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Jason R. How
Edith Cowan University

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The biology and ecology of epinephilidae speciea and their implications to fisheries management

Jason R. How
Edith Cowan University, jason.how@fish.wa.gov.au

The Biology and Ecology of Epinephilidae Species and Their Implications to Fisheries Management

Jason Richard How

B.Sc. (Hons)

This thesis is presented for the degree of

Doctor of Philosophy at Edith Cowan University

Faculty of Computing and Health Science

School of Natural Science

Centre for Marine Ecosystems Research

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Dedication

This thesis is dedicated to my 'girls'

Two of whom have not known me without my Ph.D. while the other gave me two of my greatest gift during that time and not to mention immense support.

So, to Sandy, Charlotte and Alyssa, I dedicate this thesis to you and look forward to spending more time with my 'girls'.

Charlotte's drawing of Daddy's fish

Abstract

This thesis examines the biological and ecological aspects of members of the globally exploited Epinephilidae with reference to fisheries management and in particular the use of closures. The leopard coralgrouper *Plectropomus leopardus* is a common reef fish throughout the Indo-Pacific region and a major target of both commercial and recreational fisheries. There has been limited research on this species in the eastern Indian Ocean, where there are only two populations. An examination of the species found a number of significant biological differences to their eastern Australian con-specifics. Most noticeable was the strong size-related cue over their protogynous sex change. The lack of the typical social mechanisms regulating sex change for this family ultimately resulted on a spatially explicit pattern in spawning omission. After an assessment of a number of anthropogenic and environmental parameters, fishing pressure was found to be the likely cause behind spawning omission. Spawning was restricted to areas where fishing pressure was light (i.e. within protected areas), and fish were able to attain a size where they could become male. The strong link between spawning sites and males, coupled with historical samples of spawning fish at locations where current extensive sampling revealed none, re-affirms the hypothesis of fishing pressure causing spawning omission. Histology, underwater visual census and acoustic tracking were used to determine the spawning aggregation dynamics of leopard coralgrouper at some of these spawning sites. Results yielded interesting differences from the GBR population and a number of other epinephelids. At the Abrolhos Islands, spawning was not limited to the new moon, but also encompassed the full moon, with a distinct difference between male and female attendances at aggregation sites. Females only attended aggregations during these lunar phases, while males moved to the aggregations almost daily during the spawning season. The formation of aggregations in the morning and their cessation by the afternoon provided a major departure from almost all other examples of spawning aggregations in Epinephelidae, which are considered to undertake a dusk spawning. Acoustic telemetry of leopard coralgrouper at the Abrolhos Islands indicated the species is generally sedentary, which is consistent with the suite of epinephelids that were tracked at Ningaloo Reef. Twenty fish from six species of Epinephelidae were tracked for up to two years revealing highly sedentary, habitat attached movement patterns. There was limited movement between habitats, with channels bisecting the reef slope, providing a natural barrier to fish movement. The findings of the thesis are all discussed with reference to fisheries management and in particular spatial and temporal closures. They all provide evidence toward a common finding that management measures must take into account local species-specific biological and ecological characteristics.

USE OF THESIS

Declaration

I certify that this thesis does not, to the best of my knowledge and belief:

i. Incorporate, without acknowledgement, any material previously submitted for a degree or diploma in any institution of higher education

ii. Contain any material previously published or written by another person except where due reference is made in the text; or

iii. Contain any defamatory material

I also grant permission for the Library at Edith Cowan University to make duplicate copies of my thesis as required

Sign ……..

A selection of the people that helped in the field along the way

Acknowledgements

This thesis has been an extremely testing process at times and its completion is due in no small part to a number of people's assistance, guidance and support.

Firstly, a huge debt of gratitude is owed to Dr Glenn Hyndes. Glenn was my primary supervisor, and as the rest fell away, he kept the thesis and me on track. Thank you Glenn for all of your support both academically and politically to get this thesis through. I truly wouldn't have been able to do it without you. And now after 3 years as your RA and too many to mention as your student, you are finally free. Cheers mate.

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Being a collaborative project with the Department of Fisheries and having so many good friends there was of immeasurable help in this project. I think during this thesis I have utilized peoples expertise from every section: Biodiversity, Finfish, Invertebrates and SADA. I have gotten a lot of support from almost everyone in the building asking how I was going and encouraging me when my motivation was low. However, there were several people that have been particularly helpful that I should mention, so I may as well go through it by section

To the Biodiversity team, and particularly Scott Evans and Lynda Bellchambers, thank you for allowing me to come on your Abrolhos Islands trips. Without being able to come on these trips I wouldn't have been able to get this project anywhere close to where it is now. Being able to collect fish and even undertake underwater visual surveys while on your research trips made a massive difference, so thank you both very much for being so accommodating on what were already busy trips. Scott, you have been not only a fantastic mate but an awesome colleague to help out with all of the various projects and make them a whole heap of fun and light-hearted even when they were far from that. I really appreciate it mate!

