Investigation of coral trout (*Plectropomus* spp.) movement patterns and resource use: a multidisciplinary approach using acoustic telemetry and dietary indicators

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To the best of my knowledge and belief, the thesis contains no material previously published by any other person except where due acknowledgment has been made. Permission from external copyright holders and collaborators has been obtained when necessary.

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Abstract

Understanding how co-occurring species within comparable trophic guilds (sympatry) partition resources provides fundamental information about their ecological roles within an ecosystem. Despite morphological and biological similarities, resources may be selected and exploited independently, leading to alternative interactions and influences within the ecosystem. Studying movement and dietary patterns directly relates to an animal's resource use, and is a valuable approach to characterise preferred prey and habitat within and between sympatric species. Expanding knowledge of resource use is essential to address how animals are affected by, and how they might respond to, an increasingly variable environment, and is necessary to implement ecosystem-based management practices.

Coral trout or coralgrouper (*Plectropomus* spp.) are iconic and economically significant mesopredatory reef fishes within Australia's Great Barrier Reef (GBR) and throughout the Indo-Pacific region. Despite the importance of *Plectropomus* spp. in the Queensland Coral Reef Fin Fish Fishery, investigations focussed on their ecology are surprisingly limited. Much of the behavioural-based research has been conducted in scenarios of captivity, is biased by confounding sampling limitations, or only provides short-term, data-poor perspectives. Consequently, interpretations of findings are often applicable only to certain periods or locations, or are only based on patterns from a small number of individuals. This hinders the ability of managers to evaluate how fishing pressure, protection initiatives, and environmental fluctuations or disturbances might impact populations. Furthermore, research is overwhelmingly directed at *P. leopardus* (or grouped as *Plectropomus* spp.) which forms the majority of commercial catches on the GBR. Nevertheless, other species such as *P. maculatus* and *P. laevis* are readily captured by both recreational and commercial sectors, but their resource selection patterns and interactions with *P. leopardus* are unknown.

The research in this thesis employed two methodological approaches – passive acoustic telemetry and stable isotope analysis, to study movement and dietary patterns, respectively, in three exploited species of coral trout – *P. leopardus*, *P. maculatus*, and *P. laevis*. The research was conducted at three primary locations – Orpheus Island, four mid-shelf/offshore reefs in the Townsville region (Townsville reefs), and the Marine and Aquaculture Research Facilities Unit (MARFU) at James Cook University. Samples and data were collected over the course of three years (2013-2015) providing extensive ecological and behavioural information from more than 300 individual *Plectropomus*. The overall aim of this research was to quantify, qualify, and compare long-term movement and dietary patterns of sympatric *Plectropomus* spp.

By using multiple approaches, this thesis showed that broad resource selection trends differ between sympatric species, but interestingly, the way they differ is unique to each species pairing. At the Townsville reefs, *P. laevis* moved greater distances and had increased variability in depth use compared to *P. leopardus*. Movement patterns were correlated with distinct dietary niches between species, particularly when colour phases of *P. laevis* (footballer and blue-spot) were separated. The limited isotopic niche overlap between species was not correlated with fish size, indicating alternate prey selection, feeding styles, or energetic requirements engrained at a species level. Based on results from an aquarium-based stable isotope feeding trial, the trophic position of *P. leopardus* in the wild varied little between sampling locations and time periods. Similarly, the isotopic niches between species remained constant for several tissues (a proxy to feeding timeline) and at several reefs, suggesting feeding pressures exerted by each species is consistent within the region. Consequently, it is hypothesised that both *P. laevis* and *P. leopardus* will respond to environmental or human-induced disturbances in similar ways within and across compatible reefs. At Orpheus Island, *P. maculatus* shared the same home range size as *P. leopardus*, however *P. maculatus* remained deeper in the water column throughout daily and monthly periods. These spatial patterns were correlated to overlapping isotopic niches - or similar prey selection. These trends indicate a high potential for competition that may be mediated by spatial or habitat partitioning.

Overall, this research highlights the need for greater species-specific consideration relative to conservation and management initiatives since *Plectropomus* spp. readily demonstrate distinct behavioural patterns, and will likely respond to disturbances differently. Without fundamental knowledge of how co-occurring species select and partition resources, their interactions and impacts throughout the reef ecosystem remain unknown. Not only did this thesis provide new information about each species, it produced preliminary evidence that interactions between species may shape how resources are utilised on coral reefs.

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Chapter 1

General Introduction

The co-occurrence of closely related and morphologically similar species within the same environment (sympatric species) has important ecological implications within coral reef ecosystems. Due to similar but often isolated evolutionary lineages (e.g., allopatric speciation; see Rocha and Bowen (2008) for review of speciation in coral reef fishes), sympatric species commonly share biological (e.g., growth/size, physiology, or reproduction) and behavioural (e.g., diet, habitat, or defence) attributes (e.g., Randall et al.1997). As a result, sympatric species commonly exploit similar resources (e.g., food and habitat) within the same environment (Sale 1977). Depending on the abundance and availability of resources, competition may occur. The potential outcomes are numerous, but limiting resources may lead to competitive interactions resulting in alternate resource pools (or niches) being targeted (Connell 1980; Munday et al. 2004). These interactions can have multiplicative and cascading effects throughout the ecosystem, ultimately influencing community structure and the functional role of organisms (Finke and Denno 2005; Harmon et al. 2009). The timelines for these events are context-dependent and do not strictly occur within evolutionary temporal scales. Therefore, knowing how sympatric species partition resources provides fundamental information relating to the specificity of their ecological roles and the impact agonistic interactions could have throughout an ecosystem. Additionally, understanding how prey and habitat are selected by sympatric species provides a baseline for determining responses to human or environmental stressors. From this perspective, studying movement and dietary patterns is particularly valuable because they are directly linked to resource use.

Studying the movements of animals can reveal an array of life history and behavioural traits related to resource use at different spatial and temporal scales. For example, in the marine environment movement patterns have provided insight into habitat use (Udyawer et al. 2016) and connectivity (Lédée et al. 2015), ontogeny (Knip et al. 2011), diel behaviour patterns (Espinoza et al. 2015a), mortality rates (Heupel and Simpfendorfer 2011), home ranges (Currey et al. 2014), environmental impacts (Schlaff et al. 2014), reproductive behaviour (Waldie et al. 2016), and conservation and management (Chin et al. 2012), among others. There are a variety of ways to study animal movement in the marine environment, mainly underwater visual census (UVC), mark-recapture, satellite telemetry, and acoustic telemetry (active and passive). Each approach has inherent limitations which bias data collection and

interpretation. For example, UVC only covers a relatively small area, and may not represent reef-wide trends, observer presence may alter animal behaviour, and surveys are often depth-limited (Davies 1996; Zeller 1997; Thompson and Mapstone 2002; Miller et al. 2012). Mark-recapture has low recapture rates (e.g., <10%, Davies 1996; Sumpton et al. 2008) and short retention times of markings (e.g., freeze-brands visible for 140 days, Samoilys 1997; 38% of t-bar anchor tags lost in first year, Davies 1996). Satellite telemetry provides large-scale tracking data but fine-scale movement patterns are difficult to discern (Eckert and Steward 2001; Kuhn et al. 2009), and tagging is typically limited to larger animals (e.g., seals, sharks, and whales). Active acoustic telemetry provides fine-scale movement data, however it is sampling intensive and is typically only capable of tracking tagged individuals one at a time (Zeller 1997; Zeller 2002). Passive acoustic telemetry samples tagged individuals located within range of moored receivers (fixed positions) (Heupel et al. 2006). Individuals can be sampled simultaneously and long-term when located within detection ranges of receivers. As a result, detections are collected 'off-effort' and thus increase the amount data that can be obtained from individuals. Additionally, the resolution of data can be monitored and receiver numbers and positioning adjusted depending on research questions. The limitation of this approach is geographic specificity of receiver locations. If an individual travels beyond a receiver's detection range, data collection declines or ceases. Nevertheless, this methodology is a considerable improvement over techniques that collect fragmented and depauperate data points, especially for animals that are resident to specific areas.

Studying the diet of organisms also provides a wide range of temporal and spatial information associated with resource use because food acquisition is one of the main biological drivers of behaviour. Dietary knowledge of animals living on coral reefs provides insight into ontogeny (Artero et al. 2015), foraging behaviour (Kramer et al. 2016), prey behaviour (Lönnstedt et al. 2012), movement patterns (McMahon et al. 2012), ecological specialisation (Brandl et al. 2015), habitat selection (Brooker et al. 2013), trophic structure (Heithaus et al. 2013), and management concerns (Nash et al. 2013), among others. Prey selection by reef organisms has traditionally been investigated using visual observations of feeding in both captivity and the wild. This method is advantageous for resident species because prey types and energy intake can be quantified. However, it is sampling intensive and limited to restricted periods (e.g., daytime) or depths. Alternatively, dietary patterns are commonly determined by visually identifying prey remains from gut contents. Again, short-term (hours-days) prey selection patterns can be interpreted, however high digestion rates limit identification, the occurrence of empty stomachs is often frequent, digestion rates differ for different prey types, and lethal sampling is generally necessary (St. John 1995). Stable isotope analysis is an increasingly used approach in ecology to study diet and food web dynamics because stable isotopes reflect tissue

assimilation from prey and are not hindered by many biases associated with stomach content analysis (Hobson et al. 1996). Carbon stable isotopes ($\delta^{13}C$) are used because they reflect sources of primary production in the diet (e.g., <1‰ enrichment between predator and prey; Michener and Schell 1994; Sweeting et al. 2007a), while nitrogen stable isotopes ($\delta^{15}N$) estimate trophic level (e.g., ~3.4‰ enrichment between predator and prey; Minigawa and Wada 1984; Sweeting et al. 2007b). Since the isotopic turnover (i.e., the time tissue of prey takes to be integrated into consumer tissue) is influenced by metabolic activity, temporal trends can be elucidated by sampling different tissues (Tieszen et al. 1983; Kurle 2002). For example, the turnover of stable isotopes is higher in the metabolically more active liver and represent more recent food intake compared to muscle due to tissue-specific fractionation (Hobson and Clark 1992). Therefore, stable isotopes provide information relating to temporal dietary regimes, as well as identify the resource pools (e.g., algal vs. plankton) responsible for driving energy pathways.

Both passive acoustic telemetry and stable isotope analysis retain numerous advantages to inform about resource use of marine organisms, especially in comparison with other approaches. However, independently, they are still limited in a few ways. For example, passive acoustic telemetry is a 'blind' observation tool describing animal movement. Without on-site observations, environmental sensors, or supplementary techniques, the drivers of movement and the mechanisms leading to movement decisions remain correlative and elusive. Similarly, stable isotopes provide broad-scaled dietary assimilation patterns and sources of prey selection, but do not strictly identify the characteristics relating to prey acquisition (e.g., diel patterns, depth preferences, and specific foraging behaviour). The application of both passive acoustic telemetry and stable isotope analysis in conjunction greatly reduces these limitations because dietary patterns help to explain movement patterns and vice versa (e.g., Cunjak et al. 2005; Papastamatiou et al. 2010; Speed et al. 2012; Matich and Heithaus 2014; Carlisle et al. 2015).

In addition to ecological implications associated with interactions between sympatric species, there are potential management concerns, particularly in multispecies fisheries. Due to similar morphology and distribution, sympatric species are often grouped together in stock assessments and research (Heupel et al. 2010). However, despite similarities, life history traits and population demographics are often distinct and removal may affect species differently (Currey et al. 2013). Without species-specific information on catch history and population dynamics, conservation tools such as size and catch limits or fishery closures (e.g., seasonal/spawning or marine protected areas) may be biased to more abundant or better studied species. By studying dietary and movement patterns of sympatric species, a greater

understanding of temporal resource and distribution overlap (or segregation) can be gained, which can mediate vulnerabilities of each population or species.

A group of sympatric species that have particular ecological and management importance within the Great Barrier Reef Marine Park (GBRMP) are coral trout (or coralgrouper), mainly *Plectropomus leopardus*, *P. maculatus*, and *P. laevis*. Coral trout are the primary target species for the recreational and commercial sectors of the Queensland Coral Reef Fin Fish Fishery, generating ~$30 million p.a. in Australia, and more abroad in the live reef food fish trade (Sadovy de Mitcheson et al. 2013). In many parts of their distribution, *Plectropomus* spp. are locally depleted due to over-exploitation (Scales et al. 2007; Sadovy de Mitcheson et al. 2013). Due to high demand and consequences of increasingly variable environmental conditions (e.g., extreme weather events, habitat degradation, prey availability, and temperature-driven distribution shifts), there is concern that populations within the GBRMP could be negatively impacted (Leigh et al. 2014; Johansen et al. 2015). The implications of population declines are far-reaching because coral trout are a ubiquitous and abundant mesopredator on reefs throughout the GBRMP. Several studies have highlighted that the presence, density, and behaviour of coral trout (particularly *P. leopardus*) are intrinsically linked to the composition and abundance of organisms at lower trophic levels in the food web (Graham et al. 2003; Rizzari et al. 2014; Boaden and Kingsford 2015; Palacios et al. 2015). At a broad scale, species of coral trout inhabit different geographical regions on reefs but do co-occur (Williams and Russ 1994); consequently, behavioural traits (e.g., movement and diet) likely differ between species. Surprisingly, the extent to which sympatric coral trout compete for or share resources (e.g., diet, habitat, and distribution) is unknown. Indeed, comprehensive evaluations relating to their ecology primarily exists for only *P. leopardus*. Further still, species of coral trout are commonly grouped together for research (e.g., St. John 1995; Graham et al. 2003; Williamson et al. 2004) and stock or strategic assessments (e.g., Leigh et al. 2014; GBRMPA 2014) despite evident distributional, demographic, and life history discrepancies. Without species-specific biological and ecological information, directed managements goals will not necessarily address issues pertinent to all species. Clearly, a greater knowledge of species-specific resource use is needed to effectively manage the coral trout complex long-term.

With the above in mind, the overall aims of this research were to:

1. Quantify and qualify movement and dietary patterns of three coral trout species (*P. leopardus*, *P. maculatus*, and *P. laevis*) at different temporal scales.
2. Compare movement and dietary patterns between sympatric species of coral trout (i.e., *P. leopardus* and *P. maculatus* – inshore; *P. leopardus* and *P. laevis* – mid-shelf/offshore).

3. Explore the potential ecological and management implications of movement and/or dietary differences between species.

Each data chapter within this thesis addresses certain aspects of the described aims, but can be broadly categorised as either movement (passive acoustic telemetry) or dietary (stable isotope analysis) investigations. As a result, chapters have been modified to minimise redundant material, particularly with relation to methodology. Each data chapter represents a manuscript either published, in review, or to be submitted.

First, **Chapter two** provides a comprehensive overview of the animals studied for this thesis, including a synthesis of the biological and ecological information known to date. This chapter also describes the main sampling approaches used throughout the thesis. **Chapter three** described an aquarium feeding trial conducted to better understand the assimilation of stable isotopes from prey to tissues of *P. leopardus*. This chapter addressed two major limitations associated with stable isotope analysis: unknown tissue-specific turnover rates and discrimination factors (i.e., the isotopic enrichment value between consumer and prey). **Chapter four** applies the stable isotope information gathered in **Chapter three**, to investigate spatial and temporal dietary patterns within *P. leopardus*, and between *P. leopardus* and *P. laevis* at four different mid-shelf/offshore reefs within the Townsville region (Townsville reefs). Next, **Chapter five** incorporates passive acoustic telemetry at two of the reefs studied in **Chapter four** to compare movement and space use patterns between *P. leopardus* and *P. laevis* at several temporal scales. **Chapter six** combines passive acoustic telemetry and stable isotope analysis to explore niche overlap and competition between *P. leopardus* and *P. maculatus* at inshore reefs adjacent to Orpheus Island. Finally, **Chapter seven** consists of the general discussion of the thesis, in which the main findings are synthesised and the overall implications of the research, in ecological and management terms, are summarised.

Chapter 2

General Methodology

2.1 Study species

This thesis investigated movement and dietary trends of 'coral trout' or 'coralgrouper' (family Epinephelidae) from the genus *Plectropomus* (Oken 1817). The term 'coral trout' incorporates several species including: *Plectropomus leopardus* (Lacépède 1802), *P. laevis* (Lacépède 1801), *P. maculatus* (Bloch 1790), *P. areolatus* (Rüppell 1830), *P. oligacanthus* (Bleeker 1855), *Variola louti* (Forsskål 1775), and *V. albimarginata* (Baissac 1953). For the purpose of this thesis, the term 'coral trout' or '*Plectropomus* spp.' refers non-discriminately to two or more of the seven species listed above, although there is typically bias towards *P. leopardus* when pooled in the literature. Alternatively, species are identified directly when appropriate. The three main species found within the Great Barrier Reef Marine Park (GBRMP) were studied in this thesis: *P. maculatus, P. leopardus,* and *P. laevis* (Figure 2.1).

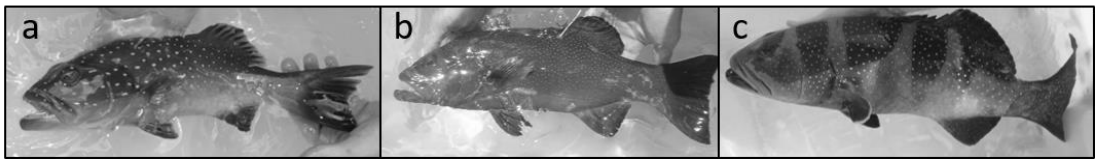

Figure 2.1: The coral trout (*Plectropomus* spp.) studied in this thesis including: *P. maculatus* (a), *P. leopardus* (b), *P. laevis* (c).

2.1.1 Fisheries importance

Plectropomus spp. form the basis of commercial, recreational, subsistence, and artisanal fisheries in the south-western Pacific including Australia, Indonesia, and Fiji, among others (Sadovy de Mitcheson and Colin 2012). The importance of each species varies at a regional scale, however, in the commercial Queensland Coral Reef Fin Fish Fishery (CRFFF) within the GBRMP, *P. leopardus* dominates coral trout catch (>80%, Sadovy de Mitcheson and Colin 2012). The commercial CRFFF is regulated by an annual Total Allowable Commercial Catch (TACC; ~1300 t since 2004), accessible to license/transferable quota owners (~250 licenses). Stocks of *P. leopardus* appear to be healthy in the GBRMP, and are under-fished (<1000 t p.a. since 2004; i.e., TACC not reached) for reasons such as increasing operating costs, limited gear type (hook and line), spawning season closures, marine park zoning, and the effects of cyclones and social learning on catch rates (Little et al. 2008; Tobin et al. 2010; Leigh et al.

2014; Thébaud et al. 2014). The current commercial sector gross value production within Queensland is ~$30 million each year based on license sales (Leigh et al. 2014). The majority of commercial catch is exported live to Asia where *Plectropomus* spp. comprise ~34% of the market (Frisch et al. 2016a). In the recreational sector, which is typically limited to nearshore/inshore reefs, the catch is mainly split between *P. leopardus* and *P. maculatus* (Leigh et al. 2014). The recreational sector is regulated by bag (seven coral trout) and size (*P. leopardus* and *P. maculatus*: <38 cm total length; *P. laevis*: <50 cm and >80 cm total length) limits, as well as marine park zoning. Total recreational coral trout landings are considerably lower than in the commercial sector at <250 t p.a. since 2004 (Leigh et al. 2014). Subsistence catch of coral trout by indigenous communities is ~11 t p.a. (Henry and Lyle 2003; Leigh et al. 2014).

2.1.2 Biology, abundance, and distribution

The biological and life history traits of *P. leopardus*, *P. maculatus*, and *P. laevis* are relatively well studied. All species are protogynous hermaphrodites, transitioning from female (immature or mature) to male (immature or mature). The timing of sex-change is variable, occurring over a wide range of sizes and ages (Ferreira 1993, 1995; Heupel et al. 2010). Similarly, the sex ratio varies between populations but is typically female-biased (see Table 2 – Frisch et al. 2016a). Spawning occurs between September and December (once every 4.3 days) throughout the GBRMP, and may vary regionally by temperature and marine park designation (see Table I and III – Carter et al. 2014). Unlike many large groupers that move long distances during spawning periods for site-specific aggregative reproductive activity, *P. leopardus* appear to use a combination of strategies (e.g., transient aggregator, resident aggregator, and resident non-aggregator; Sadovy de Mitcheson and Colin 2012). Overall, the majority of *P. leopardus* individuals appear to reproduce locally in small groups (Zeller 1998; Tobin et al. 2013; Matley et al. 2015), although exceptions do occur (Samoilys 1997; Zeller 1998; Kingsford 2009). Maternal age does not influence the quality of eggs or offspring survival of *P. leopardus*, however offspring from larger females may have increased chance of survival through enhanced egg provisioning (Carter et al. 2015). Larvae are planktonic for the first ~30 days of life, after which they gain the ability to actively swim and begin to settle on suitable habitat (Wright et al. 2008; Wen et al. 2013). Larval dispersal patterns vary but have been demonstrated to replenish local reefs (<30 km; Harrison et al. 2012), and support genetic heterogeneity over larger scales (>100 km; DH Williamson pers. comm.).

Plectropomus spp. most closely fit 'r-type' life history traits, characterised by rapid growth relative to maximum body size, high mortality, and short-life spans, although this is only relative to other grouper species sampled from a variety of locations (see Table 1 – Frisch et al.

2016a). *Plectropomus leopardus* and *P. maculatus* reach maturity at the same size and age (~300 mm fork length (FL) and 3 years), and typically live for 12-16 years, growing up to 600-680 mm FL (Adams 2002; Ferreira and Russ 1992; Ferreira and Russ 1994; Ferreira 1995; Williams et al. 2008). *Plectropomus laevis* grow larger (~1200 mm FL) and mature earlier (~1 year; ~300 mm FL) than *P. leopardus* and *P. maculatus* (Heupel et al. 2010). Additionally, *P. laevis* undergo a dramatic colour transition from footballer (white/yellow/black) to blue-spot (dark with large blue spots) phase upon reaching ~30 cm (~1:1 footballer:blue-spot at 50 cm; Heupel et al. 2010), although the timing of phase transition is variable (Figure 2.2).

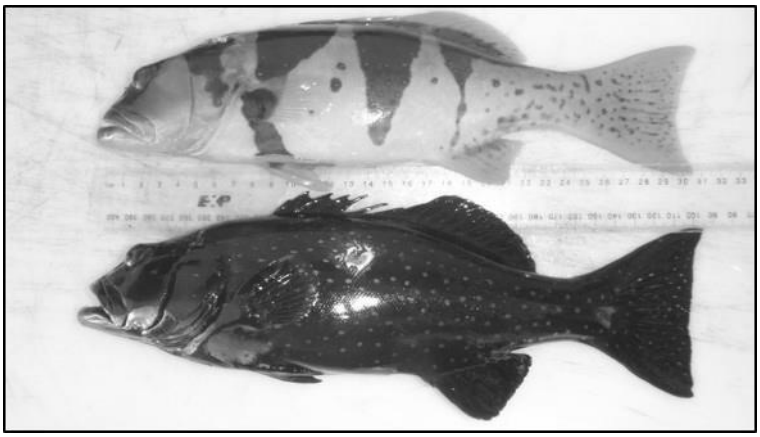

Figure 2.2: The two colour phases of *P. laevis*: footballer (top) and blue-spot (bottom).

The broad distribution of *P. leopardus*, *P. maculatus*, and *P. laevis* differs drastically between species. *Plectropomus leopardus* are abundant throughout the GBRMP including inshore/nearshore, mid-shelf, and offshore reefs (Kingsford 1992; Williams and Russ 1994; Zeller 1997). *Plectropomus maculatus* are almost exclusively found at reefs with silty and turbid waters, mainly nearshore (Williams and Russ 1994). Finally, *P. laevis* occur at mid-shelf reefs, but are more abundant at offshore reefs (Ayling and Choat 2008). The specific reason for these spatial differences is unknown. Fine-scale distribution is not readily known, however *P. leopardus* and *P. maculatus* co-occur at nearshore reefs, and *P. leopardus* and *P. laevis* co-occur at mid-shelf and offshore reefs. Fluctuating sea-levels causing physical barriers and limiting gene flow in the Pleistocene are attributed to speciation within *Plectropomus* spp. (van Herwerden et al. 2006, 2009).

The abundance/density of *Plectropomus* spp. in the GBRMP varies regionally and depending on marine park designation. At reefs in the Townsville sector, *P. leopardus* were ~5, 4, and 2 times more abundant than *P. laevis* in blue, pink, and green zones, respectively (Ayling and Choat 2008). The density of adult *P. leopardus* in green zones (closed to fishing; ~5.6 fish ha^{-1}) and pink zones (no access; ~14 fish ha^{-1}) was ~1.5 times and ~4 times higher than in blue

zones (open to commercial and recreational line fishing; ~3.7 fish ha^{-1}), respectively (Ayling and Choat 2008). Underwater visual census (UVC) estimates from the mid-1980s were extrapolated throughout the GBRMP to 8400 t and 3350 t of *P. leopardus* in blue and green zones, respectively (Leigh et al. 2014). *Plectropomus laevis* are also typically less abundant within management areas open to fishing. For example, *P. laevis* were ~2 times more abundant in green and pink zones (~5 fish ha^{-1}) than in blue zones (~2.5 fish ha^{-1}) at reefs offshore from Townsville (Ayling and Choat 2008). The density of *P. maculatus* compared to *P. leopardus* varies regionally; for example, within the Keppel Islands, the species composition is ~98% *P. maculatus* and 2% *P. leopardus* (~15 fish 1000 m^{-2}; Williamson et al. 2014), but further north at Orpheus Island the composition is more equitable (DH Williamson pers. comm.).

2.1.3 Ecology

Although a few exceptions exist, information about the ecology of coral trout is almost entirely based from research on *P. leopardus*. The main aspects of *P. leopardus* ecology, such as general movement patterns, habitat use, and diet selection have been investigated, but large gaps in knowledge still exist, especially long-term patterns of resource use. Additionally, only one study has been previously published addressing potential ecological interactions between different species of coral trout (i.e., Frisch et al. 2013).

In general, *P. leopardus* spend a large proportion of time at few locations for access to shelter, prey, and cleaning stations. Long-range and inter-reef movements have been documented, but they are not common. The size of *P. leopardus* home ranges vary among studies, in part, due to different sampling techniques. In general, three approaches have been used to examine movement of coral trout: UVC, mark-recapture, and acoustic telemetry (see Zeller 1997; Zeller and Russ 1998; Heupel et al. 2006 for description of methods). A large-scaled mark-recapture study in Queensland waters found low fishing recapture rates of *Plectropomus* spp. (146/2005), however the majority of recaptures were within 1 km of release site (Sumpton et al. 2008). One individual was recaptured ~30 km away. Mark-recapture and UVC research during a 12-month period at Heron Island found that 59 of 101 *P. leopardus* were resighted over periods of 4-5 months, 80% of which were visually recaptured in the same 2000m^2 area of release (Samoilys 1997). Individuals moved up to 7.5 km (mean ~2 km) along the reef slope throughout the study period. Individual ranges were between 37.5 m^2 and 4493 m^2 (n = 55), however the number of recaptures were deemed insufficient to reliably calculate home range. Using active acoustic tracking at Lizard Island, Zeller (1997) measured the home range (based on minimum convex polygons) of *P. leopardus* at a continuous fringing reef (n = 29; ~10,000 m^2) and isolated patch reef (n = 18; ~19,000 m^2). The mean daily distance moved within home ranges was ~190 m (maximum ~1,100 m). Horizontal core use (50% kernel utilisation

distribution; KUD) and movement extent (95% KUD) home ranges were estimated for *P. leopardus* at Heron Island and One Tree Island using passive acoustic telemetry throughout a 3-year period (Matley et al. 2015). Based on detections from 74 individuals (mean detection period ~286 days), the mean 50% and 95% KUDs were ~0.5 km^2 (0.1-4.0 km^2) and 2.5 km^2 (0.2-28.2 km^2), respectively.

Movement and activity patterns of *P. leopardus* vary temporally. Zeller (1997) monitored six individuals during both night and day (with active acoustic telemetry), and found that on 88.3% of monitored nights (n = 6 individuals over 10 nights) fish showed no movement. On nights where relocations occurred, the mean distance travelled was only 58m (vs. 192m during the day). Similarly, daytime movement was higher in both reproductive (~12 km d^{-1}) and non-reproductive (~16 km d^{-1}) periods compared to night movements (~8 km d^{-1}) at One Tree Island (Bunt and Kingsford 2014). Movement patterns do not change significantly throughout daylight hours. For example, Samoilys (1997) completed UVC of branded *P. leopardus* at Heron Reef and found that the size of the area they moved in was not influenced by the time of day (divided unequally as dawn, dusk, and day). Abundance counts of serranids (including *P. leopardus*) at three different times during the day (0500-0700; 1100-1300; 1700-1900) were also similar at One Tree Reef from 1991-1993 (Connell and Kingsford 1998).

The role of tide in influencing position of *P. leopardus* on the reef is not clear. Zeller (2002) found that *P. leopardus* positioned themselves in the up-current portion between their home site when abundance sampling was stratified by flood, ebb, and neutral tide. Goeden (1978) and Kingsford (1992) anecdotally described increased abundance in relation to tidal current flow to catch incoming prey. By contrast, Samoilys (1997) and Connell and Kingsford (1998) reported that home range area and abundance, respectively, were not influenced by tide. Overall, the effect of tide on the behaviour of *P. leopardus* is not clear but appears to be related to feeding.

Not surprisingly, reproduction appears to influence the movement and behaviour of *P. leopardus*, at least for part of each population. Davies (1996) found that catch per unit effort was greater in September, October, and February compared to April between 1992 and 1994 at several reefs in the Cairns section of the GBRMP, and attributed these findings to increased feeding activity and tendency to aggregate during spawning season. Further, Zeller (1998) actively tracked thirteen individuals moving 220-5210m to reach spawning sites, with the highest participation at new moon periods. Distances travelled did not differ between males and females, but males made more trips and spent more time at spawning sites. Alternatively, acoustic tracking within a small array (0.04 km^2) at One Tree Island, revealed greater mean

daily movements (~14.5 km d^{-1}) during the post-reproductive period compared to the reproductive period (10.5 km d^{-1}), possibly due to increased foraging after spawning activities (Bunt and Kingsford 2014). These studies imply that reproduction plays a role in defining the movement patterns of portions of adult coral trout populations. By contrast, Tobin et al. (2013) proposed that *P. leopardus* aggregate for reasons other than spawning (e.g., feeding) based on similar commercial fisheries catch data independent of spawning-related fishery closures throughout the year in the GBRMP. Roaming indices for *P. leopardus* increased during the summer at Heron Island and One Tree Island possibly due to active searching for reproductive partners, but differences were small and increased movements could have been related to seasonally altered prey acquisition (Kingsford 1992; St. John 1995).

Substantial evidence has highlighted the value of marine park zoning in the GBRMP to provide survival benefits to adult (Evans and Russ 2004; Russ et al. 2006; Emslie et al. 2015) and larval/juvenile (Harrison et al. 2012) coral trout populations. However, there is no significant evidence that marine protected areas influence movement of *P. leopardus*, although research is limited. For example, Zeller et al. (2003) tested the likelihood of a "spill over" effect (i.e., post-settlement movement from marine reserves to adjacent areas) by experimentally removing *P. leopardus* (up to 83% decrease in abundance at two sites) from open reefs. There was no clear pattern of directional movement from adjacent closed reefs; however, surveys were conducted only three months after the manipulations.

Habitat preference of coral trout is variable and is not strictly limited to any specific habitat type or depth. *Plectropomus leopardus* and *P. maculatus* often associate with coral cover that is structurally complex, perhaps for access to prey and/or protection (Wen et al. 2013; Bunt and Kingsford 2014; Williamson et al. 2014; Emslie et al. 2015), but this is inconsistent between studies (Connell and Kingsford 1998; Evans and Russ 2004; Ayling and Ayling 2011). Ayling and Choat (2008) found a significant effect of within-reef location (front vs back) on abundance estimates for adult *P. leopardus* in green zones at reefs in the Townsville region, while *P. laevis* showed no such trend for all age/size classes and management zones.

Depth use of *Plectropomus* spp. has been investigated irregularly. Commercial fishers often report large *P. laevis* in shallow waters, and Heupel *et al.* (2010) reported individuals mainly at depths <10m at locations around Lizard Island, Townsville, Mackay, and Storm Cay. Bunt and Kingsford (2014) examined *P. leopardus* depth use within the lagoon (max. depth ~7.5 m) at One Tree Island during both reproductive and non-reproductive periods, and found they typically remained at ~4 m deep. Individuals occurred deeper in the reproductive period at night, and were deeper during the morning and day after the reproductive period, potentially to

feed. Tide was found to not influence depth use in the same study. Long-term (~3 years) depth use of *P. leopardus* was explored at Heron Island and One Tree Island using passive acoustic tracking (Matley et al. 2015). Individuals (n = 55) were deeper during the day (~10 m), and appeared to rest in shallower waters at night (~8 m). Additionally, greater depth use coincided with summer, likely related to spawning behaviour. Indeed, several individuals made deep movements (~21 m deep) at dusk, during new moon periods between September and December – the known spawning period (Samoilys and Squire 1994, Ferreira 1995, Samoilys 1997, Zeller 1998). Live-market fishers normally catch coral trout <24 m (~85% of fishing effort), but do target individuals up to 135 m to be frozen (~15% of fishing effort) (Little et al. 2008). Therefore, the depth distribution of coral trout is difficult to ascertain due to prejudices associated with fishing depths or depth-related survey techniques.

There is only a limited amount of research studying diet of coral trout – again, primarily limited to *P. leopardus*. The diet of *P. leopardus* is varied including up to 422 prey items from 28 families (St. John 1999), primarily fish (~96%, Goeden 1978). The most common families include Pomacentridae, Scaridae, Caesionidae, Blenniidae, Clupeidae, and Labridae (Kingsford 1992; St. John 1999). St. John (1999) visually examined stomach contents of 1076 *P. leopardus* individuals collected from offshore reefs between Townsville and Cairns from 1990 and 1992, and found that Clupeidae, Pomacentridae, and Labridae families accounted for 60% of the diet (numerically). This suggests *P. leopardus* are generalist feeders with selective tendencies (St. John 1995). Overall, there is little difference in prey items between seasons and throughout the year, although pulses of abundant prey (e.g., schooling fish) affect temporal variation (Kingsford 1992; St. John 1999). Largely, it is the Clupeidae family that accounts for this variation in dietary composition as it schools near reefs during the summer (Kingsford 1992; St. John 1999). Otherwise, the same four prey families (Pomacentridae, Caesionidae, Labridae, and Scaridae) are important throughout the year representing 75% of the diet by weight, 48% by number, and 64% by index of relative importance (IRI) (St. John 1999).

Plectropomus leopardus likely do not feed every day and consume few items per feeding bout. For example, St. John (1999) found that 34% of stomachs sampled (n = 1076) were empty, and 49% contained only one prey item. Smaller individuals tended to have fewer empty stomachs and more food items than larger individuals. Frequency of feeding may be affected by tidal flow, especially during flood tide when feeding activity is greatest (Goeden 1978; Kingsford 1992). When classified into four broad habitat types (soft sediments, midwaters, benthic reef substrata and demersal reef substrata), *P. leopardus* fed from the midwater and demersal habitats four times more than other habitats (St. John 1995). Also, many report *P. leopardus*

moving out of the demersal habitat to pursue pelagic schools of forage fish, mainly Clupeidae and Caesionidae (Goeden 1978; Samoilys 1987; St. John 1995).

Ontogenetic shifts in *P. leopardus* diet usually occur at ~15 cm standard length (SL) and ~35 cm SL (St. John 1995). When <15cm SL, *P. leopardus* consumed ~60% benthic prey, primarily crustaceans (e.g., penaied shrimps). Between 15-35 cm SL demersal fishes such as Gobiidae and Trypterygiidae were consumed more frequently. When >35 cm SL piscivory was dominant (>90%) and composition of diet and prey length did not change despite the ability to grow >20 cm longer (St. John 1995). The size of prey consumed did not change as *P. leopardus* grew because individuals >15 cm SL fed on a wide size range of prey (30-100 mm SL) (St. John 1995). Wen et al. (2012) found similar size-related diet shifts for juvenile *P. maculatus* (<300 mm total length; TL) where individuals <99 mm TL consumed shrimp (Caridae), and larger individuals (200-300 mm TL) selected mainly pomacentrids. Interestingly, prey selection of juvenile *P. maculatus* appeared to shift, independent of prey availability, from crustaceans to fishes following a coral habitat degradation at the Keppel Islands, demonstrating adaptive feeding strategies to disturbances (Wen et al. 2016).

Greenwood et al. (2010) examined stable isotopes in muscle - representing long-term diet assimilation, of *P. leopardus*. There was a positive relationship between $\delta^{15}N$ values and size of *P. leopardus* (n = 41, size range = 225 mm) indicating ontogenetic diet shifts. Furthermore, based on different $\delta^{15}N$ values (equivalent to 1 trophic level) and similar $\delta^{13}C$ values between *P. leopardus* and *Chromis xanthura* (Pomacentridae), the authors concluded that pomacentrids were the main prey source of *P. leopardus*, as supported by St. John et al. (2001). Using the other three species' feeding habits and their $\delta^{13}C$ values, carbon in *P. leopardus* was derived mainly from planktonic sources (*C. xanthura*), and not coral (*Chaetodon lunulatus*) or benthic algal (*Acanthurus nigrofuscus*) sources. At Northwest Island in the southern GBRMP, Frisch et al. (2013) sampled muscle stable isotopes of co-occurring *P. leopardus* (n = 10) and *P. maculatus* (n = 10) to investigate differences in trophic ecology. Based on $\delta^{13}C$ values, they concluded that *P. leopardus* mainly derive prey from planktonic sources, and *P. maculatus* derive prey from benthic reef sources. Despite differences in $\delta^{13}C$ and $\delta^{15}N$ values between species, both were deemed to occupy similar trophic levels after adjusting $\delta^{15}N$ values of each species based on regressions between $\delta^{13}C$ and $\delta^{15}N$ values of three other large predatory species, in addition to *P. leopardus* and *P. maculatus*. Overall, the trophic position of *P. leopardus* and *P. maculatus* appear to be similar to other large mesopredators (fish and sharks) resident to coral reefs (Frisch et al. 2016b; Roff et al. 2016).

Although coral trout densities often differ between management zones, prey selection appears to be similar. There was little difference and high overlap in the diet items of *P. leopardus* between reefs closed and open to fishing, indicating that fishing pressure does not readily affect feeding behaviour or competition (St. John 1995). Similarly, line fishing does not significantly alter the diet of *P. leopardus*, as only ~1.5% of daily consumption is composed of bait from recreational fishers (St. John 1995). There were also no differences in prey selection and occurrence of empty stomachs for recruit and juvenile *P. maculatus* (<300 mm TL) between open and closed zones (Wen et al. 2012).

Based on previous research outlined above, it is evident that ecological and behavioural research within *Plectropomus* is focussed on the more abundant *P. leopardus*. In general, *P. leopardus* are opportunistic/generalist ambush predators that typically remain in a relatively small area diurnally and seasonally. By contrast, little is known about resource and habitat use of *P. laevis* or *P. maculatus*. Considering the relative importance of the 'coral trout' fishery, it is surprising that limited research has addressed the ecology of species other than *P. leopardus*.

2.2 Study sites

2.2.1 Townsville Reefs

A portion of the research for this thesis was conducted from the RV James Kirby at reefs within the Townsville region of the GBRMP (Townsville reefs) where *P. leopardus* and *P. laevis* co-occur. This included Lodestone, Helix, Yankee, and Coil reefs (18°37'25"S; 147°17'45"E; Figure 2.3). These are middle to outer shelf reefs of varying morphology (patch, crescentric, and planar) oriented in a cross-shelf northeast direction (Figure 2.3). Generally, at each reef, the reef slope, consisting of various amounts of coral cover and structures, drops to about 15 m, then changes into a gently sloping reef base of mostly sand and rubble. Lodestone Reef is open to commercial and recreational line fishing, whereas Helix, Yankee, and Coil reefs are closed to fishing. The acoustic telemetry aspect of this thesis conducted at Townsville reefs (**Chapter five**) incorporated only Lodestone and Helix reefs, whereas the dietary investigation (**Chapter four**) sampled at all four reefs.

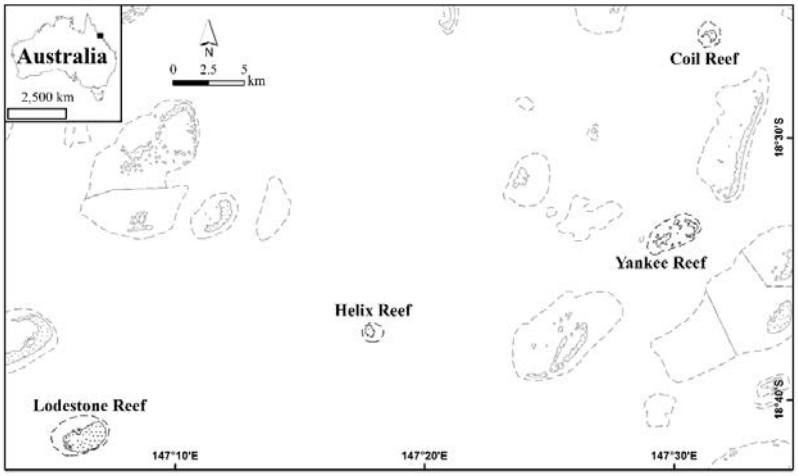

Figure 2.3: Location of reefs off of Townsville (Townsville reefs) where movement and dietary patterns of *P. leopardus* and *P. laevis* were investigated.

