During low tides, individuals moved slower, using more random paths (ROM of 3.08 m.min^{-1} ± 1.6 SE and L of 0.46 ± 0.18 SE) (Fig. 5.2). Data collected during high tide was too limited for a full evaluation as tags became entangled in prop roots and detached from stingrays. Differences in ROM and Linearity between spring and neap tides were not significant ($p = 0.793$ and $p = 0.349$, respectively). Differences in ROM and Linearity between sexes were not observed ($p = 0.987$ and $p = 0.689$, respectively).

Second order polynomial natural splines showed a significant difference in ROM between day and night ($p < 0.001$) (Fig. 5.2a). Juvenile mangrove whiprays had a mean ROM of 6.67 m.min^{-1} ± 3.5 SE during the day and 2.49 m.min^{-1} ± 0.5 SE at night. Linearity values were 0.63 ± 0.19 SE for daytime tracks and 0.54 ± 0.13 SE for night tracks. Thus, juvenile mangrove whiprays were found to move faster during the day, while moving short distances at a slower pace at night. Results of second order polynomial natural splines did not detected significant differences in Linearity between day and night periods ($p = 0.177$) (Fig. 5.2b).

66
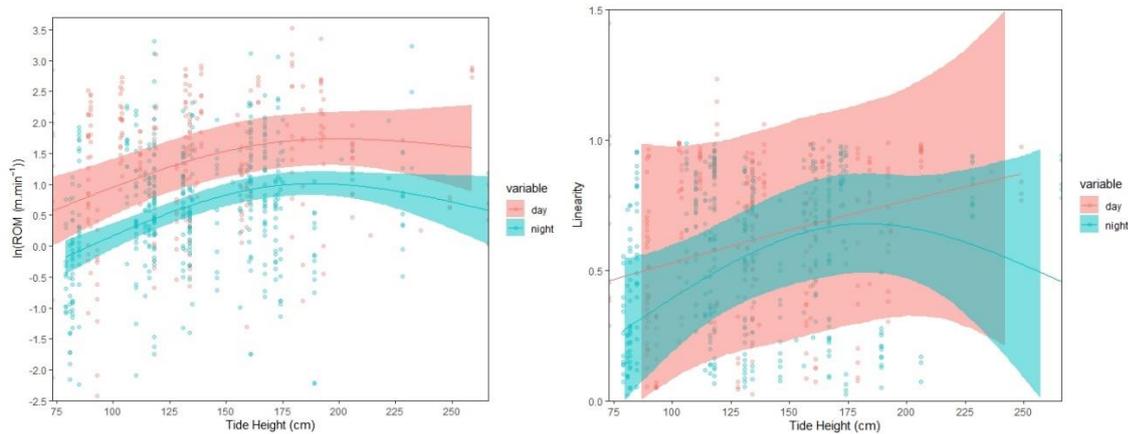
Fig. 5.2. Effects of tide and diel changes on juvenile mangrove whiprays (a) Rate of Movement (ROM) and (b) Linearity. Blue points represent individual locations at night and pink points represent individual locations during the day. 95% confidence intervals are represented for day and night in their respective colours.

Kernel utilisation distributions (KUD) revealed juvenile mangrove whiprays primarily use reef flat areas in Pioneer Bay (Fig. 5.3). Most individuals were captured under or in close proximity to mangrove patches and remained within these areas for as long as water levels permitted. Once the water receded, mangrove whiprays were forced to move, often moving toward sand patches within the reef flat and crest. This habitat functioned as their refuge during low tides. Transitions between mangrove and sand patches within the reef were exclusively made through shallow and soft bottom areas. Stingrays were not observed using rocky habitat located in the southern region of the bay. Individual activity space had no relationship with the duration of the tracks ($p = 0.311$).
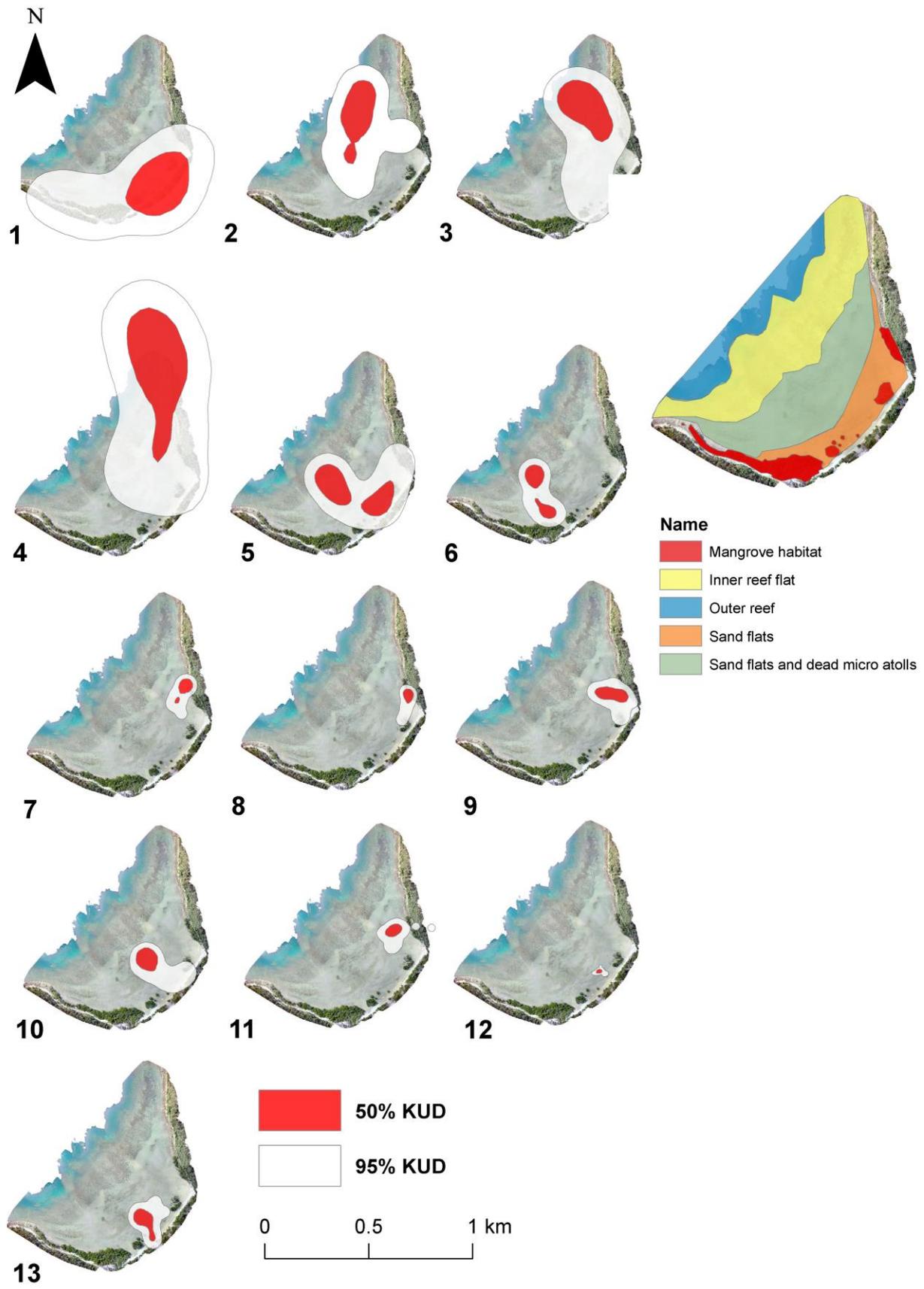
Fig. 5.3. Kernel utilisation distributions (KUD) of juvenile mangrove whiprays.

5.4 Discussion

The use of towed-float GPS telemetry employed in this study allowed for examination of aspects of stingray spatial ecology that have not previously been possible. This includes the first insights into juvenile mangrove whipray diel activity patterns and the lack of differences in movement activity between sexes. These results add to movement information provided by Davy *et al.* (2015) using manual acoustic telemetry, expanding our understanding of mangrove whipray movements and reinforcing the influence of tidal stages on its habitat use.

Juvenile mangrove whiprays showed strong site affinity to Pioneer Bay – not leaving the area over the study period. Based on general linear models, there was no significant effect of sex on juvenile mangrove whipray ROM and Linearity. As suggested by Dale *et al.* (2011), sexual segregation might be absent in juvenile stingrays until they reach sexual maturity. Therefore, changes in movement may become apparent as individuals increase in size and age, which were not evident here. Activity space of individuals varied from 0.1 to 4.7 km² and showed no significant influence of track duration. Similar results were demonstrated by Elston (2016) for juvenile porcupine rays (*Urogymnus asperriumus*), with individuals showing activity space areas smaller than 3 km² over one year of acoustic tracking, and by Cartamil *et al.* (2003) for *Bathytoshia lata*, with manual tracked individuals showing 95% KUD between 0.6 and 2.77 km² during the ~ 74 hour tracks. Freshwater whiprays (*Urogymnus dalyensis*) tracked in Cape York Peninsula (Australia) also showed movement patterns restricted to a small segment of river, less than 8 km in length (Campbell *et al.*, 2012). Even more mobile batoids, such as the cownose rays (*Rhinoptera bonosus*) often used relatively small areas (mean = 17.9 km²) during 16 months of tracking (Collins *et al.*, 2007). Thus, use of restricted areas by juvenile mangrove whiprays is consistent with information for other ray species. These small areas must offer sufficient food resources and favourable conditions for survival. Therefore, locations such as Pioneer Bay must provide ample food for juvenile mangrove whiprays – allowing them to restrict their movements to shallow and protected areas and avoid encounters with predators that occur in deeper waters (Heupel *et al.*, 2007).