Working on a finfish species, it was natural to get assistance from the finfish section. Coincidently, a lot of the projects on which I got their assistance didn’t make it to the final thesis for one reason or another, but what I learnt from those experiences certainly did. Firstly, to David Fairclough and Brett Molony for their review of chapters as they were completed, and to Dave for his assistance in further explaining the intricacies of sex change and endless support in working through some of the findings of the biological chapters. Also to Steven Newman who very kindly parted with some historical biological data of leopard coralgrouper from the Abrolhos Islands that was pivotal in the current examination of spawning omission. I should also thank Paul Lewis. Lewie was a constant companion for a lot of the work that didn’t make it into this thesis, but took up considerable field time. So Lewie, thank you very much for all your help on the Dhufish work, I just hope that we one day get it sorted so all that work wasn’t in vain.

While not working on finfish, or having an extensive field program at the Abrolhos Islands, staff from the Invertebrate section were extremely helpful. Their assistance in a lot of the work that lead to the Appendix to this thesis dealing with acoustic tracking is not forgotten. Most were involved in dropping and pulling blocks with receivers on them. Special mention must go however to Simon de Lestang (spelt it right this time, unlike my honours acknowledgements). Sime provided me not only a job when I deferred from thesis, and again when my funding finished, but also was an extremely understanding teacher. Thanks Sime for your patience in teaching me R, and not pointing out the obvious when my ‘rock lobster’ coding was clearly being used for my thesis. Your encouragement and assistance was awesome, thanks boss.

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The chapter on the movement of groupers at Ningaloo was the brainchild of Russ Babcock and Richard Pillans. Their generosity to provide tags and field time at Ningaloo as part of their program was the only way this chapter ever came about. I am very grateful for the help you guys gave me, and the experiences I had while I was up at Ningaloo with you. My only saving grace is that I haven’t had to sit through too many State of Origin games with you in recent times. Carn the Roaches.

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To Mum and Dad, your support was also invaluable. Mum your encouragement at even the smallest victory spurred me on to complete this thesis, not to mention providing me with another long standing joke. Even though the work on the red-throated emperor didn’t make it into the thesis, its new name you inadvertently gave it will forever be associated with you and this thesis. And Dad, having someone of your scientific calibre to discuss ideas with and edit my work was amazing. Your understanding of what I was going through, and support both academically and emotionally, was fantastic. Also I am indebted to you for offering to take me to the Abrolhos Islands on your field trips. To offer such help selflessly as you did is more than I could ever ask. Thank you both I have truly amazing parents.

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Chapter I

Introduction

1.1 Fisheries Management

The aim of fisheries management is to allow exploitation of fish resources at an ecologically sustainable level (King 1995). Traditional methods used to manage exploitation levels include input controls, such as effort quotas and gear restrictions, and output controls such as catch quotas, recreational bag limits and minimum legal sizes (Bohnsack 1998). In order to develop appropriate regulations for exploited species, it is essential to understand their biology, including their growth patterns, length and age compositions and reproductive biology (King 1995). For example, minimum legal lengths are preferably based on estimates of length at first maturity, so as to provide fish the opportunity to reproduce at least once prior to capture (McPhee 2008).

Increasingly, catch and effort data are being combined with biological and ecological data of exploited species in stock assessment models (e.g. de Lestang *et al.* 2012). Basic mathematical descriptions of fisheries dynamics are not new, with a simple description first proposed by Russell (1931, in Haddon 2001). Parameters in this model included weights of animals recruiting to the fishery, and the growth of individuals within the fishery, combined with catch and natural mortality rate (Russell 1931 in Haddon 2001). More expansive models extending on this concept also include immigration/emigration and geographical distribution (Haddon 2001).

Consequently, with growth and movement data pivotal to such models, they are intrinsically dependent on understanding the exploited species' biology. Where models start to include information regarding spawning stock and reproductive output, reproductive parameters such as the size and or age at which reproduction first occurs, spawning season, and the number of times females spawn within the season are also required (Haddon 2001).

An underlying assumption of most stock assessment models is that species are meant to spawn annually (iteroparus), and spawn each spawning season (Rideout *et al.* 2005). However, there is increasing evidence that iteroparus species may 'skip' a spawning season(s). The majority of cases showing 'skipped' spawning (or spawning omission) is usually based on fish exposed to limited food supply (Burton and Idler 1997) or low water temperature (Pawson *et al.* 2000) in laboratory conditions, which have resulted in females not completing oocyte maturation. In wild populations, there have been a few documented cases of spawning omission, with potential causes thought to be associated with high energetic costs associated with reproductive development and spawning migration (Pollock 1984; Bell *et al.* 1992) or individuals at the edge of their geographic range (Fennesy and Sadovy 2002). Reproductively inactive females above the size at maturity were also thought to be associated with fine-scale variations in spawning times (Adams 1996). The cause of spawning omission in wild populations, however, is often difficult to identify due to a lack of both spatial and temporal data (Rideout *et al.* 2005). Identification of spawning omission, independent of the cause, is of great importance in managing stocks. It has ramifications for estimation of spawning biomass and can lead to an overestimation of size at maturity if all mature fish are not spawning each season. Therefore, documenting spawning omission is critical for management, and identification of the cause would allow future predictions of the likelihood of spawning omission.