2.2.2 Orpheus Island

The other portion of research for this thesis was conducted from the Orpheus Island Research Station at Orpheus Island (18°36'48"S, 146°29'19"E; Figure 2.4) in the GBRMP. Sampling was conducted along the northwest side of the island (Scientific Research Zone - closed to extractive practices except with scientific permit) where *P. leopardus* and *P. maculatus* co-occur. See **Chapter six** for specific description of the study area. Both acoustic telemetry and dietary investigation aspects of this thesis were conducted within the area described (**Chapter six**).

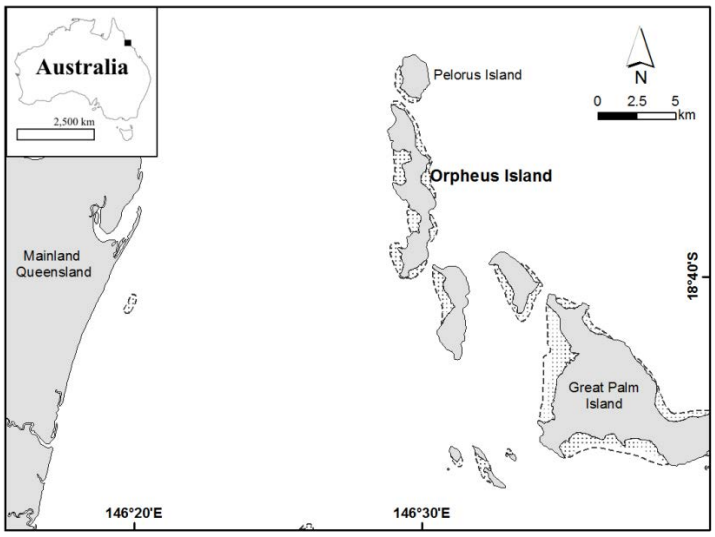

Figure 2.4: Map of Orpheus Island where movement and dietary patterns of *P. leopardus* and *P. maculatus* were investigated.

2.3 Fish collection and sampling procedures

2.3.1 Acoustic telemetry

Acoustic telemetry was conducted at Townsville reefs (i.e., Helix Reef and Lodestone Reef; **Chapter five**) and Orpheus Island (**Chapter six**). *Plectropomus leopardus* (Lodestone Reef n = 32; Helix Reef n = 51) and *P. laevis* (Lodestone Reef n = 2; Helix Reef n = 10) were tagged with acoustic transmitters between Feb 2013 and Jul 2014 at Townsville reefs. At Orpheus Island, *P. leopardus* (n = 32) and *P. maculatus* (n = 30) were tagged with acoustic transmitters between Sep 2013 and May 2014. All species were captured with a barbless hook (8/0 and 10/0) on line. At the surface, individuals were vented to avoid barotrauma, tagged externally (dart tag, PDS; Hall- print©), and placed in an anaesthetic bath (Aqui-S® diluted with seawater, 1:10000). When individuals lost equilibrium, they were moved to fresh seawater for surgery. A V13P (13 x 36 mm) acoustic transmitter (Vemco©, Halifax, Canada) was surgically inserted in the body cavity of each individual by making a small incision (~2-3 cm) in the ventral body wall using a sterile scalpel blade and forceps. The incision was closed using 2/0 synthetic absorbable sutures. Once individuals recovered from the anaesthetic (~10 min), they were released <20 m from their capture site. Each tag randomly emitted a unique identification code every 120 to 200 s with associated depth measurements (±2.5 m manufacturer estimate; < ±1.0 m field estimate – Matley et al. 2015) for an estimated tag life of 352 days.

At all sites, VR2W (69 kHz) acoustic receivers (Vemco©, Halifax, Canada) were moored ~1 m off the bottom with either a star-picket (hammered into sandy bottom) or chain and rope (attached to reef structure). The acoustic receiver array at Orpheus Island consisted of 19 receivers; eight receivers were deployed at Lodestone Reef and nine at Helix Reef. Receivers were downloaded every six months throughout the study.

2.3.2 Stable isotopes

Individuals were collected for stable isotope analysis for two separate aspects of this thesis: aquarium feeding trial (**Chapter three**) and field-based dietary investigation (**Chapter four** and **Chapter six**). For the aquarium feeding trial, 47 *P. leopardus* were collected from John Brewer Reef (18°37'52.05"S, 147° 3'21.40"E) using hook and line (described above) during 19-20 August 2013. The field-based dietary investigation sampled *Plectropomus* spp. collected by speargun while diving with SCUBA. These individuals were captured from <15 m deep along the reef slope, placed in a catch-bag, and floated to the surface for collection by an awaiting vessel. At Orpheus Island, *P. leopardus* (n = 9) and *P. maculatus* (n = 11) were sampled in May 2015. At Lodestone, Helix, Yankee, and Coil reefs, a total of 117 *P. leopardus* and 39 *P. laevis* were sampled between Aug 2013 and Feb 2014.

Both the aquarium feeding trial and field-based dietary investigation used similar sampling techniques, however the context of sampling was different. For simplicity, only the procedures for stable isotope processing are described below. Refer to representative chapters for specific collection and sampling procedures.

Five tissues from *Plectropomus* spp. were sampled for stable isotope analysis including: fin, plasma, red blood cells (RBC), liver, and muscle. A small segment (~2 cm x 1 cm) of caudal fin membrane along the exterior margin was removed with forceps and scissors (rinsed in ethanol then distilled water), washed with distilled water, and stored in a sterile 2 mL vial. Since many of the individuals were sampled on multiple occasions during the aquarium feeding trial, fin tissue collection was alternated between the lower and upper portions of the caudal fin (minimum of 35 days between sampling fin from the same portion). Next, between 1-2 mL of blood was taken from the 2nd or 3rd gill arch of each individual using a 23-gauge sterile needle. This method was chosen over sampling from the haemal arch because it was more efficient and a short trial revealed no lasting damage to the gills. Similar to fin tissues, the left and right gill arches were alternated when individuals were repeatedly sampled during the aquarium feeding trial. Whole blood was immediately transferred to a sterile 2 mL vial and centrifuged for 4-8 min using an Imbros Pty Ltd PC100 Micro Centrifuge (Cambridge, Tasmania, Australia). The plasma component was pipetted (Eppendorf Research® plus 10-100 µL; North Ryde, NSW, Australia) into a sterile 2 mL vial. The remaining plasma layer and the top layer of RBC (including white blood cells) were then discarded leaving only RBC in the vial. When lethally sampling, the gills of fish were then severed and the fish was placed in an ice-slurry to ensure mortality. Next, the liver was excised, weighed, and a small portion (~4 cm^3) removed with forceps and scissors, and placed into a sterile 2 mL vial. Similarly, a piece of dorsal muscle (no skin/scales attached) was removed and placed into a vial. Vials containing fin, plasma, RBC, liver, and muscle were immediately placed on ice after collection until they could be moved to a -20° C freezer no later than four hours after initial sampling (field-based dietary investigation), or within the hour (aquarium feeding trial).

Tissues were freeze-dried for 48 h, and ground into a fine powder with a mortar and pestle, except for fin tissues, which were cut into small pieces with sterile scissors. Lipids were removed from most tissues (see exceptions in **Chapter three**) following McMeans et al. (2009) by adding 5 mL 2:1 chloroform/methanol solvent to a <1 g subsample, vortexed for 30 s, and left for 24 h in a 30° C water bath. Afterwards, another 5mL of solvent was added, vortexed, poured out, and the tissue was left to dry for 24 h. Dry tissues were weighed (400-800 µg) into tin capsules, and $\delta^{13}C$ and $\delta^{15}N$ values were determined using a continuous flow isotope ratio mass spectrometer (Finnigan MAT Deltaplus, ThermoFinnigan©, San Jose, CA,

USA) equipped with a Costech Elemental Analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA). Stable isotope ratio values were expressed following the equation:

(1) $\delta X = [(R_{Sample}/R_{Standard}) - 1]$

where X is ^{13}C or ^{15}N, R_{Sample} is the ratio ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) in the sample, and $R_{Standard}$ is the ratio in the standard. The standard reference material was PeeDee Belemnite carbonate and atmospheric N_2 for carbon and nitrogen samples, respectively. Every 12th sample was run in triplicate to assess precision, where the standard deviations (SD) of $\delta^{13}C$ and $\delta^{15}N$ were generally <0.2 and <0.1 ‰, respectively. Further, laboratory and National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA) standards were analyzed every 12 samples. The analytical precision (standard deviation) for NIST standard 8414 (bovine liver, n = 130) and an internal laboratory standard (tilapia muscle, n = 130) for $\delta^{13}C$ was 0.05 and 0.07 ‰, respectively, and for $\delta^{15}N$ was 0.16 and 0.13 ‰, respectively. Accuracy was checked monthly using certified urea (n = 120) and was within 0.16 and 0.05 ‰ of mean calculated values for $\delta^{13}C$ and $\delta^{15}N$.

Chapter 3

Diet-tissue discrimination factors and turnover of carbon and nitrogen stable isotopes in tissues of an adult predatory coral reef fish, *Plectropomus leopardus*

3.1 Introduction

The application of stable isotope analysis (SIA) in ecosystem studies is a powerful tool that uses biogeochemical markers to explore the relationship between animals, their diet, and their environment (Peterson and Fry 1987). The use of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes in ecological research has increased significantly over the last 25 years (Newsome et al. 2010; Layman et al. 2011). SIA has been used to track the bioaccumulation of contaminants in Arctic marine megafauna (Atwell et al. 1998; Hoekstra et al. 2003), determine residency and movement patterns of tropical fish (McMahon et al. 2011; Currey et al. 2014), identify ontogenetic niche shifts of Antarctic organisms (Cherel et al. 2007; Polito et al. 2013), and quantify the dietary/energetic pathways in food webs of whole ecosystems (Vander Zanden et al. 1999; Hobson et al. 2002).

Despite its wide-spread application, there are a number of caveats that must be considered to properly interpret and apply stable isotopes in ecology (see Gannes et al. 1997; Post 2002 for reviews). For example, one of the main applications of $\delta^{13}C$ and $\delta^{15}N$ is to calculate the trophic position of organisms as a quantitative tool to measure the hierarchical role each organism has in a food web (Post 2002; Hussey et al. 2014). Additionally, $\delta^{13}C$ and $\delta^{15}N$ are often used to infer the proportional contribution of different prey items in the diet, typically via statistical mixing models (Layman et al. 2011). However, both applications are heavily biased by a user-defined input parameter, the diet-tissue discrimination factor (DTDF). Diet-tissue discrimination factors represent the difference in $\delta^{13}C$ (or $\delta^{15}N$) values between the consumer and its food ($\Delta^{13}C = \delta^{13}C_{consumer} - \delta^{13}C_{food}$; $\Delta^{15}N = \delta^{15}N_{consumer} - \delta^{15}N_{food}$). This metric is informative because it is a quantitative tool to estimate trophic pathways via mixing models, which can account for variation in parameter estimates. Most studies rely on experimentally-derived DTDFs found in the literature, and often use values that have been determined from species with different life history traits or that inhabit dissimilar environments (Caut et al. 2009). However, there can be considerable inter- and intra-specific variability in DTDFs

caused by a number of factors such as diet quality (Robbins et al. 2005; Montanari and Amato 2015), tissue type (Dalerum and Angerbjörn 2005; MacNeil et al. 2006), growth/size (Gaye-Siesseggar et al. 2003; Trueman et al. 2005), and temperature and feeding rates (Barnes et al. 2007). Furthermore, applying fixed DTDFs based on constant ^{15}N enrichment at each trophic level (e.g., 3.4‰ is commonly used) may bias top predator trophic position/DTDF estimates because dietary $\delta^{15}N$ is inversely related to $\Delta^{15}N$ (Overmyer et al. 2008; Caut et al. 2009; Dennis et al. 2010; Hussey et al. 2014). Therefore, instead of using potentially inaccurate and inappropriate values, DTDFs characterized by relevant trophic interactions (including meaningful variation associated with estimates) to interpret isotopic data are necessary (Wolf et al. 2009; Hussey et al. 2010).

There are several advantages to using SIA to study trophic dynamics compared to traditional techniques such as gut content analysis which only provides a short-term snapshot of often highly degraded prey. First, because different tissues metabolise proteins and carbohydrates at different rates, food is incorporated into consumer tissues at rates (and DTDFs) specific to each tissue – turnover rate. By sampling multiple tissues, it is possible to obtain dietary information over a range of time periods (Dalerum and Angerbjörn 2005). Second, in addition to $\delta^{13}C$ and $\delta^{15}N$ providing information on trophic structure (described above), they also indicate the baseline source of carbon or nitrogen in a particular food chain, after accounting for DTDFs at each trophic exchange (Vander Zanden and Rasmussen 2001; Chouvelon et al. 2012). For example, in aquatic environments, consumers that feed on benthically linked dietary pathways often have higher $\delta^{13}C$ values compared to pelagic pathways (Hobson and Welch 1992; France 1995). Another advantage of SIA is that non-lethal approaches can be used (Willis et al. 2013).

As part of SIA, there are several considerations regarding tissue preparation (see Newsome et al. 2010 for review); one of the most influential is the decision whether to extract lipids prior to analysis. The common basis for this decision is that lipids are depleted in ^{13}C (lower $\delta^{13}C$) compared to proteins and carbohydrates and that there is inherent lipid variability among individuals and species, as well as among tissue types within an individual (DeNiro and Epstein 1978; Post et al. 2007). This can lead to bias when comparing the same tissues of different individuals, and different tissues from the same individual. Removing lipids chemically to reduce this bias is not always feasible because it is expensive and time-consuming, and can influence $\delta^{15}N$ values of a sample by preferentially removing isotopically lighter nitrogenous compounds (Murry et al. 2006). Adjusting stable isotope values using mathematical normalizations is an alternative method to account for lipids but remains largely

untested across ecosystems. Therefore, standardized protocols to deal with bias associated with lipids are encouraged at a species and tissue level.

The goal of this study was to determine DTDFs and turnover rates, for several tissues of an economically and ecologically important coral reef fish, *Plectropomus leopardus*, in a captive feeding trial. *Plectropomus leopardus* is a large (up to ~65 cm; 4 kg) predatory epinephelid with broad distribution on the Great Barrier Reef, Australia and throughout the Indo-Pacific region (Mapstone et al. 2008; Yin 2014). Recently, its future role in fisheries has received increased attention due to concerns relating to climate change (e.g., reduced habitat, altered prey distribution, and metabolic costs due to warmer temperatures; Johansen et al. 2013). As a result, a few pilot studies have used $\delta^{13}C$ and $\delta^{15}N$ to begin to understand their trophic relationships in the reef environment (Greenwood et al. 2010; Frisch et al. 2013). However, no study has determined DTDFs or turnover rates for *P. leopardus* or any other coral reef fish species, notwithstanding a preliminary study using four individual gag grouper (*Mycteroperca microlepis*) (Nelson et al. 2011). Given concerns about coral reef food webs and the role of key predators such as *P. leopardus*, there is need to understand stable isotope dynamics for predatory coral reef fish species. The explicit aims of this study were to (1) quantify accurate DTDFs and turnover rates in a predatory reef fish and reveal the best tissues for inclusion in ecological studies using stable isotopes, (2) investigate the utility of non-lethal sampling, and (3) evaluate the need for lipid correction approaches for specific tissues.

3.2 Methods

Sample collection

Forty-seven *P. leopardus* were collected from John Brewer Reef, Australia (18°37'52.05"S, 147° 3'21.40"E) during 19-20 August 2013. Individuals were captured using hook and line and externally tagged, following **section 2.3.1**. After capture, they were immediately placed in a live well (~350 L) with continuous seawater flow. All fish were transported to the Marine and Aquaculture Research Facilities Unit (MARFU) at James Cook University (<48 h from initial capture). Individual fish were measured (fork length, mm) and weighed (total mass, g), then placed in one of four 2000 L holding tanks. These tanks constantly received re-circulated filtered seawater, and were aerated by at least one air stone per tank.

Feeding trial

Fish were left for two days to acclimate to the holding tanks prior to commencing the feeding trial. Initially, 10 of the 47 individuals were sacrificed and their tissues sampled to provide a baseline for $\delta^{13}C$ and $\delta^{15}N$ values (Day 0). Ideally, DTDFs are calculated by measuring the

isotope change between two distinct end-members (food items) when both are at equilibrium with consumer tissues (Hesslein et al. 1993). However, due to anticipated difficulties keeping this large predatory reef fish alive for enough time for two-end-members to reach equilibrium, Day 0 samples were used as the initial end-member (Dennis et al. 2010). The ten Day 0 individuals were sampled to account for potential variation in feeding in the wild. Moreover, to reduce isotopic variation among individuals, *P. leopardus* were only captured from one reef over a short period. The remaining *P. leopardus* were fed only one food item (*Nemipterus theodorei*) for the duration of the trial and turnover rates were calculated by comparing Day 0 samples with subsequent sampling periods. After the initial sampling (Day 0), tissues were lethally and non-lethally sampled intermittently over a 196-day period (see Table 3.1 for sampling schedule and sample sizes). Due to the relatively small number of individuals obtained for this experiment, some individual fish were repeatedly sampled (non-lethally) prior to the final lethal sampling (Table 3.1). The minimum time between repeat sampling of the same individual was 14 days.

Plectropomus leopardus were fed pieces of thawed threadfin bream (*N. theodorei*) (excluding the head) to satiation every Monday, Wednesday, and Friday throughout the experiment. *Nemipterus theodorei* was selected because of its success as a feed for *P. leopardus* in the past (AJ Tobin pers. comm.; Johansen et al. 2013). This food was purchased in bulk prior to the commencement of the feeding trial to reduce variation in prey isotope signatures. *Nemipterus theodorei* is found near sand or muddy bottoms in offshore waters of the Great Barrier Reef, feeding on crustaceans, molluscs, and small fish (Pears et al. 2012). A random subsample (n = 15) of *N. theodorei* (excluding the head) was kept aside (frozen) and homogenized for SIA of the food item.

Lethal and non-lethal sampling was conducted as outlined in Table 3.1. The non-lethal approach sampled fin, red blood cells (RBC), and plasma, while liver and muscle tissues (in addition to fin, RBC, and plasma) were collected during lethal sampling. The protocol for tissue sampling was similar for both lethal and non-lethal approaches. First, using a dip net, an individual was moved from the holding tank into a ~50 L container filled with an anesthetic solution (1:10000 Aqui-S® (Lower Hutt, New Zealand):seawater). Once the animal lost equilibrium, it was weighed and measured. Tissues were then sampled as described in **section 2.3.2**.

Table 3.1: Summary of sampling regime for *Plectropomus leopardus* during experimental feeding trial, including mean (± SE) size (length range in brackets), final mass, mass change, hepatosomatic index (HSI), and condition factor (CF) for each sampling period.

Sampling day	n	Type	Tissues sampled	Fork length (mm)	Final mass (g)	Relative mass change (g)	HSI	CF
0	10	Lethal	Fin, liver, muscle, plasma, RBC	441 ± 16 (365-527)	1444 ± 199		9.4 ± 1.0	1.58 ± 0.04
7	5	Lethal	Fin, liver, muscle, plasma, RBC	439 ± 22 (385-514)	1382 ± 224	0.98 ± 0.04	9.7 ± 1.4	1.58 ± 0.08
15	5	Lethal	Fin, liver, muscle, plasma, RBC	429 ± 22 (368-500)	1319 ± 223	1.05 ± 0.03	11.0 ± 1.0	1.61 ± 0.08
21	5	Lethal	Fin, liver, muscle, plasma, RBC	441 ± 17 (377-481)	1376 ± 173	1.02 ± 0.02	10.0 ± 1.4	1.56 ± 0.04
28	5	Non-lethal[a]	Fin, plasma, RBC	427 ± 21 (392-496)	1262 ± 208	1.02 ± 0.01		1.58 ± 0.03
42	4	Non-lethal[b]	Fin, plasma, RBC	402 ± 9 (390-430)	1049 ± 82	1.10 ± 0.03		1.60 ± 0.03
49	5	Lethal[a]	Fin, liver, muscle, plasma, RBC	444 ± 20 (395-495)	1385 ± 192	1.00 ± 0.03	8.9 ± 0.7	1.53 ± 0.04
63	4	Non-lethal[b]	Fin, plasma, RBC	406 ± 9 (391-434)	1099 ± 87	1.15 ± 0.06		1.63 ± 0.05
70	5	Non-lethal[c]	Fin, plasma, RBC	441 ± 24 (366-511)	1449 ± 256	1.05 ± 0.02		1.61 ± 0.03
77	4	Non-lethal[b]	Fin, plasma, RBC	408 ± 9 (394-434)	1135 ± 99	1.19 ± 0.05		1.65 ± 0.03
92	5	Non-lethal[c]	Fin, plasma, RBC	440 ± 23 (366-504)	1435 ± 251	1.04 ± 0.03		1.60 ± 0.04
98	4	Lethal[b]	Fin, liver, muscle, plasma, RBC	409 ± 9 (395-434)	1175 ± 92	1.24 ± 0.08	12.9 ± 1.3	1.71 ± 0.06
147	4	Lethal	Fin, liver, muscle, plasma, RBC	446 ± 31 (388-507)	1379 ± 290	1.13 ± 0.04	12.9 ± 2.7	1.50 ± 0.09
196	5	Lethal[c]	Fin, liver, muscle, plasma, RBC	449 ± 22 (387-519)	1634 ± 253	1.21 ± 0.07	11.3 ± 0.8	1.75 ± 0.02

Note: three fish died (as described in results) and were not included in study, nor was one individual that showed declining weight and liver condition. Relative weight change was calculated as final weight (during that period)/initial weight. Hepatosomatic index (HSI) was calculated as (W_{liver}/W_{total}) *100 and condition factor (CF) was (W_{total}*10^5)/L_{fork} ^{3}, where W_{liver} and W_{total} are liver and total fish weight (g), respectively, and L_{fork} is fork length (cm). [a,b,c] represent repeated sampling of tissues from the same individuals.

Analysis

For tissues that demonstrated a transition in isotope values toward equilibrium during the feeding trial (i.e., $\delta^{15}N$), turnover rates were estimated for LE and bulk tissues by fitting a nonlinear least squares regression model using the following equation (Fry and Arnold 1982):

(1) $\delta_t = \delta_f + (\delta_i - \delta_f)e^{(-vt)}$

where δ_t is the stable isotope ($\delta^{15}N$) value at time t; δ_f is the asymptotic stable isotope value at equilibrium with the new diet; δ_i is the initial value for that tissue (Day 0), v is fractional rate of isotopic incorporation into the tissue, or turnover rate (Reich et al. 2008); and t is the sampling day. The primary influences of tissue turnover rates are growth and metabolism (Fry and Arnold 1982). Thus, the parameter v was further defined as the sum of tissue net growth (k_g) and tissue catabolic turnover (m) (Hesslein et al. 1993):

(2) $v = k_g + m$

The parameter k_g was estimated by fitting nonlinear least squares to an exponential growth model (Ricker 1979):

(4) $W_f = W_i e^{kgt}$

where W_f is the final wet mass of an individual at time of sampling; W_i is the initial mass; and k_g and t are defined as before.

Therefore, m was the unknown solved with this approach providing tissue turnover rates (day^{-1}) independent of growth.

The turnover rate for both growth and metabolism (v) was also presented as a half-life ($T_{0.5}$) to assist interpreting wild tissue samples in future studies (Tieszen et al. 1983):

(5) $T_\alpha = \ln(1-\alpha)/-v$

where T_α is the length of time (in days) needed to achieve a target transition state α (e.g., 50%) from initial stable isotope values (Day 0) to equilibrium values. Similarly, 95% ($T_{0.95}$) transition periods were calculated for each tissue.

Diet-tissue discrimination factors were calculated as (Minigawa and Wada 1984):

(6) $\Delta\delta = \delta_f - \delta_d$

where δ_f is the tissue-specific stable isotope value of *P. leopardus* at equilibrium with the new diet; and δ_d is the mean value of *N. theodorei* diet. Standard errors (SE) for the DTDFs were

calculated using the SE associated with model estimate δ_f and the SE of *N. theodorei* values (Buchheister and Latour 2010):

$$(7)\ SE_{\Delta\delta} = \sqrt{\mathrm{SE}_{\delta f}{}^2 + \mathrm{SE}_{\delta d}{}^2}$$

For those tissues that turnover/equilibrium could not be estimated (i.e., unable to fit with Equation 2 - $\delta^{13}C$), mean DTDFs were estimated by subtracting mean *N. theodorei* stable isotope values (δ_d) from *P. leopardus* values (δ_t) sampled between Day 98 and 196. This approach was selected because $\delta^{13}C$ values were relatively consistent throughout the feeding trial for each tissue, especially after Day 98 indicating consumer values had reached equilibrium with prey.

The effect of lipid extraction on $\delta^{13}C$, $\delta^{15}N$, %C, %N, and C:N (%C/%N) was evaluated in the different tissues by examining differences between LE and bulk values. Paired t-tests were then performed to determine if LE values differed from bulk values for each tissue sampled. The effectiveness of using lipid-normalizing models for bulk $\delta^{13}C$ was examined by comparing observed LE values with corresponding predicted values from three correction models (McConnaughey and McRoy 1979; Kiljunen et al. 2006; Post et al. 2007). The accuracy of these models was determined by calculating the percentage of estimates that fell within 0.1‰ ($P_{0.1}$) and 0.5‰ ($P_{0.5}$) of LE values. Additionally, r^2 and Akaike's Information Criterion corrected for small sample sizes (AIC_c) were determined to evaluate the precision and fit of correction models (Burnham and Anderson 2002). The resultant linear model was used to re-estimate model values to standardize them, and adjusted $P_{0.1}$ and $P_{0.5}$ were determined.

All modelling and data analyses were conducted in the R environment (R Core Team 2013). and results were considered significant when $p < 0.05$. Assumptions relating to normality of dependent variables and homogeneity of variances were verified using Q-Q plots and visual inspection of residual plots, respectively.

3.3 Results

The feeding trial lasted 196 days, during which lethal and non-lethal sampling of muscle, liver, fin, plasma, and RBC were collected at designated intervals (Table 3.1; Table 3.2). After an initial acclimation period of a few days, all individuals began feeding and displayed limited signs of stress. Three individuals died during the experiment: one after ten days, and the other two after more than a month. The first may have been stress-induced, while the two latter died after propelling themselves out of the tank through a mesh cover. On a few occasions, an individual became externally infected with bacterial/fungal growth. Infected individuals were bathed in freshwater <2 min and Betadine® was applied to the infected area, after which they

recovered fully. One of the larger individuals (sampled on Day 42, 63, 77, and 98) fed less than all others and decreased in mass by ~15% compared to initial measurements. Consequently, data from this individual were removed from all analyses to avoid bias associated with fasting/nutritional stress (Hobson et al. 1993). Additionally, examination of Cook's D (identifies outliers) was used to remove four $\delta^{13}C$ values (in plasma, RBC, and fin tissues) and three $\delta^{15}N$ values (in plasma and RBC). The mean fork length and mass of *P. leopardus* at each sampling period ranged between 402 – 449 mm, and 1049 – 1634 g, respectively (Table 3.1). The general health of individuals throughout the experiment was good, and most demonstrated increased mass and liver condition (HSI) (Table 3.1).

Table 3.2: Summary of mean (± SD) $\delta^{15}N$ and $\delta^{13}C$ values from each sampling period during experimental feeding trial.

Sampling day	n	Type	Tissues sampled	Final mass (g)[d]	Muscle		RBC		Fin		Plasma		Liver	
					$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$
0	10	Lethal	Fin, liver, muscle, plasma, RBC	1444 ± 199	11.4 ± 0.2	-16.0 ± 0.4	10.1 ± 0.2	-16.7 ± 0.4	11.4 ± 0.3	-13.4 ± 0.5	11.5 ± 0.3	-15.5 ± 0.5	10.2 ± 0.4	-16.3 ± 0.7
7	5	Lethal	Fin, liver, muscle, plasma, RBC	1382 ± 224	10.9 ± 0.2	-15.9 ± 0.4	10.0 ± 0.3	-16.8 ± 0.4	11.1 ± 0.3	-13.4 ± 0.2	11.5 ± 0.2	-15.6 ± 0.4	10. ± 0.3	-15.9 ± 0.4
15	5	Lethal	Fin, liver, muscle, plasma, RBC	1319 ± 223	11.0 ± 0.4	-15.2 ± 0.9	10.2 ± 0.5	-16.5 ± 0.9	11.3 ± 0.2	-13.2 ± 0.9	11.6 ± 0.1	-15.5 ± 0.4	10.7 ± 0.5	-15.9 ± 0.4
21	5	Lethal	Fin, liver, muscle, plasma, RBC	1376 ± 173	10.7 ± 0.4	-15.4 ± 1.1	10.0 ± 0.3	-16.2 ± 1.1	11.4 ± 0.4	-12.7 ± 0.8	11.4 ± 0.3	-15.3 ± 0.3	10.4 ± 0.4	-15.2 ± 0.5
28	5	Non-lethal[a]	Fin, plasma, RBC	1262 ± 208			10.0 ± 0.2	-16.6 ± 0.9	11.4 ± 0.2	-12.9 ± 1.0	11.5 ± 0.2	-15.3 ± 0.5		
42	4	Non-lethal[b]	Fin, plasma, RBC	1049 ± 82			10.3 ± 0.5	-16.3 ± 1.1	11.6 ± 0.3	-13.4 ± 0.8	11.6 ± 0.2	-15.4 ± 0.7		
49	5	Lethal[a]	Fin, liver, muscle, plasma, RBC	1385 ± 192	10.7 ± 0.4	-15.6 ± 0.7	10.5 ± 0.2	-16.5 ± 0.5	11.2 ± 0.4	-13.0 ± 0.6	11.5 ± 0.2	-15.6 ± 0.2	10.6 ± 0.5	-15.7 ± 0.4
63	4	Non-lethal[b]	Fin, plasma, RBC	1099 ± 87			10.7 ± 0.45	-16.7 ± 0.6	11.9 ± 0.6	-13.3 ± 0.7	11.7 ± 0.2	-15.7 ± 0.1		

70	5	Non-lethal[c]	Fin, plasma, RBC	1449 ± 256			10.8 ± 0.2	-16.5 ± 0.5	11.9 ± 0.2	-13.2 ± 0.6	11.5 ± 0.1	-15.7 ± 0.3		
77	4	Non-lethal[b]	Fin, plasma, RBC	1135 ± 99			11.1 ± 0.6	-16.7 ± 0.2	11.7 ± 0.6	-13.6 ± 0.5	11.8 ± 0.2	-15.6 ± 0.2		
92	5	Non-lethal[c]	Fin, plasma, RBC	1435 ± 251			11.0 ± 0.2	-16.8 ± 0.4	12.1 ± 0.2	-13.1 ± 0.7	11.7 ± 0.2	-15.6 ± 0.2		
98	4	Lethal[b]	Fin, liver, muscle, plasma, RBC	1175 ± 92	11.4 ± 0.2	-15.5 ± 0.3	11.3 ± 0.3	-16.4 ± 0.5	11.6 ± 0.5	-13.7 ± 0.3	11.9 ± 0.2	-15.3 ± 0.3	10.9 ± 0.4	-15.1 ± 0.4
147	4	Lethal	Fin, liver, muscle, plasma, RBC	1379 ± 290	11.7 ± 0.1	-15.6 ± 0.7	11.4 ± 0.4	-16.5 ± 0.4	11.7 ± 0.3	-13.1 ± 0.4	11.8 ± 0.2	-15.3 ± 0.2	11.2 ± 0.5	-15.0 ± 0.2
196	5	Lethal[c]	Fin, liver, muscle, plasma, RBC	1634 ± 253	11.9 ± 0.2	-15.3 ± 0.6	11.4 ± 0.2	-16.3 ± 0.3	11.6 ± 0.2	-13.2 ± 0.3	11.7 ± 0.1	-15.3 ± 0.2	10.6 ± 0.4	-14.8 ± 0.3

[a,b,c] represent repeated sampling of tissues from the same individuals

[d] variation in final mass is provided as ± SE.

Tissue turnover for $\delta^{15}N$ and $\delta^{13}C$

Lipid-extracted and bulk fin, liver, plasma, muscle, and RBC stable isotope parameters were estimated for the time-based $\delta^{15}N$ model (Eq. 2) (Figure 3.1; Table 3.3). For muscle $\delta^{15}N$, initial values were elevated and could not be fitted to the model above. Under the assumption that prey tissues take >15 days to be incorporated into consumer muscle tissues (Buchheister and Latour 2010; Nelson et al. 2011) and acknowledging inherent isotopic variability in wild-caught fish, the model was adapted to only incorporate sampling periods between Day 21 and 196 (Figure 3.1). The nonlinear model described changes in $\delta^{15}N$ values over time relatively well for muscle and RBC ($r^2 = 0.70$-0.76; Table 3.3). Tissue-specific metabolic turnover rates (v) were calculated after the exponential growth model estimated the net growth constant (k_g) to be 0.00084 day^{-1} (Table 3.3; individuals ranged between -0.00786 and 0.00906 day^{-1}). The half-lives ($T_{0.5}$) for LE and bulk $\delta^{15}N$ of liver, fin, plasma, RBC, and muscle ranged between 10 and 126 days, and 95% incorporation rates ($T_{0.95}$) were between 43 and 543 days (Table 3.3). None of the $\delta^{13}C$ tissues could be fit to the time-based nonlinear model to estimate turnover rates.

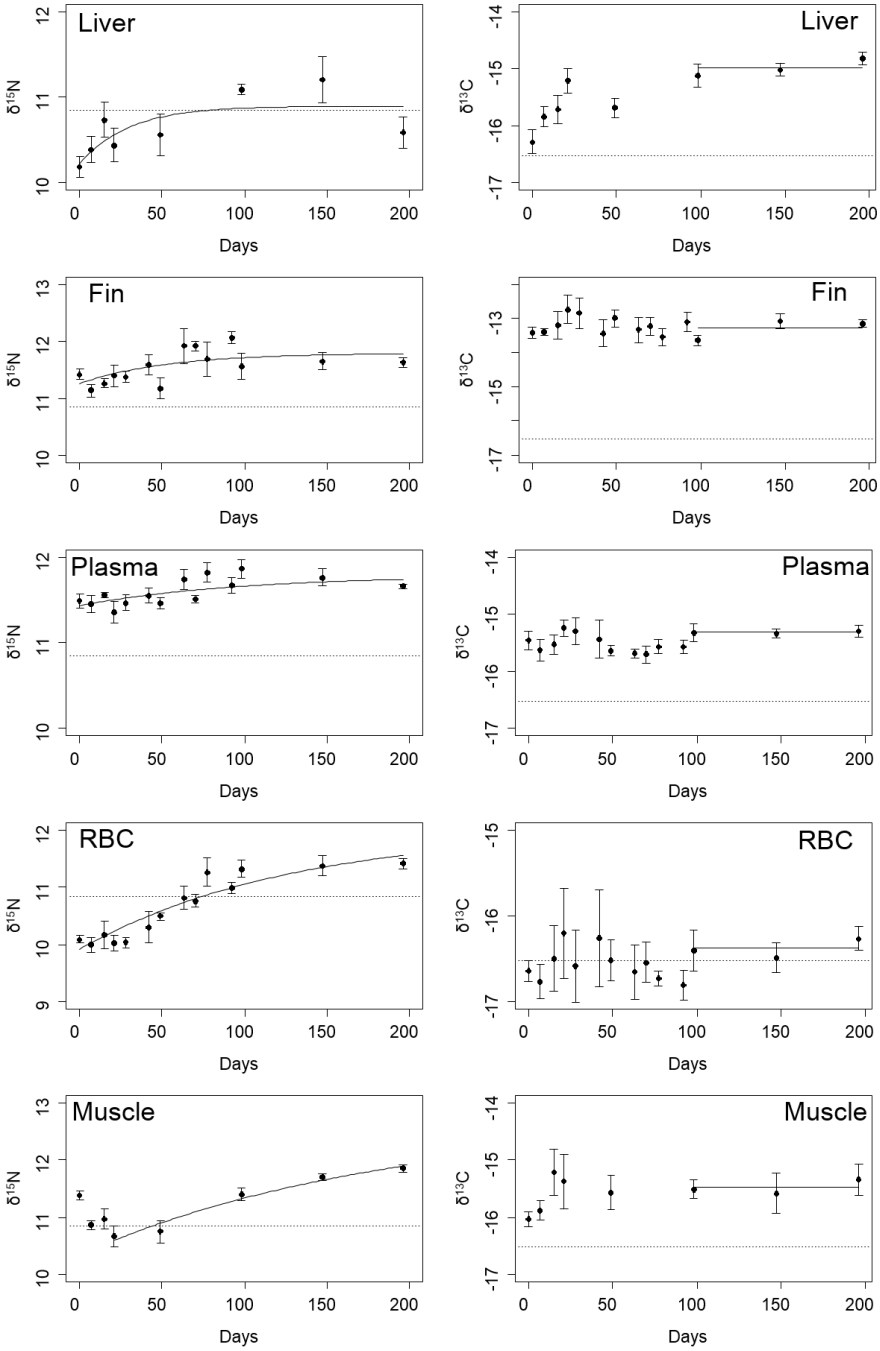

Figure 3.1: Mean (± SE) $\delta^{15}N$ and $\delta^{13}C$ estimates for lipid extracted tissues during feeding trial. Plots for $\delta^{15}N$ (left) contain the least-squares regression from the time-based isotope models for liver, fin, plasma, RBC, and muscle tissues. Solid horizontal lines in $\delta^{13}C$ plots (right) represent the mean $\delta^{13}C$ values of *P. leopardus* tissues between Days 98 and 196. The dotted horizontal lines on each plot represent the mean value of *Nemipterus theodorei* ($\delta^{15}N$ = 10.9‰; $\delta^{13}C$ = -16.5‰).

Table 3.3: Parameter estimates from nonlinear least squares time-based lipid extracted (LE) and untreated (Bulk) $\delta^{15}N$ models for liver, fin, plasma, RBC, and muscle tissues, including the initial $\delta^{15}N$ value for that tissue (i.e., Day 0; δ_i, ‰), equilibrium value (δ_f; ‰), turnover rate constant (v; day^{-1}), tissue catabolic turnover (m; day^{-1}), proxy to model fit (r^2), tissue half-life and 95% incorporation time ($T_{0.5}$, $T_{0.95}$; days), and mean diet-tissue discrimination factor (DTDF or Δ_{tissue}; ‰; ± SE, SD*; also estimated for LE and bulk $\delta^{13}C$).

Isotope	Type	Tissue	δ_i	δ_f	v	m	r^2	$T_{0.5}$	$T_{0.95}$	Δ_{tissue}
		Liver	10.2	10.9	0.034	0.033	0.27	21	89	0.0 ± 0.2, 0.4
		Fin	11.3	11.8	0.019	0.018	0.17	37	158	0.9 ± 0.2, 0.2
	LE	Plasma	11.4	11.8	0.011	0.010	0.20	66	283	0.9 ± 0.2, 0.1
		RBC	9.9	12.0	0.008	0.007	0.70	88	380	1.1 ± 0.5, 0.2
		Muscle	10.3	12.7	0.006	0.005	0.74	126	543	1.8 ± 1.5, 0.2
$\delta^{15}N$										
		Liver	10.1	10.2	0.069	0.068	0.01	10	43	-0.2 ± 0.2, 0.2
		Fin	11.3	11.5	0.016	0.015	0.05	44	191	1.2 ± 0.2, 0.3
	Bulk	Plasma	11.3	11.7	0.018	0.017	0.18	39	170	1.3 ± 0.2, 0.1
		RBC	10.0	12.0	0.008	0.007	0.74	90	388	1.7 ± 0.4, 0.1
		Muscle	9.8	11.8	0.008	0.007	0.76	83	360	1.5 ± 0.6, 0.2
		Liver								1.5 ± 0.1, 0.3
		Fin								3.2 ± 0.1, 0.4
	LE	Plasma								1.2 ± 0.1, 0.2
		RBC								0.1 ± 0.1, 0.4
		Muscle								1.1 ± 0.2, 0.5
$\delta^{13}C$										
		Liver								0.4 ± 0.2, 0.6
		Fin								3.9 ± 0.1, 0.4
	Bulk	Plasma								1.0 ± 0.1, 0.4
		RBC								1.6 ± 0.1, 0.3
		Muscle								2.3 ± 0.2, 0.5

Note: Tissue catabolic turnover (m) was estimated by subtracting a tissue net growth (k_g) value of 0.00084 day^{-1} from the turnover rate constant (v). To calculate Δ_{tissue} the mean stable isotope values of *Nemipterus theodorei* (LE: $\delta^{15}N$ = 10.9‰; $\delta^{13}C$ = -16.5‰, Bulk: $\delta^{15}N$ = 10.3‰; $\delta^{13}C$ = -17.8‰) were subtracted from equilibrium (δ_f) estimates for $\delta^{15}N$ and mean $\delta^{13}C$ values of *Plectropomus leopardus* tissues between day 98 and 196. $\delta^{15}N$ values from day 0, 7, and 15 were not included in muscle estimates. *For $\Delta^{15}N$ Δ_{tissue}, SE was calculated using Equation 7; SD (and SE for $\Delta^{13}C$) was calculated by subtracting *N. theodorei* $\delta^{13}C/\delta^{15}N$ values from *P. leopardus* values (between Day 98 and 196 for $\delta^{13}C$; Day 196 for $\delta^{15}N$) for use in isotopic mixing models.

Diet-tissue discrimination factors for $\delta^{15}N$ and $\delta^{13}C$

Food (*N. theodorei*) $\delta^{15}N$ values varied slightly for lipid extracted (n: 15; mean ± SE: 10.9‰ ± 0.1; range: 9.8‰ – 11.7‰) and untreated (n: 15; mean ± SE: 10.3‰ ± 0.1; range: 9.5‰ – 11.1‰) samples. The range in mean DTDFs for LE and bulk $\delta^{15}N$ among tissues was 0.0 – 1.8 and -0.2 – 1.7, respectively (Table 3.3).