Tide type (spring and neap) were found to have no effect on ROM and Linearity, but juvenile mangrove whipray movements were strongly influenced by the tide, with all tracks following tidal current direction. The same pattern was found by Brinton & Curran (2017) for the Atlantic stingray (*Hypanus sabina*). This behaviour of moving with the tidal flow is likely to

provide energetic benefits by reducing the energy costs of swimming and allowing the use of shallow waters, except during high tides. Tide height also influenced juvenile mangrove whipray ROM and Linearity. The ROM of individuals was significantly higher in tidal heights during outgoing and incoming tidal stages. These are the times when tidal movement are the fastest, so it is likely that juvenile mangrove whiprays needed to move faster and in a more directed manner to remain in shallow water and also avoid becoming stranded as water receded. Similar patterns were found by Davy *et al.* (2015) and George *et al.* (2019) for juvenile mangrove whiprays and blacktip reef sharks, respectively. In both cases, results suggest that juveniles could be more exposed to predation or stranding during these tides, thus the observed movement patterns would reduce these risks.

Predation risk is likely to play a major role in juvenile stingray activity patterns and habitat use (Cerutti-Pereyra *et al.*, 2013; Davy *et al.*, 2015; Kanno *et al.*, 2019). The presence of small-bodied stingrays is often associated with habitats that provide soft-bottom for burying or physical barriers for protection, such as mangrove root systems (Stump *et al.*, 2017). This type of habitat use pattern has been observed for juvenile southern stingrays (*Hypanus americanus*) (Tilley, 2011) and juvenile porcupine rays (*Urogymnus asperriumus*) (Elston, 2016). In Pioneer Bay, deeper waters are often visited by blacktip reef (*Carcharhinus melanopterus*) and lemon sharks (*Negaprion acutidens*) (Kanno *et al.*, 2019; Schlaff *et al.*, 2017) which could serve as predators for juvenile stingrays. Juvenile mangrove whiprays were observed to avoid these deeper water areas most of the time, therefore reducing their risk of encountering potential predators. However, juvenile stingrays could not always avoid deeper waters, especially during the high tide. Unfortunately, performing telemetry within complex mangrove habitats during high tides is not an easy task, so it is difficult to determine what methods stingrays employ to reduce predation risk. Recently, Kanno *et al.* (2019) used stationary video monitoring to better understand the use of mangrove habitats by juvenile stingrays in Pioneer Bay. This study showed mangrove areas of Pioneer Bay primarily serve as a refuge from predation, highlighting the high use of the mangrove root structure by stingrays during high tide and lack of predatory sharks in these areas. Our tracking results reinforce these findings with individuals moving into mangrove areas during high tide periods prior to entangling and loss of GPS tags. In addition, Kanno *et al.* (2019) also identified mangrove patches as potential feeding grounds for juvenile mangrove whiprays suggesting this habitat may serve multiple purposes. Juvenile mangrove whiprays were also occasionally seen in groups, but these appeared to be random events not linked to any

specific conditions. Thus, further research is needed to evaluate the use of grouping as a strategy to avoid predation in juvenile mangrove whiprays by increasing their ability to detect predators as demonstrated by Semeniuk and Dill (2004).

In contrast to what has been found for other stingray species (Brinton & Curran, 2017; Cartamil *et al.*, 2003; Corcoran *et al.*, 2013), juvenile mangrove whiprays were more active during the day. The reasons for this behaviour are unknown, and more research is required to fully understand these patterns. Hypotheses involving higher abundance of predators or prey at night time were discarded since increased predator avoidance or feeding activities at night would likely lead to significant changes in linearity – which was not observed. However, a few hypotheses still can be considered. First, predators could be less common at night, so stingrays would be more relaxed and possibly reduce ROM. Second, Higgins (2018) demonstrated that juvenile mangrove whiprays preferred to use areas with water temperatures ranging from 30 ℃ to 36 ℃, avoiding temperatures below 25 ℃. Drops in water temperature at night to close to or below 25 ℃ could reduce juvenile stingray body temperature and consequently reduce their movements. Moreover, further research for predicting longer-term behaviour of juvenile mangrove whiprays in Pioneer Bay are needed for a better understanding of the species movement patterns and habitat use over time.

5.5 Conclusions

In this study, I extend our understanding of juvenile mangrove whipray diel-tidal activity patterns. Juvenile mangrove whipray activity patterns and space use were strongly influenced by tidal stage and diel period. Individuals moved faster and chose more direct paths during outgoing and incoming tides. Activity space was significantly larger during the day than at night time, but more research is needed to reveal the causes of this behavior. Mangrove areas were identified as primary habitat for the species, followed by sand patches within reef flats. These areas are thought to provide physical protection against predators during the high and low tides. Only soft-bottom areas were used during the transition between mangroves and reef flat. These areas can also provide refuge, by allowing burying behavior, and also access to food resources. Data presented in this study demonstrate fine-scale details of how juvenile mangrove whiprays use reef flat environments. Additional research is needed to fully understand the year-round abundance and movement patterns and ontogenetic shifts of mangrove whiprays. The results of this research, however, provide useful information to

examine the implications of anthropogenic impacts (e.g. mangrove loss) on this species and other juvenile species that inhabit similar complex habitats.

Chapter 6: Trophic ecology of sympatric juvenile elasmobranchs within a nursery area

6.1 Introduction

Communal nurseries are known to have great importance to the life histories of many elasmobranchs (Martins *et al.*, 2018). Overlapping distributions of juvenile elasmobranch species within a relatively small area can provide benefits such as more successful predator avoidance (Simpfendorfer & Milward, 1993). However, overlapping distributions can also require species to develop strategies to reduce competition for resources (Pianka 1974, 2011). To reduce competition and allow coexistence, similar sympatric species often partition or exploit different food resources (Albo-Puigserver *et al.*, 2015; Schoener, 1983; White *et al.*, 2004), which results in distinct ecological niches and/or trophic positions (Munroe *et al.*, 2014). Understanding how sympatric species exploit resources can provide important information on their ecological role in the community as well as on ecosystem dynamics (Matley *et al.*, 2017; Navia *et al.*, 2007; Yick *et al.*, 2011). Unfortunately, only a limited number of studies have focused on understanding juvenile elasmobranch trophic ecology and resource partitioning within communal nursery environments (Bethea *et al.*, 2004; Kinney *et al.*, 2011).

Stable isotope analysis (SIA) has been successfully applied to explore elasmobranch trophic ecology in marine ecosystems (Espinoza *et al.*, 2015; Estrada *et al.*, 2003; Fisk *et al.*, 2002; Kinney *et al.*, 2011; Macneil *et al.*, 2005; Mcmeans *et al.*, 2013). This technique uses $\delta 13C$ and $\delta 15N$ as biological tracers of an animal's diet and habitat use since the concentration of these isotopes increases consistently from primary producers to top predators within food webs (Caut *et al.*, 2009). Values of $\delta 15N$ increase from prey to predator in a predictable amount, providing relative trophic positions. Values of $\delta 13C$ increase in smaller amounts than $\delta 15N$, but differ between primary producers (e.g., pelagic vs benthic) and provide an estimate of habitat use (Hussey *et al.*, 2012). Taken together, $\delta 13C$ and $\delta 15N$ in an organism can provide important insights into its habitat use and movements, diet, and trophic position (Hussey *et al.*, 2014).

Thus, SIA can also provide insights into carbon sources of individuals within food webs and ecosystems (France & Peters, 1997; Post, 2002). For example, mangroves are commonly recognized as highly productive habitats, playing an important role as a primary source and

store of carbon in coastal and estuarine areas (Alongi, 2014; Kristensen et al., 2008). These highly productive habitats can therefore provide resources to marine and estuarine organisms (Kathiresan, 2012), from microbial to large elasmobranch populations. The importance of mangroves for juvenile teleosts has been well explored over time (Lee et al., 2014; Whitfield, 2017), however it has not been fully explored for juvenile elasmobranchs. In fact, some juvenile elasmobranchs are known to use mangroves as nursery areas (Heupel et al., 2019; Martins et al., 2018), but their dependence on mangrove derived resources is unknown. Thus, understanding carbon flow in these areas could provide previously unknown information on the relationship between juvenile elasmobranchs and mangroves.

Turnover of isotopes vary between tissues, and can provide a temporal assessment of diet in organisms (Hussey et al., 2012). Active tissues, such as plasma, incorporate dietary markers much faster than low-turn over tissues, such as muscle, which reflect feeding events over longer periods (Buchheister & Latour, 2010). The comparisons between tissues with different turnover rates can reveal whether an animal's foraging habitats have shifted over time (Eberts et al., 2015) and provide insights into temporal variations in diet (Matley et al., 2017).

Comparing isotope values between species can provide insights into differential resource use or partitioning of resources due to, for example, competition. However, using mean values for isotopes can mask differences in the feeding ecology of species, in part because they do not account for individual variation. As $\delta 13C$ and $\delta 15N$ represent different aspects of an animal's diet, the area or space occupied by isotopic values provides an estimate of the dietary niche of a population (Newsome et al., 2007), which is referred to as an isotopic niche. Isotopic niche has become a useful metric for assessing resource partitioning in co-occurring species or populations.