Typically, models and management practices are based on gonochoristic (separate sex) species (Bannerot *et al.* 1987), however, a large number of tropical reef fish are hermaphroditic (Sadovy 1996). This has important implications for management, as the cue for sexual transition determines the ability of a population to respond to increases in fishing pressure. Species with an exogeneous (social) control on sexual transition will have reduced mean fecundity, as bigger females compensate the loss of males through fishing by transitioning to males (Shapiro 1987). In contrast, species with an endogenous control would be more susceptible to sperm limitation caused by the loss of larger individuals through selective removal. Hence, an understanding of reproductive mode (gonochoristic or hermaphroditic) and the patterns of reproductive development are important for the success of management measures.

A demographic feature of a number of reef fish species is the aggregation of a large number of individuals to spawn (Thresher 1984; Choat 2012). Exploitation of spawning aggregations has led to local depletions at a number of spawning aggregations sites in the Caribbean and Indo-Pacific regions (Colin 1992; Claydon 2004; Sadovy and Domeier 2005). Depletions through over-exploitation of spawning aggregations can lead to declines in spawning biomass and potentially to an over-fished stock (King 1995). This over-exploitation has been facilitated by the temporal and spatial predictability of those spawning aggregations (Colin 1996; Johannes *et al.* 1999). Conversely, the predictability that has led to the demise of spawning aggregations can also be utilised to appropriately manage/protect spawning aggregations through temporal or spatial closures.

1.2 Management through fisheries closures

Closures, typically temporal, have been used to protect spawning aggregations from over-exploitation through local tribal law when traditional groups are prohibited from capturing reef fish during the spawning season (Johannes *et al.* 1999), or by legislative fishing bans over specific lunar periods when aggregations form (DEEDI 2010). For example, a seasonal spawning closure is in place for *Pagrus auratus* (Sparidae) during its four-month spawning period in two marine embayments in Western Australia where the species spawns (Wakefield 2010). In comparison, two nine day closures around the new moons in October and November on the Great Barrier Reef, were designed to protect the spawning aggregations of primarily *Plectropomus leopardus* (Epinephelidae), which aggregates on the new moons from September to December (Samoilys 1997b). These two examples highlight the variation in the temporal component of a closure from either months (*P. auratus*) to days (*P. leopardus*). The duration of a temporal closure requires different levels of information relating the spawning aggregation dynamics of the exploited species. Information necessary for seasonal closures can be attained through biological data, however, when specific lunar bans are implemented, a more detailed assessment of spawning aggregation dynamics is required.

An extension of the temporal closure concept has been the introduction of marine reserves, which have also been referred to as either sanctuaries, refugia, marine protected areas or no–take zones (Halpern 2003). Such reserves are used throughout the world to denote specific areas where the extraction of some or all marine organisms is illegal (Newman *et al.* 2002, Gell and Roberts 2003, Halpern 2003). This is a more contemporary tool used for the management of fish resources and conservation of marine biodiversity, through the removal of exploitation pressure in defined areas (Newman *et al.* 2002, Roberts *et al.* 2003). For fisheries management, marine reserves are considered an effective tool for heavily exploited reef fish populations that do not benefit from traditional management measures (Plan Development Team 1990) and in regions that lack any other form of fisheries management, or where such measures are unable to be enforced (Newman *et al.* 2002).

Marine reserves are implemented to provide a refuge from fishing pressure, and thereby increase biomass of fish populations (Willis *et al.* 2003a). This may provide a benefit to adjacent fished areas through export of larval or post settlement individuals (Gell and Roberts 2003). Reviews of studies have shown that marine reserves do create an area of increased abundance and biomass, that on average, doubles the density and triples the biomass of a species, and increases the size of fish for a species (Gell and Roberts 2003, Halpern 2003). Although critical examination of published empirical studies has shown that there can be shortfalls in the design of those studies (Willis *et al.* 2003b), there have been a number of examples of rigorous empirical studies that have shown a positive effect of marine reserves (see Willis *et al.* 2003b). In some cases, the increase within a marine reserve has led to density dependent export of post-settlement fish to adjacent fished areas (Gell and Roberts 2003, Abesamis and Russ 2005, Goñi *et al.* 2008).

The success of marine reserves in the protection of exploited species, and their potential benefits, is dependent on the degree of protection afforded to the species. The amount of protection can be viewed as the proportion of time spent by an individual within a reserve (Kramer and Chapman 1999). Effective protection, therefore, requires the area of the marine reserves to be related to the home range of the exploited species. Barrett (1995) suggested that the reserve size should be an order of magnitude greater than the distance of the daily movements of the widest ranging individual tracked for a species. However, a meta-analysis of marine reserves literature examining 89 studies showed most marine reserves were small (median size 4km^2; Halpern 2003), and therefore were more likely to benefit only sedentary species. The sedentary nature of many exploited species often leads to increased exploitation as they can be targeted, but their limited movement would also afford them good protection in marine reserves.