Values of LE $\delta^{13}C$ of food (n: 15; mean ± SE: -16.5‰ ± 0.1; range: -17.1‰ – -15.7‰) were less variable than untreated samples (n: 15; mean ± SE: -17.8‰ ± 0.3; range: -20.3‰ – -16.0‰). The range in mean DTDFs among tissues for LE and bulk $\delta^{13}C$ was 0.1 – 3.2 and 0.4 – 3.9, respectively (Table 3.3).

Bulk vs Lipid extracted tissues

The t-tests comparing LE and bulk values of $\delta^{13}C$, $\delta^{15}N$, %C, %N, and C:N showed that lipid extraction produced generally different outputs than untreated/bulk samples. Only $\delta^{15}N$ values of RBC (*t*-test, $t_{71} = 0.34$, $p = 0.73$) and %C in fin (*t*-test, $t_{61} = -1.34$, $p = 0.19$) were similar between LE and bulk. Differences between LE and bulk parameters also showed marked differences (Figure 3.2a), although values were consistent for some tissues, particularly $\delta^{15}N_{LE\text{-}Bulk}$ (mean ± SD) for muscle (0.5 ± 0.1), plasma (0.1 ± 0.2), and RBC (0.0 ± 0.2); and $\delta^{13}C_{LE\text{-}Bulk}$ (mean ± SD) for muscle tissue (-0.1 ± 0.2) (Figure 3.2b). Lipid extraction reduced C:N for all tissues and food, however the C:N in LE liver tissue remained relatively high (mean ± SD: 6.9 ± 1.7; Table 3.4) even after multiple extractions. The lipid-normalizing models that were examined produced relatively similar outputs (Table 3.5). Based on r^2, ΔAIC (values ≤ 2 show strongest support for model fitting; Burnham and Anderson 2002) and adjusted $P_{0.1}$ and $P_{0.5}$, the best models varied for each tissue (indicated in Table 3.5). Overall, muscle and RBC were the tissues best described by the correction models (Table 3.5).

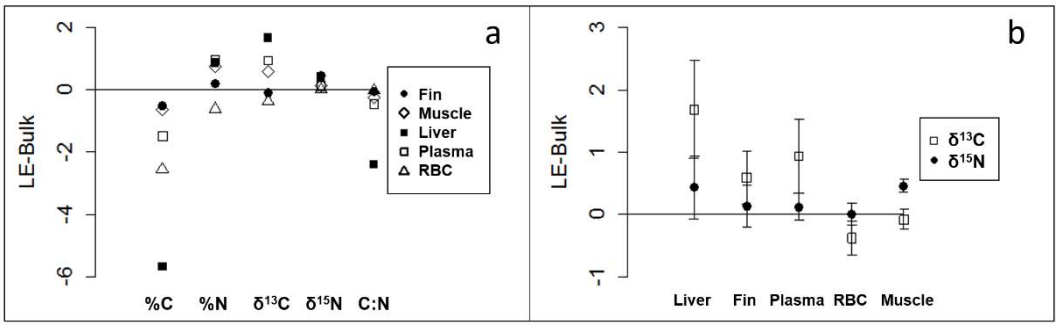

Figure 3.2: Comparison of mean %C, %N, $\delta^{15}N$, $\delta^{13}C$, and C:N for several tissues after subtracting untreated (Bulk) values from lipid extracted (LE) values (a). Mean (± SD) $\delta^{15}N$ and $\delta^{13}C$ are plotted again at a finer scale (b).

Table 3.4: Mean (± SD) ratio of %Carbon to %Nitrogen (C:N) from stable isotope analysis conducted for lipid-extracted (LE) and untreated tissues (Bulk).

Tissue	n	C:N (LE)	C:N (Bulk)	*p*
Liver	44	6.9 ± 1.7	9.3 ± 2.8	<0.001
Fin	62	2.9 ± 0.1	3.2 ± 0.1	<0.001
Plasma	69	3.6 ± 0.1	4.1 ± 0.3	<0.001
RBC	72	3.4 ± 0.1	3.5 ± 0.1	<0.001
Muscle	45	3.2 ± 0.1	3.3 ± 0.1	<0.001
Food (*Nemipterus theodorei)*	15	3.2 ± 0.1	4.0 ± 0.7	<0.001

Note: *p*-values were calculated from paired t-tests between LE and Bulk samples

Table 3.5: Linear relationship between LE $\delta^{13}C$ and lipid-normalized $\delta^{13}C$ values from three predictive models for each tissue. Output includes the following metrics to interpret the best fitting models: the percent of predicted $\delta^{13}C$ values that fall within 0.1‰ ($P_{0.1}$) and 0.5‰ ($P_{0.5}$) of LE $\delta^{13}C$ values (%); the linear model equation comparing LE $\delta^{13}C$ and lipid-normalized $\delta^{13}C$; r^2 of the linear model; and AIC_c and ΔAIC_c for model selection.

Tissue	Lipid correction approach	$P_{0.1}$	$P_{0.5}$	Equation	r^2	AIC_c	ΔAIC_c	$P_{0.1}$ (adjusted)	$P_{0.5}$ (adjusted)
Muscle	Post et al. (2007)*	55.6	97.8	y = 0.984x - 0.270	0.943	-32.8	0	57.8	100
	McConnaughey and McRoy (1979)*	0	2.2	y = 0.992x + 0.757	0.941	-31.0	1.8	51.1	100
	Kiljunen et al. (2006)	44.4	97.8	y = 0.994x - 0.148	0.939	-29.7	3.1	51.1	100
	This study (LE vs bulk)	44.4	97.8	y = 0.971x - 0.541	0.946	-32.2	0.6	51.1	100
Fin	Post et al. (2007)*	4.8	29.0	y = 0.722x - 3.121	0.692	47.6	1.7	17.7	88.7
	McConnaughey and McRoy (1979)*	0	0	y = 0.738x - 2.145	0.699	46.0	0.1	16.1	88.7
	Kiljunen et al. (2006)*	3.2	14.5	y = 0.742x - 2.775	0.700	45.9	0	14.5	88.7
	This study (LE vs bulk)	6.5	43.5	y = 0.686x - 3.736	0.664	52.9	7	27.4	87.1
Plasma	Post et al. (2007)	27.5	71.0	y = 0.569x - 6.505	0.375	65.8	5.9	23.2	89.9
	McConnaughey and McRoy (1979)	2.9	5.8	y = 0.595x - 5.707	0.399	63.1	3.2	24.6	89.9
	Kiljunen et al. (2006)*	21.7	85.5	y = 0.624x - 5.934	0.426	59.9	0	24.6	89.9
	This study (LE vs bulk)	1.4	21.7	y = 0.341x - 9.836	0.200	82.8	22.9	24.6	87.0
RBC	Post et al. (2007)*	2.8	59.7	y = 0.974x - 0.888	0.841	15.4	0	23.6	97.2
	McConnaughey and McRoy (1979)*	15.3	65.3	y = 0.974x - 0.077	0.839	16.6	1.2	25.0	97.2
	Kiljunen et al. (2006)	0	34.7	y = 0.973x -1.057	0.837	17.5	2.1	27.8	95.8
	This study (LE vs bulk)	8.3	72.2	y = 0.967x - 0.902	0.840	16.1	0.5	20.8	95.8
Liver	Post et al. (2007)	0	6.8	y = 0.153x - 13.816	0.370	74.0	10	11.4	63.6
	McConnaughey and McRoy (1979)*	6.8	36.4	y = 0.485x - 8.347	0.498	64.0	0	18.2	72.7
	Kiljunen et al. (2006)*	0	2.3	y = 0.449x - 9.564	0.488	64.9	0.9	18.2	72.7
	This study (LE vs bulk)	0	2.3	y = 0.395x - 8.750	0.249	81.7	17.7	13.6	63.6

Note: adjusted $P_{0.1}$ and $P_{0.5}$ are taken after the linear equation was used to standardize lipid-normalized $\delta^{13}C$ values. * represent best models for each tissue. Results for 'this study' are based on regressions between LE $\delta^{13}C$ and bulk $\delta^{13}C$ values for comparison.

3.4 Discussion

The 196-day feeding trial that consisted of sampling five tissues lethally and non-lethally from 43 individual *P. leopardus* revealed expected variation in stable isotope dynamics and associated metrics, which have implications for their use in studies with this species. Overall, RBC and muscle tissues produced the least variable and most reliable estimates of DTDFs and turnover rates associated with the captive diet, as well as comparisons between LE and bulk C:N, and accounting for lipid-related bias. By contrast, stable isotope trends in lipid-rich liver were variable independent of lipid extraction suggesting caution is needed when used in future work with this species, and others like it. Stable isotope values in plasma and fin, both non-lethal sampling methods, reflected short-term dietary patterns (half-life <70 days), while diet-assimilation was slowest in muscle and RBC (half-life >80 days). Diet-tissue discrimination factors for $\delta^{15}N$ were <2‰ for all tissues – lower than values commonly reported in the literature (e.g., ~3.4‰; see Post 2002). By contrast, DTDFs for $\delta^{13}C$ ranged between 0‰ and 4‰, demonstrating that stepwise-enrichment in ^{13}C was not negligible for some tissues.

Tissue turnover for $\delta^{15}N$ and $\delta^{13}C$

As expected, given *P. leopardus* in this study were medium-large sized adults (mature at ~36 cm; Ferreira 1995) with slow growth rates compared to juveniles (Ferreira and Russ 1994) most $\delta^{15}N$ incorporation was driven by metabolism, as opposed to growth. Growth contributed <10% of turnover in the metabolically slower tissues such as muscle and RBC, and ~1% in tissues with fast turnover such as liver. A few studies have examined the contribution of growth to isotope incorporation in larger slow-growing species and also found that metabolic processes such as tissue catabolism and protein synthesis were the main drivers of turnover rates (Suring and Wing 2009; German and Miles 2010; Nelson et al. 2011). By contrast, growth contributed more to turnover rates in smaller juveniles with faster relative growth (Marcogliese 2001; Suzuki et al. 2005; Reich et al. 2008). For example, in hatchery-reared juvenile summer flounder (*Paralichthys dentatus*) with growth rates of 0.00816 day^{-1} (compared to 0.00084 day^{-1} in this study), growth contributed ~11% in liver and >50% in blood and muscle (Buchheister and Latour 2010). Since *P. leopardus* have a minimum retain size of 38 cm in commercial and recreational fisheries, only adults were examined to address stable isotope ecology in the context of fisheries management.

In LE tissues, $\delta^{15}N$ turnover rates from quickest to slowest were liver, fin, plasma, RBC, and muscle with half-lives between 21 and 126 days. In bulk tissues, the order was liver, plasma, fin, muscle, and RBC with half-lives between 10 and 90 days. The differences in turnover rates and estimated half-lives between tissues, independent of tissue treatment approach, match

relatively well with the few studies using medium-large sized fish (Table 6). For example, half-lives of $\delta^{15}N$ in plasma and fin were relatively short in the adult catfish *Pterygoplichthys disjunctivus* (<35 days; German and Miles 2010) and similar rates have been determined in liver for juvenile species of goby (*Pomatoschistus minutus*) and *P. dentatus* (Guelinckx et al. 2007; Buchheister and Latour 2010). Plasma and liver are hypothesized to have similar turnover rates because plasma proteins are mainly synthesized in the liver (Turner and Hulme 1970; Adkins et al. 2002; Reich et al. 2008). However, in this study, for LE and bulk treatments $\delta^{15}N$ turnover in liver was quicker than in plasma (i.e., half-life up to 45 days earlier in liver), and may indicate different catabolic processes involved, although the large amount of liver $\delta^{15}N$ variation throughout the feeding trial may have confounded the estimate. Muscle and RBC $\delta^{15}N$ values fitted the turnover rate models best for both LE and bulk tissues. Not surprisingly, estimates of RBC $\delta^{15}N$ turnover in this study (half-life ~90 days) were considerably higher than determined for smaller and faster growing adult *P. disjunctivus* (half-life ~10 days, $k_g = 0.0017$ day^{-1}; German and Miles 2010). Nevertheless, $\delta^{15}N$ incorporation rates in RBC are commonly slower than plasma solutes and faster than (or similar to) muscle (Fischer et al. 1998; Dalerum and Angerbjörn 2005; Kim et al. 2012a). Turnover rates of $\delta^{15}N$ in muscle vary between studies but are slower than other tissues because protein synthesis and degradation rates are slow (Table 3.6; Smith 1981; Houlihan et al. 1988; de la Higuera et al. 1999). For example, the estimated $\delta^{15}N$ half-life in muscle of leopard shark (*Triakis semifasciata*), is ~225 days compared to RBC and plasma which are ~100 and 40 days, respectively (Nelson et al. 2011). Muscle is also the tissue commonly sampled for isotopic studies because values are less variable within and between individuals (Pinnegar and Polunin 1999; Kelly et al. 2006). A longer sampling period would have improved turnover estimates, particularly for tissues with slower turnover such as muscle but logistically was not possible.

Table 3.6: Summary of previously published nitrogen turnover (reflecting growth and metabolic incorporation) and diet-tissue discrimination factors ($\Delta^{15}N$ and $\Delta^{13}C$) in different fish tissues.

Source	Species	Tissue	Temperature (°C)	Maturity	Mass (g)	Length (mm)	$\delta^{15}N$ Turnover rate (day^{-1})	$\delta^{15}N$ Half life (days)	$\Delta^{15}N$	$\Delta^{13}C$
Herzka and Holt (2000)	Red drum (*Sciaenops ocellatus*)	Whole	24,28	Larvae	<0.1	<7	0.25	2.8[a]	1.5-4.2	0.2-1.9
Herzka et al. (2001)	Red drum (*Sciaenops ocellatus*)	Whole	16-30	Larvae	<0.1	<7	>0.058	<12[a]	6	
Vander Zanden et al. (1998)	Smallmouth bass (*Micropterus dolomieu*)	Whole		Larvae/juvenile	<0.1	<50	0.14-0.23[a]	3-5[a]		
Bosley et al. (2002)	Winter flounder (*Pseudopleuronectes americanus*)	Whole	13,18	Juvenile	<0.1		0.18,0.22	3.9,3.1	-0.3-2.2	2-2.5
Witting et al. (2004)	Summer flounder (*Paralichthys dentatus*)	Whole	13,22	Larvae	<0.1		0.09, 0.22[a]	8,3[a]	3.8,2.9	0.2,0.6
		Whole	13,22	Larvae	<0.1		0.05,0.11[a]	14,6[a]	2.8,3.1	0.5,0.9
		Whole	13,22	Juvenile	0.34,0.43	37.8-61.3	0.01,0.01[a]	63,99[a]	12.2,3.7	-0.2,0.2
Maruyama et al. 2001)	Goby (*Rhinogobius* sp.)	Muscle	13,22	Juvenile	0.1-0.9		0.007-0.021[a]	33-99[a]	5.1	
Logan et al. (2006)	Mummichog (*Fundulus heteroclitus*)	Muscle	18	Juvenile	0.84-1.75		-2.33[b]		-1.0,0.2	
		Liver	18	Juvenile	0.84-1.75		-5.85[b]		0,1.2	
McIntyre and Flecker (2006)	Armoured catfish (*Ancistrus triradiatus*)	Muscle	20-27	Juvenile	0.24-2.64		0.038	18.2		
		Blood	20-27	Juvenile	0.24-2.64		0.041	16.9		
		Fin	20-27	Juvenile	0.24-2.64		0.057	12.2		
Guelinckx et al. (2007)	Sand goby (*Pomatoschistus minutus*)	Muscle	17	Juvenile	~5-15	>42	0.025	27.8	3.4	1
		Heart	17	Juvenile	~5-15	>42	0.026	26.6	1	0.84
		Liver	17	Juvenile	~5-15	>42	0.251	2.8	1.35	-3.92
Sweeting et al.	European sea bass	Muscle	4-17	Juvenile	8.0-48.5		0.0140, 0.0215	49.5, 32.2		

(2005)	(*Dicentrarchus labrax*)	Heart	4-17	Juvenile	8.0-48.5		0.0202, 0.0507	34.3, 13.7		
		Liver	4-17	Juvenile	8.0-48.5		0.0193, 0.0196	35.9, 35.4		
German and Miles (2010)	Catfish (*Pterygoplichthys disjunctivus*)	Fin	25	Adult	>51	>128	0.021	33[c]	1.29	-0.93
		RBC	25	Adult	>51	>128	0.0715	9.7[c]	5.17 ± 0.13[e]	0.24 ± 0.56[e]
		Plasma	25	Adult	>51	>128	0.0905	7.7[c]	4.39 ± 0.05[e]	0.06 ± 0.08[e]
Suzuki et al. (2005)	Japanese temperate bass (*Lateolabrax japonicus*)	Fin	23	Juvenile	9.87-94.1	84.0-178.0	0.031	22.4	2.21	3.66
		Muscle	23	Juvenile	9.87-94.1	84.0-178.0	0.036	19.3	2.41	2.4
		Liver	23	Juvenile	9.87-94.1	84.0-178.0	0.048	14.4	0.59	0.34
Hesslein et al. (1993)	Broad whitefish (*Coregonus nasus*)	Muscle	10	Juvenile	5.1-325	51-210	0.032-0.072	9-22	3.8	2
		Liver	10	Juvenile	5.1-325	51-210	0.033-0.073	9-22	3.8	2
MacAvoy et al. (2001)	Channel catfish (*Ictalurus punctatus*)	Muscle	11-19	Spawning adult			0.017-0.023	30-39		1.3
		Blood	11-19	Spawning adult			0.022-0.027	25-32		1.5
		Barbel	11-19	Spawning adult			0.039-0.044	15-18		
MacNeil et al. (2006)	Ocellate stingray (*Potamotrygon motoro*)	Cartilage	26	Juvenile/adult	>106		0.005	133.3		
		Muscle	26	Juvenile/adult	>106		0.007	97.6		
		Blood	26	Juvenile/adult	>106		0.011	61.3		
		Liver	26	Juvenile/adult	>106		0.018	38.5		
Harvey et al. (2002)	Lake trout (*Salvelinus namaycush*)	Muscle	10.6	Juvenile	55-196		0.0005[a]	69[a]	~ -0.7	~ 3.0
Trueman et al. (2005)	Atlantic salmon (*Salmo salar*)	Liver		Juvenile	48.5-341.7	132-334	22[d]	2.25[d]	0.0 ± 0.3[f]	1.6 ± 0.3[f]
		Muscle		Juvenile	48.5-341.7	132-334	40[d]	1[d]	2.3 ± 0.3[f]	2.1 ± 0.1[f]
Tarboush et al. 2006)	Zebra danio (*Danio rerio*)	Muscle	28.5	Adult			0.0047	147	7.4	~ 2
Buchheister and Latour (2010)	Summer flounder (*Paralichthys dentatus*)	Muscle	20	Juvenile/early adult	26.3-446.0	130-325	0.0082,0.0065	84.9,106.5	2.13 ± 0.12[e]	3.10 ± 0.51, 4.79 ±

										0.36[e]
		Blood	20	Juvenile/early adult	26.3-446.0	130-325	0.0158, 0.021	43.8,33.0	2.26 ± 0.32, 3.86 ± 0.29[e]	3.11 ± 0.17[e]
		Liver	20	Juvenile/early adult	26.3-446.0	130-325	0.0632	10	1.45 ± 0.10, 2.23 ± 0.09[e]	2.86 ± 0.09[e]
This study (lipid extracted)	Leopard coralgrouper (*Plectropomous leopardus*)	Muscle	27-29	Adult	660-2730	365-527	0.006	126	1.8 ± 1.5[e]	1.1 ± 0.2[e]
		RBC	27-29	Adult	660-2730	365-527	0.008	88	1.1 ± 0.5[e]	0.1 ± 0.1[e]
		Plasma	27-29	Adult	660-2730	365-527	0.011	66	0.9 ± 0.2[e]	1.2 ± 0.1[e]
		Fin	27-29	Adult	660-2730	365-527	0.019	37	0.9 ± 0.2[e]	3.2 ± 0.1[e]
		Liver	27-29	Adult	660-2730	365-527	0.034	21	0.0 ± 0.2[e]	1.5 ± 0.1[e]
Kim et al. (2012a)	Leopard shark (*Triakis semifasciata*)	Muscle	13-17	Juvenile/adult	1000-4250	600-1000	0.00307	225.8[c]	5.5 ± 0.4[f]	3.5 ± 0.6[f]
		RBC	13-17	Juvenile/adult	1000-4250	600-1000	0.00687	100.9[c]	4.6 ± 0.3[f]	2.8 ± 0.6[f]
		Plasma	13-17	Juvenile/adult	1000-4250	600-1000	0.0172	40.3[c]	4.2 ± 0.3[f]	3.7 ± 0.4[f]

[a] represents estimates calculated by McIntyre and Flecker (2006)

[b] calculation derived from a growth-based model, where if c=-1 growth is entirely responsible for turnover and lower values have increasingly greater metabolic contribution

[c] half-life value estimated from turnover rate using $T_{\alpha} = \ln (1- \alpha)/-v$

[d] study estimates were monthly instead of daily

[e] is standard error (SE)

[f] is standard deviation (SD)

Tissue turnover for $\delta^{13}C$ could not be determined due to the lack of consistent temporal trends in $\delta^{13}C$ values. There are a few reasons why this may have been the case. First, variation in dietary *N. theodorei* $\delta^{13}C$ may have resulted in a variable exposure to $\delta^{13}C$ values in *P. leopardus*. Second, there appeared to be more inherent $\delta^{13}C$ variability in tissues compared to $\delta^{15}N$, particularly plasma, RBC, and muscle, which made fitting models more difficult (see also Post 2002; Pinnegar and Polunin 1999). Finally, the most likely reason why turnover could not be calculated was because $\delta^{13}C$ of *N. theodorei* was similar to $\delta^{13}C$ values of prey consumed on the reef in the wild. Hence no significant isotopic change was found over time because $\delta^{13}C$ values in the wild did not vary sufficiently compared to aquarium values.

Although equilibrium values could not be confirmed for $\delta^{13}C$ in different tissues, mean values throughout the trial remained similar across sampling dates, especially after day 98 of the experiment; thus $\Delta^{13}C$ values appear to be suitable for most tissues. Furthermore, $\delta^{13}C$ turnover is commonly faster than $\delta^{15}N$ turnover in fish tissues (MacAvoy et al. 2001; Suzuki et al. 2005; Guelinckx et al. 2007; Buchheister and Latour 2010), suggesting the elapsed time before calculating $\Delta^{13}C$ was more than adequate to represent equilibrium values.

Diet-tissue discrimination factors for $\delta^{15}N$ and $\delta^{13}C$

Diet-tissue discrimination factors of $\delta^{15}N$ varied between tissues. Muscle and RBC had the highest DTDFs, followed by plasma and fin, and liver had the lowest $\Delta^{15}N$ values, between -0.2 and 0.0‰, indicating relatively little change in $\delta^{15}N$ values between consumer and prey. This order in tissue $\Delta^{15}N$ matches well with other studies (Table 3.6). For example, previous work has found that liver $\delta^{15}N$ and $\Delta^{15}N$ are usually lower than in muscle for fish (MacNeil et al. 2006; Buchheister and Latour 2010; Matley et al. 2013). Pinnegar and Polunin (1999) hypothesized that fish muscle is typically more ^{15}N enriched because of the high abundance of the non-essential amino acid taurine. By contrast, fish liver contains less taurine and more essential amino acids, which fractionate less during tissue catabolism (Wilson and Poe 1974; Pinnegar and Polunin 1999; McMahon et al. 2010). Similarly, the order of $\Delta^{15}N$ values in liver, fin, and muscle of juvenile bass (*Lateolabrax japonicas*; Suzuki et al. 2005) followed the present study (i.e., $\Delta^{15}N_{liver} < \Delta^{15}N_{fin} < \Delta^{15}N_{muscle}$). Although few studies have compared DTDFs in blood components with other tissues in fish, variation in biochemical composition, specifically the relative abundance of amino acids appears to be the main factor responsible for different DTDFs among tissues (Gaebler et al. 1966; Pinnegar and Polunin 1999).

In general, $\delta^{15}N$ DTDFs in this study were lower (range: -0.2 – 1.8‰) than commonly used or reported values in fish, particularly for muscle (~2-5‰) (Table 3.6; see also Sweeting et al. 2007b). Muscle $\Delta^{15}N$ values had reduced precision in DTDF estimates due to the relatively

large standard errors associated with the consumer $\delta^{15}N$ not being equilibrated to the diet (see also Buchheister and Latour 2010). Consequently, DTDFs for muscle may have been underestimated as demonstrated by predicted $\Delta^{15}N$ values (2.1 - 2.8‰, Caut et al. 2009; 3.0‰, Hussey et al. 2014) from linear relationships with dietary $\delta^{15}N$ for muscle/whole fish tissue in the literature. A longer sampling period would have increased the precision of muscle $\delta^{15}N$ equilibrium estimates but was beyond the scope of this study. Plasma and RBC $\Delta^{15}N$ values from this study were within the lower range estimated by Buchheister and Latour (2010) for whole blood in *P. dentatus* (1.1 – 2.8‰) and lower than plasma (4.4‰) and RBC (5.2‰) values determined for the herbivore *P. disjunctivus*. Fin $\Delta^{15}N$ values of *P. leopardus* were also lower compared to juvenile bass (*L. japonicas;* 2.2 – 2.5‰; Suzuki et al. 2005). Despite these differences, variation in $\Delta^{15}N$ is common within the same tissues of different fish species (see Appendix A in Robbins et al. 2010), largely because dietary protein content and quality affects $\Delta^{15}N$ (Bosley et al. 2002; Robbins et al. 2010). Also, most DTDF estimates are based on temperate species, and several studies have found a significant relationship between decreasing $\Delta^{15}N$ and increasing water temperature (Nagai and Suzuki 2000; Olive et al. 2003; Trueman et al. 2005). Therefore, DTDFs of tropical species may not be readily comparable to those in temperate ecosystems.

Diet-tissue discrimination factors for LE and bulk $\delta^{13}C$ were between 0‰ and 4‰ among tissues. Removing lipids chemically altered $\delta^{13}C$ and $\Delta^{13}C$ values compared to untreated samples (see below). Lipid extraction also changed the order of enrichment between tissues, likely in response to the adjusted lipid content in relation to other biochemical fractions (Pinnegar and Polunin 1999). Similar to the $\Delta^{15}N$ tissue order determined for *L. japonicas*; Suzuki et al. 2005), $\Delta^{13}C$ also matched this study ($\Delta^{13}C_{liver} < \Delta^{13}C_{muscle} < \Delta^{13}C_{fin}$) for untreated samples. The high lipid content in liver resulted in lower $\delta^{13}C$ values and hence lower $\Delta^{13}C$ compared to other tissues. By contrast, fin tissue, which consists of mainly collagen (Hanisch et al. 2010), had the highest $\Delta^{13}C$ compared to other tissues for both LE and bulk samples. Fin tissue is often ^{13}C enriched because of its protein content (Willis et al. 2013), and is unrelated to lipid effects - C:N ratios were low for LE and bulk samples (see Post et al. 2007; Sweeting et al. 2007a).

Commonly, $\delta^{13}C$ DTDFs are assumed to be <1‰ because of limited fractionation between diet and consumer (DeNiro and Epstein 1978; Vander Zanden and Rasmussen 2001; Post 2002). However, Sweeting et al. (2007a) found that $\Delta^{13}C$ in fish tissues such as liver, muscle, heart, and whole body are often between 1‰ and 2‰ (see also Table 6). Based on the negative linear relationship between $\Delta^{13}C$ and dietary $\delta^{13}C$ (Caut et al. 2009), $\Delta^{13}C$ values for all LE tissues in this study were predicted to be ~0.7‰, however this estimate is based only on liver, muscle

and whole body tissues. Nevertheless, only a few tissues had $\Delta^{13}C$ values larger than 2‰ (i.e., LE and bulk fin and bulk muscle) in this study, demonstrating that $\Delta^{13}C$ estimates were consistent with other studies. Compared to $\Delta^{13}C$ values in fin of *L. japonicas* (bulk: 3.1 – 3.7‰), the findings of this study (LE: 3.2‰; bulk: 3.9‰) were similar. The sampling of fin membranes resulted in relatively consistent $\delta^{13}C$ values and improves on other studies where fin tissues were composed of varying tissue elements (e.g., bone, hard spines, and soft rays) which differ in fractionation (Suring and Wing 2009; Willis et al. 2013). Plasma (LE: 1.2‰; bulk: 1.0‰) and RBC (LE: 0.1‰; bulk: 1.6‰) $\Delta^{13}C$ estimates were lower than in leopard sharks (*Triakis semifasciata*) (plasma: 2.8 – 3.7‰, RBC: 2.3 – 2.8‰, Kim et al. 2012a; b), and whole blood $\Delta^{13}C$ values in *P. dentatus*, (bulk: 2.3 – 3.3‰, Buchheister and Latour 2010), yet were similar to published values in marine mammals (see Caut et al. 2011). Further studies are necessary to understand these contrasts, however it may be related to how differences in amino acids affect $\delta^{13}C$ in different blood components and organisms (Kurle 2002; Caut et al. 2011).

Bulk vs Lipid extracted tissues

Tissues that are rich in lipids are often ^{13}C depleted, resulting in lower $\delta^{13}C$ estimates compared to tissues high in proteins or carbohydrates (DeNiro and Epstein 1977; McConnaughey and McRoy 1979). Additionally, there can be considerable heterogeneity in lipid content among species, individuals, and tissues (Hobson and Clark 1992; Sweeting et al. 2006). To reduce bias associated with tissue lipid content, chemical removal of lipids is common, however it may cause fractionation in $^{15}N/^{14}N$ and it is more laborious to process tissues (Pinnegar and Polunin 1999; Sotiropoulos et al. 2004). In this study, it was evident that removing lipids affected both $\delta^{13}C$ and $\delta^{15}N$ values (i.e., only RBC $\delta^{15}N$ values did not significantly change). This was surprising because bulk C:N of three of the five tissues was <3.5, an amount which is considered to produce negligible lipid bias for $\delta^{13}C$ (Post et al. 2007). Other studies have also detected higher muscle $\delta^{15}N$ values after removing lipids (Ingram et al. 2007; Logan et al. 2008; Hussey et al. 2010), and proposed that leaching of nitrogenous metabolites or waste occurs during lipid extraction (Sotiropoulos et al. 2004; Murry et al. 2006). Yurkowski et al. (2015) found that lipid extracts contained small amounts of ^{15}N-depleted nitrogen in liver and muscle tissues of Arctic marine mammals correlating to higher $\delta^{15}N$ values after chemical lipid extraction. Nevertheless, for $\delta^{15}N$, the difference between LE and bulk samples was small (mean $\delta^{15}N_{LE\text{-}Bulk}$ <0.5‰ in all tissues) and often varied little (e.g., muscle, plasma, and RBC), signifying limited influence of lipid extraction on $\delta^{15}N$ in these tissues; lipid extraction should still be considered depending on the specific study. The large change in liver %C, $\delta^{13}C$, and C:N indicated a high amount of lipids in liver which should be treated with caution (see below); while lipid extraction may not be necessary

for RBC and fin which had low lipid content based on small $\delta^{13}C$ and C:N differences, consistent with other studies (Bone and Roberts 1969; Hussey et al. 2010).

Lipid-normalizing models for $\delta^{13}C$, specifically those proposed by Post et al. (2007) and McConnaughey and McRoy (1979) were useful at predicting LE $\delta^{13}C$ in muscle of *P. leopardus*. All three models are derived from various temperate and sub-arctic aquatic invertebrate and vertebrate organisms (McConnaughey and McRoy 1979; Kiljunen et al. 2006; Post et al. 2007). To a large extent, these models are based on measurements from fish muscle tissue, which provides reasoning for the strong correlation with LE muscle $\delta^{13}C$ values in this study. Additionally, it explains why other lean tissues such as RBC and fin were well supported by models due to the small variation in lipid-free C:N ratios in these tissues (Post et al. 2007). For most tissues, corrections using regression models from this study were as informative as other models. Also, muscle tissue does not necessarily require lipid correction as bulk and LE $\delta^{13}C$ values were relatively similar, although accuracy was marginally better using the correction model suggested by Post et al. (2007).

The main purpose of this experiment was to better understand species- and tissue-specific $\delta^{15}N$ and $\delta^{13}C$ values and patterns of tropical coral reef fish so that future ecological studies can interpret isotopic data meaningfully. In general, decisions relating to tissue preparation (e.g., lipid extraction) and tissue selection should be based on the specific goals of the study. For example, if research questions are addressing a particular time period or season, sampling must account for temporal variation in tissue turnover. Also, the feasibility of lethal/non-lethal sampling needs to be considered, especially for species that are facing or are at risk of population declines. Muscle and RBC provided the most reliable ^{15}N turnover estimates and represented similar isotopic incorporation periods. Additionally, LE $\delta^{15}N$, $\delta^{13}C$, and C:N values in muscle and RBC had little variation when compared with bulk values and these tissues worked well with lipid-normalizing models. Therefore, for a relatively long-term representation of feeding habits, RBC or muscle should be used. Both tissues can be sampled non-lethally, however if lethal approaches are deemed necessary, muscle is often more amenable because it can be sampled post-mortality. Similarly, if chemical lipid extraction is deemed too expensive or time-consuming, lipid-normalizing techniques described here can easily be utilized with comparable success.

For future work interested in determining short-term feeding ecology, we suggest plasma or fin, both non-lethal approaches. Both performed similarly in non-linear $\delta^{15}N$ equilibrium models with relatively quick turnover periods (half-life <70 days). Also, bulk $\delta^{15}N$ and C:N values changed little when lipid extracted, and correction models predicted LE $\delta^{13}C$ adequately. Liver is often used in stable isotope studies due to its quick turnover (Pinnegar and

Polunin 1999; Buchheister and Latour 2010; Matley et al. 2013), however this study demonstrated that, at least for this species, it is not likely to be a suitable selection for most studies. Its high lipid content confounded isotopic interpretation, as has been seen in other marine fish (Hussey et al. 2010). For example, when C:N values >3.5, the presence of lipids will likely bias $\delta^{13}C$ estimates (Post et al. 2007). Even after chemical lipid extraction, liver C:N values remained high, suggesting the lipid extraction methods used were not effective for high lipid content. Previous studies have also found high C:N values in fish liver after chemical lipid extraction and cautioned about the difficulty in effectively standardizing high lipid content tissues (Hobson and Clark 1992; Ingram et al. 2007; Hussey et al. 2010).

Estimates of $\delta^{13}C$ and $\delta^{15}N$ DTDFs for many tissues were within range of previous studies, despite some variation within sampling periods. The finding that muscle $\Delta^{15}N$ was less than the commonly used range of 3 to 4‰ (Peterson and Fry 1987; Hobson and Welch 1992; Sweeting et al. 2007b) is important for estimating trophic position and prey proportions in tropical ecosystems more accurately in the future. Based on the estimated $T_{0.95}$, an experimental period of at least twice as long as used in this study would have improved $\Delta^{15}N$ estimates. Nevertheless, isotopic mixing models can account for deviation in parameter estimates to simply provide more conservative outputs (Parnell et al. 2010). Tissue-specific estimates of this kind are not readily available, especially for tropical species, and are necessary to interpret isotope data in feeding ecology studies.

There are limited studies that have calculated DTDFs and turnover rates for medium-large sized or adult fish, particularly for tropical reef fish. Fewer still have additionally sampled numerous different tissues or explored the utility of lipid correction techniques. It is not known how applicable the patterns and estimates from this study are to wild individuals or other species and locations. For example, the composition of macromolecules (i.e., proteins, lipids, carbohydrates) in prey, as well as prey itself (i.e., multiple diet items) will vary for *P. leopardus* in the wild, which could lead to differential DTDFs (McMahon et al. 2015). Some studies have found differences between laboratory and field isotopic estimates (Vander Zanden and Rasmussen 2001; Buchheister and Latour 2010), while other values appear to be robust and applicable in the field (Sweeting et al. 2007a; b). There is also concern that 'unrestricted' laboratory feeding rates may bias stable isotope signatures because they are not representative of natural conditions (e.g., reduced prey availability and increased competition can lead to restricted feeding and growth rates) (Sweeting et al. 2007b). However, during this study, growth was comparable to wild individuals (Ferreira and Russ 1994), they were fed at similar intervals as in the wild, and wild adult *P. leopardus* feed almost exclusively on fish (i.e., high protein diet) (St. John 1999). This study is one of the first to provide experimentally-derived

stable isotope data for an adult tropical fish and is an important step to validate metrics to understand the ecology of this and similar species, as well as reef trophic structure.

Chapter 4

Niche specialisation and spatio-temporal comparisons of feeding ecology in sympatric coral trout (*Plectropomus leopardus and P. laevis*)

4.1 Introduction

Investigating resource use among closely related species with overlapping distributions (sympatry) helps define how competition and prey selection influence ecosystem dynamics (Harper et al. 1961). Sympatric species generally compete for resources, share resources, or exploit different resources to survive and reproduce (Schoener 1983). Resultant interactions shape food web structure, population trends, and habitat selection (e.g., McPhail 1993; Christiansen et al. 2012; Gaston and Elliott 2014) and ultimately the functional role of species within an ecosystem (Harmon et al. 2009).

By incorporating different resource pools or partitioning them to reduce competition, similar species are able to co-exist within distinct ecological niches (Schoener 1974; Ross 1986). The niche of an organism delineates an animal's place in its community, mainly in relation to interactions between its predators and prey (Elton 1927). Therefore, an ecological niche is primarily driven by competition for resources and feeding interactions among organisms, although other factors may contribute (Hilborn and Stearns 1982; Ross 1986). Given the close tie between trophic structure and the functional role of an animal in the community, studying foraging patterns is useful to differentiate ecological (or trophic) niches between sympatric species.

Stable isotope analysis is an increasingly utilised technique to investigate foraging patterns and estimate the niche of aquatic animals (e.g., Espinoza et al. 2015b; Munroe et al. 2015). Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes are used as biological tracers of diet and habitat because they track tissue assimilation from prey to consumer. Specifically, there is a predictable change in bulk $\delta^{13}C$ and $\delta^{15}N$ values at each trophic level, which are used to estimate trophic position (Michener and Schell 1994; Hussey et al. 2014), prey composition (Layman et al. 2011), and basal sources of carbon and nitrogen in a particular food chain (Hobson 1999). For example, organisms with higher $\delta^{15}N$ values consume prey at higher trophic levels because consumer tissues are ^{15}N-enriched (i.e., $\delta^{15}N$ increases at each trophic exchange) (Minigawa and Wada 1984). Also, $\delta^{13}C$ can provide an indication of feeding habitat

because carbonates (^{13}C) of primary producers fractionate at different rates (i.e., $\delta^{13}C_{C4\ plants} > \delta^{13}C_{C3\ plants}$) and organic deposition can increase $\delta^{13}C$ values in the benthic environment (Hobson et al. 1995; France 1995; Vander Zanden and Rasmussen 1999). As a result, isotopic niche space can be determined (e.g., $\delta^{13}C$ - $\delta^{15}N$ bi-plot) to reflect trophic structure and habitat selection. Moreover, isotopic niche provides an indication of the breadth (or isotope range) of resource use and provides insights on specialist and generalist feeding tendencies (Bearhop et al. 2004). Overlap in bulk isotopic niche space can be compared between species (or populations) to quantify differences in resource exploitation. Insight into temporal or spatial variation in foraging within species can also be elucidated by comparing niche space at different time periods or locations, or within the same individual by using different tissues with variable turnover rates (see Newsome et al. 2010). The utility of spatial or temporal comparisons, however, is limited unless baseline $\delta^{13}C$ and $\delta^{15}N$ are standardised between periods/locations (Tamelander et al. 2009).

Knowledge of resource-use overlap between sympatric species is important from an ecological context, but also has significant bearing on management and conservation of exploited species. Closely related species are often treated as single stocks due to similar morphology and distribution (Heupel et al. 2010). Grouping sympatric species for management purposes can be problematic because life history traits and population dynamics are often different (Currey et al. 2013). Therefore, fishery assessments (e.g., based on the demography and population status) such as calculating catch and size limits, or delineating essential habitats may be erroneous or biased to the more abundant or 'better' studied species. The coral trout (or coralgrouper) species complex is a prime example. Coral trout (*Plectropomus* spp. and *Variola* spp.) are the main commercial fishery target in the Great Barrier Reef, Australia, and other Indo-Pacific regions (Mapstone et al. 2004). The complex consists of several species, but fishery data are often compiled as one stock despite varying life history traits. For example, *P. laevis* matures at a younger age (~1 year) and grows larger (~100 cm max length) than the more abundant and widespread *P. leopardus* (matures at 2-3 years; ~60 cm max length) (Ferreira 1995; Heupel et al. 2010). Additionally, *P. laevis* undergoes a dramatic colour transition from footballer (white/yellow/black) to blue-spot (dark with large blue spots) phase upon reaching ~30 cm (~1:1 footballer:blue-spot at 50 cm; Heupel et al. 2010). Both species overlap in distribution but little is known about resource and habitat selection, particularly for *P. laevis*, which remains poorly studied.

This study investigated ecological niche specialisation between *P. leopardus* and *P. laevis* to better understand how agonistic interactions affect resource partitioning in closely related and co-occurring species. Variation in feeding ecology within and between *P. leopardus* and *P.*

laevis, was quantified using $\delta^{13}C$ and $\delta^{15}N$. Multi-tissue (muscle, red blood cells, plasma) sampling took place at four reefs in the Great Barrier Reef Marine Park (GBRMP) during three sampling periods to examine spatial and temporal influences on isotopic niche overlap and breadth. This study is the first to compare resource use of these two sympatric species, both integral parts of reef fisheries in the Indo-Pacific. Knowledge of how predatory species select resources over time and space is critical to understanding competition, community structure, and the functional role of predators in complex ecosystems. A species-specific understanding of the ecological niche of sympatric fishes is necessary to determine how they will be affected by human or environmental disturbances, and will contribute to more directed biodiversity and conservation initiatives (e.g., marine protected areas).