This study aimed to evaluate the relative trophic relationships of four juvenile elasmobranch species within a previously established nursery area using SIA from multiple tissues (red blood cells, plasma and muscle). In addition, the study aimed to: (1) identify possible influences of size, season or sex on relative trophic position, (2) better understand dietary resource partitioning of four juvenile elasmobranchs using isotopic niches, and (3) identify the main source of primary carbon (mangrove/terrestrial, benthic or pelagic) used by elasmobranchs within the communal nursery. Results presented in this study bring new insights to the feeding ecology of four sympatric elasmobranch species and their ecological roles within a communal nursery area.

6.2 Methods and Materials

6.2.1 Study area and data collection

Pioneer Bay, located on the western side of Orpheus Island, central region of the Great Barrier Reef, Australia, is a well-established nursery area for sharks and rays. Tidal reef flats and mangrove habitats are dominant in nearshore areas, providing protection and foraging opportunities for juvenile elasmobranch development (Davy *et al.*, 2015; George *et al.*, 2019). Four main species are known to inhabit the area year-round: Mangrove whipray *Urogymnus granulatus*, cowtail stingray *Pastinachus ater*, blacktip reef shark *Carcharhinus melanopterus* and giant shovelnose ray *Glaucostegus typus*. However, many elasmobranch species have been reported in the 400-m wide reef flat of Pioneer Bay including lemon shark *Negaprion acutidens*, blue-spotted maskray *Neotrygon kuhlii*, blue-spotted ribbontail ray *Taeniura lymma*, eagle ray *Aetobatus ocellatus*, reef manta ray *Mobula alfredi*, great hammerhead *Sphyrna mokarran*, pink whipray *Himantura fai* and reticulate whipray *Himantura australis*. Here I have focused on the most commonly encountered species since their use of and reliance on this habitat is likely to be high.

Mangrove whipray, cowtail stingray and giant shovelnose specimens were captured in shallow waters (<1m) of Pioneer Bay using seine and dip nets. Blacktip reef sharks were similarly caught nearshore with seine and gill nets, or with rod and reel from the shore. All animals were caught between April 2016 and March 2018; and all were juveniles. Upon capture individuals were sexed and measured (disc width (DW) for stingrays; stretch total length (STL) for sharks and shovelnose). To ensure that the same animal was not sampled multiple times, all individuals were tagged with a uniquely numbered spiracle tag (spiracle of stingrays) or Dalton Rototag (first dorsal fin of sharks and shovelnose rays). Approximately 1 g of white muscle was removed via a 4-mm biopsy punch and 1.5 ml of blood was taken from the caudal vein of individuals and stored in sterile vials. Sample collection depended on animal's response to capture and handling, therefore not all individuals were sampled for all tissue types. Samples were immediately taken to Orpheus Island Research Station for further processing. Specifically, muscle samples were transferred to a −20°C freezer; whole blood samples were centrifuged for 5 minutes (Imbros PC100 Micro Centrifuge), then plasma solutes were separated from red blood cells (RBC) and transferred to another vial, and finally stored in a −20°C freezer.

Tissue samples from a variety of organisms in Pioneer Bay were also collected to explore possible sources of carbon and prey in the diet of juvenile elasmobranchs. Organisms that incorporated major habitat or feeding types within the bay were selected including: mangrove root (collected by hand), mangrove leaf (collected by hand), algae (collected by hand nearshore – various species pooled (e.g. green, red and brown algae)) plankton (collected offshore with 63 µg net – phytoplankton and zooplankton pooled), annelid worm (collected in sand using shovel and sieve), snail (collected by hand on mangrove), crab (collected by hand in elasmobranch nets) and baitfish (collected nearshore using dip net – family Gerreidae). All samples were collected between April 2016 and March 2018, and frozen (−20°C) until further processing.

6.2.2 Stable isotope analysis

Prior to stable isotope analysis, all tissue samples were frozen at −80°C for 24 hrs, freeze-dried, then ground into a fine powder with mortar and pestle. All tools were sterilized with ethanol between samples. Samples were not lipid-treated because C:N values were <3; therefore, the low lipid content was not expected to bias isotope values (Post *et al.*, 2007). Between 400 and 1500 µg of the crushed tissue were weighed and placed into tin or silver capsules. Isotope ratios 13C:12C and 15N:14N were then quantified from these samples at the Great Lakes Institute of Environmental Research, University of Windsor, using a continuous flow isotope ratio mass spectrometer (Finnigan MAT Deltaplus, Thermo - Finnigan) equipped with a Costech Elemental Analyzer (Costech Analytical Technologies). Isotope ratios were expressed as δ13C and δ15N following: δ13C or δ15N = (R^{sample}/ $R^{standard}$ − 1) × 1000 (‰), where R^{sample} is the ratio of heavy to light isotope in the sample, and $R^{standard}$ is the ratio of heavy to light isotope in the reference standard. As per laboratory protocol, the 12th sample was run in triplicate to assess precision – standard deviation (SD) of δ13C and δ15N was generally <0.2 and <0.1‰, respectively. Additionally, laboratory and National Institute of Standards and Technology (NIST; Gaithersburg, MD) standards were analysed every 12 samples to assess analytical precision for NIST standard 8414 (bovine liver, n = 130) and an internal laboratory standard (tilapia muscle, n = 130) – SD of δ13C was 0.05 and 0.07‰, respectively, and 0.16 and 0.13‰ for δ15N, respectively. Accuracy was validated using certified urea (n = 120) and was within 0.16 and 0.05‰ of mean calculated values for δ13C and δ15N, respectively.

6.2.3 Data analysis

Analytical approaches aimed to determine the effect of several biological parameters on multi-tissue isotope values, characterise isotopic niche across elasmobranchs and tissues, and estimate sources of dietary contribution. All analyses were completed in R (R Development Core Team 2019). Preliminary investigations revealed a broad range of elasmobranch $\delta13C$ and $\delta15N$ values within sampling periods that were equivalent to the isotopic ranges across periods. Consequently, independent of possible differences, sampling periods were pooled for isotopic niche investigations, as well as when estimating sources of dietary contribution. This was also done because sample sizes of elasmobranchs and baseline sources/prey were not sufficient to comprehensively address potential seasonal differences (i.e., calculate baseline-derived trophic position each season). The giant shovelnose ray was not included in the analyses listed below because only two individuals were caught.

First, general linear models (GLMs) were used to test whether sampling period, elasmobranch size, and sex influenced $\delta13C$ and $\delta15N$ values. To facilitate more representative comparisons with other studies, the trophic position (TP) metric was used to represent trophic level instead of $\delta15N$. Trophic position for each individual/tissue was calculated following the scaled approach described by Hussey *et al.* (2014). Small crabs were selected as the baseline organism in TP calculations because they had one of smallest isotopic niche sizes of all prey reflecting minimal variation in isotope values from detrital feeding (trophic level of 2 was designated). Separate models were used for each elasmobranch species because analysis of variance (ANOVA) revealed significant species differences in $\delta13C$ and $\delta15N$ values across tissues (see Results). Plasma, RBC, and muscle tissues were also analysed independently because they represent dietary assimilation during different periods and could result in unique patterns. Diagnostic plots confirmed data normality and heterogeneity, and results were considered significant when $p<0.05$. When significant, within-variable differences were explored using Tukey's HSD.

Next, several tissue-specific isotopic niche metrics were determined as a proxy for feeding patterns over different time periods. Niche size (or breadth) was initially calculated based on the corrected standard ellipse area (i.e., ~40% of data points incorporated; SEA_C) and convex hull area (i.e., the polygon enclosing all data points; TA). The SEA_C and TA commonly represent the 'core' niche and niche 'extent' of consumers, respectively (Jackson *et al.*, 2011). Niche size comparisons were made between elasmobranchs (from the same tissue)

based on 104 posterior draws of SEA$_C$, yielding Bayesian estimates from the posterior distribution of the covariance matrix for each species/tissue. If ≥95% of these estimates for one species were smaller (or larger) than estimates for another species, niche size differences were deemed significant. Typically, a larger niche size indicates a broader resource pool contributing to the diet of consumers directly through prey or indirectly through species lower in the food chain (Layman *et al.*, 2012).

Niche overlap between species (from the same tissue) was calculated to quantify the degree in which similar resources were shared. Two approaches were used to quantify niche overlap: overlap between SEA$_C$ and overlap between Bayesian posterior distributions. The former was calculated directly from each species' ellipse and quantified as the proportion of overlapping area between two ellipses relative to the non-overlapping area (SEA$_C$-Overlap = SEA$_C$-Shared overlap / (SEA$_C$-Species1 + SEA$_C$-Species2 - SEA$_C$-Shared overlap). The Bayesian approach utilized the first 1000 posterior draws of SEAC for each species, however 95% prediction ellipses were used as opposed to 40%, and following the same equation as above produced 1000 overlap values which were summarized in a histogram. The R package SIBER (Jackson *et al.*, 2011) was used to calculate niche metrics described above and rjags (Plummer, 2013) was implemented for Bayesian approaches. Isotopic niche ellipses (40%) of two meso-predatory fishes (leopard coral grouper *Plectropomus leopardus* and bar-cheek coral trout *P. maculatus*) and one pelagic planktivore (fusilier *Caesio teres*) sampled within and adjacent to Pioneer Bay (Matley *et al.*, 2017) were included in plots for comparative purposes.