The placement (location) of a marine reserve has two main features that can greatly alter its effectiveness: the habitats contained within the marine reserve, and the location of the marine reserve boundaries in relation to these habitats (Kramer and Chapman 1999). Given the diversity in the habitat requirements and movement patterns of exploited species (King 1995), the placement of an marine reserve that is suitable to all species is essentially impossible. Placement is further complicated by variations in habitat use within species. Estimates of home range size, or home range configuration (shape) has been shown to be significantly different for species in the different habitats within the same locations (Zeller 1997, Eristhee and Oxenford 2001). The rate of movement across reserve boundaries can also be different depending on whether the boundary was located along contiguous reef habitat or separated by channels (Eristhee and Oxenford 2001). Since reef fish are generally sedentary, with reluctance to cross sand channels to adjacent reefs (Barrett 1995, Chapman and Kramer 2000), placing marine reserve boundaries on natural habitat boundaries has major influences on the effectiveness of the marine reserve (Topping *et al.* 2005).

If the aim of a marine reserve is to benefit an exploited species, it is critical to assess the movement patterns of that species in planning new, or assessing existing, marine reserves. This also involves an understanding of habitat utilisation and potential natural barriers to movement that, if included, may augment the effects of protection. Teleost and elasmobranch fisheries are often non-selective and thus capture a range of species as opposed to a single species (Welch *et al.* 2008). Therefore, if marine reserves were to be used in the management of a suite of exploited species, a multi-species spatial assessment of the likely benefits of a proposed marine reserve would be required. With a spatial understanding inherent in marine reserve design, examples of multi-species movement studies, particularly those using acoustic telemetry, have been rare (e.g. Finstad *et al.* 2005, Lindholm *et al.* 2005).

1.3 Epinephelidae

The family Epinephelidae (Groupers), formerly Serranidae (Smith and Craig 2007), is a heavily exploited group of fish in world fisheries (Morris *et al.* 2000). Studies on the biology of epinephelids have been conducted on species around the world, including the Atlantic (Colin 1992; Boldon 2000), Indian (Grandcourt 2002, Grandcourt *et al.* 2009) and Pacific Oceans (Ferreira and Russ 1994, Ferreira 1995, Adams *et al.* 2000). Epinephelids are generally sedentary and ambush their prey, and have a predominantly piscivorous diet on tropical coral reefs (Heemstra and Randle 1993). Typically, they are protogynous hermaphrodites that tend to form spawning aggregations (Heemstra and Randle 1993). Due to their biological characteristics, such as longevity and late sexual maturation, and their tendency to form spawning aggregations, epinephelids are vulnerable to fishing (Colin 1992; Claydon 2004; Sadovy and Domeier 2005, Sadovy de Mitcheson *et al.* 2012). The susceptibility of this family to exploitation highlights the need for appropriate fisheries management. However, epinephelids, likely due to their sedentary nature, have shown to benefit from reserves (Nardi *et al.* 2004, Evans and Russ 2004, Williamson *et al.* 2004). In Western Australia, they form part of the Gascoyne Demersal Scalefish Fishery among others, which abuts the Ningaloo Marine Park (Jackson *et al.* 2011). The Ningaloo Marine Park is a multiuse marine park incorporating multiple sanctuary zones which were recently expanded (Anon. 2005). Given the expansion of the sanctuary zone, and the exploitation of this family, a multi-species assessment of the movement of this exploited family would benefit the assessment of these new sanctuary zones.

1.4 Leopard Coralgrouper (*Plectropomus leopardus)*

One member of Epinephilidae, the leopard coralgrouper *Plectropomus leopardus*, is listed as “Near Threatened” as part of the IUCN “Red List” (Cornish and Kiwi 2004). Its distribution extends from Japan in the north, to both the west and east coasts of Australia in the south, as well as extending as far eastwards into the Pacific Ocean to Fiji and the Caroline Islands (Heemstra and Randle 1993) (Figure 1.1)