4.2 Methods

Study area and sample collection

Plectropomus leopardus (n = 117) and *P. laevis* (n = 39) were collected at Lodestone, Helix, Yankee, and Coil reefs in the GBRMP between August 2013 and February 2014 (see **section 2.2.1**; Table 4.1). Coral trout were collected using a speargun while diving with SCUBA and five tissues were sampled for stable isotopes as described in **section 2.3.2**. Visual inspection of gut contents was conducted as a supportive tool to stable isotope results.

In addition, coral (*Pocillopora damicornis* and *P. verrucosa*), macroalgae (*Chlorodesmis* spp. and *Halimeda* spp.), plankton, and the planktivorous fusilier - *Caesio teres*, were collected at sampling reefs/periods to control for variation in baseline stable isotope values (Table 4.2). Ancillary species collection was limited by sampling/time constraints, therefore, only a fragmented representation of the reef food web was examined. Coral was collected by removing a small fragment from independent colonies with pliers. A handful of macroalgae was similarly collected with cutters. Plankton (phytoplankton and zooplankton) was collected in 20 ml vials using a 63 μm net held ~5 m deep for 15 min when the main vessel was stationary. Muscle tissue was collected from speared fusiliers (processed the same as coral trout tissues). Samples were frozen (-20° C) after collection until laboratory processing. In the laboratory, coral tissue (zooxanthellae and animal tissue) was removed from the frozen coral skeleton with a modified airbrush connected to a dive cylinder containing compressed air. Tissues were air-brushed into a small bag containing distilled water. Water was removed from the sample after contents were poured onto 70 mm filter paper (Whatman Glass Microfilters GF/F; Piscataway, NJ, USA) placed in a vacuum filtration system. Similarly, vials containing plankton were thawed and rinsed (distilled water) onto filter paper. Coral tissue, plankton (on

filter paper) and macroalgae (in petri dishes) were oven-dried (60° C) for 48 h then ground into a powder.

Based on findings from **Chapter 3**, plasma, RBC, and muscle were selected for stable isotope analysis because they best represented medium- (plasma $\delta^{15}N$ half-life <70 days) and long-term (RBC and muscle $\delta^{15}N$ half-life >80 days) diet assimilation. Prior to analysis, some tissues were chemically treated to remove lipids or inorganic carbonates, known to bias $\delta^{13}C$ values (see Post et al. 2007; Schlacher and Connolly 2014). Lipids were removed from plasma, RBC, muscle (of coral trout and *C. teres*), and coral tissues following McMeans et al. (2009) by adding a 5 ml 2:1 chloroform/methanol solvent to a <1 g subsample, vortexed for 30 s, and left for 24 h in a 30° C water bath. Afterwards, another 5 ml of solvent was added, vortexed, poured out, and the tissue left to dry for 24 h. Coral, macroalgae, and plankton samples were acid-treated following Kennedy et al. (2005) to remove inorganic carbonates by adding a few drops of 1 M hydrochloric acid into silver cups (with sample) until effervescence ceased. If carbonates remained (as indicated by effervescence), the procedure was repeated the next day after oven-drying samples overnight. A subsample of *Chlorodesmis* spp. was HCl-treated but showed no presence of carbonates; as a result, they were not acid-treated. The addition of HCl to samples might bias $\delta^{15}N$ values (Schlacher and Connolly 2014); therefore, untreated coral, macroalgae, and plankton samples were also analyzed separately for $\delta^{15}N$ values.

Table 4.1: Mean (± SE) stable isotope ($\delta^{15}N/\delta^{13}C$) values (‰) from *Plectropomus leopardus* and *P. laevis* muscle, red blood cells (RBC), and plasma tissues sampled at four reefs between August 2013 and February 2014 (with corresponding sample size in brackets).

Tissue	Species	Date	Reef	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Plasma	*P. leopardus*	November 2013	Lodestone	-15.8 ± 0.2 (n=14)	10.9 ± 0.1 (n=14)
			Helix	-16.3 ± 0.1 (n=14)	11.7 ± 0.1 (n=14)
			Yankee	-15.1 ± 0.4 (n=15)	11.0 ± 0.2 (n=15)
			Coil	-14.6 ± 0.3 (n=10)	10.7 ± 0.2 (n=10)
		February 2014	Lodestone	-15.5 ± 0.4 (n=8)	10.3 ± 0.1 (n=8)
			Helix	-15.8 ± 0.3 (n=19)	10.7 ± 0.1 (n=19)
	P. laevis (blue-spot)	November 2013	Helix	-13.2 ± 0.9 (n=3)	10.6 ± 0.3 (n=3)
			Yankee	-12.7 ± 0.3 (n=9)	10.3 ± 0.1 (n=9)
			Coil	-12.4 ± 0.3 (n=16)	10.3 ± 0.1 (n=16)
	P. laevis (footballer)	November 2013	Helix	-16.4 (n=1)	11.3 (n=1)
			Yankee	-13.3 ± 0.3 (n=4)	10.2 ± 0.2 (n=4)
			Coil	-15.5 (n=1)	10.5 (n=1)
RBC	*P. leopardus*	November 2013	Lodestone	-16.4 ± 0.4 (n=14)	9.8 ± 0.1 (n=14)
			Helix	-17.0 ± 0.1 (n=18)	10.3 ± 0.1 (n=18)
			Yankee	-15.3 ± 0.2 (n=16)	9.7 ± 0.1 (n=16)
			Coil	-15.4 ± 0.3 (n=15)	9.8 ± 0.2 (n=15)
		February 2014	Lodestone	-16.6 ± 0.3 (n=10)	9.3 ± 0.1 (n=10)
			Helix	-17.0 ± 0.2 (n=20)	9.8 ± 0.1 (n=20)
	P. laevis (blue-spot)	November 2013	Helix	-14.5 ± 0.6 (n=3)	9.7 ± 0.2 (n=3)
			Yankee	-13.9 ± 0.3 (n=9)	9.2 ± 0.1 (n=9)
			Coil	-12.8 ± 0.3 (n=15)	9.2 ± 0.1 (n=15)
	P. laevis (footballer)	November 2013	Yankee	-14.9 ± 0.2 (n=5)	9.3 ± 0.1 (n=5)
			Coil	-14.5 ± 1.1 (n=2)	9.7 ± 0.5 (n=2)
Muscle	*P. leopardus*	August 2013	Helix	-14.8 ± 0.4 (n=11)	11.1 ± 0.1 (n=11)
			Lodestone	-15.2 ± 0.4 (n=16)	10.8 ± 0.1 (n=16)
		November 2013	Helix	-16.0 ± 0.1 (n=19)	11.4 ± 0.1 (n=19)
			Yankee	-14.8 ± 0.4 (n=19)	10.6 ± 0.1 (n=19)
			Coil	-14.7 ± 0.3 (n=20)	10.6 ± 0.1 (n=20)
		February 2014	Lodestone	-15.3 ± 0.3 (n=11)	11.2 ± 0.1 (n=11)
			Helix	-15.8 ± 0.2 (n=21)	10.9 ± 0.1 (n=21)
	P. laevis (blue-spot)	November 2013	Helix	-13.8 ± 0.7 (n=3)	10.3 ± 0.1 (n=3)
			Yankee	-13.4 ± 0.4 (n=9)	10.3 ± 0.1 (n=9)
			Coil	-12.5 ± 0.2 (n=16)	9.9 ± 0.1 (n=16)
	P. laevis (footballer)	November 2013	Yankee	-14.2 ± 0.2 (n=5)	10.4 ± 0.1 (n=5)
			Coil	-13.6 ± 0.6 (n=3)	10.1 ± 0.2 (n=3)

Table 4.2: Mean (± SE) stable isotope ($\delta^{15}N/\delta^{13}C$) values (‰) of baseline consumers/producers from designated periods/locations, and corresponding baseline-consumer carbon ratios (BCCRs) calculated for *P. leopardus* (corresponding sample size in brackets).

Species	Date	Reef	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	BCCR
Coral (*Pocillopora verrucosa*)	November 2013	Lodestone	-15.9 ± 0.4 (n=3)	4.8 ± 0.2 (n=3)	0.2 ± 0.1 (n=16)
		Helix	-15.4 ± 0.2 (n=4)	5.2 ± 0.1 (n=4)	-0.1 ± 0.1 (n=19)
		Yankee	-15.6 ± 0.4 (n=4)	5.0 ± 0.1 (n=4)	0.3 ± 0.1 (n=18)
		Coil	-14.1 ± 0.2 (n=3)	5.0 ± 0.2 (n=3)	-0.2 ± 0.1 (n=20)
Coral (*Pocillopora damicornis*)	November 2013	Lodestone	-16.5 ± 0.2 (n=3)	4.9 ± 0.2 (n=3)	0.3 ± 0.1 (n=16)
	February 2014	Lodestone	-16.5 ± 0.2 (n=3)	4.1 ± 0.2 (n=2)	0.3 ± 0.1 (n=11)
		Helix	-15.9 ± 0.2 (n=4)	4.2 ± 0.1 (n=4)	0.0 ± 0.1 (n=21)
Algae (*Chlorodesmis* spp.)	November 2013	Lodestone	-22.4 ± 1.1 (n=2)	3.0 ± 0.1 (n=2)	1.4 ± 0.1 (n=16)
		Helix	-23.2 ± 0.4 (n=3)	2.7 ± 0.1 (n=3)	1.4 ± 0.1 (n=19)
		Yankee	-23.4 ± 0.6 (n=4)	2.7 ± 0.2 (n=4)	1.8 ± 0.1 (n=18)
		Coil	-23.6 ± 0.8 (n=3)	2.3 ± 0.5 (n=3)	1.8 ± 0.1 (n=20)
	February 2014	Lodestone	-23.4 ± 0.4 (n=2)	2.2 ± 0.1 (n=2)	1.6 ± 0.1 (n=11)
		Helix	-22.6 ± 0.1 (n=2)	2.9 ± 0.1 (n=2)	1.4 ± 0.1 (n=21)
Algae (*Halimeda* spp.)	November 2013	Lodestone	-16.8 ± 0.5 (n=4)	3.3 ± 0.2 (n=4)	0.3 ± 0.1 (n=16)
		Helix	-18.6 ± 1.4 (n=2)	3.5 ± 0.1 (n=2)	0.5 ± 0.1 (n=19)
		Coil	-21.6 ± 0.1 (n=4)	2.8 ± 0.3 (n=4)	1.5 ± 0.1 (n=20)
	February 2014	Lodestone	-17.9 ± 0.6 (n=4)	2.7 ± 0.3 (n=4)	0.5 ± 0.1 (n=11)
		Helix	-17.9 ± 0.7 (n=2)	2.1 ± 0.4 (n=2)	0.4 ± 0.1 (n=21)
Plankton	August 2013	Lodestone	-21.3 ± 0.1 (n=2)	6.8 ± 0.2 (n=2)	NA
		Helix	-21.4 ± 0.1 (n=3)	6.6 ± 0.1 (n=3)	2.1 ± 0.1 (n=11)
	February 2014	Lodestone	-19.6 ± 0.6 (n=3)	4.9 ± 0.8 (n=3)	1.4 ± 0.1 (n=11)
		Helix	-20.3 ± 0.2 (n=3)	6.1 ± 0.2 (n=3)	1.5 ± 0.1 (n=21)
		Yankee	-21.2 ± 0.4 (n=3)	4.9 ± 0.2 (n=3)	NA
Fusilier (*Caesio teres*)	November 2013	Lodestone	-17.8 ± 0.1 (n=9)	9.9 ± 0.1 (n=9)	3.5 ± 0.5 (n=16)
		Helix	-17.8 ± 0.1 (n=10)	10.0 ± 0.1 (n=10)	2.4 ± 0.1 (n=19)
	February 2014	Lodestone	-17.9 ± 0.1 (n=5)	9.8 ± 0.1 (n=5)	3.5 ± 0.4 (n=11)
		Helix	-17.8 ± 0.1 (n=4)	9.6 ± 0.1 (n=4)	2.8 ± 0.3 (n=21)

Note: baseline-consumer carbon ratios close to 1 represent an idealised source of carbon in the *P. leopardus* food web (see Eq. 2).

Analysis

Initially, to explore the relationship between muscle $\delta^{13}C$ and $\delta^{15}N$ with fish size (fork length) where *P. leopardus* and *P. laevis* (footballer and blue-spot phases) co-occurred (Helix, Yankee, and Coil reefs), a least-squares linear regression was done for each species/phase. Colour phases of *P. laevis* were investigated separately based on correlations with size (Heupel et al. 2010) which may influence feeding patterns. Stable isotope niche size and overlap was assessed using the R package SIAR (Parnell et al. 2010) in R, version 3.0.2 (R Core Team 2014), following Jackson et al. (2011). Bayesian standard ellipses, representing the "typical" niche breadth of individuals (Jackson et al. 2011; Layman and Allgeier 2012), were plotted on an isotope bi-plot for each species/phase at three reefs (Helix, Yankee, Coil) and for three tissues (muscle, RBC, plasma). Areas of each standard ellipse (SEA_B) were calculated to determine niche breadth overlap between species/phases (presented as % of shared isotopic space). Differences in SEA_B size were considered significant when ≥95% of posterior draws (10^4) for one species/phase were smaller than the other.

To facilitate comparison of *P. leopardus* stable isotopes at different locations and periods, values were standardised using baseline producers/consumers. To determine which baseline values were appropriate (i.e., best represented the source(s) of carbon within the *P. leopardus* food web), a baseline-consumer carbon ratio (BCCR) was calculated for coral, macroalgae, plankton, and *C. teres* and corresponding *P. leopardus* (i.e., from same sampling location and period) following (Nawrocki et al. *In review*):

$$(1)\ \text{Baseline-consumer carbon ratio (BCCR)} = \frac{\Delta\delta^{13}\mathrm{C}_{consumer-baseline}/\mathrm{DTDF}_{\delta^{13}C}}{\Delta\mathrm{TP}_{consumer-baseline}}$$

where $\Delta\delta^{13}C_{consumer\text{-}baseline}$ is the difference in $\delta^{13}C$ values between *P. leopardus* and the corresponding mean baseline value; $DTDF_{\delta^{13}C}$ is the $\delta^{13}C$ diet-tissue discrimination factor (DTDF) calculated for *P. leopardus* (muscle: 1.1‰; **Chapter 3**); and $\Delta TP_{consumer\text{-}baseline}$ represents the difference in trophic position (TP) between *P. leopardus* and each baseline organism. Baseline TPs were selected based on their assumed discrete trophic level within the reef ecosystem. Coral and macroalgae were designated as primary producers with TP = 1 (acknowledging coral also consists of animal tissue), plankton TP = 2 because, in addition to phytoplankton, samples consisted of small zooplankton, and *C. teres* TP = 3 as a zooplanktivore. The TP of *P. leopardus* was determined for each individual using the following equation:

$$(2)\ \mathrm{TP}_{consumer} = \frac{\Delta\delta^{15}\mathrm{N}_{consumer-baseline}}{\mathrm{DTDF}_{\delta^{15}N}} + \mathrm{TP}_{baseline}$$

where $\Delta\delta^{15}N_{consumer\text{-}baseline}$ is the difference in $\delta^{15}N$ values between *P. leopardus* and the corresponding mean baseline value; $DTDF_{\delta^{15}N}$ is the $\delta^{15}N$ diet-tissue discrimination factor calculated for *P. leopardus* (muscle: 1.8‰; **Chapter 3**); and $TP_{baseline}$ is the assigned trophic position of baseline organisms as described above. The BCCRs from Eq. 2 provide an approximation of the $\delta^{13}C$ sources of the consumer and its effective food chain when values are close to 1 (assuming a singular prey source). Baseline organisms which, of the limited range sampled, best represented carbon sources for *P. leopardus* were used to adjust the TP at each sampling location and period when available. Additionally, scaled TP (TP_{scaled}) estimates were explored as an alternative to $TP_{consumer}$ (calculated in Eq. 3) because the latter calculation does not account for the proportional decrease in $\Delta\delta^{15}N_{consumer\text{-}baseline}$ with increasing trophic transfer/prey $\delta^{15}N$, and simply uses a constant DTDF (see Hussey et al. 2014):

$$(3)\quad TP_{scaled} = \frac{\log(\delta^{15}N_{lim} - \delta^{15}N_{baseline}) - \log(\delta^{15}N_{lim} - \delta^{15}N_{consumer})}{k} + TP_{baseline}$$

where $\delta^{15}N_{lim}$ represents the saturating isotope limit as TP increases, occurring when rates of ^{15}N and ^{14}N uptake equal those of ^{15}N and ^{14}N elimination, and determined through meta-analysis for fish (21.93‰); and k is the rate at which $\delta^{15}N_{consumer}$ approaches $\delta^{15}N_{lim}$ per trophic transfer (0.14; Hussey et al. 2014). For application, TP_{scaled} was designed using discrete trophic levels of baseline consumers ($TP_{baseline}$ = 2 or 3) from different marine ecosystems, consequently the absolute values of TP_{scaled} may not be accurate for all baseline groups used in this study (e.g., $TP_{baseline}$ = 1). Nevertheless, TP_{scaled} and $TP_{consumer}$ were used to standardise *P. leopardus* $\delta^{15}N$ values across locations and periods, and not as distinct identifiers of TP in the reef ecosystem. A general linear model (GLM) investigated the influence of location, period, and fork length (and its interactions with location and period) on adjusted TPs to determine if feeding regime differed. Tukey's HSD was applied to adjusted TPs to identify where periods/locations differed. Model residuals were verified for normality and heterogeneity using diagnostic plots, and analyses were considered significant when $p < 0.05$.

4.3 Results

There was not a significant relationship between muscle $\delta^{15}N$ (regression: $df = 56$, $r^2 = 0.02$, $p = 0.17$) or $\delta^{13}C$ (regression: $df = 56$, $r^2 = 0.01$, $p = 0.18$) and fork length in *Plectropomus leopardus* at Helix, Yankee and Coil reefs during November 2013 (Figure 4.1a, b). Size of *P. laevis* had no effect on $\delta^{15}N$ (regression: $df = 35$, $r^2 = 0.01$, $p = 0.55$) (Figure 4.1c). There was a significant positive trend in muscle $\delta^{13}C$ (regression: $df = 35$, $r^2 = 0.10$, $p = 0.04$) and fork length, however when colour phases were separated neither was significant ($p > 0.05$; Figure 4.1d).

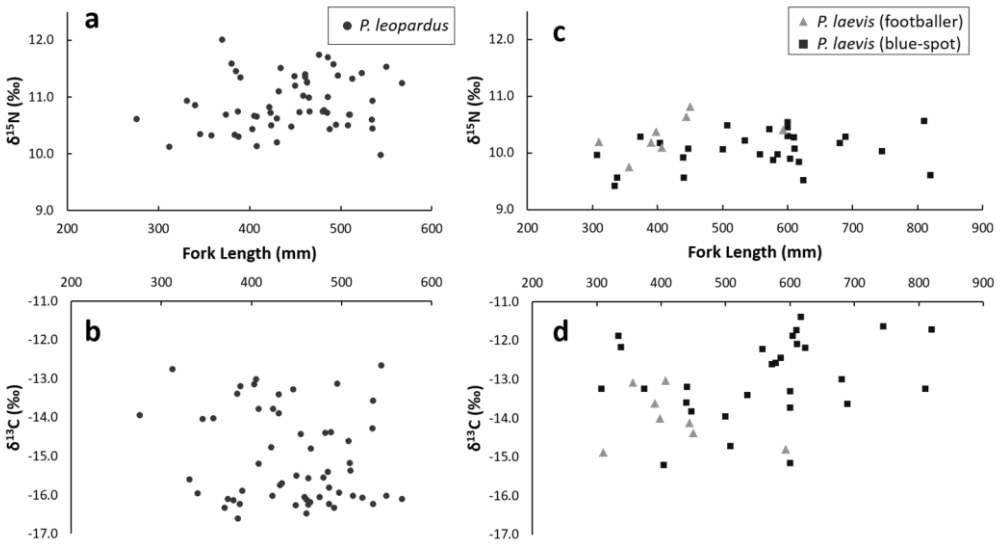

Figure 4.1: Muscle $\delta^{15}N$ (a, c) and $\delta^{13}C$ (b, c) values (‰), plotted with fork length of *P. leopardus* (a, b) and *P. laevis* (c, d) at Helix, Yankee, and Coil reefs (where they co-occurred). Blue-spot and footballer *P. laevis* are separated. A significant positive relationship was only determined for $\delta^{13}C$-fork length of *P. laevis* when colour phases were pooled ($df = 35$, $r^2 = 0.10$, $p = 0.04$).

Overall, *P. leopardus* had higher $\delta^{15}N$ and lower $\delta^{13}C$ values compared to both phases of *P. laevis* (Figure 4.2). Isotopic niche breadth of *P. leopardus* and *P. laevis* (footballer and blue-spot analyzed separately) at each location were mostly similar, except *P. leopardus* SEA_B was smaller than *P. laevis* (blue-spot) at Helix Reef for all tissues, and SEA_B of blue-spot was smaller than *P. leopardus* at Yankee Reef for plasma (Figure 4.3). Shared % overlap between standard ellipses of *P. leopardus* and *P. laevis* (blue-spot) rarely overlapped (except at Yankee Reef where overlap was <23%; Table 4.3). In contrast, *P. laevis* (footballer) shared up to 62% and 53% of isotopic niche breadth with *P. leopardus* and *P. laevis* (blue-spot), respectively (Table 4.3). In general, isotopic patterns between tissues were the same at each reef (Figure 4.3), consequently only muscle was used for analysis.

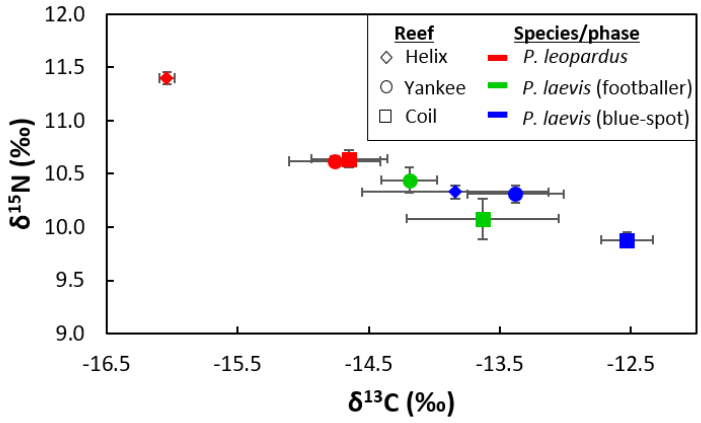

Figure 4.2: Mean (± SE) $\delta^{15}N$-$\delta^{13}C$ bi-plot of sympatric coral trout (including colour phases of *P. laevis*) muscle tissue sampled during November 2013 at Helix, Yankee, and Coil reefs.

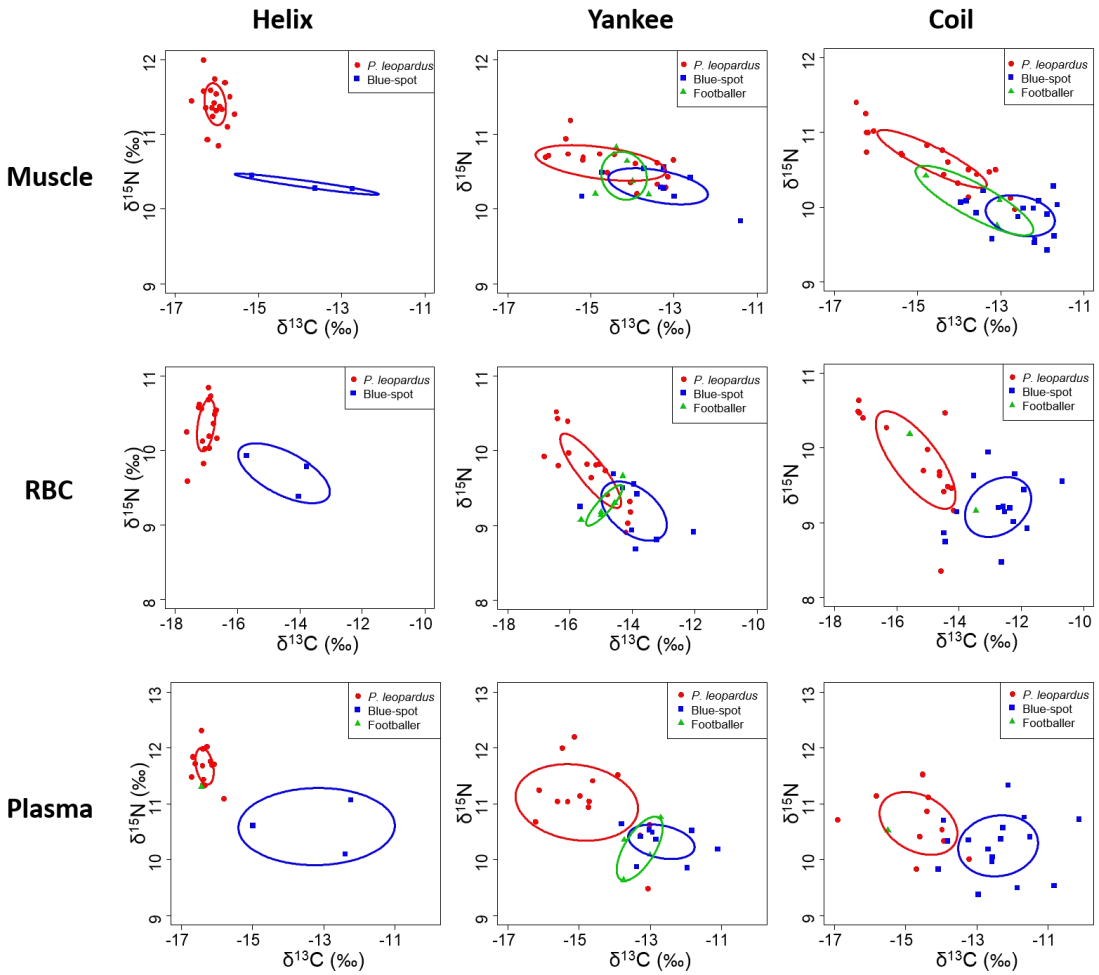

Figure 4.3: Stable isotope ($\delta^{15}N/\delta^{13}C$) niche breadth of sympatric *P. leopardus* and *P. laevis* (footballer and blue-spot colour phases separated) sampled from muscle, red blood cells (RBC), and plasma tissues at Helix, Yankee, and Coil reefs during November 2013 as indicated by Bayesian standard ellipses.

Table 4.3: Shared percent (%) overlap between Bayesian standard ellipses (derived from $\delta^{15}N/\delta^{13}C$ values) of *P. leopardus* and *P. laevis* (including footballer and blue-spot colour phases).

Reef	Tissue	*P. leopardus*/ *P. laevis* (footballer)	*P. leopardus*/ *P. laevis* (blue-spot)	*P. laevis* (blue-spot)/ *P. laevis* (footballer)
Helix	Muscle	NA	0/0	NA
	RBC	NA	0/0	NA
	Plasma	NA	0/0	NA
Yankee	Muscle	32/62	18/23	33/49
	RBC	11/36	19/13	11/53
	Plasma	0/0	0/0	44/43
Coil	Muscle	16/11	0/0	53/32
	RBC	NA	0/0	NA
	Plasma	NA	0/0	NA

Note: the first value in each column represents the total area of overlap of the first species/phase with the second species/phase divided by the total area of the first species/phase. The second value is the total shared area divided by the total area of the second species/phase.

Mean *P. leopardus* muscle $\delta^{15}N$ and $\delta^{13}C$ differed among sampling period/location as much as ~1‰ and 1.5‰, respectively (Figure 4.4; Table 4.1). *Halimeda* spp. (sometimes consumed by herbivores; Mantyka and Bellwood 2007) was selected as the most appropriate baseline organism to standardise TP among reefs and sampling dates based on the BCCR (mean value closest to 1; Table 4.2). *Caesio teres* (planktivore) was also selected despite elevated BCCRs because it is a secondary consumer and less likely to be influenced by inherent baseline $\delta^{15}N/\delta^{13}C$ variability (Post 2002); additionally, *C. teres* are readily consumed by *Plectropomus* spp. (see below). Analysis of *Halimeda* spp. TP was conducted using TP_{scaled} (sufficient samples were collected from November 2013 (Lodestone, Helix, and Coil reefs) and February 2014 (Lodestone and Helix reefs)) to account for narrowing $\delta^{15}N$ discrimination with increasing prey $\delta^{15}N$ (Hussey et al. 2014). By contrast, $TP_{consumer}$ was used for *C. teres* TP comparisons (sufficient samples were collected from November 2013 and February 2014 at Lodestone and Helix reefs) because DTDFs have specifically been calculated for *P. leopardus* when consuming a similar diet of fish (**Chapter 3**). The GLM for *Halimeda* spp. TP_{scaled} showed that location was a significant factor (GLM: $F_{2,86} = 11.5$, $p < 0.01$), whereas for *C. teres* $TP_{consumer}$ location (GLM: $F_{1,66} = 4.60$, $p = 0.04$) and sampling period (GLM: $F_{1,66} = 4.80$, $p = 0.03$) were significant. Length was not a contributing factor to either TP estimate when applied as a single factor (Figure 4.5) or as an interaction with sampling location and period (*p*

> 0.05). Tukey *post hoc* tests for both *Halimeda* spp. TP_{scaled} and *C. teres* $TP_{consumer}$ showed that TP at Lodestone Reef in November 2013 was lower than Lodestone Reef in February 2014 and Helix Reef during both sampling periods (Figure 4.6). Similarly, muscle $\delta^{15}N$ values of *P. leopardus* were lower in November 2013 at Lodestone Reef, but higher in November 2013 at Helix Reef compared to February 2014 (Figure 4.7). At Lodestone Reef, $\delta^{15}N$ values were typically higher in February 2014 in muscle, however in RBC and plasma, the opposite pattern occurred where November 2013 $\delta^{15}N$ values were higher (Figure 4.7). Only a small number of individuals had identifiable gut contents (18/141 (13%) *P. leopardus*; 5/43 (12%) *P. laevis*), of which, the primary items were Labridae, Pomacentridae, and Caesionidae (Table 4.4).

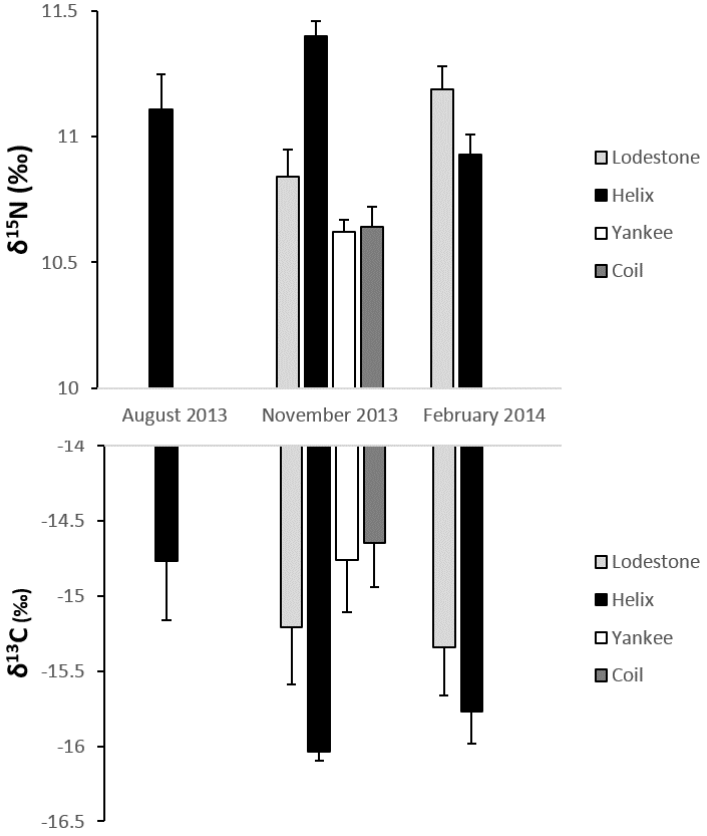

Figure 4.4: Mean (± SE) muscle $\delta^{15}N/\delta^{13}C$ values (‰) of *P. leopardus* sampled at Lodestone, Helix, Yankee, and Coil reefs during three sampling periods in August and November 2013, and February 2014.

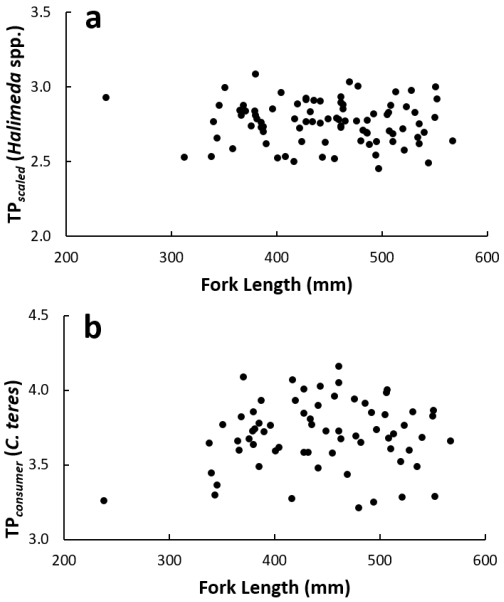

Figure 4.5: Linear regression examining the relationship between *Halimeda* spp. (a; TP_{scaled}) and *Caesio teres* (b; $TP_{consumer}$), and fork length of *P. leopardus*. Neither relationship was significant ($p < 0.05$).

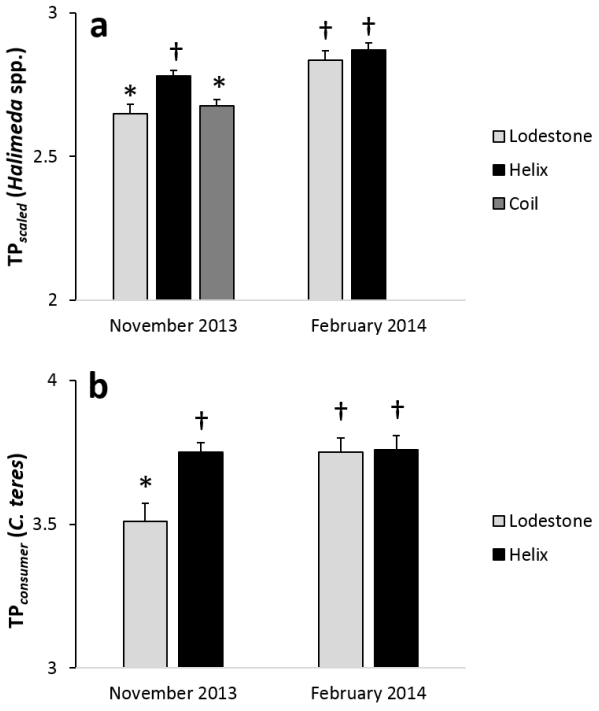

Figure 4.6: Mean (± SE) trophic position of *P. leopardus* calculated from *Halimeda* spp. (a; TP_{scaled}) and *Caesio teres* (b; $TP_{consumer}$) as baseline organisms (see equation 3 and 4).

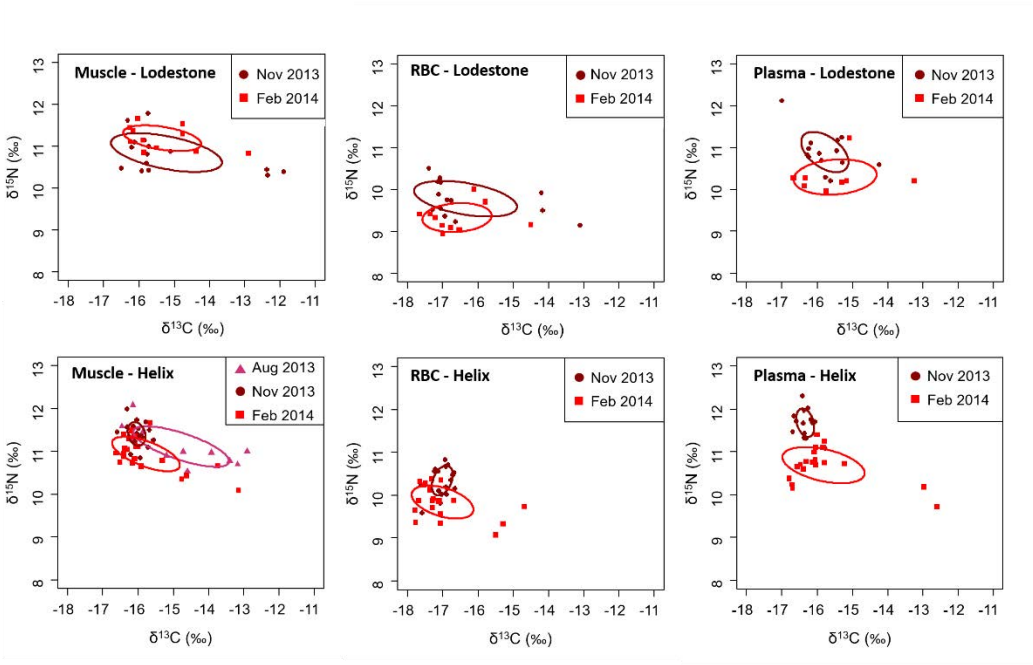

Figure 4.7: Stable isotope ($\delta^{15}N/\delta^{13}C$) niche breadth of *P. leopardus* sampled from muscle, red blood cells (RBC), and plasma tissues at Lodestone and Helix reefs during August 2013, November 2013, and February 2014 as indicated by Bayesian standard ellipses.

Table 4.4: Summary of identifiable gut contents collected from *P. leopardus* and *P. laevis*

Species	Reef	Date	Family	Final ID	Feeding mode
P. laevis (blue-spot)	Coil	Nov-13	Pomacentridae	*Acanthochromis polyacanthus*	Planktivore
P. laevis (blue-spot)	Helix	Nov-13	Labridae	*Choerodon fasciatus*	Herbivore, carnivore
P. laevis (blue-spot)	Helix	Nov-13	Siganidae	Siganidae	Herbivore
P. laevis (blue-spot)	Yankee	Nov-13	Caesionidae	*Caesio* sp.	Planktivore
P. laevis (footballer)	Helix	Feb-14	Pomacentridae	Pomacentridae	Herbivore, planktivore
*P. leopardus**	Coil	Nov-13	Caesionidae	Caesionidae	Planktivore
*P. leopardus**	Coil	Nov-13	Pomacentridae	Pomacentridae	Herbivore, planktivore
P. leopardus	Coil	Nov-13	Crustacea	Crustacea	Detrivore, planktivore
P. leopardus	Helix	Aug-13	Acanthuridae	Acanthuridae	Herbivore, detrivore, planktivore
*P. leopardus***	Helix	Aug-13	Labridae	Labridae	Herbivore, corallivore, carnivore
*P. leopardus***	Helix	Aug-13	Labridae	*Thalassoma hardwicke*	Herbivore, corallivore, carnivore
P. leopardus	Helix	Aug-13	Labridae	*Cirrhilabrus punctatus*	Planktivore
P. leopardus	Helix	Aug-13	Labridae	*Thalassoma hardwicke*	Herbivore, corallivore, carnivore
P. leopardus	Helix	Nov-13	Apogonidae	*Cheilodipterus quinquelineatus*	Carnivore, planktivore
*P. leopardus****	Helix	Nov-13	Caesionidae	Caesionidae	Planktivore
*P. leopardus****	Helix	Nov-13	Pomacentridae	*Pomacentrus mollucensis*	Planktivore
P. leopardus	Helix	Nov-13	Caesionidae	*Pterocaesio* sp.	Planktivore
P. leopardus	Helix	Nov-13	Caesionidae	Caesionidae	Planktivore
P. leopardus	Helix	Nov-13	Labridae	Labridae	Herbivore,

					corallivore, carnivore
P. leopardus	Helix	Feb-14	Crustacea	Crustacea	Planktivore
P. leopardus	Helix	Feb-14	Labridae	*Cirrhilabrus punctatus*	Planktivore
P. leopardus	Lodestone	Aug-13	Caesionidae	*Pterocaesio marri*	Planktivore
P. leopardus	Lodestone	Nov-13	Caesionidae	*Pterocaesio marri*	Planktivore
P. leopardus	Lodestone	Nov-13	Caesionidae	*Pterocaesio* sp.	Planktivore
*P. leopardus*****	Lodestone	Feb-14	Pomacentridae	*Chromis* sp.	Planktivore
*P. leopardus*****	Lodestone	Feb-14	Pomacentridae	*Chromis* sp.	Planktivore
P. leopardus	Yankee	Nov-13	Gobiidae	Gobiidae	Detrivore, herbivore, carnivore

Note: asterisks (*) represent prey items collected from the same individuals. Feeding modes were based from Randall et al. (1997), Depczynski and Bellwood (2003), Barnett et al. (2006), and Green and Bellwood (2009).

4.4 Discussion

In addition to biological and life history differences that may disproportionally influence exploitation of co-occurring fish species (e.g., Schindler et al. 2002; Currey et al. 2013), ecological and behavioural patterns can alter exposure and susceptibility to human-induced pressures (e.g., Januchowski-Hartley et al. 2011; Tobin et al. 2013). As a result, knowing how sympatric species select resources is critical for effective management of multi-species fisheries. Niche specialisation of two sympatric coral trout species revealed distinct separation in foraging patterns that was consistent across reefs and time periods (as indicated by stable isotopes of multiple tissues). Moreover, by accounting for baseline isotopic variation in the reef ecosystem, spatio-temporal investigation of *Plectropomus leopardus* feeding ecology was possible.