Finally, potential prey or prey sources were investigated for each elasmobranch species (and tissue). Initially, stable isotope mixing models were applied, however diagnostics showed that prey isotopes were not sufficiently different to discriminate between sources. Also, diagnostic plots suggested that at least one important prey item was not incorporated in our samples based on expected δ15N values relative to consumer δ15N values (and diet-tissue discrimination factors; DTDFs). Consequently, a more simplified (and conservative) approach exploring possible sources of δ13C in consumer tissues was implemented. Specifically, tissue-specific DTDFs were subtracted from consumer δ13C values resulting in adjusted consumer δ13C values that were equivalent to potential prey. An idealized 'δ13C-prey range' was created for each individual consumer by incorporating a measure of uncertainty associated with the adjusted consumer δ13C values. A relatively high level of uncertainty was incorporated (±1‰) because of the possibility of seasonal variation, as well

as inherent uncertainty with DTDF estimates. For each individual, the proportion of each prey within this 'δ13C-prey range' was calculated. Distributions of prey proportions for all individuals were summarised with a histogram to quantify the prey δ13C values that are consistent with contributing to the diet of elasmobranchs. Only a subset of prey were incorporated in this analysis representing distinct feeding types/habitats in Pioneer Bay; these included snail (mangrove/terrestrial), plankton (offshore pelagic), baitfish (nearshore pelagic), crab (nearshore benthic), and worm (nearshore benthic). Because δ13C values increase successively at each trophic level, 'δ13C-prey range' for each consumer-prey pairing was corrected for the difference in trophic levels. For example, the trophic level of plankton was assumed to be 2 and that of blacktip reef sharks was 4. Therefore, the adjusted consumer δ13C values reflected a change of two trophic levels (i.e., 2*DTDFs). Based on exploratory plots and known feeding ecology, the trophic level of elasmobranchs was chosen to be 3, baitfish were set to 3, and the remaining prey (snail, plankton, crab, and worm) were considered to be at trophic level 2. Given the relatively low $\delta^{15}N$ values of cowtail stingrays, baitfish were not included in their '$\delta^{13}C$-prey range' estimates. Also, because mangrove whiprays had a large range of $\delta^{15}N$ values, iterations of this approach were done with and without baitfish included, since baitfish had higher $\delta^{15}N$ values for some individuals. Tissue-specific DTDFs were selected from (Matley *et al.*, 2016), despite being based on adult reef fish, because estimates for all three tissues were available and species were from similar geographic locations.

6.3 Results

A total of 60 juvenile elasmobranchs (36 mangrove whiprays, 17 cowtail stingrays, 5 blacktip reef sharks and 2 giant shovelnoses) and potential prey items were sampled in Pioneer Bay from 2016 to 2018 (Table 6.1). Sampled stingrays ranged from 25 to 65 cm DW, while giant shovelnose rays and blacktip reef sharks ranged from 40 to 64 cm STL. All three tissues (plasma, RBC, and muscle) had considerable variation in $\delta^{13}C$ (e.g., between -16‰ and -8‰) and $\delta^{15}N$ (e.g., between 3‰ and 13‰) values across species, particularly for mangrove whiprays and cowtail stingrays (Fig. 6.1). All juvenile elasmobranchs exhibit trophic position ~3. Potential prey and baseline organisms also demonstrated a wide range of $\delta^{13}C$ values (e.g., between -30‰ and -1‰), although $\delta^{15}N$ values were mostly between 0‰ and 5‰ (except baitfish: ~6 – 9‰; Fig. 6.2). The ANOVA testing for elasmobranch species differences in $\delta^{13}C$ and $\delta^{15}N$ values showed that all species had different $\delta^{15}N$ values for all

tissues (p<0.001), while $\delta^{13}C$ values were also different, except in plasma and RBC where mangrove whiprays and cowtail stingrays had similar values (p>0.05).

Table 6.1. Stable isotope ($\delta^{13}C$ and $\delta^{15}N$) values of elasmobranch, prey and environmental samples collected from Pioneer Bay and their respective mean ± SE. Tissue types: Muscle (M), Red blood cells (RBC) and Plasma (P).

ID	N (animals)	N (tissue)	Mean ± SE $\delta^{13}C$ ‰	Mean ± SE $\delta^{15}N$ ‰
Mangrove whipray	36	M = 16	-12.13 ± 2.38	9.14 ± 1.94
		P = 30	-11.69 ± 1.96	8.37 ± 2.39
		RBC = 34	-12.30 ± 1.69	8.27 ± 2.02
Cowtail stingray	17	M = 13	-10.16 ± 1.46	6.37 ± 1.82
		P = 15	-10.71 ± 1.17	4.20 ± 1.93
		RBC = 15	-11.64 ± 1.02	4.28 ± 1.59
Blacktip reef sharks	5	P = 5	-13.14 ± 0.97	10.05 ± 0.34
		RBC = 5	-12.16 ± 0.93	10.40 ± 0.24
Giant shovelnose	2	P = 2	-9.36 ± 0.31	5.89 ± 0.05
		RBC = 2	-9.92 ± 0.54	6.60 ± 0.10
Bar-cheek coral trout	11	M = 10	-14.08 ± 1.08	10.85 ± 0.38
		P = 4	-14.14 ± 1.52	10.60 ± 0.51
		RBC = 4	-14.60 ± 1.71	9.97 ± 0.72
Leopard coral grouper	11	M = 11	-14.24 ± 1.54	10.74 ± 0.42
		P = 3	-11.56 ± 1.09	10.28 ± 0.61
		RBC = 3	-13.36 ± 0.60	9.18 ± 0.55
Fusilier	10	M = 10	-17.54 ± 0.12	9.79 ± 0.18
Baitfish	40	M = 40	-13.79 ± 2.53	8.00 ± 0.83
Crab	12		-13.07 ± 3.42	3.91 ± 0.74
Mangrove leaf	17		-27.54 ± 0.89	1.44 ± 1.97
Mangrove root	4		-23.94 ± 6.34	1.42 ± 1.75
Soft coral	20		-13.45 ± 3.74	2.83 ± 0.85
Hard coral	10		-5.25 ± 1.86	4.35 ± 0.53

Algae (inshore)	44	-8.37 ± 3.68	2.22 ± 1.10
Algae (offshore)	3	-22.36 ± 0.36	2.66 ± 0.36
Annelid worm	12	-8.34 ± 2.26	2.79 ± 1.19
Snail	18	-22.33 ± 3.98	2.16 ± 2.13
Prawn	2	-9.21 ± 0.73	4.26 ± 0.08
Plankton	8	-19.87 ± 0.89	4.55 ± 1.21
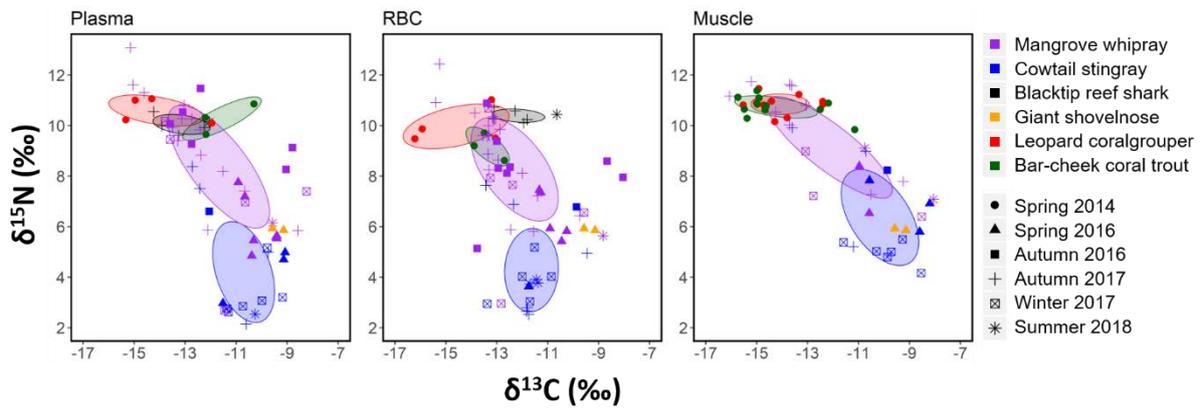
Fig. 6.1. Muscle, red blood cells (RBC) and plasma stable isotope niche breadth ($\delta^{13}C$ and $\delta^{15}N$) of sympatric elasmobranch and teleost predators sampled from in Pioneer Bay. Ellipses represent niche breadth of each species.
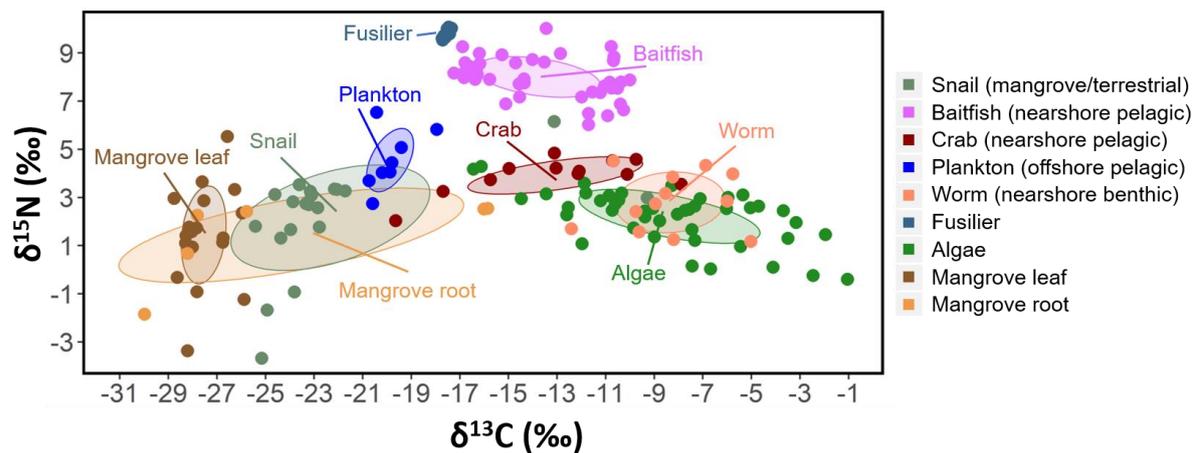
Fig. 6.2. Stable isotope niche breadth ($\delta^{13}C$ and $\delta^{15}N$) of juvenile elasmobranchs' potential prey item and primary producer organisms in Pioneer Bay.