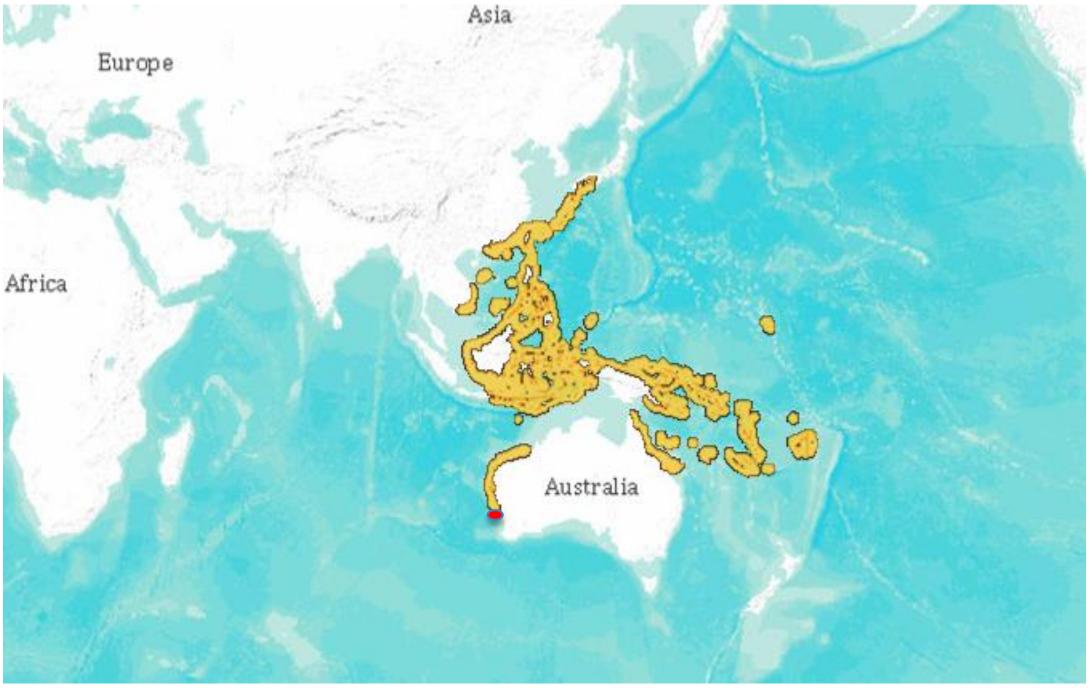

Figure 1.1 Distribution of *P. leopardus* from IUCN Red-list (http://maps.iucnredlist.org/map.html?id=44684; Accessed 17 Apr. 12) and the location of Houtman Abrolhos Islands (red circle)

Genetic samples from four regions within *P. leopardus'* range (Taiwan, West Australia, East Australia and New Caledonia) revealed six distinct populations, with a possibility of an additional six populations likely from other areas of the species' range not sampled (van Herwerden *et al.* 2009). The major genetic separation occurs between eastern Australia, including the Great Barrier Reef (GBR) and New Caledonia, and Western Australia-Taiwan, and is a likely consequence of the Torres Strait land bridge (van Herwerden *et al.* 2009). Current oceanographic features suggest Scott Reef is a source region for stocks of *P. leopardus* at the Abrolhos Islands off the mid-west coast of Australia, however, population subdivision implies high levels of self-recruitment at each location to replenish their biomass (van Herwerden *et al.* 2009).

P. leopardus is commercially exploited throughout almost its entire range (see Cornish and Kiwi 2004), with increased exploitation driven by the live reef fish trade, for which it is a preferred species (Sadovy *et al.* 2003). Hong Kong is a major importer of live fish (Lee and Sadovy 1998), with catch figures revealing an increasing contribution from Australian populations to this live fish market (Cornish and Kiwi 2004). The Australian catch comes predominantly from the GBR, where *P. leopardus* forms a major component of commercial as well as recreational reef-fish fisheries (Mapstone *et al.* 2001).

Due to its particular importance in the GBR, there has been considerable research on *P. leopardus* in eastern Australia. Living up to 14 years (Ferreira and Russ 1994), this diandric protogynous hermaphrodite species (Adams *et al.* 2000), spawns at dusk on the new moon (Samoilys 1997b) during a September to December spawning season (Ferreira 1995). It is sedentary, with home ranges of around one to two hectares (Davies 1996, Samoilys 1997a, Zeller 1997, 1998, Zeller and Russ 1998, Zeller 1999, Zeller *et al.* 2003), within which it ambushes its predominantly fish prey (Zeller 1997, St. John *et al.* 2001). Such biological data have fed into management of *P. leopardus* on the GBR, which is achieved through a legal minimum size of 38cm total length that exceeds the size at maturity (Ferreira 1995), and a bag limit of seven fish (combined *Plectropomus* spp.) (DEDDI 2012). Additionally, there are two nine day fishing closures centred on the new moon in October and November to protect *P. leopardus* spawning aggregations (DEEDI 2010). Finally, *P. leopardus* is protected from fishing pressure within the sanctuary zones within the Great Barrier Reef Marine Park. Research examining the effectiveness of these MPAs for *P. leopardus* has shown that these sanctuary zones increase density and biomass of the species (Evans and Russ 2004, Williamson *et al.* 2004).

In contrast to the GBR, there has been little research conducted on *P. leopardus* in Western Australia, where they are known to be abundant at both the Houtman Abrolhos Islands (Figure 1.1; hereafter referred to as the Abrolhos Islands) and Scott Reef (Allen and Swainston 1988, Allen 2002). The Abrolhos Islands is the only location where leopard coralgrouper is commercially fished in Western Australia (St John and King 2005), but it is also taken by the recreational and charter sectors at the Abrolhos Islands (Sumner 2008). However, previous studies of fish species at the Abrolhos Islands have focussed primarily on the response of fished species (including leopard coralgrouper) to fisheries protection (Nardi *et al.* 2004 Watson *et al.* 2007, 2009, McLean *et al.* 2010). The protection of leopard coralgrouper at the Abrolhos Islands comes in the form of Reef Observation Areas (ROAs), where fin-fish capture is prohibited, but the capture of western rock lobster *Panulirus cygnus* is still permitted (Anon. 1998). There are four ROAs within the Abrolhos Islands, which range in size from 514-2744 ha and are located in the eastern part of each island group (Figure 2.1). Other than these ROAs, the other management regulations for *P. leopardus* are a recreational bag limit of 1 fish per day with a minimum legal size of 45 cm total length. The commercial sector, which is a multispecies fishery has a maximum effort of 24 398 hours annually in the "Mid-West Area", which contains the Abrolhos Islands (Anon. 2007). The commercial catch of *P. leopardus* in Western Australia however is small, and as a result, is not reported on in the state's annual fishery status reporting (Anon. 2007). The leopard coralgouper specific management restrictions were put in place despite the lack of biological information for the genetically distinct *P. leopardus* in Western Australia. This a significant knowledge gap for effective management of this exploited population.