The size of predatory fishes often has significant bearing on the type and size of prey consumed. Conventional foraging theory predicts predators will select prey that maximises energetic gains while minimising costs (energetic or survival) (Pyke et al. 1977). Thus predators commonly select larger, energetically profitable food items, while concomitantly being constrained by gape-size (Mittelbach and Persson 1998), prey encounter rates (Stephens and Krebs 1986), and experience/learned behaviour (Kieffer and Colgan 1992), among others. Within the size range of coral trout sampled in this study (~25-80 cm FL), there was no

evidence that $\delta^{15}N$ or TP was significantly affected by size for either species, indicating prey from similar trophic levels were selected independent of predator size. The positive relationship between fork length and $\delta^{13}C$ in *P. laevis* suggests size-related changes in foraging habitat/carbon sources, but there was a high degree of variation (i.e., $r^2 = 0.10$) and different $\delta^{13}C$ patterns existed between blue-spot and footballer phases. *Plectropomus leopardus* and *P. laevis* mature (~50% of population) at ~32 cm FL (Ferreira 1995) and ~45 cm FL (Heupel et al. 2010), respectively, indicating that ~95% of *P. leopardus* and ~60% of *P. laevis* sampled were likely mature. St. John (1999) similarly found that diet composition and prey size did not vary in *P. leopardus* >35 cm, because larger individuals fed on a wide selection of piscivore prey. In the Solomon Islands, $\delta^{15}N$ values of *P. leopardus* were positively related to size, but only within ~17-36% of maximum length (Greenwood et al. 2010). Studies have also found that size does not affect movement patterns and space use of adult *P. leopardus* (Zeller 1997; Bunt and Kingsford 2014; Matley et al. 2015). The lack of TP-size (adult) effects has important implications for management in the GBR, because the adult population will probably respond similarly to changes in prey composition. Also based on these data, recreational or commercial fishers who can legally retain *P. leopardus* ≥ 38 cm and *P. laevis* between 50-80 cm in length in the GBRMP, may not negatively impact predator-prey dynamics by unwittingly removing size-classes with different trophic ecology since adult size-related feeding regimes appear to be ubiquitous. However, this needs to be confirmed as only multi-species comparisons were made at reefs closed to fishing (i.e., Helix, Yankee, and Coil reefs).

Separation in $\delta^{15}N$ and $\delta^{13}C$ between *P. leopardus* and *P. laevis* demonstrated that resource selection differed widely between species. Not only were patterns similar between reefs, but also for tissues sampled. Since sampling for species comparisons was only conducted in November 2013 there was no confounding temporal factor associated with the findings. Although sampling only included one period, use of multiple tissues provided an indication of dietary changes through time. For example, the time needed for adult *P. leopardus* to incorporate between 50-95% of prey $\delta^{15}N$ values (i.e., turnover rate) is ~ 66-283, 88-380, and 126-543 days for plasma, RBC, and muscle tissue, respectively (**Chapter 3**). Based on these estimates, it is reasonable to conclude that general feeding patterns/differences between species were comparable throughout the year prior to sampling. Differences in $\delta^{13}C$ values between species/phase indicated baseline carbon sources/feeding habitat varied. Lower $\delta^{13}C$ values suggest *P. leopardus* consumed a larger proportion of prey with pelagic carbon sources compared to the benthic-derived diet of *P. laevis*. The extent of these prey selection patterns could not strictly be verified by gut content analysis given the low rate of identifiable remains (both algal- and planktonic-derived prey found) in both species. Supplementary diet data for *P.*

laevis is limited, but pelagic prey (e.g., Caesionidae, Clupeidae, Engraulidae, and some Pomacentridae) are commonly consumed by *P. leopardus* (Kingsford 1992; St. John 1999; Frisch et al. 2013). Despite the limited dietary data, differences in movement patterns and space use between *P. leopardus* and *P. laevis* are major – *P. laevis* typically uses ~3x the horizontal area and is often shallower than *P. leopardus* throughout the day (see **Chapter 5**). The distinct movement patterns suggest separate energetic requirements or modes to select/capture prey, both of which could influence dietary/isotopic differences.

The two colour phases of *P. laevis* (footballer and blue-spot) were separated to explore whether colour transition influenced isotopic niche space. Bayesian standard ellipses for the footballer phase were typically intermediary between blue-spot phase and *P. leopardus* with contiguous area generally overlapping equally between the two. This pattern may indicate a transition between feeding regimes, where footballers feed similarly to *P. leopardus* prior to changing colour and occupying a distinct niche as a blue-spot. Interestingly, this isotopic difference appears to occur independent of fish size. For example, all three species/phases consistently overlapped in size, yet different isotopic patterns emerged. Also, length did not affect *P. laevis* $\delta^{15}N$ or $\delta^{13}C$ values when colour phases were separated. Therefore, differences in *P. laevis* feeding patterns do not appear to be related to size, but a colour phase-mediated shift. Changes in prey selection and foraging behaviour associated with colour phase may be a result of foraging success associated with altered conspicuousness on the reef (i.e., bright yellow/white/black vs. dark brown). These results suggest a behavioural component associated with the phase transition in *P. laevis*; whether this is driven by age/sexual maturity (Davies et al. 2006), energetic/physiological factors, or environmental cues remains to be tested.

The BCCR approach was used to infer the main carbon source in the coral trout food web to select the most appropriate baseline producer/consumer to estimate trophic position. As expected, there was a lot of variation in BCCR values among baseline groups due to different fractionation/accumulation of carbon in the ecosystem (e.g., France and Peters 1997; Clementz and Koch 2001; Wyatt et al. 2012; Briand et al. 2015). Coral reef food webs are complex and coral trout consume a variety of fish species that derive carbon from several different sources (St. John 1999). Therefore, BCCRs reflected multiple confounding prey sources. Baseline-consumer carbon ratio estimates are also dependent on appropriate diet-tissue discrimination factors, which have only been calculated for *P. leopardus* in a laboratory trial using one food item (**Chapter 3**), and may vary with prey type. Nevertheless, the application of BCCRs is useful for narrowing down predominant baseline carbon sources (e.g., Fisk et al. 2003; Nawrocki et al. *In review*). Despite the large discrepancy in BCCRs of *C. teres* (mean ~3) compared to the idealised value of 1, it was selected as a supplementary baseline organism to

Halimeda spp. because it is a common prey item of *P. leopardus* and because turnover rates would be more congruent (as opposed to primary producers; Martínez del Rio et al. 2009). Farmer and Wilson (2011) calculated a higher TP of *P. leopardus* (4.5) based on gut contents, and TP estimated by Frisch et al. (2013) using stable isotopes was 4.1 (weighted mean), although it varied based on the baseline group selected (3.4-4.5). Bias can arise several ways when estimating TP including selection of appropriate baseline groups, $TP_{baseline}$ values, and DTDFs (see Post 2002; Hussey et al. 2014). The $TP_{consumer}$ values presented in this study employed known prey of coral trout that are specialist zooplanktivores (i.e., TL = 3), and used species-specific DTDFs calculated for a similar trophic transfer as *P. leopardus* - C. *teres* **Chapter 3**). Additionally, *P. leopardus* mainly consume herbivores (TL = 2) and secondary (non-piscivorous) consumers (TL = 3), thus from a conventional framework, estimates of TP in this study (3.51-3.76 based on *C. teres* as baseline group) fit well with observed prey selection patterns and improve TP estimates compared to previous work.

Using either *Halimeda* spp. or *C. teres* as baseline species provided similar *P. leopardus* TP relationships among sampling locations and periods. Specifically, TP was similar at Helix (November 2013 and February 2014) and Lodestone reefs (February 2014); but lower at Lodestone Reef in November 2013. Since TP estimates were based on adjusted baseline $\delta^{15}N$ values, these results indicate a temporal dietary change for *P. leopardus* at Lodestone Reef, but not Helix Reef. Interestingly, this temporal foraging shift was further demonstrated at Lodestone Reef when muscle $\delta^{15}N$ values were higher in February 2014 compared to November 2013, but for RBC and plasma, the opposite pattern occurred. Other studies have not found differences in *P. leopardus* prey items between seasons and throughout the year, although spatial sampling was limited and pulses of abundant prey (e.g., schooling fish) affect temporal prey selection to an extent (Kingsford 1992; St. John 1999). Extensive identification of prey could not be completed in this study, making it difficult to ascertain the degree to which temporal or fine-scale influxes of different prey occurred at Lodestone Reef, and whether TP differences were ecologically relevant or fishing-induced. Reef-wide differences in niche metrics were also apparent based on Bayesian ellipses. For example, the isotopic niche breadth of *P. leopardus* at Helix Reef in November 2013 was smaller than other reefs, indicating *P. leopardus* at Helix Reef consumed prey that were isotopically similar. Whether this was a result of specialised prey selection, reduced isotopic variation at a reef-level (i.e., less isotopically diverse prey assemblages), or a combination of both is unclear. However, the few samples obtained from blue-spots at Helix Reef (which encompassed a larger niche breadth) indicate specialised prey selection by *P. leopardus* during that period. Predators on the reef are intrinsically linked to their prey, both having potential to influence community or trophic structure (Bornt et al. 2015; Palacios et al. 2015). Therefore, understanding isotopic

variation of coral trout and their prey both within and between reefs and seasons warrants further study.

Stable isotope comparisons between locations and periods are valuable as they highlight species-specific differences for a species group that is often treated as a single entity. The findings also suggest niche partitioning and competition between species, although confirmation would require extensive field manipulations (Sale 1974; Connell 1980). Behavioural differences between these two species, in addition to life-history and biological dissimilarities, emphasise the need for population/stock assessments at a species level, but also management that accounts for different functional roles. Furthermore, the impact of external stressors (e.g., warming surface waters, cyclones, altered prey composition) and species' responses to them (e.g., Tobin et al. 2010; Johansen et al. 2015) will likely vary and should be considered. Adjusting for baseline isotope values across spatial and temporal sampling periods to compare dietary patterns has not been commonly applied; this study demonstrated how localised baseline $\delta^{15}N$ variation can be used to trace changes in feeding. There are limitations to this approach; for example, baseline adjustments do not necessarily accurately account for isotopic discrimination when carbon sources other than those applied to baseline estimates are consumed (Post 2002), although the relative similarities between TP_{scaled} and $TP_{consumer}$ support that overall changes were represented by relevant baseline groups. Recent advances in compound specific isotope analysis of amino acids which reduce the need for independent baseline $\delta^{15}N$ sampling (McMahon et al. 2013) should be a consideration for future studies involving spatio-temporal investigations relating to TP.

Overall, this study highlights the need for greater diversification when considering the coral trout complex; the two species studied displayed contrasting behavioural patterns utilising different prey sources, and likely have different functional roles on the reef. Furthermore, the coral trout complex consists of several additional species that may also have distinct prey selection patterns. As human and environmental impacts continue to change reef habitats, knowledge of how exploited species will respond is critical for management; but also important is whether species will respond in similar ways at different reefs and throughout the year.

Chapter 5

Contrasting patterns of vertical and horizontal space use of two exploited sympatric coral reef fish (*Plectropomus leopardus and P. laevis*)

5.1 Introduction

Coral reef fish have important ecological and economic value, but are increasingly at risk of population declines from human (Sadovy 2005; Newton et al. 2007) and environmental (Emslie et al. 2015; Mellin et al. 2016) disturbances. To better understand how species will respond to, or be affected by, direct and indirect stressors such as fishing, habitat degradation, increased water temperature, or altered prey composition, it is essential to know how they obtain resources, meet energetic requirements, and interact within their environment (Botsford et al. 1997; Roessig et al. 2004).

Furthermore, by comparing patterns of activity and habitat use of closely related species that overlap in distribution (sympatry), insight into behaviour such as agonistic interactions and niche segregation can be explored (Dance and Rooker 2015; Guzzo et al. 2015). Competitive interactions between sympatric species (e.g., for habitat and prey) can have a multitude of effects on how biological and energetic requirements are met (Zaret and Rand 1971; Mueller et al. 2016). These interactions can also have far-reaching implications for predators (Braune et al. 2014) and prey (see Estes et al. 2011) ultimately affecting predator-prey relationships and the movement of energy within an ecosystem. Therefore, studying behavioural patterns related to space use of sympatric species – particularly those with commercial and recreational importance – can help interpret partitioning of resources, and provide fundamental information regarding appropriate management strategies and anticipated vulnerabilities.

Coral trout (or coralgrouper; *Plectropomus* spp.) form the basis of commercial, recreational, and artisanal fisheries in the south-western Pacific including Australia, Indonesia, and Fiji, among others (Sadovy de Mitcheson and Colin 2012). The term ‘coral trout’ incorporates several species of primary (*P. leopardus*, *P. laevis*, and *P. maculatus*) and secondary (*P. areolatus*, *P. oligacanthus*, and *Variola* spp.) fishery significance, however the importance of each species varies at a regional level. In the Queensland (Australia) Coral Reef Fin Fish Fishery (CRFFF), commercial fishers primarily catch ‘coral trout’ <24 m deep (Little et al. 2008) so they can be transported live to Asia for greater profit (Sadovy de Mitcheson et al.

2013). *Plectropomus* spp. comprise ~35-55% of all commercial catch in the Queensland CRFFF (Mapstone et al. 2004), which is predominantly *P. leopardus* (>80%, Sadovy de Mitcheson and Colin 2012). Consequently, scientific research, stock assessments, and commercial logbooks (e.g., for fish sold in Australia) have concentrated on *P. leopardus* or grouped all species together, despite biological differences. For example, *P. laevis*, the second most abundant 'coral trout' species at mid-shelf and offshore reefs (Ayling and Choat 2008), grows larger (~120 cm max length) and matures earlier (~1 year) than *P. leopardus* (matures at 2-3 years; ~60 cm max length) (Ferreira 1995; Heupel et al. 2010). *Plectropomus leopardus* are sedentary opportunistic/generalist ambush predators that associate with reef structure for protection or camouflage, although mid-water feeding also occurs (Goeden 1978; St. John et al. 2001). They rarely make inter-reef movements (Davies 1996; Sumpton et al. 2008) and typically remain in a relatively small area diurnally and seasonally (<0.5 km^2, Zeller 1998; Matley et al. 2015). Unlike other large epinephids, spawning activity of *P. leopardus* appears to be more localized to small groups between Sep-Dec (Zeller 1998; Tobin et al. 2013; Carter et al. 2014), but the prevalence of long-range movements and large spawning aggregations is not well known. Nevertheless, seasonal spawning-related closures in the fishery occur during 5-day new moon periods in Oct and Nov. No study has specifically investigated resource and habitat use, or movement patterns of *P. laevis*. Considering the relative importance of the 'coral trout' fishery, it is surprising that limited research has addressed the ecology of species other than *P. leopardus*.

Evidence supports the ecological role of 'coral trout' as a high order predator influencing population dynamics of prey species (Graham et al. 2003; Rizzari et al. 2014; Boaden and Kingsford 2015). The degree or strength of this influence is not known, but coral trout likely play an integral functional role in coral reef ecosystems (Heithaus et al. 2008). Although *P. leopardus* stocks appear to be healthy (Leigh *et al.* 2014), there is growing concern that over-exploitation (Little et al. 2005, McLean et al. 2011), climate change (Johansen et al. 2015), and extreme weather events (Tobin et al. 2010) will adversely affect the sustainability of populations. Similarly, *P. laevis* is currently listed as 'Vulnerable' on the IUCN Red List and without sufficient data, effective management strategies are not possible (Heupel et al. 2010). The main goal of this study was to determine if and how patterns of space use differ between co-occurring species of coral trout, in particular for *P. leopardus* and *P. laevis*. Low sample sizes of *P. laevis* were expected, so the secondary goal of this study was to provide baseline knowledge on *P. laevis* behaviour, as well as supplement past research on *P. leopardus* space use (e.g., Zeller 1997; Bunt and Kingsford 2014; Matley et al. 2015). Passive acoustic telemetry (see Hussey et al. 2015 for review) was used to track the movements of 83 *P. leopardus* and 12 *P. laevis* providing long-term and continuous horizontal and vertical space

use data. Findings will help determine if this multi-species fishery requires greater species-specific attention and will provide novel information about how coral trout partition resources.

5.2 Methods

Study area and sample collection

Plectropomus leopardus (Lodestone Reef – n: 32; Helix Reef – n: 51) and *P. laevis* (Lodestone Reef – n: 2; Helix Reef – n: 10) were tagged with acoustic transmitters (**see section 2.3.1**) during 2013-2014 at Helix and Lodestone Reefs (Table 5.1). Helix and Lodestone are mid-shelf reefs off of Townsville (Townsville reefs; see **section 2.2.1**). At each location, the reef slope drops from 5 m to 20 m deep within a few hundred meters (Figure 5.1).

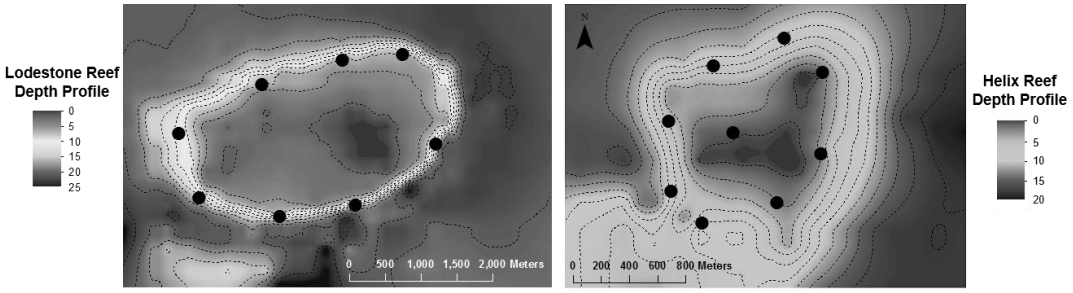

Figure 5.1: Map (including contour depth lines) of study sites at Lodestone Reef (left) and Helix Reef (right). Black circles represent the location of moored acoustic receivers.

Table 5.1: Sampling summary of *P. laevis* and *P. leopardus* including mean (± SE) size, days at liberty, and the number of days detected (numbers in brackets represent the range) for those individuals that were analysed. Only individuals that were detected ≥25 times and for ≥15 d were analysed.

Species	Reef	n (sampled)	n (analysed)	Fork Length (mm)[e]	Days at liberty	Days detected
P. laevis	Helix	10	8[a]	588 ± 62 (316-860)	252 ± 51 (32-375)	192 ± 52 (15-375)
P. laevis	Lodestone	2	2[b]	469 ± 129 (340-598)	232 ± 98 (134-330)	97 ± 13 (97-110)
P. leopardus	Helix	51	32[c]	461 ± 12 (332-564)	290 ± 18 (25-376)	198 ± 22 (20-373)
P. leopardus	Lodestone	32	16[d]	460 ± 26 (366-610)	241 ± 38 (44-377)	153 ± 31 (21-344)
Total		95	58	480 ± 14 (316-860)	270 ± 16 (25-377)	183 ± 17 (15-375)

[a]One additional individual was not analysed because it was mainly detected on the reef flat.
[b]These two individuals were not used in inter-reef comparisons or presence measurements.
[c]Two additional individuals were not analysed because they were mainly detected on the reef flat. Another individual was removed because it appeared dead <15 days after release.
[d]One additional individual was removed due to low detections after being captured by a fisher. Lodestone individuals were not included in inter-reef comparisons.
[e]The size of maturity of *P. leopardus* and *P. laevis* is ~300 mm (Ferreira 1995; Heupel et al. 2010).

The acoustic receiver array (2013-2015) consisted of eight receivers at each reef (Figure 5.1). Additionally, one receiver was deployed within the reef flat at Helix Reef (~1-3 m deep) for ~one year to examine frequency of use and cross-reef movements. The average detection range of receivers was ~250 m (Espinoza et al. 2015a), but ranges vary depending on physical and environmental interference around each receiver (Kessel et al. 2014; Huveneers et al. 2016). As a result, the detection coverage of acoustic receivers was greater at Helix Reef (>75%) compared to Lodestone Reef (~50%) (see Espinoza et al. 2015a).

Analysis

For all analyses, only individuals detected ≥25 times and for ≥15 d were included to avoid individuals with low detections biasing outputs (e.g., fishery captures, mortality events, or moving outside receiver range). If mortality events were apparent during exploratory analysis (e.g., depth sensors followed tidal influences alone), the affected portion of data was removed. Logistic regression (binomial family with logit function) tested whether the inclusion (presence) or removal (absence) of individuals from data analysis were influenced by release distance from a receiver, the size of fish, reef, or release location (i.e., each reef divided into four sections, each containing two receivers: northeast, southeast, northwest, southwest). Validated detections were grouped into 2-hr intervals to reduce effects of autocorrelation between consecutive time periods and to estimate individual locations using a position averaging algorithm (see below). Data were verified for normality and heterogeneity using diagnostic plots, and analyses described throughout were considered significant when $p \leq 0.05$. All analyses were completed in R version 3.2.4 (R Core Team 2016).

To explore diel detection patterns within the arrays, diel receiver efficiency (i.e., proportion of detections each two hours) was determined at Helix Reef using a range test tag deployed ~125 m away (unobstructed) from a moored receiver for ~3 months. Receiver efficiency was not tested at Lodestone Reef, however a similar deployment was conducted at John Brewer Reef (~200 m distance between tag and receiver), which is located adjacent to Lodestone Reef and has similar acoustic characteristics (e.g., reef morphology and boating/fishing activity). The mean proportion of *P. leopardus* and *P. laevis* detections that occurred during each 2-hr interval throughout the study were also calculated for each individual and pooled within-species at Helix Reef and Lodestone Reef.

Monthly measures of residency and roaming were calculated to determine presence within the range of receivers. Proportional residency indices were calculated as the number of days an individual was detected on any receiver each month divided by the total number of days in that month. Roaming was defined as the number of receivers an individual was detected on each

month. The first and last month of detections were removed for each individual when measuring residency and roaming unless detections consisted >25 days in that month (at which point residency was calculated accordingly).

The occurrence of individual movements between receivers for consecutive 2-hr detection periods was tested (generalised linear mixed-effects model (GLMM); binomial distribution) using the *glmer* function in the *lme4* package (Bates et al. 2014). The aim of this logistic regression approach was to test whether the presence (movement between receivers) or absence (detected on same receiver) of movements was affected by variables such as tide, spawning period, lunar phase, fish size, and time of day. However, due to low sample size of *P. laevis* and rare movements of *P. leopardus*, models were unable to converge, however hourly movements are presented (see *results*).

Horizontal kernel utilisation distributions (hKUD) were calculated to compare space use patterns within and between species. Location estimates were based on 2-dimensional positions determined using a 2-hr mean position algorithm to derive centres of activity (COAs) (Simpfendorfer et al. 2002). The 2-hr period was selected as opposed to the 1-hr period used by Simpfendorfer et al. *(*2002) for sharks because *P. leopardus* are sedentary in comparison. Therefore, the 2-hr period was chosen as a compromise between optimizing position estimates (i.e., more time allotted to be detected on multiple receivers) and maximizing daily data points (i.e., longer binned periods would reduce temporal resolution of data). Horizontal KUDs representing the core home range (50%) of positions and home range extent (95%) of individuals were calculated using the *adehabitatHR* package (Calenge 2006). A smoothing parameter (*h*) of 100 was used to estimate hKUDs based on successive visual trials testing different values (e.g. values that were too high overlapped too much with reef flat areas; values too low underestimated receiver detection ranges). For individuals only detected on one receiver, hKUDs were estimated relative to the average detection range for receivers (250 m - 95% hKUD; 125 m – 50% hKUD). Horizontal KUDs were calculated at weekly, monthly, and pooled (all detections for each individual) levels. At the monthly level, the first and last month of detections were removed for each individual unless detections consisted >25 days in that month. Species differences were tested (log-transformed) using repeated measures (RM) ANOVAs with individual (tag ID) as a random factor. To test whether the size of individuals or time of year influenced space use estimates, linear mixed effects (LME) models (*nlme* in R; Pinheiro et al. 2013) were used for each species (separately) with weekly hKUDs as response variables, fork length (mm), season (summer: Dec-Feb; autumn: Mar-May; winter: Jun-Aug; spring: Sep-Nov), and reef (for *P. laevis* data from Lodestone Reef and Helix Reef were pooled) as explanatory variables, and tag ID as a random factor. The *varExp* variance structure

(nlme; Pinheiro et al. 2013) was used at a monthly level to weight hKUD models to improve homogeneity of variances (Zuur et al. 2009). When categorical factors were significant, contrasts were fitted using the *gmodels* package (Warnes et al. 2015)

Transmitter depth data were used to explore vertical space use. A repeated measures ANOVA tested if overall depth use differed between species throughout the study using the 2-hr estimates (log-transformed; tag ID as random effect). Depth values (and their standard deviations) were similarly pooled to compare depth differences between *P. leopardus* and *P. laevis* at each hour (paired *t*-test), and to compare within-individual day/night depth differences (paired *t*-test) for each species. Linear mixed effects models were applied to determine if fish size, season, and/or reef influenced depth use (log-transformed; monthly *varID* variance structure - Pinheiro et al. 2013) for each species (tag ID as random effect). Similarly, the proportion of depth use >20 m was tested as a response variable (exp(1/2)-transformed) with fish size, season, reef, and location (i.e., northeast, southeast, northwest, southwest) as explanatory variables (tag ID as random effect) to investigate vulnerability of individuals to capture for the live reef food fish trade (~20 m depth cut-off). Proportional estimates were determined at each 2-hr COA time period, and grouped for all detections each month, and also for each 5-day new moon period (to investigate spawning-related movements) for each species.

5.3 Results

Of the 95 individuals tagged with acoustic transmitters, 39 were removed from analyses due to low numbers of detections (Table 5.1). Of these, one was reported caught by a commercial fisher at Lodestone Reef and another appeared to have been eaten based on its depth profile. Another three individuals were not incorporated in analyses because they were tagged at and mainly detected on the reef flat receiver (>75% of detections), which was only present for part of the study. No other individuals were detected on the lagoon receiver indicating movements across the reef likely did not occur. On average, individuals were tagged ~110 m (range: 0-650 m) from a receiver. None of the factors (i.e., release distance, fish size, reef, and location) significantly explained whether *P. leopardus* with low detection data were included in analyses or not. *Plectropomus laevis* were not analysed in this instance because of their small sample size and high detections at both reefs (e.g., 10/12 individuals had sufficient data).

Detection efficiencies calculated from stationary transmitters at Helix Reef and John Brewer Reef were also higher during the day, although more pronounced at Helix Reef (Figure 5.2a). Receiver detections from tagged fish were more common during the day (~7:00-17:00) for both species (Figure 5.2b). Both species had relatively high residency indices typically

remaining within the receiver array >50% of days at Lodestone Reef (*P. leopardus*) and >70% of days at Helix Reef (for both species) each month (Figure 5.3a). The mean number of receivers with detections (i.e., a proxy to roaming area or extent) per individual was higher for *P. laevis* (~4 receivers) compared to *P. leopardus* (~1-2 receivers) throughout monthly detection periods (Figure 5.3b).

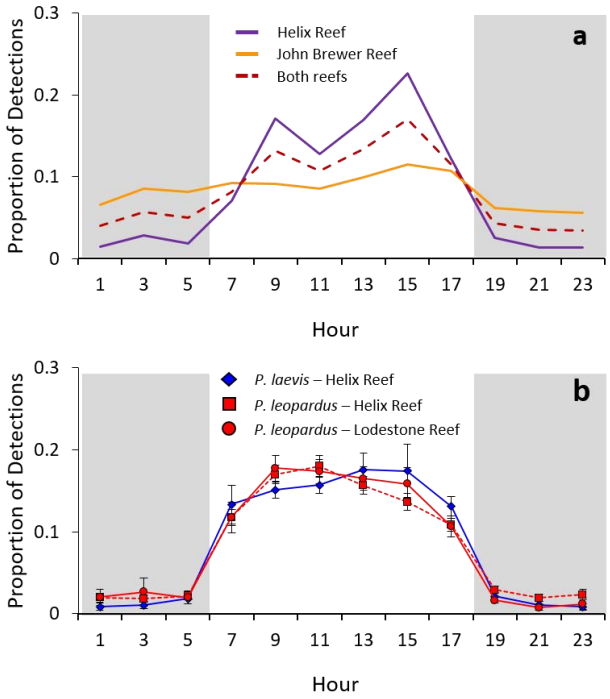

Figure 5.2: The mean (± SE) proportion of detections grouped in 2-hr intervals for and range test tags at Helix Reef and John Brewer Reef (a) and *P. leopardus* and *P. laevis* at Helix Reef and Lodestone Reef (b). Shaded areas represent night-time detections.

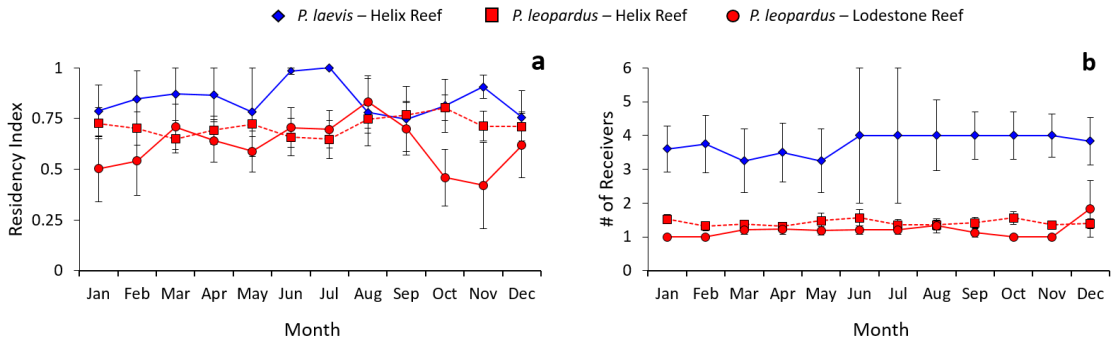

Figure 5.3: The mean (± SE) residency indices (a) and number of receivers with detections per individual (b) of *P. leopardus* and *P. laevis* grouped monthly throughout detection periods.

Movements between receivers were more common during the day; this was particularly evident for *P. laevis*, which moved between adjacent receivers in equal proportion to remaining near the same receiver for consecutive detections (Figure 5.4). Comparisons of mean hKUDs between species at weekly, monthly, and pooled levels showed that *P. laevis* used more horizontal area than *P. leopardus* ($p < 0.01$ for all comparisons; Figure 5.5). There was a gradual increase in the size of 95% hKUDs as temporal resolution decreased, particularly for *P. laevis*; 50% hKUDs remained constant (Figure 5.5). The size of individual *P. laevis* was positively related to weekly 95% hKUD estimates ($F_{1,221} = 5.64$, $p = 0.05$; Figure 5.6a).

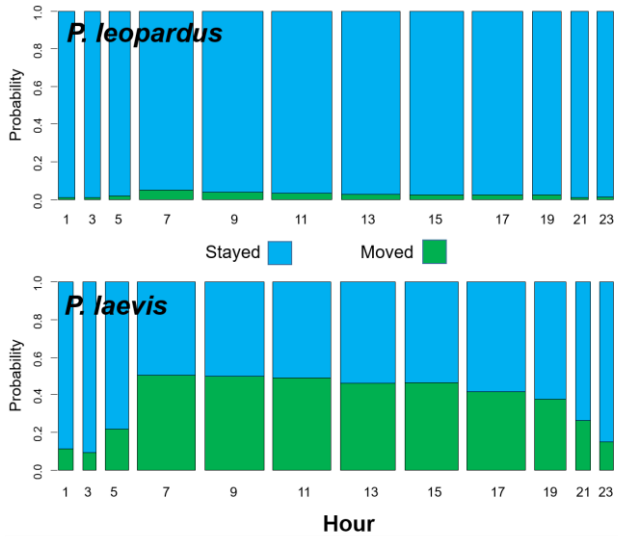

Figure 5.4: Likelihood of movements occurring between receivers for consecutive detections binned by 2-hr periods for *P. leopardus* (n = 39,579 centres of activity estimates) and *P. laevis* (n = 8,107 centres of activity estimates). The width of bar plots represents the relative sample size for each species.

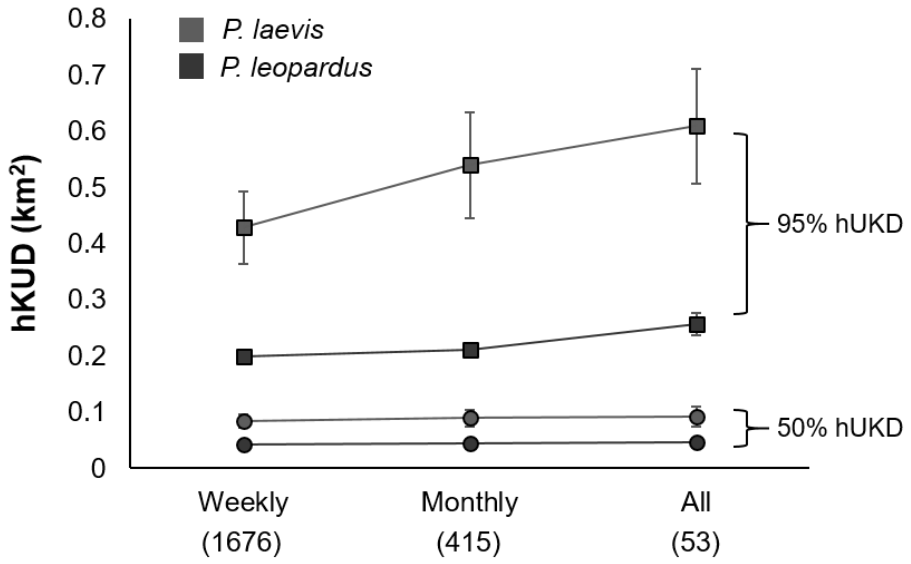

Figure 5.5: Mean (± SE) 50% and 95% hKUDs pooled weekly, monthly, and for all detections throughout each individual's detection period. Comparisons between species revealed that at each level, hKUDs differed ($p < 0.05$). The numbers in brackets represent the total sample size for each comparison.

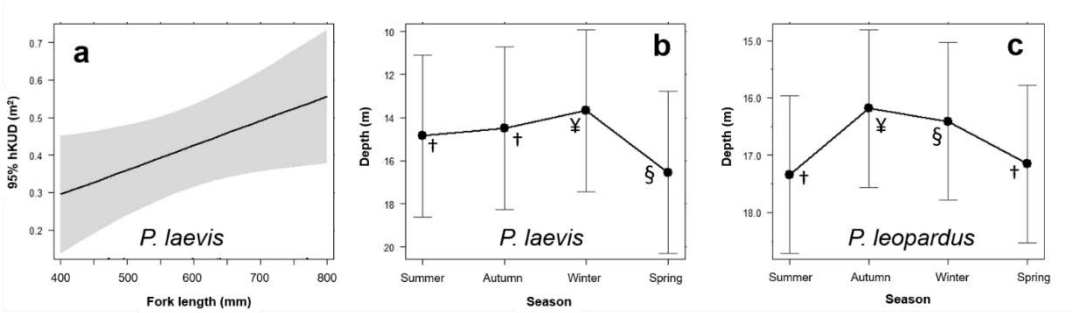

Figure 5.6: Significant size and seasonal effects plots from linear mixed effects models for 95% hKUDs (a; $F_{1,221} = 5.64$, $p = 0.05$) and depth (b; $F_{3,8186} = 71.85$, $p < 0.01$) for *P. laevis*, respectively, and seasonal depth differences for *P. leopardus* (c; $F_{3,33670} = 159.27$, $p < 0.01$). Symbols above each plot represent statistically different categories based on contrasts following the mixed effects models.

Based on all 2-hr COA estimates, depth use did not differ between *P. leopardus* and *P. laevis* (RM ANOVA: $F_{1,41865} = 2.14$, $p = 0.15$); individuals from both species were primarily positioned between 13-18 m throughout the year (Figure 5.6b, c). When comparing mean hourly depth use between species, *P. laevis* moved shallower than *P. leopardus* (*t*-test, $t_{11} = -7.21$, $p < 0.01$; Figure 5.7a), and had greater variation in depth use (*t*-test, $t_{11} = 3.61$, $p < 0.01$; Fig 5.7b) throughout each day. *Plectropomus leopardus* was detected deeper throughout the night compared to the day (*t*-test, $t_{27} = -2.95$, $p < 0.01$; Figure 5.7a), while the variation of *P.*

laevis' depth use was greater during the day (*t*-test, t_5= 2.61, p = 0.05; Figure 5.7b). Season was a significant factor affecting depth use for both species independently; *P. laevis* tended to be deeper during spring (Sep-Nov; Figure 5.6b) and *P. leopardus* moved deeper in spring and summer (Sep-Feb; Figure 5.6c). Similarly, the analysis investigating the effect of fish size, season, reef, and location on the proportion of detections >20 m identified season as significant for *P. laevis* (LME: $F_{3,57}$ = 4.61, p < 0.01; proportion of deep movements greatest in winter and spring: Jun-Nov) and *P. leopardus* (LME: $F_{3,270}$ = 8.80, p < 0.01; proportion of shallow movements greatest in autumn: Mar-May) when all days within each month were included (Figure 5.8). Only reef location of *P. leopardus* (LME: $F_{3,267}$ = 5.26, p < 0.01) influenced the proportion of detections >20 m when 5-day new moon periods were analysed. Specifically, deeper detections were more common in the southeast sections at Lodestone Reef and Helix Reef; however, whether this is related to habitat characteristics or individual variability is unclear as individuals in this section were not detected elsewhere. For all mixed effects models the random factor (individual) accounted for ~50-60% of variation.

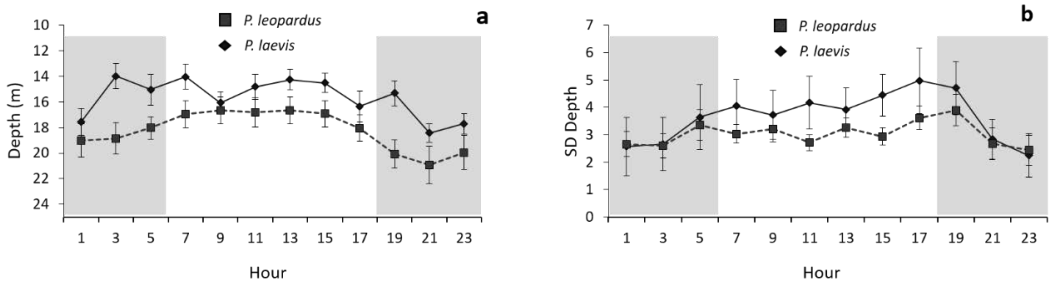

Figure 5.7: Hourly mean (± SE) depth (a) and mean standard deviation (SD) ± SE of depth (b) for *P. leopardus* and *P. laevis* calculated from 2-hr averages. Shaded areas represent night-time detections.

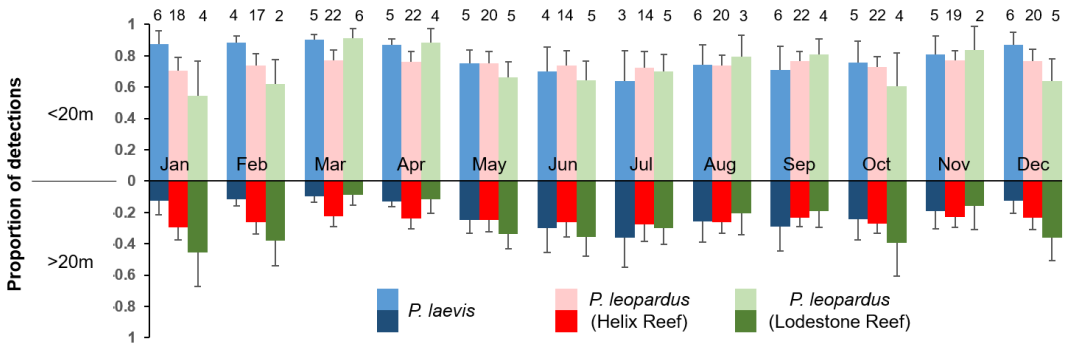

Figure 5.8: Summary of monthly mean (± SE) proportions of detections occurring <20 m and >20 m for *P. leopardus* and *P. laevis* (Helix Reef and Lodestone Reef pooled). Mean proportions of deep/shallow detections were calculated within each 2-hr centres of activity period for each individual before averaging for final results. The numbers above each estimate represent the number of individuals with detections for that month.

5.4 Discussion

Understanding how sympatric fish species access resources and partition space is important for developing directed management plans (Chin et al. 2012; Espinoza et al. 2015c). *Plectropomus* species form an integral part of fisheries in the Great Barrier Reef and Indo-Pacific region, but their behavioural interactions and long-term reef-use patterns are not well known, especially for species other than *P. leopardus*. Passive acoustic telemetry provided a powerful tool to investigate long-term patterns of space use and activity of co-occurring *P. leopardus* and *P. laevis*. Different seasonal and diel depth use patterns by each species were demonstrated, however, in general, vertical space use was similar. By contrast, horizontal space use differed between species at several temporal scales. This study provides a preliminary examination of space use patterns for these co-existing species, and help to inform how each exploits resources on the reef.

Horizontal space use varied between species indicating differences in the amount of reef habitat used. *Plectropomus laevis* was detected on ~2-3 more receivers each month than *P. leopardus* and hKUD size was ~2 times larger for both core home range and extent at all temporal scales (i.e., weekly, monthly, all detections). *Plectropomus laevis* also moved between receivers along the reef slope more readily than *P. leopardus*. The stark difference in horizontal space use between *P. leopardus* and *P. laevis* is indicative of separate behavioural, energetic, and/or physiological requirements. In support of this concept, *P. laevis* were on average ~125 mm larger than *P. leopardus*, therefore they may be more energetically driven to

find prey (Schoener 1968). However, even small *P. laevis* (<500 mm, FL) were detected on >4 receivers and there was considerable fish size overlap between species, suggesting other factors may also be responsible for the observed movement patterns. For example, the bright colouration of smaller *P. laevis* (i.e., footballer phase – see Heupel et al. 2010) may increase conspicuousness, making it a less efficient ambush predator compared to similar sized *P. leopardus*. Interestingly, **Chapter 4** showed that the isotopic niche space of *P. laevis* differed significantly from that of *P. leopardus*, again, when size overlapped, and that *P. laevis* likely fed on benthic prey to a greater extent. This further suggests that feeding regime (and any associated interactions) is a strong factor defining space use between species.