6.3.1 Biological influences on δ^{13}C and trophic position

General linear models showed no significant trends between δ^{13}C and TP values and elasmobranch size ($p > 0.05$), sex ($p > 0.05$) or season ($p > 0.05$) for all three tissues.

6.3.2 Isotopic niche metrics

Isotopic niche size of blacktip reef sharks was smaller than both cowtail stingrays (plasma: $p = 0.002$ (or 99.8% of posterior draws); RBC: $p < 0.001$) and mangrove whiprays (plasma: $p = 0.003$; RBC: $p < 0.001$) in two of the sampled tissues (Table 6.2; Fig. 6.3). Bayesian iterations comparing SEA_C of cowtail stingrays and mangrove whiprays typically resulted in mangrove whiprays having the larger niche size (plasma: 90.8%, RBC: 95.9%, and muscle: 70.1% of posterior draws; Table 6.2).

Table 6.2. Isotopic niche metrics of juvenile elasmobranch species. SEA: standard ellipse area, SEAc: corrected standard ellipse area and TA: convex hull area.

Species	Plasma			Red blood cell			Muscle		
	SEA	SEAc	TA	SEA	SEAc	TA	SEA	SEAc	TA
Blacktip reef shark	0.98	1.31	1.03	0.67	0.84	0.88	-	-	-
Cowtail stingray	6.58	7.04	11.00	5.10	5.46	14.80	7.20	7.85	15.50
Mangrove whipray	9.91	10.29	34.07	8.85	9.09	36.73	7.91	8.48	18.48
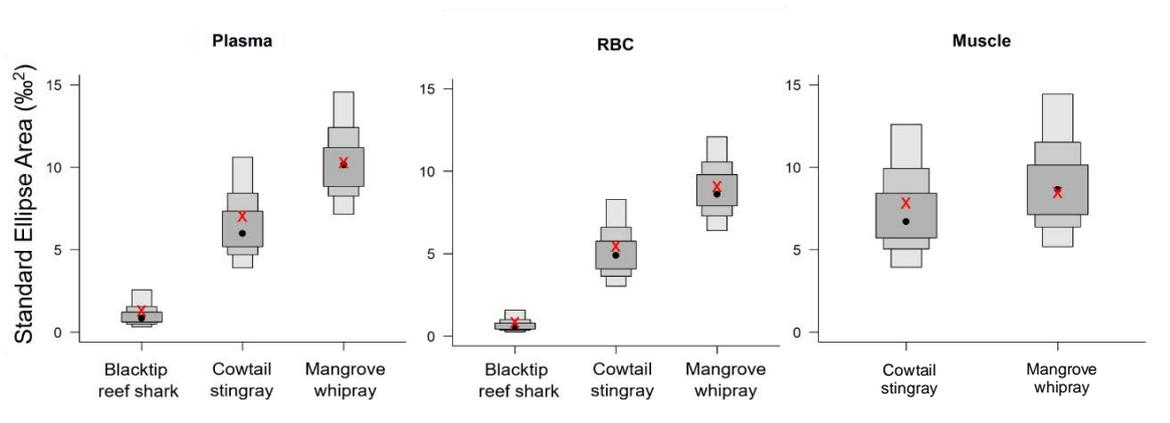
Fig. 6.3. Standard ellipse size (SEA) estimates of juvenile elasmobranchs based on numerous iterations for each tissue types. Red 'x' represents the corrected standard ellipse area (SEAc) estimate.

Table 6.3. Percent (%) overlap and the probability of size differences between Bayesian standard ellipses derived from δ15N and δ13C values of juvenile elasmobranchs. Niche size differences were considered significant when ≥95% of estimates for one species were smaller or larger than estimates for another species. Blacktip reef sharks (BTS), Cowtail stingrays (CWT) and Mangrove whiprays (MWR).

Ellipse size difference	Plasma			Red blood cell			Muscle		
	BTS	**CWT**	**MWR**	**BTS**	**CWT**	**MWR**	**BTS**	**CWT**	**MWR**
BTS		0.998	0.999		1	1			
CWT			0.908			0.958			0.700
Ellipse overlap (area)									
BTS		0	1.030		**<0.001**	**<0.001**			
CWT			**<0.001**			**<0.001**			1.464
Ellipse overlap (% standardized)									
BTS		0	0.097		**<0.001**	**<0.001**			
CWT			**<0.001**			**<0.001**			0.098
95% predictions overlap (area)									
BTS		**<0.001**	7.842		**<0.001**	4.745			
CWT			23.240			17.714			29.719
95% predictions overlap (% standardized)									
BTS		<0.001	0.127		<0.001	0.086			
CWT			0.288			0.255			0.436

Niche overlap calculated using SEA$_C$ (with 40% prediction ellipses) resulted in negligible overlap for all species pairings except for plasma and muscle where the niche space of blacktip reef sharks and mangrove whiprays overlapped ~10% (Table 6.3; Fig 6.1). Niche overlap calculated using 1000 Bayesian posterior distributions (with 95% prediction ellipses) resulted in distributions with higher overlap between cowtail stingrays and mangrove whiprays across all tissues (~20-50%; Fig. 6.4); blacktip reef sharks and mangrove whiprays had overlap ~0-30% (Fig. 6.4), while blacktip reef sharks and cowtail stingrays rarely overlapped (<10%; Fig. 6.4). The difference in overlap between the two methods above is primarily driven by greater variation in cowtail stingray and mangrove whipray isotopes leading to larger values in posterior distributions, especially with the larger prediction ellipses used (i.e., 95% vs 40%). Visual comparisons of elasmobranch and *Plectropomous* spp. isotopic niche space showed that the latter species often had higher $\delta^{13}C$ values, however $\delta^{15}N$ values of the reef fishes were similar to blacktip reef sharks.
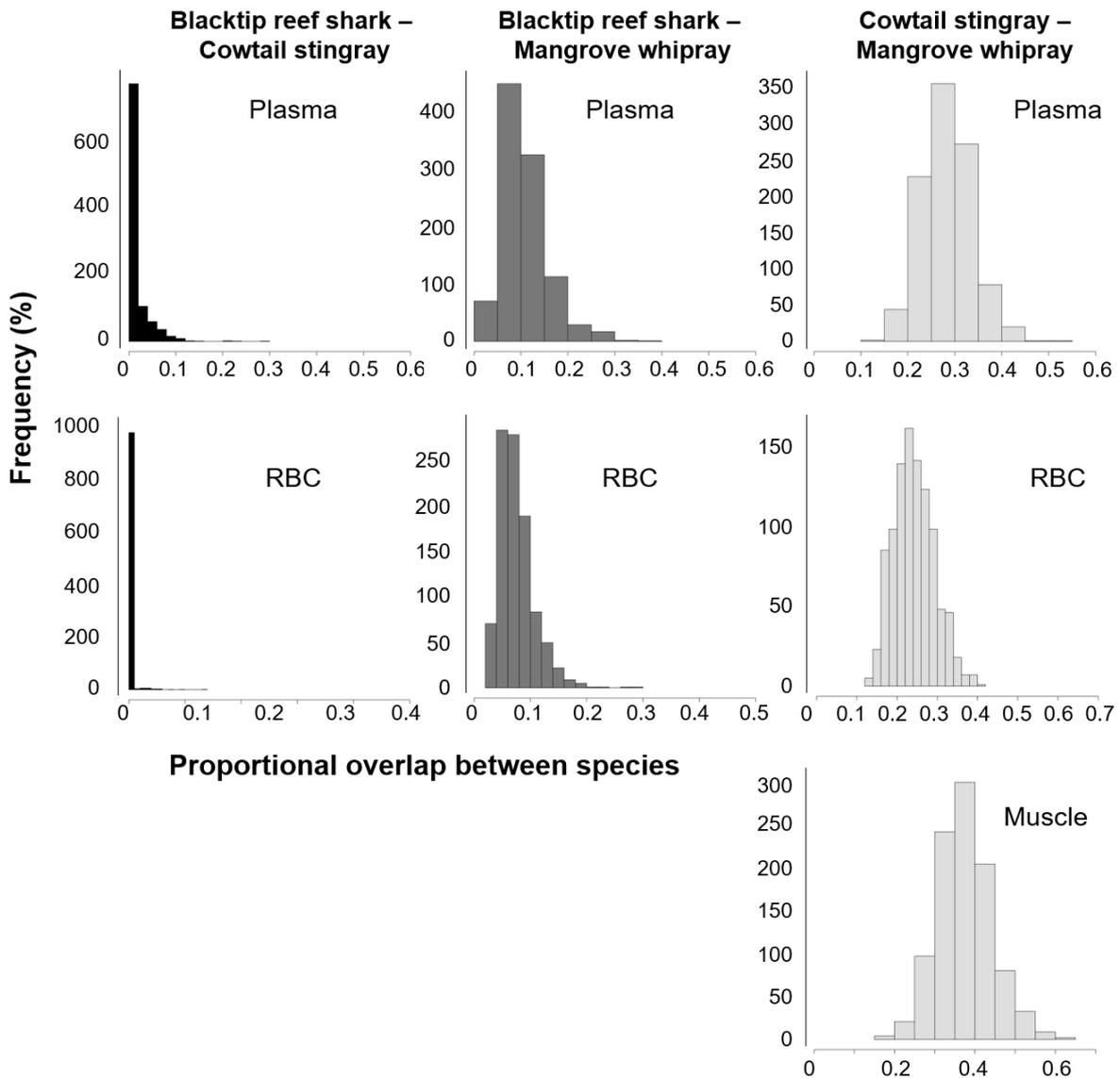
Fig. 6.4. Niche overlap calculated using 1000 Bayesian posterior distributions.