1.5 Thesis Aims and Structure

This thesis addresses a number of key aspects of epinephelid biology and ecology that are critical for effective assessment and planning of fisheries management, particularly through fisheries closures. As focal taxa, epinephelids were chosen as they represent a ubiquitous and highly exploited family on coral reefs globally. The research focus was on leopard coralgrouper, *P. leopardus*, at a high latitude coral reef, the Abrolhos Islands, off Western Australia (28^{o}S) (Figure 2.1). A family based assessment of movement patterns and habitat use was also conducted on a suite of epinephelids at the northern Western Australian fringing coral reef of Ningaloo.

Thesis Aims

The broad aim of this thesis was to describe key aspects of the biology and ecology of epinephelids in the eastern Indian Ocean that provide important data for fisheries management, and compare these to existing data for relevant species in other Indo-Pacific and Atlantic Ocean regions. It then examines the importance of applying locally derived biological and ecological information into fisheries management, including closures. The specific objectives of the thesis were to:

1. To examine the reproductive pattern of the leopard coralgrouper *P. leopardus* at Abrolhos Islands;
2. To determine if spawning omission is apparent in the leopard coralgrouper at the Abrolhos Islands, and its potential causes;
3. To document the biology of *P. leopardus* at the Abrolhos Islands and the effects of failing to account for spawning omission on reproductive parameter estimates;
4. To examine the movement patterns and spawning aggregation dynamics of *P. leopardus* at the Abrolhos Island; and
5. To assess the suitability of a MPA for a suite of Epinephelidae species through acoustic tracking

Thesis Structure

To achieve the above aim, the study has quantified the following critical aspects of the life history characteristics of *P. leopardus*, in the Abrolhos Islands. Chapter 2 determines the reproductive patterns of *P. leopardus*, highlighting the strong endogenous (size- related) control over sexual transition in two parts of the population exposed to very different fishing pressures. The fine spatial-scale patterns in reproductive development and evidence for spawning omission in this species is documented in Chapter 3. Patterns in reproductive activity were then related to a range of environmental and anthropogenic effects to establish the possible cause of spawning omission. Chapter 4 focuses on determining growth and reproductive parameters that are critical to traditional fisheries management, and highlighting the need to examine a broad section of the population to account for spawning omission and lack of effectiveness of using non-locally derived biological parameters for the management of that species at another location. Chapter 5 then examines, through using acoustic telemetry, the movement parameters and spawning aggregation dynamics of *P. leopardus* at the Abrolhos Islands. Data on movement and aggregation patterns are critical to evaluate the appropriate size of MPAs and timing of temporal closures for effective protection of this species. The movement study is then expanded by tracking a suite of epinephelids, including *P. leopardus*, within the Ningaloo Marine Park in Chapter 6. This allowed consideration of the variability of movement patterns among epinephelids in relation to the use of MPAs to protect multiple species. The longevity (two years of tracking) of the study provides a robust assessment of movement patterns for selected species in this highly exploited family. Chapter 7 then synthesises the results from the different chapters, and examines and discusses them in the context or fisheries management, and particularly closures as a management tool. Finally, since acoustic telemetry formed a significant part of this study (Chapters 5 and 6), and is often used to examine movements in other marine organisms (Heupel *et al.* 2006), Appendix 1 examines the nature of acoustic detections with distance, and how this changes with transmitter power and environmental conditions. This work was published as: How JR, de Lestang SN (2012) Acoustic tracking: issues impacting design, analysis and interpretation of data from movement studies. Mar Freshw Res 63:312-324.

Leopard coralgrouper Plectropomus leopardus *at the Abrolhos Islands*

Chapter II

An endogenous sex change cue for a diandric protogynous hermaphrodite

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2.1 Abstract

Leopard coralgrouper, *Plectropomus leopardus*, is a common coral-reef fish throughout the Indo-Pacific, though there has been no examination of its reproductive biology in the western extent of its range. This study aims to determine the reproductive patterns of *P. leopardus*, and potential cues for sexual transition by examining populations inside and outside of marine protected areas. In the south-western extent of its range in the eastern Indian Ocean, *P. leopardus* was confirmed as a diandric protogynous hermaphrodite, with histological confirmation of secondary males confirming functional hermaphrodism and primary males developing from immature females, which provided the alternative route for male development. The transition to male, through either primary or secondary development, had a strong endogenous control, which contrasts with the common socially-mediated mechanism for sexual transition for most protogynous hermaphrodites. Sex change for *P. leopardus* in eastern Indian Ocean occurred over a narrow length range with 90% of females estimated to become male within a 17cm size range, about a L_{50} of 55cm fork length. The strong endogenous length-related cue for transition highlights the importance of fish having sufficient time to attain a length to become male. Protected areas served to reduce fishing pressure, allowing females to grow, and transition to

males. This serves to have a more balanced sex ratio reducing the potential for spawning failure.