As stated above, the size of fish readily influences activity patterns since individuals optimise energetic budgeting; for example, larger fish swimming greater distances to find food or smaller fish remaining local to reduce costs of movements (Nash et al. 2015). In this study, 95% hKUDs were positively correlated to the size of *P. laevis* indicating a higher proclivity for larger individuals to travel outside of focal home ranges. The lack of seasonal changes in horizontal space use suggests this behaviour is independent of spawning or temperature, and may be foraging-based. Alternatively, larger individuals may spend less time seeking refuge from potential predators enabling them to explore further from home sites. Nevertheless, this increased roaming by large *P. laevis* only occurred for home range extent (i.e., 95% hKUDs) and did not influence the size of core area space use (i.e., 50% hKUDs). Similarly, home range sizes were not affected by the size of *P. leopardus*. Therefore, independent of fish size and at the spatial resolution of the acoustic array, *P. laevis*, as well as *P. leopardus*, exploited consistent local horizontal areas, providing evidence that adult energetic and social requirements are met at a fine-scale relevant to each species.

Vertical movements along the reef slope were common for both species. It was apparent that *P. leopardus* and *P. laevis* shifted their depth use daily and seasonally. During the day, *P. leopardus* were shallower than at night, which differed from *P. leopardus* at Heron Island (shallower depth use during the night; Matley et al. 2015) indicating patterns may vary by location or based on interactions with other species (i.e., fewer *P. laevis* at Heron Island). *Plectropomus leopardus* are mainly sedentary at night seeking refuge among reef structure (Zeller 1997), consequently differences between studies are likely a reflection of suitable resting habitat. There was greater variation in *P. laevis* depth use during the day compared to night, which is also demonstrative of reduced activity during the night. Caution must be applied when comparing day-time and night-time detections, however, because receiver detection efficiency is often reduced during the night (Payne et al. 2010). Despite this, detections at night were expected to be low due to the nocturnal behaviour of *P. leopardus*

described above. The reduced movement between receivers at night further supports resting behaviour. Vertical movements also showed seasonal patterns when 2-hr COA estimates were used in analyses with continuous depth values and proportion of movements >20 m (monthly level). The results varied between approaches but detections were generally deeper in spring for both species. Deeper movements during this period may have been associated with spawning-related behaviour as they often occurred during documented spawning periods (at dusk during new moon periods between Sep-Dec; Samoilys and Squire 1994, Ferreira 1995, Samoilys 1997, Zeller 1998) and has been postulated previously (see Matley et al. 2015). However, occurrences of deep movements varied daily and seasonally between individuals of both species. Also, season was not a significant factor when only 5-day new moon periods were used, suggesting movements >20 m deep were not strictly driven by spawning or that movements relating to spawning (e.g., courtship or recovery) are not limited to these periods. Based on the proportion of vitellogenic *P. leopardus* females at reefs off Townsville, Carter et al. (2014) determined that spawning occurred every few days throughout the spawning season; there is also evidence for regional variation in spawning season. Therefore, behavioural patterns of *Plectropomus* spp. associated with reproduction may still be associated with new moon periods but likely are not restricted to a 5-day period.

Depth use throughout the day showed species differences with *P. leopardus* remaining deeper and changing depths less frequently than *P. laevis*. These differences may be associated with more dedicated search for food by *P. laevis*, dietary specialization, and/or niche partitioning/competition (Davis et al. 2015). Alternatively, preferred depth ranges may differ due to species-specific adaptations to metabolic costs associated with daily energy expenditure (Nilsson et al. 2009). Specifically, the energetic cost of greater space use by *P. laevis* may be reduced by inhabiting warmer waters near the surface, thus optimizing metabolic scope (Johansen et al. 2013; 2015). The restricted movements of *P. leopardus* may enable individuals to remain deeper in more desirable habitats. Depth segregation between species requires further investigation and patterns will likely become more pronounced as coral reef ecosystems continue to face rapid thermal and habitat changes.

Overall, depth differences between species were notably small (usually <5 m) and species commonly shared the same space along the reef slope, suggesting similar habitat selection. For sympatric species to co-exist, each typically has to exploit an alternate habitat or resource (Connell 1980). Different home ranges and dietary patterns may provide the resource partitioning needed for these species to survive together. When deeper waters are selected by either species, the risk of capture inevitably decreases, particularly when fishers target for live-trade and seek to reduce barotrauma by fishing shallow (~85% of fishing effort; Little et al.

2008). In this study, both *P. laevis* and *P. leopardus* were caught <20 m deep, and these individuals remained within this depth range ~75% of detections, indicating that both species are consistently vulnerable to capture throughout the day. Understanding spatial and temporal behavioural trends in congenerics to a greater extent will better equip fishers for more efficient operation and/or managers for more successful management (e.g., identifying temporal or spatial exploitation risk.

A relatively large number of *P. leopardus* were not included in the analyses (~35% and ~50% at Helix Reef and Lodestone Reef, respectively) due to limited detections. Although some of the removals had detection periods >15 days, were reported captured by fishers, or had died (based on depth profiles), the majority of these individuals were only detected for a few days (and not often), leaving uncertainty as to why. The lack of data could not be attributed to the size of fish, reef, or cardinal location. The release distance from a receiver also did not affect individual inclusion in this dataset, however, a release distance <200 m is suggested to maximise probably of detections. This loss of individuals is not necessarily surprising considering the receiver array was originally designed for inter-reef mobile predators. Individual variability was an important random effect in movement patterns (accounted for ~50% of model variability), which follows behaviour reported for *P. leopardus* (Sadovy de Mitcheson and Colin 2012; Matley et al. 2015) and other reef fish (O'Toole et al. 2011; Currey et al. 2014). Even so, the rarity of *P. leopardus* inter-reef movement (Davies 1996; Sumpton et al. 2008) and broad receiver coverage at Helix Reef suggests individuals do not move far regardless of dietary, physiological, reproductive and/or refuging needs. Considering the high residency of both species, it is more likely that individuals were nearby but simply outside of detection ranges, transmissions were blocked due to structural complexity of coral reef habitat, or they had died. A denser receiver array designed specifically for more sedentary reef fish is required to better understand the behaviour of all individuals in a population (Currey et al. 2015).

There are concerns that anthropogenic impacts will have detrimental effects on population demographics, energetic capacity/budgeting, and prey availability among large predatory reef fish (Johansen et al. 2015; Mellin et al. 2016). The adaptive capacity of reef fish will be a main determinant of population persistence in an ever-changing environment (Munday et al. 2008). Species-specific knowledge of movement patterns is integral to understanding the extent and timing of energy expenditure, direct or indirect interactions, and the ability to modify behaviour in response to environmental stressors. This study used acoustic telemetry to show that two congenerics – often grouped together in research and management, have distinct activity patterns on coral reefs, mainly, *P. laevis* was more mobile than *P. leopardus*, using

habitat surrounding half of Helix Reef. Also, *P. laevis* typically remained in shallower water throughout the day compared to *P. leopardus*, however both were less vulnerable to capture (i.e., >20 m deep) in warmer months. Unfortunately, given the population status of *P. laevis* and receiver array locations (i.e., mid-shelf reefs as opposed to offshore reefs), their sample size was small, and consequently, these results need to be interpreted cautiously. Nevertheless, these findings, in conjunction with dietary inferences (**Chapter 5**) that show dietary segregation, indicate that environmental changes and human exploitation will likely impact each species differently. How each species will respond is unknown, but based on these findings *P. laevis* may be more vulnerable to stressors based on its larger size, lower abundance, and greater energetic requirements.

Chapter 6

Niche overlap between co-occurring *Plectropomus leopardus* and *P. maculatus* using acoustic telemetry and stable isotopes

6.1 Introduction

Similar species often co-exist in the same area (sympatry) and use behavioural adaptations such as reducing spatial and dietary overlap with competitors (e.g., López et al. 2016), altering the timing of feeding (e.g., Kronfeld-Schor and Dayan 2003), or suppressing negative impacts of intraguild interactions (e.g., Mueller et al. 2016) to reduce negative impacts from sympatry. Understanding interactions among co-occurring species is necessary for developing informed strategies relating to how human (e.g., marine protected areas; fishing pressure), environmental (e.g., extreme weather events; shifting temperature regimes), or biological (e.g., competition; spawning) factors impact population dynamics, connectivity, trophic pathways, habitat use and distribution (Sadovy 2005; Bornt et al. 2015; Emslie et al. 2015; Espinoza et al. 2015c). As a result, knowing if, and how, sympatric species select different resources and habitat is integral to establish effective species-specific conservation and management practices in aquatic environments (Botsford et al. 1997; Johnson and Welch 2016).

An increasingly common approach to explore spatial and temporal behavioural differences between sympatric fish species is acoustic telemetry (e.g., Farmer and Ault 2011; Knip et al. 2012; Speed et al. 2015). Acoustic telemetry systems frequently consist of a transmitter that is surgically implanted into an individual and emits a unique acoustic signal, and a moored receiver that records the acoustic signal. Acoustic telemetry is advantageous because relatively long-term data can be obtained at a spatial scale relevant to the study animal(s) or system. Not only can the geographic position of an individual be located (resolution depends on study design), other parameters such as depth, temperature, and acceleration can also be recorded, depending on transmitter specifications. Resultant data can provide information about a species' residency and spatial extent (Afonso et al. 2016), focal areas of use (Lédée et al. 2015), diel activity (Papastamatiou et al. 2010), individual/seasonal variability (Kessel et al. 2015), habitat selection (Wolfe and Lowe 2015), environmental drivers (Heupel and Simpfendorfer 2014), and migration patterns (Eldøy et al. 2015). While increasingly capable of characterizing a diverse array of spatial and temporal patterns, use of acoustic telemetry to track the movements of aquatic species is often limited in its capacity to directly link space-use with causal behavioural drivers (e.g., spawning, thermoregulatory or foraging behavior). Stable

isotope analysis (e.g., $\delta^{15}N$ and $\delta^{13}C$) is a complimentary approach to acoustic telemetry because animal $\delta^{15}N$ and $\delta^{13}C$ values change in predictable ways based on regional-scale habitat use or consumed prey (see Newsome et al. 2010 for review). Specifically, variation in $\delta^{13}C$ or $\delta^{15}N$ values in consumer tissues can be indicative of different habitat selection or migratory patterns due to altered uptake and fractionation of organic or nitrogenous compounds at the base of the food web (Hobson et al. 1995; Vander Zanden and Rasmussen 2001; Montoya 2007). Different $\delta^{13}C$ or $\delta^{15}N$ values also provide insight about the primary sources of prey (e.g., plankton vs. algae), prey composition, and trophic position (see Layman et al. 2011) because there is commonly a step-wise enrichment or depletion at each trophic level that can be measured (e.g., scaled or constant enrichment; see Hussey et al. 2014). Using stable isotopes to study resource specialization in sympatric species readily supplements acoustic telemetry because $\delta^{13}C$ and $\delta^{15}N$ values can determine whether a spatial overlap (identified using telemetry) also results in a dietary overlap (identified using stable isotopes).

Coral trout (*Plectropomus* spp.) form the major portion of recreational and commercial catch within the Great Barrier Reef Marine Park (GBRMP) (Mapstone et al. 2008), and are also a significant fishery species throughout the Indo-Pacific region (see Frisch et al. 2016a). Two of the main species captured are *P. leopardus* (leopard coralgrouper/common coral trout) and *P. maculatus* (bar-cheek coral trout). Both species have similar biology, reaching maturity at the same size and age (~300mm fork length (FL) and 3 years), and typically live for 12-16 years, growing up to 600-680mm FL (Adams 2002; Ferreira and Russ 1992; Ferreira and Russ 1994; Ferreira 1995; Williams et al. 2008). They co-occur at silty and turbid reefs, mainly nearshore (Williams and Russ 1994). Despite their similarities, interactions between these species and their environment are primarily unknown. Trophic pathways (using $\delta^{13}C$ and $\delta^{15}N$) have previously been explored for these species in sympatry (see Frisch et al. 2013), but never in conjunction with long-term acoustic telemetry. Consequently, important information about the spatial and temporal scales at which they co-occur and partition resources and habitat is unknown. The goal of this study was to determine if and how resource and habitat use differ between two sympatric species of coral trout using a multidisciplinary approach (acoustic telemetry and stable isotope analysis).

6.2 Methods

Study area and sample collection

The study was conducted along the fringing reef at the northwest side of Orpheus Island (Figure 6.1). Within this area, the northern portion mainly comprises a narrow strip of fringing reef along the coast no more than 50m perpendicular to shore, dropping relatively steeply

(especially at the northern tip of Orpheus Island) to sand at ~12-18 m. The reef slope in the southern portion descends gradually (to ~5-10 m) and is more expansive with some shallower segments exposed as the tide falls, particularly in Pioneer Bay (Figure 6.1). A total of 32 *P. leopardus* and 30 *P. maculatus* were tagged and monitored as described in **section 2.3.1**. Muscle tissue was sampled (*P. leopardus*: n = 9; *P. maculatus*: n = 11) for stable isotope ($\delta^{13}C$ and $\delta^{15}N$) values following **section 2.3.2**.

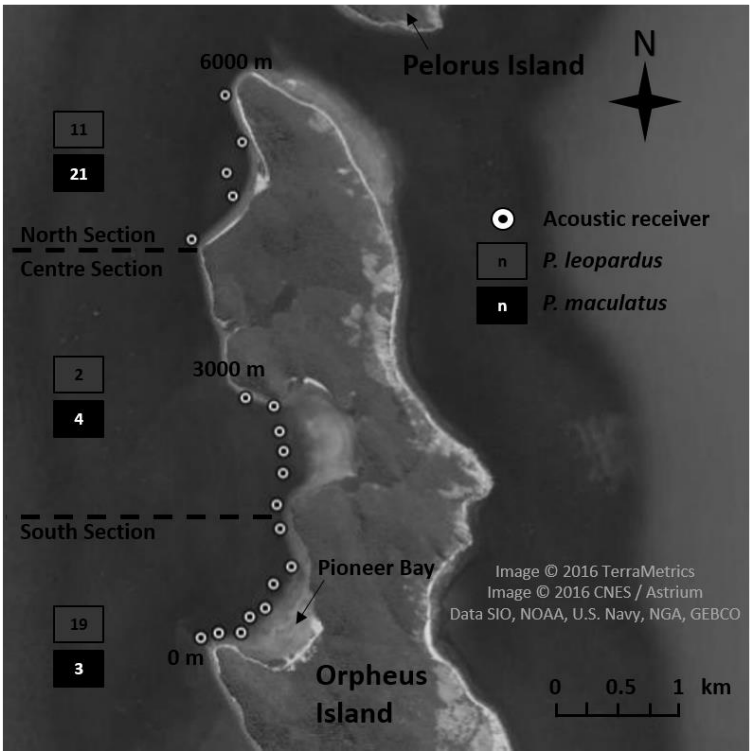

Figure 6.1: Study area north-west of Orpheus Island. Acoustic receiver deployment locations and number of tagged *Plectropomus* spp. are indicated within the North, Centre, and South sections of the study area. The distance along the reef (e.g., 0 m, 3000 m, 6000 m) used to calculate vertical kernel utilisation distributions (vKUDs) is also identified.

Analysis

Analysis of tracking data was only completed for individuals that were within range of receivers for an extended period, following Matley et al. (2015). This approach consisted of only analysing data from individuals detected ≥25 times and for ≥15 days to ensure individuals with infrequent detections did not bias outputs. Additionally, exploratory analysis of monthly depth profiles was used to investigate possible mortality events (e.g., predation from shark). If mortality was apparent (e.g., detected on multiple new receivers in the last few days of detection periods or depth patterns reflected tidal changes only), that portion of data was removed from analyses. For all movement analyses, horizontal and vertical positions were

estimated using a 2-hr mean positioning algorithm to estimate centres of activity (COAs; see Simpfendorfer et al. 2002). This approach reduces temporal autocorrelation of consecutive detections by using the mean depth of an individual each 2-hr period and a mean horizontal position estimate weighted by the number of detections accrued at each receiver during that period. To explore daily patterns of transmitter detections for *P. leopardus* and *P. maculatus*, the mean proportion of detections in each 2-hr period were calculated. Additionally, because of the high number of *P. maculatus* captured in the north of the study area, direct comparisons between species were only made in the North section (Figure 6.1).

Vertical space use

Vertical space use was examined using 2-hr depth measurements, as well as vertical kernel utilization distributions (vKUDs; m^2) calculated at weekly intervals. Vertical KUDs indicate areal space use from a linear perspective, where the horizontal axis represents the linear distance between consecutive receivers along the reef of Orpheus Island and the vertical axis represents the depth (e.g., Currey et al. 2014; Heupel and Simpfendorfer 2014). Although the layout of receivers made detecting cross-slope movements more difficult, this approach was selected because the reef is narrow with unsuitable (sandy) habitat in deeper water and receiver numbers were limited. Furthermore, six additional acoustic receivers were deployed within the shallow intertidal area of Pioneer Bay to detect inshore movements. Detections on these receivers were rare (<0.1% of detections) providing additional support that the linear arrangement of receivers (and vKUD analysis), as well as receiver coverage was adequate to detect movements throughout the area. The R version 3.2.2 (R Core Team 2015) package *ks* (Duong 2007) was used incorporating each 2-hr position with a fixed bandwidth matrix (adapted from Heupel and Simpfendorfer 2014) to calculate 50% (the area of core use) and 95% (the area of an individual's main extent) vKUDs. For individuals that were only detected on one receiver (or only had two position estimates), vKUDs were approximated based on their median (50% vKUD) and maximum (95% vKUD) depth range and an expected horizontal movement radius of ~150 m (50% vKUD) and ~300 m (95% vKUD) (see **Chapter 5**). A repeated measures (RM) ANOVA tested if the explanatory variables species and/or season (summer (Dec-Feb); autumn (Mar-May); winter (Jun-Aug); and spring (Sep-Nov)) resulted in different depth use and weekly vKUDs (response variables; analysed separately) for individuals detected in the North section (where species readily co-occurred). When significant ($p < 0.05$), Tukey's multiple comparison *post hoc* test identified pairwise differences. Next, a linear mixed effects (LME) model determined if season and/or fish size influenced depth use and weekly vKUDs for each species (analysed separately). Individual (i.e., transmitter ID) was treated as a random effect in these analyses. The *varExp* variance structure (nlme; Pinheiro et

al. 2013) was used to weight within-week depth and vKUD models to ameliorate homogeneity of variances after examining model residuals in exploratory plots (Zuur et al. 2009). Similarly, depth use and vKUD estimates were log-transformed prior to statistical tests to normalise data that were skewed.

Individual and species overlap

To explore how space use varied within/between individuals and species, monthly vKUDs were calculated for each individual. To compare space use patterns between individuals (and species), monthly vKUD percent overlap was calculated for all individual pairings that co-occurred on at least one receiver during the same month. The percent overlap between a pair of individuals was calculated as the mean value of overlap of one individual with the other and vice versa. These estimates were derived by dividing the area of vKUDs (50% and 95%) for an individual by areal estimates of another individual located within the same coordinate space based on kernel approximations in the *ks* package (Duong 2007). Individuals and months that had <3 position estimates were excluded from this analysis. Also, the first and last month of detections were removed for each individual unless detections consisted >25 days in that month. A general linear model (GLM) was used to test if the percent overlap of 50% and 95% vKUDs (log-transformed; *varExp* variance structure at monthly level) differed by season and/or species grouping (i.e., *P. leopardus*-*P. leopardus*, *P. leopardus*-*P. maculatus*, *P. maculatus*-*P. maculatus*). When significant ($p < 0.05$), Tukey's multiple comparison *post hoc* test identified pairwise differences. To explore individual space use variability between months, the percent overlap of vKUDs between consecutive months was calculated for each individual. Due to small sample sizes in the North section (i.e., monthly detections common on only one receiver), species comparisons were not made, and all individuals were pooled to visualise consistency in space use.

Stable isotopes

Muscle $\delta^{13}C$ and $\delta^{15}N$ niche size and overlap between species was assessed by Bayesian standard ellipses with the R package SIAR (Parnell et al. 2010). Bayesian standard ellipses represent the "typical" niche breadth of individuals (Jackson et al. 2011) plotted on an isotope bi-plot. Areas of each standard ellipse (SEA_B) were calculated to determine niche breadth overlap between species. Differences in SEA_B size between species were considered significant if ≥95% of posterior draws (10^4) were smaller than the other. Stable isotope differences between species and tagging locations were also tested using ANOVA ($\delta^{13}C$ and $\delta^{15}N$ analysed separately), followed by Tukey's HSD test. Finally, the effect of fish size was investigated using a linear regression with $\delta^{13}C$ and $\delta^{15}N$ as response variables.

6.3 Results

Of the 62 individuals fitted with acoustic transmitters, eight (*P. leopardus*: n = 6; *P. maculatus*: n = 2) were removed from analyses due to low numbers of detections, four (*P. leopardus*: n = 1; *P. maculatus*: n = 3) were removed because of evident mortality, and a small portion of data was ignored for three individuals (*P. maculatus*) because mortality occurred at the end of detection periods. Residency indices were high for both species - each individual was typically detected for >150 days throughout the study (Table 6.1). The majority of *P. maculatus* were caught and released in the North section, while *P. leopardus* were captured in the North and South sections (Figure 6.1). Exploration of daily detection patterns showed the same general trend of greater detection during the day compared to night for both species (Figure 6.2a, b, c). For individuals tagged in the North section, *P. maculatus* had more detections in the afternoon compared to the morning (Figure 6.2b). Also, *P. leopardus* detected in the South section had more night detections than *P. leopardus* in the North (Figure 6.2c).

Table 6.1: Summary of *Plectropomus* spp. tagged with acoustic transmitters and their detection information (mean ± SE; numbers in brackets represent the range).

Species	n (sampled)	n (analysed)	Fork Length (mm)	Detection period (d)	Days detected	# of receivers
P. leopardus	32	25	484 ± 12 (408-621)	317 ± 22 (32-376)	297 ± 22 (29-376)	6.9 ± 0.8
P. maculatus	30	25	375 ± 10 (308-474)	242 ± 24 (25-376)	167 ± 23 (23-376)	4.1 ± 0.7
Total	62	50	430 ± 11 (308-621)	280 ± 17 (25-376)	232 ± 18 (23-376)	5.5 ± 0.5

Note: eight (*P. leopardus*: n = 6; *P. maculatus*: n = 2) individuals were removed from analyses due to low numbers of detections, and four (*P. leopardus*: n = 1; *P. maculatus*: n = 3) were removed because of evident mortalities.

In the North section, species (RM ANOVA: $F_{1,5687} = 6.02$, $p = 0.02$) was a significant predictor of depth use, but only season was significant for 50% (RM ANOVA: $F_{3,956} = 2.62$, $p = 0.05$) and 95% (RM ANOVA: $F_{3,956} = 4.38$, $p < 0.01$) vKUD sizes (Table 6.2; Table 6.3). *Plectropomus maculatus* consistently used deeper water than *P. leopardus* (Figure 6.2d, e, g, h) and no apparent difference was found in *P. leopardus* depth use between North and South sections (Figure 6.2f, i). When each species was analysed separately, season affected depth use in both species, as well as 50% (*P. leopardus*; RM ANOVA: $F_{3,1195} = 5.92$, $p < 0.01$) and 95% (*P. maculatus*; RM ANOVA: $F_{3,696} = 3.68$, $p = 0.01$) vKUD size (Table 6.3). Based on *post hoc* tests, *P. maculatus* depth and vKUD values were typically lower in spring (Sep-Nov) and summer (Dec-Feb) compared to autumn (Mar-May) and winter (Jun-Aug), but were higher in spring and summer for *P. leopardus* (Fig 6.3). The FL of *P. leopardus* had a positive influence on increasing depth use (LME: $F_{1,7043} = 6.20$, $p = 0.05$; Table 6.3). The random factor for these models contributed a large amount of variation: for depth, ID contributed ~50% and ~75% in *P. leopardus* and *P. maculatus*, respectively; for vKUDs, ID contributed ~25-35% in each species.

Table 6.2: Summary of weekly sample sizes and vKUD estimates (mean ± SE) for *P. maculatus* and *P. leopardus* within the study area.

Species		n (total)	n (> 2 receivers)	n (≤ 2 receivers)	50% vKUD (m^2)	95% vKUD (m^2)
P. leopardus						
	All	1195	903	292	359.9 ± 5.4	1952.7 ± 26.5
	North section	333	96	237	398.1 ± 12.5	1522.2 ± 48.3
P. maculatus						
	All	696	258	438	490.5 ± 9.6	1879.9 ± 34.6
	North section	623	194	429	509.7 ± 10.4	1882.7 ± 37.7

Table 6.3: Summary of *p*-values for analyses exploring the effect of season, species, and size (explanatory variables) on depth and vKUD estimates (response variables) in the North section of Orpheus Island Reef. Values in bold were considered significant ($p < 0.05$). Values in brackets represent the sample size of 2-hourly (Depth) and weekly (vKUDs) estimates.

		Depth	**50% vKUD**	**95% vKUD**
North section		(5687)	(956)	(956)
	Season	0.270	**0.050**	**0.005**
	Species	**0.021**	0.087	0.122
P. leopardus		(1951)	(333)	(333)
	Season	**<0.001**	**<0.001**	0.850
	Size	**0.047**	0.159	0.102
P. maculatus		(3736)	(623)	(623)
	Season	**<0.001**	0.419	**0.012**
	Size	0.315	0.297	0.162

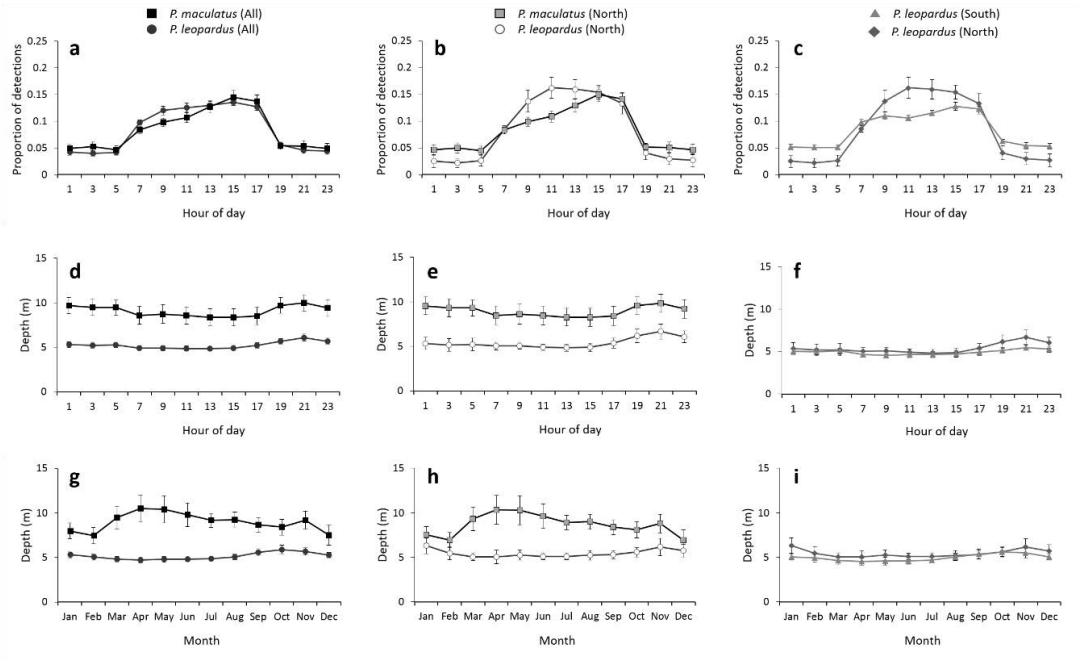

Figure 6.2: Summary of mean ± SE proportion of daily detections (a-c) and depth values (daily: d-f; monthly: g-i) for individual *P. leopardus* and *P. maculatus* tagged in all sections (a, d, g) and the North section (b, e, h), and for *P. leopardus* tagged in the North and South sections (c, f, i).

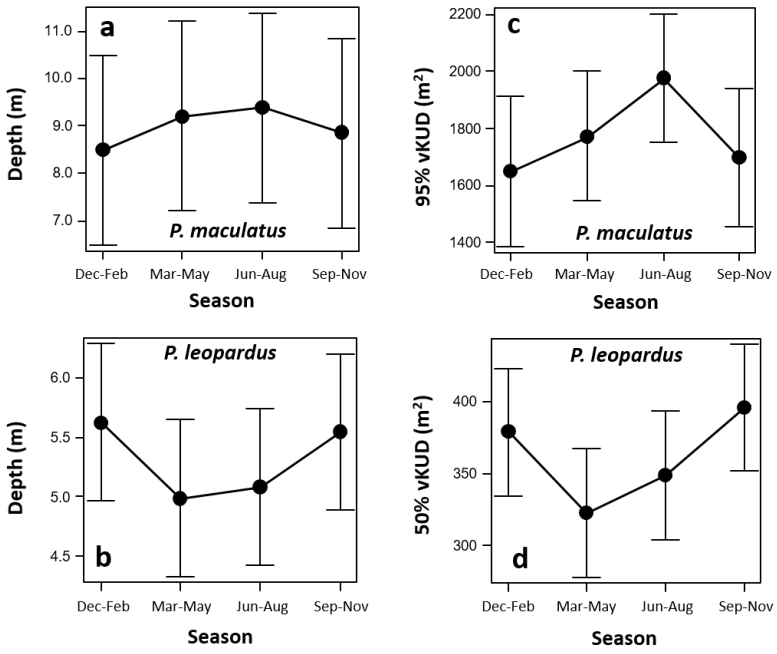

Figure 6.3: Effects plots (mean ± SD) of seasonal differences in depth use (a – *P. maculatus*, b – *P. leopardus*), and 95% (c - *P. maculatus*) and 50% (d - *P. leopardus*) vKUD sizes for individuals tagged in the North section.

Monthly vKUD overlap varied by species pairings (50% vKUD; GLM: $F_{1,1104} = 29.84$, $p < 0.01$; 95% vKUD: $F_{1,1104} = 27.24$, $p < 0.01$) and not season (50% vKUD; GLM: $F_{1,1104} = 1.28$, $p = 0.28$; 95% vKUD: $F_{1,1104} = 2.47$, $p = 0.06$); specifically, the percent vKUD (50% and 95%) overlap between individual *P. leopardus* was higher than between *P. maculatus* individuals and comparison between species (Tukey's *post hoc* test, $p < 0.05$; Figure 6.4). No within-individual analysis was conducted for monthly vKUD overlap (due to lack of sufficient sample sizes in North section), however the mean 50% and 95% vKUD overlap between consecutive months for each individual was consistently >55% and >80%, respectively (Figure 6.5).

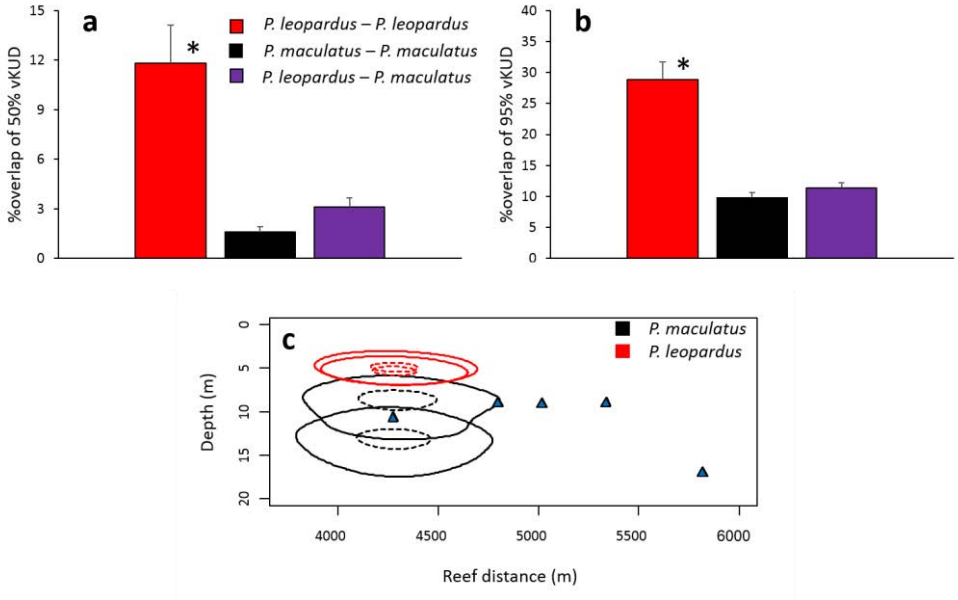

Figure 6.4: Comparisons of monthly (all combined) % vKUD (50% (a) and 95% (b)) between-individual overlap in the North section of the study area (asterisk represents significantly different species groups), including an example of between/within species overlap for two *P. leopardus* and two *P. maculatus* in July 2014 (c; triangles represent receiver locations).

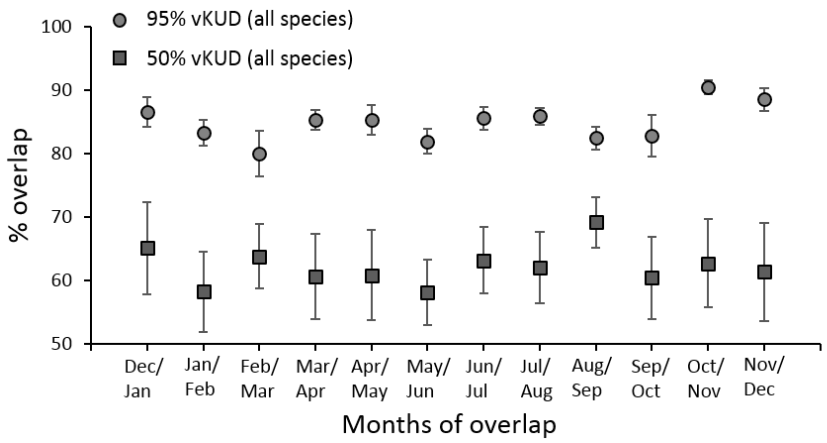

Figure 6.5: Mean ± SE of consecutive monthly % vKUD (50% and 95%) overlap for each individual in the North section. Both species were grouped due to small sample sizes.

Bayesian standard ellipses ($\delta^{13}C$ and $\delta^{15}N$) of *P. leopardus* and *P. maculatus* were similar in size and isotopic space (Figure 6.6). For example, the isotopic niche size (SEA_B) of *P. maculatus* was smaller than *P. leopardus* for only 27% of the 10^4 iterations. Additionally, percent overlap was relatively high with ~59% of the *P. maculatus* ellipse overlapping with the *P. leopardus* ellipse, and ~86% of the *P. leopardus* ellipse overlapping with the *P. maculatus* ellipse (Figure 6.6). Similarly, species did not differ when tested against $\delta^{13}C$ (ANOVA, $F_{1,18} = 0.08$, $p = 0.78$) and $\delta^{15}N$ (ANOVA, $F_{1,18} = 0.27$, $p = 0.61$) values, however $\delta^{13}C$ values ($F_{2,17} = 4.82$, $p = 0.02$) in the North section were lower than in the South (Tukey's *post hoc* test, $p < 0.05$). Finally, FL of *P. leopardus* was positively related to $\delta^{13}C$ values (regression: $\delta^{13}C = 0.013*FL - 20.26$, $n = 10$, $R^2 = 0.52$, $p = 0.02$; Fig 7).

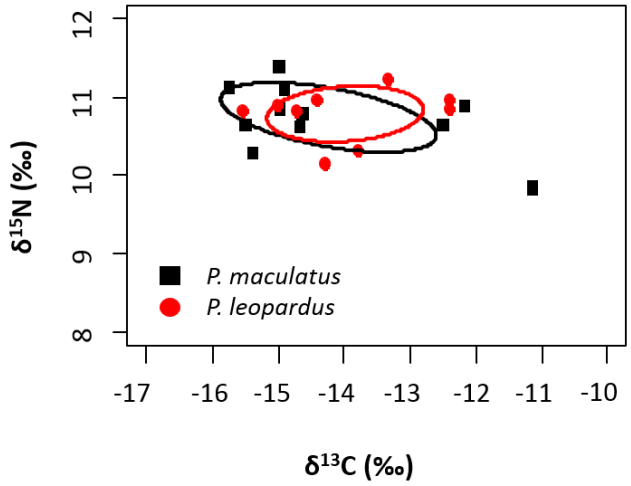

Figure 6.6: Bayesian standard ellipses of stable isotope data ($\delta^{13}C$ and $\delta^{15}N$ bi-plot) from muscle tissue of *P. maculatus* (n = 11) and *P. leopardus* (n = 9) captured on the north-west side of Orpheus Island.

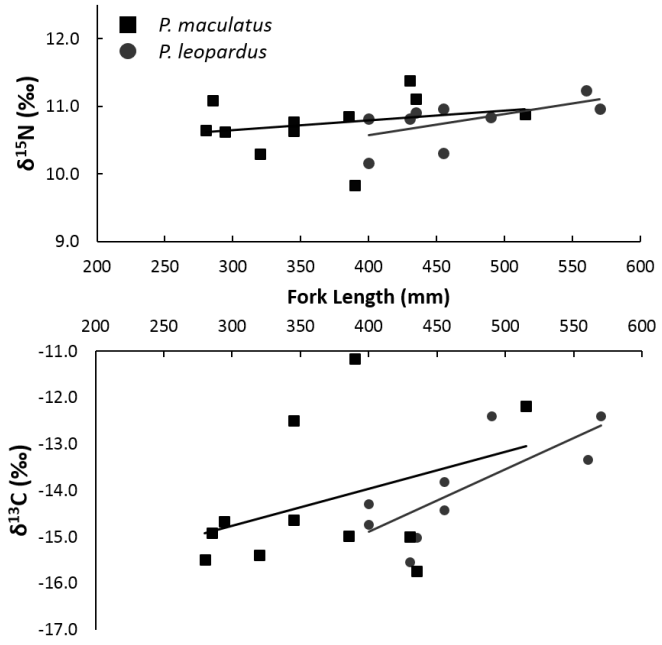

Figure 6.7: Linear regressions of stable isotope data ($\delta^{13}C$ and $\delta^{15}N$) from muscle tissue of *P. maculatus* (n = 11) and *P. leopardus* (n = 9) associated with fork length (mm).

6.4 Discussion

Spatial differences between species

Intraguild interactions among predators are important in structuring the functional and trophic roles of organisms within an ecosystem (Finke and Denno 2005; Harmon et al. 2009). Understanding these interactions, in addition to species-specific dietary and space use preferences, provides fundamental information about how different predators will impact or be impacted by their environment. Multi-year acoustic tracking of *P. leopardus* and *P. maculatus* at Orpheus Island revealed distinct space use segregation; specifically, *P. maculatus* remained at greater depths than *P. leopardus* throughout the study and spatial overlap between species was rare. Also, differences in horizontal distribution between species were evident at the scale of the study area - *P. maculatus* mainly inhabited the North section and *P. leopardus* was more widespread. Interestingly, spatial partitioning appeared to have no effect on long-term feeding patterns as demonstrated by similar muscle $\delta^{13}C$ and $\delta^{15}N$ values between species. These patterns raise important concerns about potential competition between species if vertical distribution or energetic requirements shift due to warming surface waters, degraded habitat, or altered prey composition/density (Johansen et al. 2015; Mellin et al. 2016).

Among the spatial differences between species was diel activity patterns - *P. leopardus* were consistently detected throughout daylight hours but *P. maculatus* were more commonly

detected in the afternoon. This pattern was also found for *P. laevis* at Helix Reef, where it co-occurred with *P. leopardus* (**Chapter 5**). It is difficult to know if this was a result of competitive interactions, innate diurnal behaviour or a by-product of species-specific depth preferences and receiver efficiency along the reef (Welsh et al. 2012). Diel detection frequencies during range testing were consistent throughout all periods at Orpheus Island Reef (Welsh et al. 2012), demonstrating that low proportional detections at night correspond to inactivity/resting which matches previous reports (Zeller 1997; Matley et al. 2015). Alternatively, low detections at night could be related to movements to deeper offshore water, similar to *Lethrinus miniatus* (Currey et al. 2014), however previous tracking studies (e.g., Zeller 1997; Bunt and Kingsford 2014) contradict this behaviour.

There were also clear depth use patterns specific to each species. *Plectropomus leopardus* was consistently shallower than *P. maculatus* throughout the study period indicating significant spatial segregation that could be related to a variety of factors such as habitat preference, thermal tolerance, or competitive interactions. As adults, both species often associate with coral cover that is structurally complex for access to prey and/or protection (Wen et al. 2013; Williamson et al. 2014; Emslie et al. 2015), but this is inconsistent between studies (Connell and Kingsford 1998; Evans and Russ 2004). Depth measurements of *P. leopardus* did not differ between North and South sections, suggesting vertical positioning was independent of reef structure, at least for *P. leopardus*. Movement of ectotherms within the water column can also be influenced by water temperature to optimize physiological functions such as energetic budgeting (i.e., aerobic scope; Johansen et al. 2013) or digestion (Sun et al. 2014). There was evidence of seasonal changes in depth use of *P. leopardus*, in which deeper water was used during the warmer austral spring and summer. However, this is confounded by spawning behaviour during the same period. Additionally, *P. maculatus* showed the opposite trend and other studies have demonstrated that water temperature is not a persistent factor that drives broad patterns of depth use in *P. leopardus* (Bunt and Kingsford 2014; Matley et al. 2015). Perhaps more likely, behavioural interactions between species (historic or current), such as competitive exclusion or niche partitioning, may have caused species-specific depth tendencies. By altering patterns of space use, sympatric species with similar feeding ecology are able to reduce competition and/or exploit different resources (e.g., Davis et al. 2015; Guzzo et al. 2015). Based on the stable isotope results, prey selection was similar between species, suggesting spatial segregation could moderate competition for food (see also below). The specific mechanisms driving distinct distribution (both small- and large-scale) patterns require further investigation.