6.3.3 Sources of δ¹³C in ecosystem

Based on prey distributions of corrected 'δ^{13}C-prey ranges' the organisms reflecting the nearshore pelagic (baitfish) and benthic (crab and worm) habitats were dominant among groups sampled (Fig. 6.5). Approximately 10–40% of all baitfish and crab sampled were present in the 'δ^{13}C-prey ranges' of the blacktip reef sharks in plasma and RBC tissues. Worm also appeared to contribute (10% of samples) in RBC. The contribution of prey for cowtail stingrays were relatively similar for all tissues, with worm being incorporated in most individuals with ~10-30% of worm samples being within 'δ^{13}C-prey ranges'. A similar distribution existed for crab contribution to cowtail stingray δ^{13}C values, however it was

more common than worm. Crab was also generally more contributory to mangrove whipray $\delta^{13}C$ values, however worm was still present for at least half of individuals. When baitfish was included as a possible prey item in mangrove whipray diet, two distinct groups were formed. In the first group, baitfish was present in the majority of individuals and had similar proportions within '$\delta^{13}C$-prey ranges' as crab (which contributed to all mangrove whipray individuals). The second group of mangrove whiprays did not appear to have baitfish as a prey item. Snail (representing mangrove/terrestrial carbon input) and plankton (representing offshore pelagic sources) contributed very little or nothing at all to the diet of elasmobranchs (Fig. 6.5) due to $\delta^{13}C$ values smaller than estimated '$\delta^{13}C$-prey ranges'. Therefore, our results suggest that $\delta^{13}C$ source for juvenile elasmobranchs in Pioneer Bay has little direct input from mangroves or offshore sources.
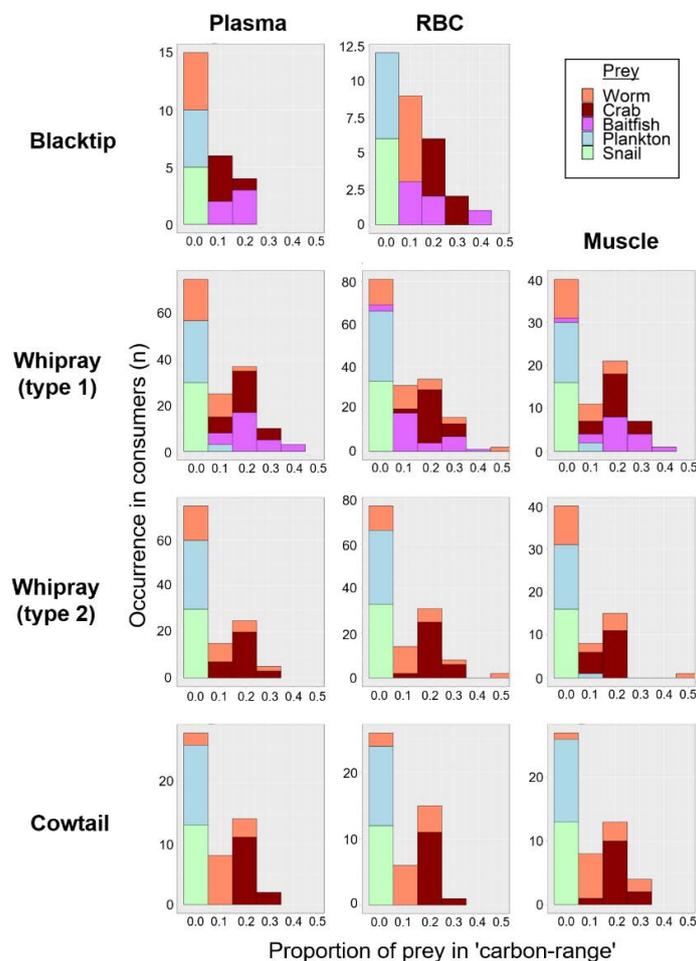
Fig. 6.5. The contribution of prey items for all elasmobranch species. Values were calculated by subtracting diet-tissue discrimination factors (DTDFs) from consumer δ13C, resulting in adjusted consumer δ13C values equivalent to potential prey. Baitfish was included in the

analysis for Whipray (type 1) and not included for Whipray (type 2). Incorporated level of uncertainty was ±1‰.

6.4 Discussion

The results of this study have provided a unique insight into the trophic ecology of elasmobranchs that directly utilise mangrove habitats. Recent studies have shown that a suite of species not only occur in habitats adjacent to mangrove habitats, but in fact utilise mangrove roots as a means of reducing predation directly (Davy *et al.*, 2015; George *et al.*, 2019; Kanno *et al.*, 2019; Martins *et al.*, 2019). Newman *et al.* (2010) and Hussey *et al.* (2017) also found a strong relationship between juvenile lemon shark *Negaprion brevirostris* diet and mangrove-associated prey fauna. However, whether other elasmobranch species also use prey that are supported by the food webs derived from mangrove primary productivity remains uncertain. The results presented here clearly demonstrate that the carbon isotope values of all four elasmobranch species examined had little or no evidence of mangrove derived carbon. This demonstrates that the food webs from which they feed are supported by other sources of primary productivity. This is despite at least one of these species (mangrove whipray) being shown to feed while within the mangrove root habitat (Kanno *et al.*, 2019).

To date, only a limited number of studies have focused on understanding juvenile elasmobranch trophic ecology (Bethea *et al.*, 2004; Kinney *et al.*, 2011; Yick *et al.*, 2011). Results presented here provide strong evidence of isotopic niche distinction between elasmobranchs within a communal nursery. Mangrove whiprays showed the largest SEAc for all tissues, followed by cowtail stingrays and blacktip reef sharks. Therefore, mangrove whiprays in Pioneer Bay had more variability in individual feeding behaviour compared to other elasmobranch species. It is important to point out that the sample size for blacktip reef sharks was small and more samples could increase its ellipse area. Cowtail stingray niche did not overlap with any other species, whereas mangrove whipray and blacktip reef shark niches overlapped at different levels between tissues, and also overlapped with the isotopic niche space of the bentho-pelagic fishes (*Plectropomus* spp.) used in this study for comparison. Some studies have shown significant dietary overlap between sympatric elasmobranch species (e.g. Navarro-González *et al.*, 2012; Treloar *et al.*, 2007). For example, Kinney *et al.* (2011) showed evidence for both partitioning and niche overlap of juvenile elasmobranchs in a communal nursery, but in a much larger system that allowed for spatial partitioning (Heupel *et al.*, 2019).

Niche partitioning between species was shown in this study, but it does not seem to lead to a complete spatial partitioning. In fact, juvenile mangrove whiprays and blacktip reef sharks have been reported feeding inside mangrove patch areas and on the sand flats in Pioneer Bay, whereas juvenile cowtail stingrays were only recorded feeding on sand flats (George *et al.*, 2019; Kanno *et al.*, 2019). Despite the differences in feeding strategy, Chapters 3 and 5 demonstrated mangrove whiprays and cowtail stingrays still share sand and reef flat areas for most of the day – except during high tides when mangrove whiprays seek protection within mangrove patches and cowtails prefer to rest in areas adjacent to mangroves (Kanno *et al.*, 2019, Chapters 3 and 5)

Nearshore pelagic (baitfish) and benthic (crab and worm) items were isotopically consistent with being prey for juvenile elasmobranchs. Some previously published studies also showworms and crustaceans as dominant food sources for stingray species. For example, Yick *et al.* (2011) reported that two coexisting species, the banded stingaree *Urolophus cruciatus* and Tasmanian numbfish *Narcine tasmaniensis*, had distinct dietary patterns – the first preferably preying on crustaceans and the second on polychaetes. Likewise, O'shea *et al.* (2013) found cowtail stingray diet was largely composed of polychaetes. Interestingly, mangrove whiprays from Pioneer Bay divided into two groups with distinct feeding habits – both groups preying upon crabs and annelid worms. However, the cause for this intra-specific differentiation is yet to be explored. Results for blacktip reef shark dietary patterns were also supported by previous studies.

Blacktip reef sharks have been reported driving schools of baitfish into shallow waters in Pioneer Bay (George *et al.*, 2019), reinforcing their reliance on small pelagic prey sources (Papastamatiou *et al.*, 2010). Crustaceans have previously be found to constitute a large part of giant shovelnose diet (Vaudo, 2011; White *et al.*, 2004), but unfortunately the small sample size in this study does not allow further conclusions about their diet. Further diet data would be necessary to draw a thorough picture of species dietary range. Ultimately, none of the juvenile elasmobranch species examined here appear to prey upon snails – an abundant prey source in the mangrove areas of Pioneer Bay. This could be related to their small jaws and that are not yet powerful enough to break open the hard shells of these gastropods.