2.2 Introduction

Functional hermaphrodism, where individuals function as both sexes over their lifetime, can either be simultaneous or sequential. Fish that are simultaneous hermaphrodites can reproduce as both a male and female, either at the same time or within a very short period of time (Sadovy de Mitcheson and Lui 2008). In comparison, sequential hermaphrodites function either as a male (protandrous) or female (protogynous) first, before changing sex (Sadovy de Mitcheson and Lui 2008). However, there can be considerable variation in the timing or cue for sex change in this latter group (see Levin and Grimes 2002).

The timing of sex change by individuals of protogynous species may be induced by exogenous and/or endogenous cues. Exogenous, or socially-induced, cues are often based around maintaining appropriate sex ratios within social groups (Shapiro 1980, Mackie 2003). This is attained by the replacement of a male through sexual transition of a larger female (Shapiro 1987, Warner and Swearer 1991, Platten *et al.* 2002), though this isn't always the largest female (Muñoz and Warner 2004). Endogenous cues often relate to sex change occurring at a specific size or age, usually a function of their maximum size or age at maturity (Allsop and West 2003a). These different cues for sexual transition have a significant impact on the species' response to fishing pressure.

With fishing targeting larger fish, there is often a reduction in the mean size of fish in targeted stocks (Jackson *et al.* 2001). In the case of protogynous hermaphrodites, the larger fish are generally males (Sadovy de Mitcheson and Lui 2008). Removal of these large fish from exogenous controlled populations would see larger females transitioning to males to maintain appropriate sex ratios (Shapiro 1980, Mackie 2003), which would lead to a reduced fecundity through the loss of larger females (Coleman *et al.* 1996). Where endogenous controls of sexual transition are important, a lack of a compensatory mechanism would lead to an altered, female dominated sex ratio, and possible sperm limitation (Petersen and Warner 2002). This results in protogynous hermaphroditic species being more susceptible to fishing pressure than gonochoristic species (Bannerot *et al.* 1987).

While computer simulations have shown that fishing pressure has a similar result, irrespective of the means by which sex change is induced (Armsworth 2001), empirical studies have shown endogenous controls for sex change resulting from altered sex ratios from high fishing pressure can lead to collapse of the mating structure and spawning omission in some species (Coleman *et al.* 1996, Chapter 3). As a large number of exploited tropical reef fish are functional protogynous hermaphrodites (Sadovy 1996), understanding the cues for sexual transition in such species is important for their effective management.

Transition from female to male in protogynous hermaphrodites can be via one or two pathways, and it is the number of pathways for transition that dictates whether a species is a monandric (one) or diandric (two) protogynous hermaphrodite (Sadovy de Mitcheson and Lui 2008). For monandric species, all males are derived from previously functioning females and therefore classified as secondary males (Sadovy de Mitcheson and Lui 2008). However, in addition to secondary males, populations of diandric species also possess primary males, those individuals that sexually mature directly as males. The number of pathways for sexual transition can be of considerable importance, particularly when there is a relatively invariant control over sexual transition.

This study aims to examine the sexual pattern of the leopard coralgrouper *P. leopardus* at Abrolhos Islands, a coral-reef system in the eastern Indian Ocean. The species is a common tropical reef Epinephilidae found throughout the Indo-Pacific (Heemstra and Randle 1993), which was initially classed as monandric (Ferreira 1995) though subsequently confirmed as a diandric protogynous hermaphrodite (Adams 2003) on the Great Barrier Reef (GBR). Potential controls over sexual transition are also investigated. Furthermore, the size/age at sex change for this species on the GBR was thought to be a combination of both endogenous and exogenous controls (Ferreira 1995). However, a subsequent regional comparison of size/age at sex change incorporating the effects of fishing through sampling in and out of marine protected areas (MPAs) found no clear exogenous or endogenous cue for sexual transition (Adams *et al.* 2000). Comparisons of sexual transition of *P. leopardus* inside and outside MPAs in this study permits a re-examination of the potential cues for sexual transition and its response to the effects of fishing on the sexual pattern of this ubiquitous, exploited Indo-Pacific epinephelid.

2.3 Methods

Study Site

The Abrolhos Islands consists of around 170 low lying islands and islets, with many associated reefs, concentrated into three major island groups (Harvey *et al.* 2001) that occur approximately 60 km offshore of Geraldton on the mid west coast of Australia. Its location near the edge of the continental shelf, sees the Abrolhos Islands ‘bathed’ in the southward flowing, warm tropical water of the Leeuwin Current (Pearce 1997).

This warm water penetrates through the Abrolhos Islands and is at its peak flow in the austral autumn and winter (Pearce 1997), resulting in the Abrolhos Islands being the southernmost major coral reef in the eastern Indian Ocean (Wells 1997).