The contrast in space use between *P. leopardus* and *P. maculatus* was further evidenced by the low degree of monthly vKUD overlap between species in the North section. Both home range extent (95% vKUD) and core home range (50% vKUD) overlapped significantly less between species than between conspecific *P. leopardus*. Similar patterns of high overlap in horizontal and vertical activity space have been demonstrated for juvenile blacktip sharks (*Carcharhinus tilstoni*; Munroe et al. 2016) and adult grey reef sharks (*C. amblyrhynchos*; Heupel and Simpfendorfer 2015), respectively, in the GBRMP. Conspecific *P. maculatus* also had low vKUD overlap, possibly due to the greater individual variability in space use or within-species competitive interactions. The latter is supported by the large isotopic breadth in *P. maculatus* muscle, particularly for $\delta^{13}C$ values, which identifies a potentially diverse diet, but this requires further investigation. Both species typically had high individual core use (>50%) and extent (>80%) vKUD overlap between months. This aligns with reportedly high site fidelity of *P. leopardus* (Zeller 1997; Bunt and Kingsford 2014), *P. laevis* (**Chapter 5**), *P. areolatus* (Hutchinson and Rhodes 2010), and other grouper species (e.g., Frias-Torres 2006; Pastor et al. 2009; Waldie et al. 2016).

Spatial differences associated with season and fish size

Depth and vKUD size varied seasonally for each species. For *P. leopardus*, depth and 50% vKUD values were higher during spring and summer (Sep-Feb), corresponding to warmer water temperatures and spawning season (see Matley et al. 2015). By contrast, *P. maculatus* depth and 95% vKUDs were lower during the same period. The contrary vKUD findings indicate that *P. leopardus* expanded its core home range possibly to locate nearby spawning partners (Matley et al. 2015) or as a result of increased local foraging activity associated with thermoregulatory demands (Johansen et al. 2015) or prey availability mid-water (St. John et al. 2001); meanwhile *P. maculatus* made fewer movements outside of core home ranges supporting local spawning. The opposing seasonal space use patterns between species, particularly depth use, provide further evidence for competitive exclusion and species-dependent behaviours at Orpheus Island Reef.

Contrary to a number of previous studies (e.g., Zeller 1997; Hutchinson and Rhodes 2010; Matley et al. 2015), there was evidence that FL of *P. leopardus* had an effect on behaviour patterns. The mean depth increased ~2-4 m between the smallest (~400 mm FL) and largest (~625 mm FL) individuals. Transition between sexual stages readily occurs between these sizes (Ferreira 1995). Whether hermaphroditic changes alter energetic requirements or behaviour is not clear in *Plectropomus* spp. (e.g., Ferreira 1995), but size of females has a significant role in spawning success (Carter et al. 2015). Body size has also been positively correlated with home range size in coral reef fish and may reflect the need to travel greater

distances to find food and balance metabolic costs (Nash et al. 2015). The greater temporal and spatial resolution provided by acoustic receivers in this study compared to others is an important and necessary step to validate how allometry influences behaviour for these and other epinephelid species.

Stable isotope similarities between species

The degree of overlap in $\delta^{13}C$ and $\delta^{15}N$ values between species indicated that food items - assimilated into muscle tissue over a period of ~126-543 days (50%-95% incorporation times - **Chapter 3**), were similar. Therefore, at a broad temporal scale diet was not distinct between *P. leopardus* and *P. maculatus*, which may be a result of feeding on locally abundant prey with varying $\delta^{13}C$ values (i.e., large $\delta^{13}C$ niche width). The analogous and relatively small mean vKUD sizes of individuals at Orpheus Island Reef further support congruent foraging patterns as individuals of both species use the same area (and likely volume) to obtain resources and meet energetic demands. In contrast, based on muscle stable isotope data, Frisch et al. (2013) concluded that feeding regimes differed between *P. maculatus* (benthic sources; n = 10) and *P. leopardus* (planktonic sources; n = 10) at Northwest Island Reef (coral cay; southern extent of GBRMP). Differences in habitat and prey availability between reefs are likely responsible for these conflicting results. Additionally, isotopic niche segregation was correlated to different areal space use between *P. leopardus* and *P. laevis* at reefs offshore from Orpheus Island (**Chapter 4**). These findings indicate that foraging patterns not only differ between *Plectropomus* species but may vary within-species and at the reef-level as well. Explicit spatial and temporal sampling (e.g., multiple tissues and sampling periods) is required to further explore how feeding regimes and diet assimilation change where species co-occur.

Body size was positively correlated to $\delta^{13}C$ values in muscle tissue of *P. leopardus* suggesting a size-related shift in the types but not trophic level of prey. It is possible that as *P. leopardus* grow larger, benthic prey or prey deriving carbon from benthic producers (Hobson et al. 1995; Vander Zanden and Rasmussen 1999) become more predominant in the diet. Body size of *P. leopardus* also influenced depth use, therefore, larger individuals may be feeding on benthic sources of prey deeper in the water column.

The combination of stable isotopes and telemetry as complimentary approaches to study ecological and behavioural interactions within and between species is a powerful approach (e.g., Cunjak et al. 2005; Papastamatiou et al. 2010; Speed et al. 2012; Matich and Heithaus 2014; Carlisle et al. 2015). By incorporating stable isotope analysis, greater resolution is available to interpret the drivers of movement, particularly foraging-related spatial trends. Telemetry results revealed distinct and prolonged segregation in space use (depth and vKUD

overlap) between two sympatric species with similar biology. Meanwhile stable isotopes indicated feeding regimes of both species were broadly similar. Consequently, differences in space use did not reflect dissimilar prey selection, but are more likely a consequence of direct competition for prey. A similar hypothesis was formed by Davis et al. (2015) after they found two sympatric snappers in the Gulf of Mexico segregated by depth, but shared common prey items. Thus integration of telemetry and stable isotopes has the capacity to provide insights into species ecology not apparent with a single approach.

This study demonstrated different space use patterns between two economically important coral reef mesopredators, and provided the first examination of movement behaviour of co-occurring *P. maculatus* and *P. leopardus* at an inshore reef. *Plectropomus* species are an integral component of fisheries within the GBRMP and more broadly throughout the Indo-Pacific. They are highly sought for local consumption and live export, representing an economy worth more than US$1 billion year^{-1} worldwide (all groupers; Sadovy de Mitcheson et al. 2013). In many parts of their distribution, *Plectropomus* spp. are locally depleted and over-exploited (Scales et al. 2007; Sadovy de Mitcheson et al. 2013). In addition to concerns of fishing pressure causing stock depletion, changing environmental conditions are expected to be a major factor affecting population sustainability (Leigh et al. 2014), energetic demands (Johansen et al. 2015), and trophic structure/species composition in reef ecosystems (Heithaus et al. 2008; Rizzari et al. 2014; Boaden and Kingsford 2015). Changing ocean temperatures are particularly concerning for large predators with small horizontal ranges (Mellin et al. 2016). At Orpheus Island, the shallow depth use of *P. leopardus* may exceed optimal thermal thresholds for this species (e.g., Johansen et al. 2013), causing a shift in vertical distribution patterns. Given the relatively narrow (~100m across) and shallow (2-15 m) corridor of reef habitat in many parts of Orpheus Island Reef, this in turn could lead to direct competitive interactions with *P. maculatus* due to similar diets. Other large predators, such as reef sharks (Heupel et al. 2014; Frisch et al. 2016b) also occupy similar trophic positions as *Plectropomus* spp. further intensifying potential antagonistic interactions and top-down pressure on reef communities. Overall, this study found that the majority of *P. leopardus* and *P. maculatus* remain in the same general area throughout the year and do not readily make distant migrations (e.g., for spawning). These consistent patterns of space use are positive from a conservation context because directed management goals can be implemented at scales relevant to appropriate management tools specific to each species (e.g., Bode et al. 2016; Waldie et al. 2016.

Chapter 7

General Discussion

7.1 Summary and synthesis of findings

This thesis provided a comprehensive examination of resource use for three sympatric species of coral trout (*Plectropomus* spp.). The combination of long-term acoustic telemetry monitoring and spatio-temporally variable stable isotope sampling was used to learn new aspects of coral trout ecology, specific to each of the main species that live in the Great Barrier Reef Marine Park (GBRMP) (**Aim one**). By interpreting movement and dietary behaviour of >300 individual sympatric *Plectropomus*, novel information regarding agonistic interactions and resource selection between species was also investigated (**Aim two**). Finally, the implications of movement and dietary patterns for an ecologically and economically significant species group, were identified throughout the thesis (**Aim three**).

7.1.1 Aim One – Movement and dietary patterns of coral trout

Plectropomus leopardus

At both Orpheus Island (**Chapter six**) and mid-shelf/offshore reefs within the Townsville sector of the GBRMP (i.e., Townsville reefs) (**Chapter five**), it was evident that *P. leopardus* remain within a small area throughout a year, and likely throughout their entire juvenile and adult lives. *Plectropomus leopardus* were readily captured throughout both sampling areas, and only a few individuals moved outside of areas where they were tagged. These limited home ranges highlight that necessary energetic (e.g., prey acquisition) and reproductive (e.g., locating spawning partners) requirements are readily achieved in a relatively small area. Depth use of *P. leopardus* at Orpheus Island was comparatively shallower than at Townsville reefs, however this is likely a product of dissimilar reef and habitat types. Nevertheless, individuals at both locations exhibited the same seasonal depth use trend where deeper water was frequented during the austral spring/summer (Sep-Feb). Whether this behaviour was associated with spawning behaviour (Samoilys 1997; Carter et al. 2014), foraging activity (St. John 1999; Tobin et al. 2013), or temperature (Nilsson et al. 2009; Johansen et al. 2013) is not clear, but indicates that seasonal drivers affect behaviour independent of broad reef type differences (e.g., inshore vs offshore). Similarly, repetitive movements to deep water centred around new moon periods and with highest density of occurrences during the austral spring/summer were

detected for a number of *P. leopardus* at Orpheus Island and Townsville reefs. Based on prior knowledge, this behaviour is likely associated with spawning (Samoilys and Squire 1994; Ferreira 1995; Samoilys 1997; Zeller 1998) and may be due to aggregating or locating partners, increased foraging activity to recoup energy spent during spawning, or behavioural thermoregulation after spawning. Unfortunately, on-site observations were not possible to verify this behaviour; nevertheless, these types of movements appear to be pervasive throughout populations.

Stable isotope comparisons were not made directly between *P. leopardus* at Orpheus Island and Townsville reefs because $\delta^{13}C$ values from end-member sources and prey assemblages likely varied between these locations (Tamelander et al. 2009). At Orpheus Island, the size of *P. leopardus* was positively correlated to $\delta^{13}C$ values, suggesting different prey were selected as individuals grew larger. Again, differences in prey assemblages may explain why this pattern was not detected at Townsville reefs, but it may also be an artefact of sampling since fewer individuals (with a smaller size range) were captured at Orpheus Island. Based on diet-tissue discrimination factors from the aquarium feeding trial (**Chapter three**), the trophic position of *P. leopardus* was estimated at Townsville reefs during two sampling periods (**Chapter four**), revealing consistent estimates independent of fish size. Furthermore, the trophic position of *P. leopardus* was, for the most part, similar among sampling periods and locations, suggesting congruent prey selection patterns throughout the year at mid-shelf reefs.

Plectropomus maculatus

At Orpheus Island, *P. maculatus* were highly resident to the location they were tagged and regularly detected throughout the one-year battery life of transmitters (**Chapter six**). Interestingly, captures of *P. maculatus* were concentrated in the north of the sampling area; whether this was due to habitat characteristics, an artefact of sampling, or proximity to the channel separating Orpheus Island and Pelorus Island is not clear. Still, this finding suggests small scale or local influences at a reef-level affect distribution of this species. Based on detection patterns, *P. maculatus* appeared to be more active during the day, particularly the afternoon, and rested in deeper water at night within reef structure for protection. Seasonal changes also affected depth use, depicted by more common detections in shallower waters during the spring/summer (Sep-Feb). A similar pattern occurred when depth was combined with linear estimates of space use (i.e., 95% vKUDs), indicating that more reef area was exploited when water temperatures were cooler. The specific reasoning for these changes in movement still require further research but may be related to increased foraging activity when ephemeral prey (e.g., clupeids abundant in summer) are absent (Kingsford 1992; St. John

1999), or more efficient aerobic scope at lower temperatures (Nilsson et al. 2009; Johansen et al. 2013). Overall, the size of *P. maculatus* had no bearing on movement or prey selection patterns suggesting most adults exert uniform predatory pressures on inshore reefs. Finally, low spatial overlap between conspecifics and variable $\delta^{13}C$ values provide preliminary evidence that competition for resources is present within *P. maculatus*.

Plectropomus laevis

At Townsville reefs, primarily Helix Reef, the acoustic telemetry data showed that each *P. laevis* individual was detected on approximately four of the receivers deployed along the reef slope (**Chapter five**). This means that half the reef was exploited by each *P. laevis* throughout one year. Additionally, movements appeared to be made exclusively along the reef slope, and not across the reef flat. *Plectropomus laevis* typically remained at depths between 14-18 m, often making intermittent forays to depths as much as 40 m. For a few individuals, these deep movements coincided with spawning periods, but for others, the movements extended such time frames, raising questions surrounding the spawning duration for *P. laevis* and potential alternate drivers. Season did not affect home range size for *P. laevis* supporting resident spawning activity and localised movements throughout the year. The size of *P. laevis* was positively correlated to 95% hKUDs indicating that larger individuals have larger home range extent, however size did not affect core use areas (i.e., 50% hKUDs), suggesting larger individuals do not commonly make these forays further away from focal areas. The larger size of some individuals make them less vulnerable to predation, but may also reduce their ability to use stealth to catch prey. As result, greater areas may be used when food is limiting. By contrast, size did not influence dietary patterns (based on $\delta^{15}N$ and $\delta^{13}C$ values; **Chapter four**) when colour phases were separated. Indeed, colour phase was a more important factor influencing stable isotope patterns than size; for example, despite size (i.e., fork length) similarities, isotopic niche space overlap between colour phases was typically less than 50%. Therefore, dietary differences between colour phases do not appear to be driven by size, but by some other factor such as foraging efficiency (e.g., footballer *P. laevis* are colourful and conspicuous).

7.1.2 Aim Two – Comparison of movement and dietary patterns between sympatric species

The use of two complimentary sampling approaches (passive acoustic telemetry and stable isotope analysis), revealed contrasting patterns of resource use between sympatric species of *Plectropomus*. Interestingly, the characteristics of species differences varied between Orpheus

Island (**Chapter six**) and Townsville reefs (**Chapter four** and **five**). More specifically, at Orpheus Island, stable isotope values, although variable, were not distinct between *P. leopardus* and *P. maculatus*, suggesting similar dietary contributions. Acoustic telemetry provided ancillary support for this finding because the size of weekly space use was similar for both species, suggesting similar energetic budgeting was allocated for survival requirements such as foraging. However, the location of space use varied between species at different spatial scales; most poignant were the daily and monthly vertical contrasts, highlighting independent habitat selection preferences. At Townsville reefs, *P. laevis* regularly travelled greater distances around the reef and moved vertically (up and down) in the water column more frequently compared to *P. leopardus*. Dietary preferences elucidated from stable isotopes also differed between species, and despite overlapping size (i.e., fork length) ranges, *P. leopardus* typically shared no stable isotope niche overlap with blue-spot *P. laevis*. Isotopic overlap was greater between *P. leopardus* and footballer *P. laevis*, suggesting some shared prey selection, but differences were more common (usually <40% niche overlap), again, despite overlapping size distributions. Interestingly, patterns of isotopic niche overlap among *P. leopardus* and *P. laevis* (footballer and blue-spot) were consistent at all Townsville reefs sampled and for all tissues sampled, suggesting consistent seasonal prey selection (*P. leopardus*: planktonic-derived food chain; *P. laevis*: algal-derived food chain) and interactive processes between species/colour phases can be inferred to mid-shelf/offshore reefs in that region.

Whether contrasting resource use patterns between species is due to competitive processes such as niche partitioning is difficult to definitely conclude because this thesis did not specifically test competition (e.g., Sale 1974). However, conceptual theory states that in order for intraguild species to co-exist, the manner of resource exploitation (e.g., timing of feeding or specific habitat) or the resources themselves must differ (Macarthur and Levins 1967; Schoener 1974). From this perspective, limited spatial overlap between *P. leopardus* and *P. maculatus* may provide a buffer to access similar food sources supporting co-existence. Similarly, at Townsville reefs, prey selection and roaming patterns may differ between *P. leopardus* and *P. laevis* as a result of (or to reduce) competitive interactions. These differences may also be a by-product of physiological requirements (e.g., energetic budgeting or thermo-tolerance) or prey acquisition (e.g., foraging efficiency or mode of acquisition).

It is also relevant to note that, although contrasting resource use patterns were identified, there were also many similarities in the behaviour of each species. For example, movements consistently followed diel patterns of limited activity at night and altered depth use during day, and exhibited seasonal variation. Similarly, fish size was not a strong factor influencing movement and dietary patterns for all species. Also, independent of species, individual

variability was commonly detected, particularly from passive acoustic telemetry. For example, some individuals made long-distance movements within the acoustic arrays or intermittently moved to deeper water independent of spawning season. Although this thesis focused on general population-wide movement and dietary patterns, it is important to be cognisant that within-population variation existed.

7.1.3 Aim Three – Ecological and management implications

The ecological and management implications of this research are extensive and not simply limited to the GBRMP. Knowledge of how movement and dietary patterns vary spatially and temporally is under-represented, especially considering the ecological and economic value of this species group. Furthermore, the behavioural information that has been compiled is grossly biased toward *P. leopardus* leaving large gaps about the ecology of other species and how they are or could be impacted by fishing or environmental disturbances.

Ecological implications

The main findings of this research indicated that resource use differs between species. Therefore, the ecological impact of each species within the coral reef environment is likely different. Previous research has indicated that, as an abundant mesopredator, *P. leopardus* are instrumental in controlling and shaping community composition (Graham et al. 2003; Rizzari et al. 2014; Boaden and Kingsford 2015; Palacios et al. 2015). *Plectropomus laevis* are less abundant and less widely distributed than *P. leopardus,* and consequently may not have as strong of a role structuring communities or may influence them in different ways. For example, the diet of *P. laevis* was derived from algal-based sources of primary production compared to *P. leopardus* (**Chapter four**), suggesting *P. laevis* exerts predatory pressure on distinct prey items. The finding that isotopic niche overlap between *P. leopardus* and *P. laevis* was ubiquitous (between reefs and tissues) (**Chapter four**) demonstrated that prey selection patterns are, at least to a degree, consistent. Similarly, the consistency of isotopic segregation between *P. leopardus* and *P. laevis* at each reef indicates that foraging trends between species exhibit limited within-reef variability. As a result, it is probable that different coral trout species will respond to environmental or human-induced disturbances in similar ways within and across compatible reefs. Within the context of foraging ecology, the aquarium feeding trial (**Chapter three**) assisted interpretation of stable isotope values from wild populations in this thesis, but also provides important parameter estimates (e.g., discrimination factors and turnover rates) for large-bodied tropical mesopredators. The limited spatial overlap between *P. leopardus* and *P. maculatus* (**Chapter six**) suggests that species are accessing resources in

unique habitat, likely due to competitive interactions. Nevertheless, movement and dietary patterns were similar between *P. leopardus* and *P. maculatus*, suggesting the functional role of these species may be analogous. The shallow depth use of *P. leopardus* raises concern as habitat degradation is continually increasing near the surface (Ainsworth et al. 2016). If deeper water becomes more hospitable, there will likely be direct interactions between *P. leopardus* and *P. maculatus*; the outcome and impact on inshore reefs is unknown.

Management implications

Findings from this research provide useful insight on species-specific resource use that is pertinent to implementing ecosystem-based management practices for recreational and commercial fisheries. The commercial sector mainly exploits mid-shelf and offshore reefs, while the recreational sector is usually limited to inshore areas (Leigh et al. 2014). Consequently, the findings from Townsville reefs (**Chapter four** and **five**) and Orpheus Island (**Chapter six**) are more pertinent to the commercial sector and recreational sector, respectively. The relatively small (within reef) and persistent (~ a year) home ranges for all species highlights the extent of protection no-take marine reserves provide for coral trout. The 2004 expansion of marine reserves in the GBR has led to increased coral trout biomass within protected inshore and offshore reefs (Williamson et al. 2004; Russ et al. 2006; Miller et al. 2012). The long-term spatially restricted movements of *Plectropomus* spp. found in this thesis help understand why these differences exist. Adult spill-over between management zones is probably rare considering the isolated and large-scale arrangement of marine protected areas within the GBRMP. At Townsville reefs, the larger space use of *P. laevis* compared to *P. leopardus* has implications for population assessments that often rely on underwater visual censuses (UVCs) that are biased by more mobile species (Ward-Paige et al. 2010). Additionally, because *P. laevis* are more mobile and less abundant (listed as 'vulnerable' – IUCN Red List), the removal of an individual probably affects population demographics and community impacts greater than removing a single *P. leopardus*. It is comforting that removal size limits for *P. laevis* are upwardly restricted to support reproductive potential (i.e., male and larger female contribution). The restricted seasonal changes in home range have important relevance to concerns of aggregative spawning vulnerability, particularly for *P. leopardus*. There was no evidence in this study that more than a few individuals made long-range (>500 m) spawning movements. The impact of spawning appears to be localized. Therefore, spawning-related fisheries closures protect known aggregation hotspots of *P. leopardus*, but as whole, may be counter-productive in a commercial fishery struggling to meet quotas (Tobin et al. 2013). Only one location was sampled where *P. leopardus* and *P. maculatus* co-exist, so it is difficult to know if depth segregations are ubiquitous. If they are, UVC surveys estimating

population densities will be biased by depth of sampling. Similarly, species that are captured during fishing will vary based on the depth that is targeted. Considering the ecological differences demonstrated throughout this thesis, species-specific assessments are needed but cannot be undertaken unless fisheries impacts are known for each species.

7.2 Future directions

The research in this thesis is the first to examine movement and dietary patterns between sympatric coral trout species. As such, there are numerous gaps for future research to build on and expand relative to how species interact within the coral reef ecosystem. Examination of fine-scale (e.g., <50 m resolution) space use was not possible due to limited numbers of acoustic receivers. A more compact acoustic receiver array would enable direct comparisons with reef structure and habitat composition, as well as identify species' areas for spawning activities. Bulk stable isotopes were used in this study because they can identify temporal (e.g., tissue-specific turnover) and spatial (e.g., algal- vs plankton-derived dietary sources) feeding regimes. An extensive amount of effort was made to reduce limitations associated with bulk stable isotope analysis (see **Chapter three** and **four**), however new advances, particularly compound-specific amino acid stable isotope analysis, have exciting advantages (e.g., McMahon et al. 2015) that are worth exploring. Unfortunately, high digestion rates and occurrences of empty stomachs limited visual identification of prey, and did not provide the level of support to stable isotope analysis as initially planned. Nevertheless, genetic identification of prey is an alternative and promising supplementary approach currently underway (Matley et al. In prep.).

In general, these new advances could be applied to address the main questions that remain. For example, this thesis was unable to exclusively determine if deeper/shallower movements were driven by spawning, temperature, foraging, or other factors. The impact of environmental drivers (e.g., water temperature, wind speed, rain, etc.) was conducted ancillary to the data provided in this thesis, and revealed no significant influences, but environmental sensors were limited, and fine-scale movement behaviour is needed to appropriately investigate this. Another limitation of this research was, that for the most part, data was only collected at reefs or in locations where fishing activity is restricted. Consequently, how recreational or commercial fishing pressure affects movement and dietary patterns could not be addressed. Nevertheless, the research conducted in this thesis provides an important and necessary step to improve knowledge of resource use for the iconic coral trout - at a species level.

References

Adams S (2002) The reproductive biology of three species of *Plectropomus* (Serranidae) and effects of fishing. PhD thesis, James Cook University

Adkins JN, Varnum SM, Auberry KJ, Moore KJ, Angell NH, Smith RD, Springer LD, Pounds JG (2002) Toward a human blood serum proteome: analysis by multidimensional separation coupled with mass spectrometry. Mol Cell Proteomics 1:947–95

Afonso P, Abecasis D, Santos RS, Fontes J (2016) Contrasting movements and residency of two serranids in a small Macaronesian MPA. Fish Res 177:59–70

Ainsworth TD, Heron SF, Ortiz JC, Mumby PJ, Grech A, Ogawa D, Eakin CM, Leggat W (2016) Climate change disables coral bleaching protection on the Great Barrier Reef. Science 352:338-342

Artero C, Koenig C, Richard P, Berzins R, Guillou G, Bouchon C, Lampert L (2015) Ontogenetic dietary and habitat shifts in goliath grouper *Epinephelus itajara* from French Guiana. Endanger Species Res 27:155–168

Atwell L, Hobson KA, Welch HE (1998) Biomagnification and bioaccumulation of mercury in an arctic marine food web: insights from stable nitrogen isotope analysis. Can J Fish Aquat Sci 55:1114–1121

Ayling AM, Choat JH (2008) Abundance patterns of reef sharks and predatory fishes on differently zoned reefs in the offshore Townsville region: Final Report to the Great Barrier Reef Marine Park Authority, Research Publication No. 91, Great Barrier Reef Marine Park Authority, Townsville

Ayling AM, Ayling AL (2011) The effect of cyclones Yasi and Anthony on fish and coral populations of reefs in the offshore Mackay and Townsville regions. Hydeaway Bay, Qld, Australia: Sea Research

Barnes C, Sweeting CJ, Jennings S, Barry JT, Polunin NVC (2007) Effect of temperature and ration size on carbon and nitrogen stable isotope fractionation. Funct Ecol 21:356–362

Barnett A, Bellwood DR, Hoey AS (2006) Trophic ecomorphology of cardinalfish. Mar Ecol Prog Ser 322:249–257

Bates D, Maechler M, Bolker BM, Walker S (2014) lme4: Linear mixed-effects models using Eigen and S4. R package version 1.0-6. http://cran.r-project.org/package=lme4

Bearhop S, Adams C, Waldron S, Fuller R, Macleod H (2004) Determining trophic niche width: a novel approach using stable isotope analysis. J Anim Ecol 73:1007–1012

Boaden AE, Kingsford MJ (2015) Predators drive community structure in coral reef fish assemblages. Ecosphere 6:1–33

Bode M, Sanchirico JN, Armsworth PR (2016) Returns from matching management resolution to ecological variation in a coral reef fishery. Proc Biol Sci 283:20152828

Bone Q, Roberts BL (1969) The density of elasmobranchs. J Mar Biol 49:913–941

Bornt KR, McLean DL, Langlois TJ, Harvey ES, Bellchambers LM, Evans SN, Newman SJ (2015) Targeted demersal fish species exhibit variable responses to long-term protection from fishing at the Houtman Abrolhos Islands. Coral Reefs 34:1297–1312

Bosley KL, Witting DA, Chambers RC, Wainright SC (2002) Estimating turnover rates of carbon and nitrogen in recently metamorphosed winter flounder *Pseudopleuronectes americanus* with stable isotopes. Mar Ecol Prog Ser 236:233–240

Botsford L, Castilla J, Peterson C (1997) The management of fisheries and marine ecosystems. Science 277:509–515

Brandl SJ, Robbins WD, Bellwood DR (2015) Exploring the nature of ecological specialization in a coral reef fish community: morphology, diet and foraging microhabitat use. Proc R Soc B 282:20151147

Braune BM, Gaston AJ, Hobson KA, Gilchrist HG, Mallory ML (2014) Changes in food web structure alter trends of mercury uptake at two seabird colonies in the Canadian Arctic. Environ Sci Technol 48:13246-13252 doi:10.1021/es5036249

Briand MJ, Bonnet X, Goiran C, Guillou G, Letourneur Y (2015) Major sources of organic matter in a complex coral reef lagoon: identification from isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$). PLoS One 10:e0131555

Brooker RM, Munday PL, Mcleod IM, Jones GP (2013) Habitat preferences of a corallivorous reef fish: predation risk versus food quality. Coral Reefs 32:613–622

Buchheister A, Latour RJ (2010) Turnover and fractionation of carbon and nitrogen stable isotopes in tissues of a migratory coastal predator, summer flounder (*Paralichthys dentatus*). Can J Fish Aquat Sci 67:445–461

Bunt CM, Kingsford MJ (2014) Movement, habitat utilization and behaviour of coral trout *Plectropomus leopardus* during and after the reproductive period on the southern Great Barrier Reef. Mar Ecol Prog Ser 496:33–45

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach. Springer-Verlag, New York, NY

Calenge C (2006) The package adehabitat for the R software: a tool for the analysis of space and habitat use by animals. Ecol Modell 197:516–519

Carlisle AB, Goldman KJ, Litvin SY, Madigan DJ, Bigman JS, Swithenbank AM, Kline TC, Block BA (2015) Stable isotope analysis of vertebrae reveals ontogenetic changes in habitat in an endothermic pelagic shark. Proc R Soc B 282:20141446

Carter AB, Russ GR, Tobin AJ, Williams AJ, Davies CR, Mapstone BD (2014) Spatial variation in the effects of size and age on reproductive dynamics of common coral trout *Plectropomus leopardus*. J Fish Biol 84:1074–98

Carter AB, Carton AG, McCormick MI, Tobin AJ, Williams AJ (2015) Maternal size, not age, influences egg quality of a wild, protogynous coral reef fish *Plectropomus leopardus*. Mar Ecol Prog Ser 529:249–263

Caut S, Angulo E, Courchamp F (2009) Variation in discrimination factors ($\Delta^{15}N$ and $\Delta^{13}C$): the effect of diet isotopic values and applications for diet reconstruction. J Appl Ecol 46:443–453

Caut S, Laran S, Garcia-Hartmann E, Das K (2011) Stable isotopes of captive cetaceans (killer whales and bottlenose dolphins). J Exp Biol 214:538–545

Cherel Y, Hobson KA, Guinet C, Vanpe C (2007) Stable isotopes document seasonal changes in trophic niches and winter foraging individual specialization in diving predators from the Southern Ocean. J Anim Ecol 76:826–836

Chin A, Tobin A, Simpfendorfer C, Heupel M (2012) Reef sharks and inshore habitats: patterns of occurrence and implications for vulnerability. Mar Ecol Prog Ser 460:115–125

Chouvelon T, Spitz J, Caurant F, Chappuis A, Laugier F, Le Goff E, Bustamante P (2012) Revisiting the use of $\delta^{15}N$ in meso-scale studies of marine food webs by considering spatio-temporal variations in stable isotopic signatures. The case of an open ecosystem: the Bay of Biscay (north-east Atlantic). Prog Oceanogr 1:95–102

Christiansen JS, Hop H, Nilssen EM, Joensen J (2012) Trophic ecology of sympatric Arctic gadoids, *Arctogadus glacialis* (Peters, 1872) and *Boreogadus saida* (Lepechin, 1774), in NE Greenland. Polar Biol 35:1247–1257

Clementz MT, Koch PL (2001) Differentiating aquatic mammal habitat and foraging ecology with stable isotopes in tooth enamel. Oecologia 129:461–472

Connell JH (1980) Diversity and the coevolution of competitors, or the ghost of competition past. Oikos 35:131–138

Connell SD, Kingsford MJ (1998) Spatial, temporal and habitat-related variation in the abundance of large predatory fish at One Tree Reef, Australia. Coral Reefs 17:49–57

Cunjak RA, Roussel J-M, Gray MA, Dietrich JP, Cartwright DF, Munkittrick KR, Jardine TD (2005) Using stable isotope analysis with telemetry or mark-recapture data to identify fish movement and foraging. Oecologia 144:636–646

Currey LM, Williams AJ, Mapstone BD, Davies CR, Carlos G, Welch DJ, Simpfendorfer CA, Ballagh AC, Penny AL, Grandcourt EM, Mapleston A, Wiebkin AS, Bean K (2013)

Comparative biology of tropical *Lethrinus* species (Lethrinidae): challenges for multi-species management. J Fish Biol 82:764–788

Currey LM, Heupel MR, Simpfendorfer CA, Williams AJ (2014) Sedentary or mobile? Variability in space and depth use of an exploited coral reef fish. Mar Biol 161:2155–2166

Currey LM, Heupel MR, Simpfendorfer CA, Williams AJ (2015) Assessing environmental correlates of fish movement on a coral reef. Coral Reefs 34:1267–1277

Dalerum F, Angerbjörn A (2005) Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. Oecologia 144:647–58

Dance M, Rooker J (2015) Habitat- and bay-scale connectivity of sympatric fishes in an estuarine nursery. Estuar Coast Shelf Sci 167:447–457

Davies CR (1996) Inter-reef movement of the common coral trout, *Plectropomus leopardus*. Great Barrier Reef Marine Park Authority Research Publication No. 61. GBRMPA, Townsville

Davies CR, Williams AJ, Mapstone BD, Benzie J, van Herwerden L, Choat JH, Adams S, Murchie CD, Bean K, Carlos G, Tobin A, Ackerman J (2006) Stock structure and regional variation in population dynamics of the red throat emperor and other target species of the Queensland tropical reef line fishery. CRC Reef Research Centre Technical Report No. 61, CRC Reef Research Centre, Townsville

Davis WT, Drymon JM, Powers SP (2015) Spatial and dietary overlap creates potential for competition between red snapper (*Lutjanus campechanus*) and vermilion snapper (*Rhomboplites aurorubens*). PLoS One 10:e0144051

de la Higuera M, Akharbach H, Hidalgo MC, Peragon J, Lupianez JA, Garcia-Gallego M (1999) Liver and white muscle protein turnover rates in the European eel (*Anguilla anguilla*): effects of dietary protein quality. Aquaculture 179:203–216 doi:10.1016/S0044-8486(99)00163-5

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

DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42:495–506

Dennis CA, MacNeil MA, Rosati JY, Pitcher TE, Fisk AT (2010) Diet discrimination factors are inversely related to $\delta^{15}N$ and $\delta^{13}C$ values of food for fish under controlled conditions. Rapid Commun Mass Spec 24:3515–3520

Depczynski M, Bellwood DR (2003) The role of cryptobenthic reef fishes in coral reef trophodynamics. Mar Ecol Prog Ser 256:183–191

Duong T (2007) ks: kernel density estimation and kernel discriminant analysis for multivariate data in R. J Stat Soft 21:1–16

Eckert SA, Stewart BS (2001) Telemetry and satellite tracking of whale sharks, *Rhincodon typus*, in the Sea of Cortez, Mexico, and the North Pacific Ocean. Environ Biol Fishes 60:299–308

Eldøy SH, Davidsen JG, Thorstad EB, Whoriskey F (2015) Marine migration and habitat use of anadromous brown trout *Salmo trutta*. Can J Fish Aquat Sci 72:1366–1378

Elton CS (1927) Animal Ecology. MacMillan, London

Emslie MJ, Logan M, Williamson DH, Ayling AM, MacNeil AM, Ceccarelli D, Cheal AJ, Evans RD, Johns KA, Jonker MJ, Miller IR, Osborne K, Russ GR, Sweatman HP (2015) Expectations and outcomes of reserve network performance following re-zoning of the Great Barrier Reef Marine Park. Curr Biol 25:983–992

Espinoza M, Heupel MR, Tobin AJ, Simpfendorfer CA (2015a) Movement patterns of silvertip sharks (*Carcharhinus albimarginatus*) on coral reefs. Coral Reefs 34:807–821 doi:10.1007/s00338-015-1312-0

Espinoza M, Munroe SEM, Clarke TM, Fisk AT, Wehrtmann IS (2015b) Feeding ecology of common demersal elasmobranch species in the Pacific coast of Costa Rica inferred from stable isotope and stomach content analyses. J Exp Mar Bio Ecol 470:12–25

Espinoza M, Lédée EJI, Simpfendorfer CA, Tobin AJ, Heupel MR (2015c) Contrasting movements and connectivity of reef-associated sharks using acoustic telemetry: implications for management. Ecol Appl 25:2101–2118 doi:10.1890/14-2293.1

Estes JA, Terborgh J, Brashares JS, Power ME, Berger J, Bond WJ, Carpenter SR, Essington TE, Holt RD, Jackson JBC, Marquis RJ, Oksanen L, Oksanen T, Paine RT, Pikitch EK, Ripple WJ, Sandin SA, Scheffer M, Schoener TW, Shurin JB, Sinclair ARE, Soulé ME, Virtanen R, Wardle DA (2011) Trophic downgrading of planet Earth. Science 333:301–306

Evans RD, Russ GR (2004) Larger biomass of targeted reef fish in no-take marine reserves on the Great Barrier Reef, Australia. Aquat Conserv Mar Freshw Ecosyst 14:505–519

Farmer BM, Wilson SK (2011) Diet of finfish targeted by fishers in North West Australia and the implications for trophic cascades. Environ Biol Fish 91:71–85

Farmer NA, Ault JS (2011) Grouper and snapper movements and habitat use in Dry Tortugas, Florida. Mar Ecol Prog Ser 433:169–184

Ferreira BP (1993) Reproduction of the inshore coral trout *Plectropmus maculatus* (Perciformes: Serranidae) from the Central Great Barrier Reef. J Fish Biol 42:831–844

Ferreira BP (1995) Reproduction of the common coral trout *Plectropomus leopardus* (Serranidae: Epinephelinae) from the central and northern Great Barrier Reef, Australia. Bull Mar Sci 56:653–669

Ferreira BP, Russ GR (1992) Age, growth and mortality of the inshore coral trout *Plectropomus maculatus* (Pisces: Serranidae) from the Central Great Barrier Reef, Australia. Aust J Mar Freshw Res 43:1301–1312

Ferreira BP, Russ GR (1994) Age validation and estimation of growth rate of the coral trout, *Plectropomus leopardus*, (Lacepede 1802) from Lizard Island, Northern Great Barrier Reef. Fish Bull 92:46–57

Finke DL, Denno RF (2005) Predator diversity and the functioning of ecosystems: The role of intraguild predation in dampening trophic cascades. Ecol Lett 8:1299–1306

Fischer U, Ototake M, Nakanishi T (1998) Life span of circulating blood cells in ginbuna crucian carp (*Carassius auratus langsdorfii*). Fish Shellfish Immunol 8:339–349 doi:10.1006/fsim.1998.0144

Fisk AT, Hoekstra PF, Gagnon J, Duffe J, Norstrom RJ, Hobson KA, Kwan M, Muir DCG (2003) Influence of habitat, trophic ecology and lipids on, and spatial trends of, organochlorine contaminants in Arctic marine invertebrates. Mar Ecol Prog Ser 262:201–214

France RL (1995) Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. Mar Ecol Prog Ser 124:307–312

France RL, Peters RH (1997) Ecosystem differences in the trophic enrichment of ^{13}C in aquatic food webs. Can J Fish Aquat Sci 54:1255–1258

Frias-Torres S (2006) Habitat use of juvenile goliath grouper *Epinephelus itajara* on the Florida Keys, USA. Endang Species Res. 2:1–6

Frisch AJ, Ireland M, Baker R (2013) Trophic ecology of large predatory reef fishes: energy pathways, trophic level, and implications for fisheries in a changing climate. Mar Biol 161:61–73

Frisch AJ, Cameron DS, Pratchett MS, Williamson DH, Williams AJ, Reynolds AD, Hoey AS, Rizzari JR, Evans L, Kerrigan B, Muldoon G, Welch DJ, Hobbs J-PA (2016a) Key aspects of the biology, fisheries and management of coral grouper. Rev Fish Biol Fish doi:10.1007/s11160-016-9427-0

Frisch AJ, Ireland M, Rizzari JR, Lönnstedt OM, Magnenat KA, Mirbach CE, Hobbs J-PA (2016b) Reassessing the trophic role of reef sharks as apex predators on coral reefs. Coral Reefs 35:459–472

Fry B, Arnold C (1982) Rapid 13C/12C turnover during growth of brown shrimp (*Penaeus aztecus*). Oecologia 54:200–204

Gaebler OH, Vitti TG, Vukmirovich R (1966) Isotope effects in metabolism of ^{14}N and ^{15}N from unlabeled dietary proteins. Can J Biochem 44:249–1257

Gannes LZ, Brien DMO, Martínez Del Rio C (1997) Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology 78:1271–1276

Gaston AJ, Elliott KH (2014) Seabird diet changes in northern Hudson Bay, 1981−2013, reflect the availability of schooling prey. Mar Ecol Prog Ser 513:211–223

Gaye-Siesseggar J, Focken U, Abel H, Becker K (2003) Feeding level and diet quality influence trophic shift of C and N isotopes in Nile tilapia (*Oreochromis niloticus* (*L.*)). Isotopes Environ Health Stud 39:125–134

German DP, Miles RD (2010) Stable carbon and nitrogen incorporation in blood and fin tissue of the catfish *Pterygoplichthys disjunctivus* (Siluriformes, Loricariidae). Environ Biol Fishes 89:117–133

Goeden GB (1978) A monograph of the coral trout *Plectropomus leopardus*. Queensland Fish Serv Res Bull 1:1–42

Graham NAJ, Evans RD, Russ GR (2003) The effects of marine reserve protection on the trophic relationships of reef fishes on the Great Barrier Reef. Environ Conserv 30:200–208

Great Barrier Reef Marine Park Authority (2014) Great Barrier Reef Region Strategic Assessment: Strategic assessment report, GBRMPA, Townsville

Green AL, Bellwood DR (2009) Monitoring functional groups of herbivorous reef fishes as indicators of coral reef resilience – A practical guide for coral reef managers in the Asia Pacific region. IUCN working group on Climate Change and Coral Reefs. IUCN, Gland, Switzerland.