In general, batoids exhibit trophic position between 3.4 and 4 (Bornatowski *et al.* 2014, Navia *et al.*, 2017). In this study, the trophic position of elasmobranchs was ~3. In fact, juvenile elasmobranch species in Pioneer Bay consume prey types from a range of trophic levels. In

addition, juveniles play important roles as potential prey for larger elasmobranchs that often visit the area. Although larger predators have not been sampled for comparison of trophic levels, the role of juvenile elasmobranchs as mesopredators has been demonstrated in the literature (Barría *et al.*, 2015; Ebert, 2002) and was confirmed in this study site through observation of predation attempts during data collection (A Martins personal observation).

Influences of size, sex or seasonality on relative trophic position were not observed. Studies have found trends in δ15N and δ13C between body size for elasmobranchs (Borrell *et al.*, 2011) – with an increase in TP for larger individuals and species. However, these trends appear to have less influence for immature individuals. This might be due to the small jaw size of juveniles reducing their ability to prey upon larger, higher trophic level items (Amariles *et al.*, 2017). Although sampling for elasmobranch species accounted for different seasons, sampling for prey and environment did not. In this way, variations in prey availability, distribution and composition may not reflect the distinctions that could possibly occur seasonally. More information is required for a complete picture of prey abundance and diversity year-round in Pioneer Bay to fully address seasonality effects on juvenile elasmobranchs trophic ecology.

Dietary patterns of the studied species are likely to change as juveniles grow. Growth rates are known to play an important role in determining tissue-specific isotope turnover, especially for juveniles (Hussey *et al.*, 2014). Macneil *et al.* (2006) reported that 65%–75% of muscle δ15N turnover in *Potamotrygon motoro* was accounted for by growth. It is important to point out that, depending on growth rates, the necessary time to achieve a near steady-state isotope value can vary drastically (Macneil *et al.*, 2006). Using baseline turnover rates calculated by Macneil *et al.* (2006) and Logan and Lutcavage (2010) for relatively small and growing batoids and sharks, respectively, shifts in juvenile elasmobranch diets are expected to be reflected in plasma and red blood cells samples within two or three months, while muscle samples would reveal a longer term, likely annual, isotopic signature. Considering all individuals sampled in this study were newborn or young of the year, it is likely that juvenile elasmobranchs δ15N values at Pioneer Bay may reflect at least some parental influence – especially in muscle samples (Olin *et al.*, 2011).

New born and young of the year isotopic values are affected by the mothers isotopic signature through gestation (when matrotrophy is present), postparturition maternal reserves and/or placental connectivity (Jenkins *et al.*, 2001; Mcmeans *et al.*, 2009). Maternal isotopic

signature can influence neonate and young of the year isotope signatures, and hence inferences about their diet, resulting in a complex combination of δ15N and δ13C values (Ann Pilgrim, 2007; Olin *et al.*, 2011). For a real picture of juvenile elasmobranch trophic ecology, the possibility of mixing signatures must be addressed through the analysis of multiple tissues with different turnover rates. In this study, δ15N and δ13C were similar between tissues, with small variation in ellipse size. However, accounting for maternal influence by carefully analysing multiple tissues was important to provide an accurate representation of juvenile elasmobranch dietary patterns over a broader time-period in Pioneer Bay. The lack of change in relative niches and niche overlap between tissues suggest juvenile elasmobranch diet do not vary temporally or only varied slightly.

6.5 Conclusion

This study highlighted the relative trophic relationships of juvenile elasmobranch species and their trophic position in Pioneer Bay, a communal nursery area. The results showed evidence of niche partitioning between species with differences in feeding strategy. Despite using mangrove habitats extensively, juvenile elasmobranchs did not rely on mangrove derived food webs, instead consuming prey that derive carbon from benthic algae or plankton. Nearshore pelagic and benthic items – such as crabs, annelid worms and small baitfishes – were isotopically consistent with being prey, although it is possible that additional prey were not sampled for isotopes. The influences of size, sex or seasonality on relative trophic position and carbon isotope values were not observed. The use of multiple tissues was essential to accurately reveal dietary patterns and trophic positions of juvenile elasmobranchs and account for any maternal influence on their isotopic signatures. Thus, results presented here could help better determine trophic structure and ecological connections in coastal communal nursery areas.

Chapter 7: General Discussion

Given the increasing threats to Dasyatid rays and the limited information available that hinders conservation efforts, this PhD thesis aimed to extend our understanding of juvenile stingray movement patterns and trophic relationships in coral reef habitats. Our telemetry results revealed stingray activity patterns and space use are strongly influenced by tidal stage and diel period. Both studied stingray species showed preference for sand flats and sand patches within the reef flats, and mangrove whiprays appear to more commonly use these areas during the day. These areas are thought to favor camouflage by allowing burying and also offer food resources. The trophic ecology results corroborate the findings by providing evidence of nearshore pelagic and benthic prey items – such as crabs, annelid worms and small baitfishes – as the main part of juvenile elasmobranch diets within the Pioneer Bay nursery. However, these preys are part of food webs that derive their carbon from algae and plankton rather than mangroves.

7.1 Movement Patterns and habitat use of juvenile stingrays

The complementary use of active acoustic and towed-float GPS telemetry allowed a deeper evaluation than previous studies on aspects of juvenile stingray spatial ecology that are generally poorly understood. Active acoustic telemetry has well known limitations regarding, for example, duration of tracks and influence from human presence (Chapter 3). However, its employment in Pioneer Bay was essential to better understand juvenile stingrays' behaviour – identifying feeding and resting areas, predator avoidance strategies and their relationship with tidal cycles. Towed-float GPS telemetry allowed the collection of accurate spatial data, and reduced human influence and enabling night tracks. However, information acquired from active acoustic telemetry was indispensable for interpretation of data collected with float tags. Thus, the combination of methods was a key aspect to expand our knowledge on mangrove whipray movements and describe, for the first time, cowtail stingrays' activity patterns in Pioneer Bay.

Both species remained in Pioneer Bay during tracking periods, with no recorded movements of individuals leaving the area (Chapter 3, 4 and 5). In fact, other studies have shown that batoid populations often restrict their distribution to relatively small areas (less than 5 km^2) for long periods (Campbell *et al.*, 2012; Cartamil *et al.*, 2003; Elston, 2016). Based on our results and continued monitoring of tagged stingrays (numbered spiracle ID), juvenile

stingrays appear to remain exclusively in Pioneer Bay for a minimum period of six months, and possibly much longer. This finding indicates that Pioneer Bay must therefore provide ample food throughout the year and between seasons, allowing juvenile stingrays to restrict their movements to the protected shallow areas, which provides advantages through reducing the risk of predation (Davy *et al.*, 2015). However, quantifying the longer-term patterns of movement and site fidelity would be necessary to fully understand juvenile stingray species use of this nursery ground.

Mangrove whiprays and cowtail stingrays mostly used sand flat areas. These areas provided access to food resources and soft-bottom for burying. The reef crest was used by both species as refuges during low tides. This habitat provided sand patches for burying and resting physically protected by the surrounding reef and stands of macroalgae. On a few occasions, cowtail stingrays also fed in the reef crest. Mangroves, when available during incoming and high tides, were mostly used by mangrove whiprays. Juveniles were often seen resting under the complex mangrove root system. Unfortunately, juvenile stingray behaviour within mangrove areas could not be fully addressed by telemetry methods because towed floats tangled in mangrove roots and acoustic signals were obstructed. Telemetry results were therefore combined with stable isotope analysis and available literature to provide a fuller understanding of behaviour at this tidal stage. Thus, mangrove whiprays mostly use mangroves for protection (Chapter 6) due to the exclusion of large predators, and have been recorded feeding in in these areas (Kanno *et al.*, 2019)

The dynamics of juvenile stingrays in Pioneer Bay was strongly driven by the tidal cycle (Chapter 3 and 5). Stingray movements were guided by both tidal current direction and height variations. Moving in the direction of tidal flow, as both species of stingrays studied did, is a common strategy for small-bodied elasmobranchs (e.g. Brinton & Curran, 2017), reducing swimming energy costs and allowing the use of shallow waters. Juvenile stingrays showed different rates of movement, with mangrove whiprays generally moving at a faster pace than cowtail stingrays, but with similar movement patterns. Both species moved further and used straighter paths during outgoing and incoming tides, whereas they adopted random and slower movements at low and high tide. This pattern corroborates Davy *et al.* (2015) and George *et al.* (2019) findings for juvenile mangrove whiprays and blacktip reef sharks, respectively, in the same area. Juvenile elasmobranchs are thought to adopt this movement strategy to keep themselves in shallow water, reducing exposure to predation while also avoid stranding, especially during falling tides. Differences in ROM and linearity between sexes

were not observed for any of the study species. As observed by Dale *et al.* (2011), sexual segregation might be absent in juveniles stingrays. Finally, contrary to many studies that show elasmobranch species have higher activity during night periods (e.g. Brinton & Curran, 2017; Cartamil *et al.*, 2003; Corcoran *et al.*, 2013; Garrone Neto & Uieda, 2012), my results highlight mangrove whiprays' higher movement rates during diurnal periods. The reasons for this are still to be fully understood, but this species' optimal temperature range is 30 °C to 36 °C (Higgins, 2018), therefore drops in temperature overnight could hinder juvenile mangrove whiprays ability to move at the same rate as during the day.

7.2 Trophic ecology of juvenile elasmobranchs in a communal nursery

Body size and sex had no influence on δ15N and δ13C values from the stable isotope analysis. Isotopic values are known to increase with size in elasmobranchs (Hussey *et al.*, 2011), however the increase is often related to changes in dietary patterns (Amariles *et al.*, 2017). In fact, juvenile elasmobranchs in Pioneer Bay varied in body size (25 cm to 65 cm DW or STL), however it is likely that mouth gape and manoeuvrability of individuals within the sampled size range changed relatively small amounts and so did not allow selection of prey items across a broad range of trophic levels. The addition of samples from adults of these species would have likely provided evidence of changing isotope patterns and hence trophic ecology (Papastamatiou *et al.*, 2010). In addition, juvenile elasmobranchs restricted their movements to within Pioneer Bay, possibly to avoid higher predation risk in open and deeper areas, which did not allow broader search for higher energetic value prey items (Chapters 3 and 5). Sexually immature individuals, with similar movement patterns as shown in Chapter 3 and 5, were also expected to show similar isotopic signatures. Unfortunately, prey availability, distribution and composition year-round in Pioneer Bay were not examined in this study. Therefore, a broad picture of the influence of seasonality on this juvenile elasmobranch communal nursery requires further investigation.

Mangrove whiprays and cowtail stingrays displayed distinct isotopic niches (Chapter 6). Thus, although they are similar in size and morphology and generally occur within the same habitats, the stable isotope data indicated that they have very different diets. This may have occurred because cowtail stingrays feed exclusively in sand flats, whereas mangrove whiprays were recorded feeding both on sand flats and within mangroves (Kanno *et al.*, 2019) – likely resulting in higher variability in individual feeding and therefore niche differentiation. Blacktip reef sharks also feed in both of these habitats (George *et al.*, 2019),

but the small sample size possibly restricted its ellipse area, and hence comparison to the stingrays. Despite niche distinction between stingrays shown by SIA, complete spatial partitioning was not observed. All elasmobranch species appear to be able to use similar areas for most of the day, as shown in Chapters 3 and 5. Considering the small area of Pioneer Bay, more feeding niche overlap was expected. The results thus demonstrated niche overlap between sympatric elasmobranch species at very fine spatial scale, while previous research has shown that it occurs even in systems much larger than Pioneer Bay (Kinney *et al.*, 2011; Navarro-González *et al.*, 2012; Treloar *et al.*, 2007).

The SIA data from Pioneer Bay suggests that juvenile stingrays have a preference for annelid worms and crustaceans, corroborating results from Bornatowski *et al.* (2014), Elston *et al.* (2017); O'shea *et al.* (2013); and Yick *et al.* (2011).These results are also supported by telemetry data that shows the common use of sand flats areas for both stingray species (Chapters 3 and 5) – where annelid worms and small crustaceans can be easily found.. Blacktip reef shark's dependence on small pelagic prey sources, such as baitfish, was reinforced in this study (George *et al.*, 2019; Papastamatiou *et al.*, 2010). All elasmobranchs selected prey items that were part of food webs that derived carbon from algal and planktonic sources, with no or little influence from mangroves or offshore sources. b

7.3 Ecological roles played by juvenile stingrays in coral reef habitats

Results from telemetry (Chapter 3, 4 and 5) and Stable Isotope Analysis (Chapter 6) of juvenile stingrays within the Pioneer Bay nursery highlight the roles they play in coral reef habitats and boosts our understanding on the importance of coral reefs to stingray populations. Juvenile stingrays rely on the Pioneer Bay system and are likely to have substantial impacts in shallow sand and reef flat, and mangrove, habitats – their primary and secondary areas of use. As previously identified, stingrays are thought to play three major ecological roles in aquatic ecosystems (Martins *et al.*, 2018, Chapter 2). This PhD confirmed at least two of these roles within the Pioneer Bay nursery area.

First, animal movement is a well-known source of habitat connectivity, linking trophic webs and transporting nutrients and energy (Sheaves, 2005, 2009). Acoustic and GPS telemetry results showed juvenile stingrays use most areas of Pioneer Bay – swimming across different habitats on a daily basis (Chapter 3, 4 and 5). Sand flats were consistently used for feeding, while the reef flats and mangroves were often visited during low and high tides, respectively, to avoid encounters with larger predators. Although in lower frequency, stingrays were

visually recorded feeding in reef flats (Chapter 4) and mangroves (Kanno *et al.*, 2019). Other studies have shown batoid's short term movements across different habitat types and a diel basis (Adkins *et al.*, 2016; Cartamil *et al.*, 2003; Davy *et al.*, 2015; Matern *et al.*, 2000; Silliman & Gruber, 1999). In this study, juvenile stingrays' diel migrations are thought to biologically link adjacent habitat types in Pioneer Bay - helping to connect mangrove, sand flat, and inner and outer reef habitats.

Second, SIA results showed juvenile stingrays occupy had a trophic position of ~3) in the Pioneer Bay communal nursery – potentially applying top down control over invertebrate populations through predation. As shown in Chapter 6, crabs and annelid worms are likely to have populations regulated by mangrove whiprays and cowtail stingrays. In addition, the frequent presence of large sharks in the bay and the predation attempts recorded during the study period, also reinforce juvenile stingrays' role as potential prey items. Thus, similar to finding of other studies (e.g. Navia *et al.*, 2007), juvenile stingrays in Pioneer Bay help to connect high level predators and lower trophic level organisms in the food web, playing an important role as mesopredators in this coral reef system. The absence of stingrays from this system could significantly affect the structure and function of the food web, forcing the remaining components of the web to readjust and adapt to a new state of organisation (Britten *et al.*, 2014; Navia *et al.*, 2017). Such reorganisation would potential have negative consequences for both their prey (lack of bioturbation and hence changes in sediment oxygenation) and their predators that might result in a reduction in populations.

Lastly, stingrays were frequently spotted suspending sediments and forming feeding pits across the sand flats. Given the abundance of stingrays and the small area of Pioneer Bay (0.8 km^2), juvenile stingrays feeding activities could in fact influence benthic prey communities and nutrient recycling. However, in this study, the physical and biological impact of stingrays' bioturbation were not fully investigated due to the lack of data on infauna and meiofauna abundance and distribution.

7.4 Future work and conservation

This PhD study has provided a unique insight into the spatial and trophic ecology of juvenile elasmobranchs in a coral reef flat environment. A better understanding of these topics is important to comprehend species relationships with ecosystem dynamics and develop effective strategies of management and conservation. Therefore, results presented here provide useful information to enhance our limited knowledge of movement patterns and

habitat use of juvenile stingray populations, better determine juvenile elasmobranch trophic structure and ecological connections in communal nurseries, and support estimates of anthropogenic impacts upon the juvenile stingray species and their essential habitats.

Healthy sand and reef flat areas are essential to support juvenile stingrays in Pioneer Bay (and likely many other places) – providing sufficient prey items and soft substrates for burying and/or hiding. Mangrove areas did not provide the source of primary production to the elasmobranchs in this communal nursery as initially suspected, however these areas were clearly important as physical barriers against large predator for juvenile mangrove whiprays and also for blacktip reef sharks, as demonstrated by George *et al.* (2019). The removal or significant degradation of these habitats could cause severe disturbances on the communal nursery structure (Adkins *et al.*, 2016) and lead to increases in juvenile mortality – directly affecting recruitment rates and therefore elasmobranch populations.

In light of the increasing threat faced by stingrays, especially in nursery areas, future research could focus on a number of aspects of juvenile stingray biology and ecology:

(1) Life history. Basic biological features of the study stingray species remain poorly understood, such as size at maturity, and age and growth. Growth rates, for example, could help unveil juvenile stingray energy intake requirements and consumption rates. Basic biological data could also help to predict how long juvenile stingray may need to stay in nursery areas. Thus, understanding life history strategies of these species is an important step to fully comprehend their habitat requirements.

(2) Abiotic drivers of movement and habitat selection. Aspects such as temperature, salinity, and oxygen levels can influence juvenile stingray movement patterns. Further investigation of these factors could help to predict juvenile stingray ecological responses to anthropogenic pressures, such as habitat degradation and climate change. In addition, experimental work could be applied to look at juvenile stingrays physiological responses to these stressors.

(3) Biotic drivers of movement and ecological effects. Predation risk was discussed in this study, but not fully investigated. Other biotic aspects, such as intra and inter-specific competition, and prey abundance and distribution could be further investigated to better understand juvenile stingray movement patterns and trophic

ecology. Furthermore, information on prey availability and composition at the location of feeding pits, and feeding pit size and persistence could help to extend our limited knowledge about how juvenile stingray bioturbation effects coastal habitats, and their effects on prey populations.

(4) Seasonal and ontogenetic shifts. Movement patterns and feeding habitats of stingrays are known to change over time. Juvenile stingrays certainly leave Pioneer Bay, but where they go and if they return at some point is unknown. Looking at seasonal migrations and ontogenetic shifts could help to better understand stingray ecology in different life stages.

(5) Population genetics. Studies could be applied to unveil genetic variation in juvenile elasmobranch populations within Pioneer Bay and its surroundings. These results could help to establish stingray site fidelity and/or natal philopatry to Pioneer Bay.

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