Greenwood NDW, Sweeting CJ, Polunin NVC (2010) Elucidating the trophodynamics of four coral reef fishes of the Solomon Islands using $\delta^{15}N$ and $\delta^{13}C$. Coral Reefs 29:785–792

Guelinckx J, Maes J, Van Den Driessche P, Geysen B, Dehairs F, Ollevier F (2007) Changes in delta C-13 and delta N-15 in different tissues of juvenile sand goby *Pomatoschistus minutus*: a laboratory diet-switch experiment. Mar Ecol Prog Ser 341:205–215

Guzzo MM, Blanchfield PJ, Chapelsky AJ, Cott PA (2015) Resource partitioning among top-level piscivores in a sub-Arctic lake during thermal stratification. J Great Lakes Res 42:276–285

Hanisch JR, Tonn WM, Paszkowski CA, Scrimgeour GJ (2010) $\delta^{15}C$ and $\delta^{13}N$ signatures in muscle and fin tissues: nonlethal sampling methods for stable isotope analysis of salmonids. North Am J Fish Manag 30:1–11

Harmon LJ, Matthews B, Des Roches S, Chase JM, Shurin JB, Schluter D (2009) Evolutionary diversification in stickleback affects ecosystem functioning. Nature 458:1167–1170

Harper J, Clatworthy J, McNaughton I, Sagar G (1961) The evolution and ecology of closely related species living in the same area. Evolution 15:209–227

Harrison HB, Williamson DH, Evans RD, Almany GR, Thorrold SR, Russ GR, Feldheim KA, Herwerden L Van, Planes S, Srinivasan M, Berumen ML, Jones GP (2012) Larval export from marine reserves and the recruitment benefit for fish and fisheries. Curr Biol 22:1023–1028

Harvey CJ, Hanson PC, Essington TE, Brown PB, Kitchell JF (2002) Using bioenergetics models to predict stable isotope ratios in fishes. Can J Fish Aquat Sci 124:115–124

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

Heithaus M, Vaudo J, Kreicker S, Layman C, Krützen M, Burkholder D, Gastrich K, Bessey C, Sarabia R, Cameron K, Wirsing A, Thomson J, Dunphy-Daly M (2013) Apparent resource partitioning and trophic structure of large-bodied marine predators in a relatively pristine seagrass ecosystem. Mar Ecol Prog Ser 481:225–237

Henry GW, Lyle JM (2003) The National Recreational and Indigenous Fishing Survey. Canberra: Australian Government Department of Agriculture, Fisheries and Forestry. http://eprints.utas.edu.au/2526/1/Henry_Lyle_Nationalsurvey.pdf

Herzka SZ, Holt GJ (2000) Changes in isotopic composition of red drum (*Sciaenops ocellatus*) larvae in response to dietary shifts: potential applications to settlement studies. Can J Fish Aquat Sci 57:137–147

Herzka SZ, Holt SA, Holt GJ (2001) Documenting the settlement history of individual fish larvae using stable isotope ratios: model development and validation. J Exp Mar Bio Ecol 265:49–74

Hesslein RH, Hallard KA, Ramlal P (1993) Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$. Can J Fish Aquat Sci 50:2071–2076

Heupel M, Simpfendorfer C (2011) Estuarine nursery areas provide a low-mortality environment for young bull sharks *Carcharhinus leucas*. Mar Ecol Prog Ser 433:237–244

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

Heupel M, Simpfendorfer C (2015) Long-term movement patterns of a coral reef predator. Coral Reefs 34:679–691

Heupel MR, Semmens JM, Hobday AJ (2006) Automated acoustic tracking of aquatic animals: scales, design and deployment of listening station arrays. Mar Freshw Res 57:1–13

Heupel MR, Williams AJ, Welch DJ, Davies CR, Adams S, Carlos G, Mapstone BD (2010) Demography of a large exploited grouper, *Plectropomus laevis*: implications for fisheries management. Mar Freshw Res 61:184–195

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

Hilborn R, Stearns SC (1982) On inference in ecology and evolutionary biology: the problem of multiple causes. Acta Biotheor 31:145–164

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

Hobson KA, Clark RG (1992) Assessing avian diets using stable isotopes II: factors influencing diet-tissue fractionation. Condor 94:189–197

Hobson KA, Welch HE (1992) Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. Mar Ecol Progr Ser 84:9–18

Hobson KA, Alisauskas RT, Clark RG (1993) Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analyses of diet. Condor 95:388–394

Hobson KA, Ambrose WG, Renaud PE (1995) Sources of primary production, benthic-pelagic coupling, and trophic relationships within the Northeast Water Polynya: insights from $\delta^{13}C$ and $\delta^{15}N$ analysis. Mar Ecol Prog Ser 128:1–10

Hobson KA, Schell DM, Renouf D, Noseworthy E (1996) Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. Can J Fish Aquat Sci 533:528–533

Hobson KA, Fisk A, Karnovsky N, Holst M, Gagnon J, Fortier M (2002) A stable isotope ($\delta^{13}C$, $\delta^{15}N$) model for the North Water food web: implications for evaluating trophodynamics and the flow of energy and contaminants. Deep Sea Res Part II Top Stud Oceanogr 49:5131–5150

Hoekstra PF, O'Hara TM, Fisk AT, Borgå K, Solomon KR, Muir DC (2003) Trophic transfer of persistent organochlorine contaminants (OCs) within an Arctic marine food web from the southern Beaufort-Chukchi Seas. Environ Pollut 124:509e522

Houlihan DF, Hall SJ, Gray C, Noble BS (1988) Growth rates and protein turnover in Atlantic cod, *Gadus morhua*. Can J Fish Aquat Sci 45:951–964 doi:10.1139/f88-117

Hussey NE, Brush J, McCarthy ID, Fisk AT (2010) $\delta^{15}N$ and $\delta^{13}C$ diet-tissue discrimination factors for large sharks under semi-controlled conditions. Comp Biochem Physiol A Mol Integr Physiol 155:445–453

Hussey NE, MacNeil MA, McMeans BC, Olin JA, Dudley SFJ, Cliff G, Wintner SP, Fennessy ST, Fisk AT (2014) Rescaling the trophic structure of marine food webs. Ecol Lett 17:239–50

Hussey NE, Kessel ST, Aarestrup K, Cooke SJ, Cowley PD, Fisk AT, Harcourt RG, Holland KN, Iverson SJ, Kocik JF, Mills Flemming JE, Whoriskey FG (2015) Aquatic animal telemetry: A panoramic window into the underwater world. Science 348:1255642

Hutchinson N, Rhodes KL (2010) Home range estimates for squaretail coralgrouper, *Plectropomus areolatus* (Rüppell 1830). Coral Reefs 29:511–519

Huveneers C, Simpfendorfer CA, Kim S, Semmens J, Hobday AJ, Pederson H, Stieglitz T, Vallee R, Webber D, Heupel MR, Peddemors V, Harcourt R (2016) The influence of environmental parameters on the performance and detection range of acoustic receivers. Methods Ecol Evol doi:10.1111/2041-210X.12520

Ingram T, Matthews B, Harrod C, Stephens T, Grey J, Markel R (2007) Lipid extraction has little effect on the $\delta^{15}N$ of aquatic consumers. Limnol Oceanogr 5:338–343

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

Januchowski-Hartley FA, Graham NAJ, Feary DA, Morove T, Cinner JE (2011) Fear of fishers: human predation explains behavioral changes in coral reef fishes. PLoS ONE 6: e22761

Johansen JL, Messmer V, Coker DJ, Hoey AS, Pratchett MS (2013) Increasing ocean temperatures reduce activity patterns of a large commercially important coral reef fish. Glob Chang Biol 20:1067–1074

Johansen JL, Pratchett MS, Messmer V, Coker DJ, Tobin AJ, Hoey AS (2015) Large predatory coral trout species unlikely to meet increasing energetic demands in a warming ocean. Sci Rep 5:13830 doi:10.1038/srep13830

Johnson JE, Welch DJ (2016) Climate change implications for Torres Strait fisheries: assessing vulnerability to inform adaptation. Clim Change 135:611–624 doi:10.1007/s10584-015-1583-z

Kelly MH, Hagar WG, Jardine TD, Cunjak RA (2006) Nonlethal sampling of sunfish and slimy sculpin for stable isotope analysis: how scale and fin tissue compare with muscle tissue. N Am J Fish Manag 26:921–925

Kennedy P, Kennedy H, Papadimitriou S (2005) The effect of acidification on the determination of organic carbon, total nitrogen and their stable isotopic composition in algae and marine sediment. Rapid Commun Mass Spectrom 19:1063–1068

Kessel ST, Cooke SJ, Heupel MR, Hussey NE, Simpfendorfer CA, Vagle S, Fisk AT (2014) A review of detection range testing in aquatic passive acoustic telemetry studies. Rev Fish Biol Fish 24:199–218

Kessel ST, Hussey NE, Crawford RE, Yurkowski DJ, O'Neill CV, Fisk AT (2015) Distinct patterns of Arctic cod (*Boreogadus saida*) presence and absence in a shallow high Arctic embayment, revealed across open-water and ice-covered periods through acoustic telemetry. Polar Biol 39:1057–1068 doi:10.1007/s00300-015-1723-y

Kieffer JD, Colgan PW (1992) The role of learning in fish behaviour. Rev Fish Biol Fish 2:125–143

Kiljunen M, Grey J, Sinisalo T, Harrod C, Immonen H, Jones RI (2006) A revised model for lipid-normalizing $\delta^{13}C$ values from aquatic organisms, with implications for isotope mixing models. J Appl Ecol 43:1213–1222

Kim SL, del Rio CM, Casper D, Koch PL (2012a) Isotopic incorporation rates for shark tissues from a long-term captive feeding study. J Exp Biol 215:2495–500

Kim SL, Casper DR., Galván-Magaña F, Ochoa-Díaz R, Hernández-Aguilar SB, Koch PL (2012b) Carbon and nitrogen discrimination factors for elasmobranch soft tissues based on a long-term controlled feeding study. Environ Biol Fish 95:37–52

Kingsford MJ (1992) Spatial and temporal variation in predation on reef fishes by coral trout *(Plectropomus leopardus*, Serranidae). Coral Reefs 11:193–198

Kingsford MJ (2009) Contrasting patterns of reef utilization and recruitment of coral trout (*Plectropomus leopardus*) and snapper (*Lutjanus carponotatus*) at One Tree Island, southern Great Barrier Reef. Coral Reefs 28:251–264

Knip D, Heupel M, Simpfendorfer C, Tobin A, Moloney J (2011) Ontogenetic shifts in movement and habitat use of juvenile pigeye sharks *Carcharhinus amboinensis* in a tropical nearshore region. Mar Ecol Prog Ser 425:233–246

Knip DM, Heupel MR, Simpfendorfer CA (2012) Habitat use and spatial segregation of adult spottail sharks *Carcharhinus sorrah* in tropical nearshore waters. J Fish Biol 80:767–84

Kramer MJ, Bellwood O, Bellwood DR (2016) Foraging and microhabitat use by crustacean-feeding wrasses on coral reefs. Mar Ecol Prog Ser 548:277–282

Kronfeld-Schor N, Dayan T (2003) Partitioning of time as an ecological resource. Annu Rev Ecol Syst 153–181

Kuhn CE, Johnson DS, Ream RR, Gelatt TS (2009) Advances in the tracking of marine species: Using GPS locations to evaluate satellite track data and a continuous-time movement model. Mar Ecol Prog Ser 393:97–109

Kurle CM (2002) Stable-isotope ratios of blood components from captive northern fur seals (*Callorhinus ursinus*) and their diet: applications for studying the foraging ecology of wild otariids. Can J Zool 80: 902−909

Layman C, Allgeier J (2012) Characterizing trophic ecology of generalist consumers: a case study of the invasive lionfish in The Bahamas. Mar Ecol Prog Ser 448:131–141

Layman CA, Araujo MS, Boucek R, Hammerschlag-Peyer CM, Harrison E, Jud ZR, Matich P, Rosenblatt AE, Vaudo JJ, Yeager LA, Post DM, Bearhop S (2011) Applying stable isotopes to examine food-web structure: an overview of analytical tools. Biol Rev 87:545–562

Lédée EJI, Heupel MR, Tobin AJ, Simpfendorfer CA (2015) Movements and space use of giant trevally in coral reef habitats and the importance of environmental drivers. Anim Biotelemetry 3:1–14

Leigh GM, Campbell AB, Lunow CP, O'Neill MF (2014) Stock assessment of the Queensland east coast common coral trout (*Plectropomus leopardus*) fishery. Queensland Government, Brisbane. http://era.deedi.qld.gov.au/4547

Little LR, Smith ADM, Mcdonald AD (2005) Effects of size and fragmentation of marine reserves and fisher infringement on the catch and biomass of coral trout, *Plectropomus leopardus*, on the Great Barrier Reef, Australia. Fish Manag Ecol 12:177–188

Little LR, Begg GA, Goldman B, Ellis N, Mapstone BD, Punt AE, Jones A, Sutton S, Williams A (2008) Modelling multi-species targeting of fishing effort in the Queensland Coral Reef Fin Fish Fishery. Fishing and Fisheries Research Centre Technical Report No. 2. Fishing and Fisheries Research Centre, James Cook University, Townsville

Logan J, Haas H, Deegan L, Gaines E (2006) Turnover rates of nitrogen stable isotopes in the salt marsh mummichog *Fundulus heteroclitus*, following a laboratory diet switch. Oecologia 147:1–17

Logan JM, Jardine TD, Miller TJ, Bunn SE, Cunjak RA, Lutcavage ME (2008) Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. J Anim Ecol 77:838–846

Lönnstedt OM, McCormick MI, Meekan MG, Ferrari MC, Chivers DP (2012) Learn and live: predator experience and feeding history determines prey behaviour and survival. Proc R Soc B 279:2091–2098

López N, Navarro J, Barría C, Albo-Puigserver M, Coll M, Palomera I (2016) Feeding ecology of two demersal opportunistic predators coexisting in the northwestern Mediterranean Sea. Estuar Coast Shelf Sci 175:15–23

MacArthur R, Levins R (1967) The limiting similarity, convergence, and divergence of coexisting species. Am Nat 101:377–385

MacAvoy SE, Macko SA, Garman GC (2001) Isotopic turnover in aquatic predators: quantifying the exploitation of migratory prey. Can J Fish Aquat Sci 56:923–932

MacNeil MA, Drouillard KG, Fisk AT (2006) Variable uptake and elimination of stable nitrogen isotopes between tissues in fish. Can J Fish Aquat Sci 353:345–353

Mantyka CS, Bellwood DR (2007) Direct evaluation of macroalgal removal by herbivorous coral reef fishes. Coral Reefs 26:435–442

Mapstone BD, Davies CR, Little LR, Punt AE, Smith ADM, Pantus F, Lou DC, Williams AJ, Jones A, Ayling AM, Russ GR, McDonald AD (2004) The effects of line fishing on the Great Barrier Reef and evaluation of alternative potential management strategies. CRC Reef Research Centre Technical Report No. 52, CRC Reef Research Centre, Townsville

Mapstone BD, Little LR, Punt AE, Davies CR, Smith ADM, Pantus F, McDonald AD, Williams AJ, Jones A (2008) Management strategy evaluation for line fishing in the Great Barrier Reef: Balancing conservation and multi-sector fishery objectives. Fish Res 94:315–329

Marcogliese, DJ (2001) Pursuing parasites up the food chain: implications of food web structure and function on parasite communities in aquatic systems. Acta Parasitol 46:82–93

Martínez del Rio C, Wolf N, Carleton SA, Gannes LZ (2009) Isotopic ecology ten years after a call for more laboratory experiments. Biol Rev 84:91–111

Maruyama A, Yamada Y, Rusuwa B, Yuma M (2001) Change in stable nitrogen isotope ratio in the muscle tissue of a migratory goby, *Rhinogobius* sp., in a natural setting. Can J Fish Aquat Sci 58:2125–2128

Matich P, Heithaus MR (2014) Multi-tissue stable isotope analysis and acoustic telemetry reveal seasonal variability in the trophic interactions of juvenile bull sharks in a coastal estuary. J Anim Ecol 83:199–213

Matley JK, Fisk AT, Dick TA (2013) The foraging ecology of Arctic cod (*Boreogadus saida*) during open water (July–August) in Allen Bay, Arctic Canada. Mar Biol 160:2993–3004

Matley JK, Heupel MR, Simpfendorfer CA (2015) Depth and space use of leopard coralgrouper *Plectropomus leopardus* using passive acoustic tracking. Mar Ecol Prog Ser 521:201–216

McConnaughey T, McRoy CP (1979) Food-web structure and the fractionation of carbon isotopes in the Bering Sea. Mar Biol 53:257–262

McIntyre PB, Flecker AS (2006) Rapid turnover of tissue nitrogen of primary consumers in tropical freshwaters. Oecologia 148:12–21

McLean DL, Harvey ES, Meeuwig JJ (2011) Declines in the abundance of coral trout (*Plectropomus leopardus*) in areas closed to fishing at the Houtman Abrolhos Islands, Western Australia. J Exp Mar Bio Ecol 406:71–78

McMahon KW, Fogel ML, Elsdon TS, Thorrold SR (2010) Carbon isotope fractionation of amino acids in fish muscle reflects biosynthesis and isotopic routing from dietary protein. J Anim Ecol 79:1132–41

McMahon KW, Berumen ML, Mateo I, Elsdon TS, Thorrold SR (2011) Carbon isotopes in otolith amino acids identify residency of juvenile snapper (Family: Lutjanidae) in coastal nurseries. Coral Reefs 30:1135–1145

McMahon KW, Berumen ML, Thorrold SR (2012) Linking habitat mosaics and connectivity in a coral reef seascape. Proc Natl Acad Sci 109:15372-15376

McMahon K, Hamady L, Thorrold SR (2013) Ocean ecogeochemistry: a review. Oceanogr Mar Biol An Annu Rev 51:327–374

McMahon KW, Thorrold SR, Elsdon TS, McCarthy MD (2015) Trophic discrimination of nitrogen stable isotopes in amino acids varies with diet quality in a marine fish. Limnol Oceanogr 60:1076–1087 doi:10.1002/lno.10081

McMeans BC, Olin JA, Benz GW (2009) Stable-isotope comparisons between embryos and mothers of a placentatrophic shark species. J Fish Biol 75:2464–2474

McPhail JD (1993) Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): origin of the species pairs. Can J Zool 71:515–523

Mellin C, Mouillot D, Kulbicki M, McClanahan T, Vigliola L, Bradshaw C, Brainard R, Chabanet P, Edgar G, Fordham D, Friedlander A, Parravicini V, Sequeira A, Stuart-Smith R, Wantiez L, Caley M (2016) Humans and seasonal climate variability threaten large-bodied coral reef fish with small ranges. Nat Commun 7:10491 doi:10.1038/ncomms10491

Michener RH, Schell DM (1994) Stable isotope ratios as tracers in marine aquatic food webs. In: Lathja K, Michener RH (eds) Stable isotopes in ecology and environmental science. Blackwell, New York, NY

Miller I, Cheal AJ, Emslie MJ, Logan M, Sweatman H (2012) Ongoing effects of no-take marine reserves on commercially exploited coral trout populations on the Great Barrier Reef. Mar Environ Res 79:167–70

Minagawa M, Wada E (1984) Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochim Cosmochim Acta 48:1135–1140

Mittelbach GG, Persson L (1998) The ontogeny of piscivory and its ecological consequences. Can J Fish Aquat Sci 55:1454–1465

Montanari S, Amato G (2015) Discrimination factors of carbon and nitrogen stable isotopes from diet to hair and scat in captive tigers (*Panthera tigris*) and snow leopards (*Uncia uncia*). Rapid Commun Mass Spectrom 29:1–7

Montoya JP (2007) Natural abundance of ^{15}N in marine planktonic ecosystems. In: Lathja K, Michener RH (eds) Stable isotopes in ecology and environmental science. Blackwell, New York, NY

Mueller A-K, Chakarov N, Heseker H, Krüger O (2016) Intraguild predation leads to cascading effects on habitat choice, behaviour and reproductive performance. J Anim Ecol 85:774–784

Munday PL, van Herwerden L, Dudgeon CL (2004) Evidence for sympatric speciation by host shift in the sea. Curr Biol 14:1498–1504

Munday PL, Jones GP, Pratchett MS, Williams AJ (2008) Climate change and the future for coral reef fishes. Fish Fish 9:261–285

Munroe SEM, Heupel MR, Fisk AT, Simpfendorfer CA (2015) Geographic and temporal variation in the trophic ecology of a small-bodied shark: evidence of resilience to environmental change. Can J Fish Aquat Sci 72:343–351

Munroe SEM, Simpfendorfer CA, Heupel MR (2016) Variation in blacktip shark movement patterns in a tropical coastal bay. Environ Biol Fishes 99:377–389

Murry BA, Farrell JM, Teece MA, Smyntek PM (2006) Effect of lipid extraction on the interpretation of fish community trophic relationships determined by stable carbon and nitrogen isotopes. Can J Fish Aquat Sci 63:2167–2172

Nagai T, Suzuki N (2000) Isolation of collagen from fish waste material - skin, bone and fins. Food Chem 68:277–281

Nash KL, Graham NA, Bellwood DR (2013) Fish foraging patterns, vulnerability to fishing, and implications for the management of ecosystem function across scales. Ecol Appl 23:1632–1644

Nash KL, Welsh JQ, Graham NAJ, Bellwood DR (2015) Home-range allometry in coral reef fishes: comparison to other vertebrates, methodological issues and management implications. Oecologia 177:73–83

Nelson J, Chanton J, Coleman F, Koenig C (2011) Patterns of stable carbon isotope turnover in gag, *Mycteroperca microlepis*, an economically important marine piscivore determined with a non-lethal surgical biopsy procedure. Environ Biol Fishes 90:243–252

Newsome SD, Clementz MT, Koch PL (2010) Using stable isotope biogeochemistry to study marine mammal ecology. Mar Mammal Sci 26:509–572

Newton K, Côté IM, Pilling GM, Jennings S, Dulvy NK (2007) Current and future sustainability of island coral reef fisheries. Curr Biol 17:655–658

Nilsson GE, Crawley N, Lunde IG, Munday PL (2009) Elevated temperature reduces the respiratory scope of coral reef fishes. Global Change Biol 15:1405–1412

O'Toole AC, Danylchuk AJ, Goldberg TL, Suski CD, Philipp DP, Brooks E, Cooke SJ (2011) Spatial ecology and residency patterns of adult great barracuda (*Sphyraena barracuda*) in coastal waters of the Bahamas. Mar Biol 158:2227–2237

Olive PJW, Pinnegar JK, Welch R, Richards G, Polunin NVC (2003) Trophic step fractionation is the result of a dynamic equilibrium. J Anim Ecol 72:608–617

Overmyer, JO, MacNeil MA, Fisk AT (2008) Fractionation and metabolic turnover of carbon and nitrogen stable isotopes in black fly larvae. Rapid Commun Mass Spectrom 22:694–700

Palacios M, Warren DT, McCormick MI (2015) Sensory cues of a top-predator indirectly control a reef fish mesopredator. Oikos 125:201–209 doi:10.1111/oik.02116

Papastamatiou YP, Friedlander AM, Caselle JE, Lowe CG (2010) Long-term movement patterns and trophic ecology of blacktip reef sharks (*Carcharhinus melanopterus*) at Palmyra Atoll. J Exp Mar Bio Ecol 386:94–102

Parnell AC, Inger R, Bearhop S, Jackson AL (2010) Source partitioning using stable isotopes: coping with too much variation. PLoS One 5:e9672

Pastor J, Verdoit-Jarraya M, Astruch P, Dalias N, Nelva Pasqual J-S, Saragoni G, Lenfant P (2009) Acoustic telemetry survey of the dusky grouper (*Epinephelus marginatus*) in the Marine Reserve of Cerbère-Banyuls: informations on the territoriality of this emblematic species. C R Biol 332:732–40

Payne N, Gillanders B, Webber D, Semmens J (2010) Interpreting diel activity patterns from acoustic telemetry: the need for controls. Mar Ecol Prog Ser 419:295–301

Pears RJ, Morison AK, Jebreen EJ, Dunning MC, Pitcher CR, Courtney AJ, Houlden B, Jacobsen IP (2012) Ecological risk assessment of the East Coast Otter Trawl Fishery in the Great Barrier Reef Marine Park: Data report, Great Barrier Reef Marine Park Authority, Townsville

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Syst 18:293–320

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Development Core Team (2013) nlme: Linear and nonlinear mixed effects models. R package v. 3.1-109

Pinnegar JK, Polunin NVC (1999) Differential fractionation of $\delta^{13}C$ and $\delta^{15}N$ among fish tissues: implications for the study of trophic interactions. Funct Ecol 13:225–231

Polito MJ, Reiss CS, Trivelpiece WZ, Patterson WP, Emslie SD (2013) Stable isotopes identify an ontogenetic niche expansion in Antarctic krill (*Euphausia superba*) from the South Shetland Islands, Antarctica. Mar Biol 160:1311–1323

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

Post DM, Layman CA, Arrington DA, Takimoto G, Quattrochi J, Montaña CG (2007) Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152:179–89

Pyke GH, Pulliam HR, Charnov EL (1977) Optimal foraging: a selective review of theory and tests. Q Rev Biology 52:137–154

R Core Team (2013, 2014, 2015, 2016) A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Randall JE, Allen GR, Steene RC (1997) Fishes of the great barrier reef and coral sea. University of Hawaii Press, Hawaii

Reich KJ, Bjorndal KA, Martínez Del Rio C (2008) Effects of growth and tissue type on the kinetics of ^{13}C and ^{15}N incorporation in a rapidly growing ectotherm. Oecologia 155:651–63

Ricker WE (1979) Growth rates and models. In: Hoar WS, Randall DJ, Brett JR (eds) Fish Physiology. Academic Press, New York, NY

Rizzari JR, Frisch AJ, Hoey AS, McCormick MI (2014) Not worth the risk: Apex predators suppress herbivory on coral reefs. Oikos 123:829–836

Robbins CT, Felicetti LA, Sponheimer M (2005) The effects of dietary protein quality on nitrogen isotope discrimination in mammals and birds. Oecologia 144:534–540

Robbins CT, Felicetti LA, Florin ST (2010) The impact of protein quality on stable nitrogen isotope ratio discrimination and assimilated diet estimation. Oecologia 162:571–579

Rocha LA, Bowen BW (2008) Speciation in coral-reef fishes. J Fish Biol 72:1101–1121

Roessig J, Woodley C, Cech J, Hansen L (2004) Effects of global climate change on marine and estuarine fishes and fisheries. Rev Fish Biol Fish 14:251–275

Roff G, Doropoulos C, Rogers A, Bozec Y-M, Krueck NC, Aurellado E, Priest M, Birrell C, Mumby PJ (2016) The ecological role of sharks on coral reefs. Trends Ecol Evol 31:395–407

Ross ST (1986) Resource partitioning in fish assemblages: a review of field studies. Copeia 2:352–388

Russ GR, Cheal AJ, Dolman AM, Emslie MJ, Evans RD, Miller I, Sweatman H, Williamson DH (2006) Rapid increase in fish numbers follows creation of world's largest marine reserve network. Curr Biol 18:514–515

Sadovy Y (2005) Trouble on the reef: the imperative for managing vulnerable and valuable fisheries. Fish Fish 6:167–185

Sadovy de Mitcheson Y, Colin PL (2012) Reef fish spawning aggregations: biology, research and management. In: Noakes D (ed) Fish & fisheries series, Vol 35. Springer, Dordrecht

Sadovy de Mitcheson Y, Craig MT, Bertoncini AA, Carpenter KE, Cheung WWL, Choat JH, Cornish AS, Fennessy ST, Ferreira BP, Heemstra PC, Liu M, Myers RF, Pollard DA, Rhodes KL, Rocha, LA, Russell BC, Samoilys MA, Sanciangco J (2013) Fishing groupers towards extinction: A global assessment of threats and extinction risks in a billion dollar fishery. Fish Fish 14:119–136

Sale PF (1974) Overlap in resource use, and interspecific competition. Oecologia 17:245–256

Sale PF (1977) Maintenance of high diversity in coral reef fish communities. Am Nat 337–359

Samoilys MA (1987) Aspects of the behaviour, movements and population density of *Plectropomus leopardus* (Lacepede) (Pisces: Serranidae) at Heron Island reef, southern Great Barrier Reef, Australia. MSc thesis, University of Queensland

Samoilys MA (1997) Movement in a large predatory fish: coral trout, *Plectropomus leopardus* (Pisces: Serranidae), on Heron Reef, Australia. Coral Reefs 16:151–158

Samoilys MA, Squire LC (1994) Preliminary observations on the spawning behaviour of coral trout, *Plectropomus leopardus* (Pisces: Serranidae), on the Great Barrier Reef. Bull Mar Sci 54:332–342

Scales H, Balmford A, Manica A (2007) Impacts of the live reef fish trade on populations of coral reef fish off northern Borneo. Proc R Soc B 274:989–994

Schindler DE, Essington TE, Kitchell JF, Boggs C, Hilborn R (2002) Sharks and tunas: fisheries impacts on predators with contrasting life histories. Ecol Appl 12:735–748

Schlacher TA, Connolly RM (2014) Effects of acid treatment on carbon and nitrogen stable isotope ratios in ecological samples: a review and synthesis. Methods Ecol Evol 5:541–550

Schlaff AM, Heupel MR, Simpfendorfer CA (2014) Influence of environmental factors on shark and ray movement, behaviour and habitat use: a review. Rev Fish Biol Fish 24:1089–1103

Schoener TW (1968) Sizes of feeding territories among birds. Ecology 49:123–141

Schoener TW (1974) Resource partitioning in ecological communities. Science 185:27–39

Schoener TW (1983) Field experiments on interspecific competition. Am Nat 122:240–285

Simpfendorfer CA, Heupel MR, Hueter RE (2002) Estimation of short-term centers of activity from an array of omnidirectional hydrophones and its use in studying animal movements. Can J Fish Aquat Sci 59:23–32

Smith MAK (1981) Estimation of growth potential by measurement of tissue protein synthetic rates in feeding and fasting rainbow trout, *Salmo gairdnerii* Richardson. J Fish Biol 19:213–220 doi:10.1111/j.1095-8649.1981.tb05825.x

Sotiropoulos MA, Tonn WM, Wassenaar LI (2004) Effects of lipid extraction on stable carbon and nitrogen isotope analyses of fish tissues: potential consequences for food web studies. Ecol Freshw Fish 13:155–160

Speed CW, Meekan MG, Field IC, McMahon CR, Abrantes K, Bradshaw CJA (2012) Trophic ecology of reef sharks determined using stable isotopes and telemetry. Coral Reefs 31:357–367

Speed CW, Meekan MG, Field IC, McMahon CR, Harcourt RG, Stevens JD, Babcock RC, Pillans RD, Bradshaw CJA (2015) Reef shark movements relative to a coastal marine protected area. Reg Stud Mar Sci 3:58–66

St. John J (1995) Feeding ecology of the coral trout, *Plectropomus leopardus* (Serranidae), on the Great Barrier Reef, Australia. PhD thesis, James Cook University

St. John J (1999) Ontogenetic changes in the diet of the coral reef grouper *Plectropomus leopardus* (Serranidae): patterns in taxa, size and habitat of prey. Mar Ecol Prog Ser 180:233–246

St. John J (2001) Temporal variation in the diet of a coral reef piscivore (Pisces: Serranidae) was not seasonal. Coral Reefs 20:163–170

Stephens DW, Krebs JR (1986) Foraging theory. Princeton University Press, Princeton, NJ

Sumpton W, Mayer D, Brown I, Sawynok B, McLennan M, Butcher A, Kirkwood J (2008) Investigation of movement and factors influencing post-release survival of line-caught coral reef fish using recreational tag-recapture data. Fish Res 92:189–195

Sun Z, Xia S, Feng S, Zhang Z, Rahman MM, Rajkumar M, Jiang S (2014) Effects of water temperature on survival, growth, digestive enzyme activities, and body composition of the leopard coral grouper *Plectropomus leopardus*. Fish Sci 81:107–112

Suring E, Wing SR (2009) Isotopic turnover rate and fractionation in multiple tissues of red rock lobster (*Jasus edwardsii*) and blue cod (*Parapercis colias*): consequences for ecological studies. J Exp Mar Biol Ecol 370:56–63. doi:10.1016/j.jembe.2008.11.014

Suzuki KW, Kasai A, Nakayama K, Tanaka M (2005) Differential isotopic enrichment and half-life among tissues in Japanese temperate bass (*Lateolabrax japonicus*) juveniles: implications for analyzing migration. Can J Fish Aquat Sci 678:671–678

Sweeting CJ, Jennings S, Polunin NVC (2005) Variance in isotopic signatures as a descriptor of tissue turnover and degree of omnivory. Funct Ecol 19:777–784

Sweeting CJ, Polunin NVC, Jennings S (2006) Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. Rapid Commun Mass Spectrom 20:595–601

Sweeting CJ, Barry JT, Polunin NVC, Jennings S (2007a) Effects of body size and environment on diet-tissue $\delta^{13}C$ fractionation in fishes. J Exp Mar Bio Ecol 352:165–176

Sweeting CJ, Barry J, Barnes C, Polunin NVC, Jennings S (2007b) Effects of body size and environment on diet-tissue $\delta^{15}N$ fractionation in fishes. J Exp Mar Bio Ecol 340:1–10

Tamelander T, Kivimäe C, Bellerby RGJ, Renaud PE, Kristiansen S (2009) Base-line variations in stable isotope values in an Arctic marine ecosystem: effects of carbon and nitrogen uptake by phytoplankton. Hydrobiologia 630:63–73

Tarboush RA, MacAvoy SE, Macko SA, Connaughton V (2006) Contribution of catabolic tissue replacement to the turnover of stable isotopes in *Danio rerio*. Can J Zool 84:1453–1460

Thébaud O, Innes J, Norman-López A, Slade S, Cameron D, Cannard T, Tickell S, Kung J, Kerrigan B, Williams L, Little LR (2014) Micro-economic drivers of profitability in an ITQ-managed fishery: An analysis of the Queensland Coral Reef Fin-Fish Fishery. Mar Pol 43:200–207

Thompson AA, Mapstone BD (2002) Intra- versus inter-annual variation in counts of reef fishes and interpretations of long-term monitoring studies. Mar Ecol Prog Ser 232:247–257

Tieszen LL, Boutton TW, Tesdahl KG, Slade NA (1983) Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for $\delta^{13}C$ analysis of diet. Oecologia 57:32–37

Tobin A, Schlaff A, Tobin R, Penny A, Ayling T, Ayling A, Krause B, Welch D, Sutton S, Sawynok B, Marshall N, Marshall P, Maynard J (2010) Adapting to change: minimising uncertainty about the effects of rapidly-changing environmental conditions on the Queensland Coral Reef Fin Fish Fishery. Final Report to the Fisheries Research & Development Corporation, Project 2008/103. Fishing & Fisheries Research Centre Technical Report no. 11, James Cook University, Townsville

Tobin A, Currey L, Simpfendorfer C (2013) Informing the vulnerability of species to spawning aggregation fishing using commercial catch data. Fish Res 143:47–56

Trueman C, McGill R, Guyard P (2005) The effect of growth rate on tissue-diet isotopic spacing in rapidly growing animals. An experimental study with Atlantic salmon (*Salmo salar*). Rap Comm Mass Spectrom 19:3239–3247

Turner MW, Hulme B (1970) The plasma proteins: an introduction. Pitman Medical & Scientific Publishing Company, London

Udyawer V, Simpfendorfer CA, Read M, Hamann M, Heupel MR (2016) Exploring habitat selection in sea snakes using passive acoustic monitoring and Bayesian hierarchical models. Mar Ecol Prog Ser 546:249–262

van Herwerden L, Choat JH, Dudgeon CL, Carlos G, Newman SJ, Frisch AJ, van Oppen M (2006) Contrasting patterns of genetic structure in two species of the coral trout *Plectropomus* (Serranidae) from east and west Australia: introgressive hybridisation or ancestral polymorphisms. Mol Phylogenet Evol 41:420–435

van Herwerden L, Choat JH, Newman SJ, Leray M, Hillersoy G (2009) Complex patterns of population structure and recruitment of *Plectropomus leopardus* (Pisces: Epinephelidae) in the Indo-West Pacific: implications for fisheries management. Mar Biol 156:1595–1607

Vander Zanden MJ, Rasmussen JB (1999) Primary consumer $\delta^{13}C$ and $\delta^{15}N$ and the trophic position of aquatic consumers. Ecology 80:1395–1404

Vander Zanden MJ, Rasmussen JB (2001) Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic fractionation: Implications for aquatic food web studies. Limnol Oceanogr 48:2061–2066

Vander Zanden MJ, Hulshof M, Ridgway MS, Rasmussen JB (1998) Application of stable isotope techniques to trophic studies of age-0 smallmouth bass. Trans Am Fish Soc 127:729–739

Vander Zanden JM, Casselman JM, Rasmussen JB (1999) Stable isotope evidence for the food web consequences of species invasions in lakes. Nature 401:464–467

Waldie PA, Almany GR, Sinclair-Taylor TH, Hamilton RJ, Potuku T, Priest MA, Rhodes KL, Robinson J, Cinner JE, Berumen ML (2016) Restricted grouper reproductive migrations support community-based management. R Soc Open Sci 3:150694 doi:10.1098/rsos.150694

Ward-Paige C, Flemming JM, Lotze HK (2010) Overestimating fish counts by non-instantaneous visual censuses: consequences for population and community descriptions. PLoS One 5:e11722

Warnes GR, Bolker B, Lumley T, Johnson RC (2015). gmodels: Various R Programming Tools for Model Fitting. R package v. 2.16.2.

Welsh JQ, Fox RJ, Webber DM, Bellwood DR (2012) Performance of remote acoustic receivers within a coral reef habitat: implications for array design. Coral Reefs 31:693–702

Wen C, Almany G, Williamson D, Pratchett M, Jones G (2012) Evaluating the effects of marine reserves on diet, prey availability and prey selection by juvenile predatory fishes. Mar Ecol Prog Ser 469:133–144

Wen CKC, Pratchett MS, Almany GR, Jones GP (2013) Patterns of recruitment and microhabitat associations for three predatory coral reef fishes on the southern Great Barrier Reef, Australia. Coral Reefs 32:389–398

Wen CKC, Bonin MC, Harrison HB, Williamson DH, Jones GP (2016) Dietary shift in juvenile coral trout (*Plectropomus maculatus*) following coral reef degradation from a flood plume disturbance. Coral Reefs 35:451–455

Williams DM, Russ GR (1994) Review of data on fishes of commercial and recreational fishing interest on the Great Barrier Reef. Great Barrier Reef Marine Park Authority Research Publication No. 33, Townsville

Williams AJ, Currey LM, Begg GA, Murchie CD, Ballagh AC (2008) Population biology of coral trout species in eastern Torres Strait: Implications for fishery management. Cont Shelf Res 28:2129–2142

Williamson DH, Russ GR, Ayling AM (2004) No-take marine reserves increase abundance and biomass of reef fish on inshore fringing reefs of the Great Barrier Reef. Environ Conserv 31:149–159

Williamson DH, Ceccarelli DM, Evans RD, Jones GP, Russ GR (2014) Habitat dynamics, marine reserve status, and the decline and recovery of coral reef fish communities. Ecol Evol 4:337–354

Willis TJ, Sweeting CJ, Bury SJ, Handley SJ, Brown JCS, Freeman DJ, Cairney DG, Page MJ (2013) Matching and mismatching stable isotope ($\delta^{13}C$ and $\delta^{15}N$) ratios in fin and muscle tissue among fish species: a critical review. Mar Biol 160:1633–1644

Wilson RP, Poe WE (1974) Nitrogen metabolism in channel catfish. *Ictalurus punctatus* - III relative pool sizes of free amino acids and related compounds in various tissues of the catfish. Comp Biochem Physiol B 48:545–556

Witting DA, Chambers CR, Bosley KL, Wainright SC (2004) Experimental evaluation of ontogenetic diet transitions in summer flounder (*Paralichthys dentatus*), using stable isotopes as diet tracers. Can J Fish Aquat Sci 61:2069–2084

Wolf N, Carleton SA, Martinez del Rio C (2009) Ten years of experimental animal isotopic ecology. Funct Ecol 23:17–26

Wolfe BW, Lowe CG (2015) Movement patterns, habitat use and site fidelity of the white croaker (*Genyonemus lineatus*) in the Palos Verdes Superfund Site, Los Angeles, California. Mar Environ Res 109:69–80

Wright KJ, Higgs DM, Belanger AJ, Leis JM (2008) Auditory and olfactory abilities of the Indo-Pacific coral trout *Plectropomus leopardus* (Lacepede) at settlement. J Fish Biol 72:2543–2556

Wyatt ASJ, Waite AM, Humphries S (2012) Stable isotope analysis reveals community-level variation in fish trophodynamics across a fringing coral reef. Coral Reefs 31:1029–1044

Yin, X (2014) Sustainability of coral trout, *Plectropomus leopardus*, fisheries in the Philippines and Indonesia. PhD thesis, University of Hong Kong

Yurkowski DJ, Hussey NE, Semeniuk C, Ferguson SH, Fisk AT (2014) Effects of lipid extraction and the utility of lipid normalization models on $\delta^{13}C$ and $\delta^{15}N$ values in Arctic marine mammal tissues. Polar Biol 38:131–143

Zaret TM, Rand AS (1971) Competition in tropical stream fishes: support for the competitive exclusion principle. Ecology 52:336–342

Zeller D (1997) Home range and activity patterns of the coral trout *Plectropomus leopardus* (Serranidae). Mar Ecol Prog Ser 154:65–77

Zeller DC (1998) Spawning aggregations: patterns of movement of the coral trout *Plectropomus leopardus* (Serranidae) as determined by ultrasonic telemetry. Mar Ecol Prog Ser 162:253–263

Zeller DC (2002) Tidal current orientation of *Plectropomus leopardus* (Serranidae). Coral Reefs 21:183–187

Zeller DC, Russ GR (1998) Marine reserves: patterns of adult movement of the coral trout (*Plectropomus leopardus* (Serranidae)). Can J Fish Aquat Sci 55:917–924

Zeller D, Stoute SL, Russ GR (2003) Movements of reef fishes across marine reserve boundaries: effects of manipulating a density gradient. Mar Ecol Prog Ser 254:269–280

Zuur AF, Ieno EN, Walker N, Saveliev AA, Smith GM (2009) Mixed Effects Models and Extensions in Ecology with R. Springer, New York, NY