The Abrolhos Islands contains four Reef Observation Areas (ROAs), which were gazetted in 1994 (see Figure 3.1, Anon. 1998). The ROAs provide an area of ‘no-take’ for all species with the exception of western rock lobster *Panulirus cygnus*. Studies have shown that the ROAs serve to protect *P. leopardus* from fishing with higher densities inside compared to adjacent fished areas (Nardi *et al.* 2004), though a subsequent study has shown a decline in *P. leopardus* abundance within the ROA compared to adjacent fished areas (McLean *et al.* 2011).

Sampling

Samples of *P. leopardus* were obtained from the Abrolhos Islands from commercial and recreational line fishers between 2005 and 2009. As these samples comprised fish greater than the minimum legal size of 450 mm total length, additional fishery-independent sampling inside and outside the ROAs was conducted pre-dominantly in the spawning period (December to March) during these years to obtain fish of the full length (and age) range. This also allowed for a comparison of sex transition inside and outside the ROAs to help explain the potential cues for sex change.

Laboratory processing

Total (L_T) and fork (L_F) length of each fish were measured to the nearest 1 mm and total weight (W_T) was measured to the nearest 10 g. For each fish, otoliths were removed and washed before being dried and stored in paper envelopes. In addition, gonads were removed and examined macroscopically for sex and stage of development (adapted from Mackie and Lewis 2001; Table 2.1). Testes were cut (transverse section) and squeezed to assess the amount of milt which could be extracted to determine male staging (Table 2.1). Each gonad was weighed to the nearest 0.1g and fixed in neutral buffered 10% formalin. The age of each fish was determined through double blind counts of opaque zones in sectioned otoliths (see Chapter 4 for full details).

Validation of macroscopic staging and sexing was conducted by examining histological sections of 400 macroscopically staged gonads (339♀; 46♂; 14 juveniles; 1 indeterminate). Sections were mounted and stained with hematoxylin and eosin for histological staging, which was based on the most advanced oocyte stage (West 1990) following the categories of Samoilys and Roelof (2000). Four replicate wall thickness measures were made for each of 197 ovaries that were greater than the size at first maturity (338 mm L_T) and in a pre-vitellogenic state. This was used to assess prior maturation of the resting/immature ovaries (Burton *et al.* 1997).

The incidence and location of spermatic tissue was recorded for all fish. However, males were defined as those fish with gonads containing spermatozoa within the dorsal sperm sinus (Ferreira 1995, Adams 1996, Samoilys and Roelofs 2000). When a fish matures first as a male, it is termed a primary male (Sadovy-deMitcheson and Lui 2008), whereas a secondary male arises from a fish that has been functionally a female first (Sadovy-deMitcheson and Lui 2008). Therefore, primary males show no evidence of female maturation (i.e. contain pre-vitellogenic oocytes) in addition to developed spermatozoa. In comparison, secondary males possess evidence of previous female function through the presence of vitellogenic oocytes (possibly degenerating) as well as developed spermatozoa (Sadovy-deMitcheson and Lui 2008).

Table 2.1 Gonad stage with description of macroscopic characteristics (adapted from Mackie and Lewis 2001)

	Stage	Description
Juveline	J (Juvenile)	Gonad is a small, translucent pink ribbon lying almost imperceptibly along the dorsal wall of the peritoneal cavity. Sex of the fish cannot be determined.
Female	F1 (Virgin)	Ovaries are small and usually translucent pink, apricot or ivory in colour. In smaller females, the ovaries are flattened, flaccid, and relatively inconspicuous, but they become rounded and firmer with a distinct lumen as the fish approaches maturity.
	F2 (Resting)	Resting ovaries appear round and firm and approximately 1cm in diameter. Colour is typically semi-translucent rose, or apricot. S
	F3 (Resting)	Similar to F2 though have more pronounced vascularisation
	F4 (Developing)	Ovaries are opaque, large and rotund with prominent blood vessels. Opaque oocytes are visible through the thin gonad wall and the colour is typically pale yellow or apricot.
	F5 (Spawning)	Ovaries are very large and swollen. Colour is apricot to peach with a prominent network of external blood vessels. The presence of translucent hydrated oocytes gives the ovaries a distinctive speckled or granular appearance through the thin gonad wall. Oocytes may also be released from the gonoduct when pressure is applied to the abdomen and may be present within the ovarian lumen.
	F6 (Spent)	Ovaries flaccid and usually red. Gonad wall feels thick and has a large dominant lumen when a transverse cut is made.
Male	M2 (Resting)	Testes are small, opaque and straplike. Little or no milt is extruded from the transverse section when squeezed. The section is quite angular in shape, with a speckled appearance.
	M3 (Developed)	Testes are large, opaque, and ivory or bone in colour. Internally, white sperm (milt) can usually be squeezed from the central sperm sinus. In some cases this may not be possible, although milt should be visible in the outer areas of the transverse section.
	M4 (Spawning)	Running ripe. Similar to the ripe testis but more swollen and with larger exterior blood vessels. Milt is released with little or no pressure on the abdomen or when cut

Underwater visual census

From December 2006 to February 2007, underwater visual census (UVC) at three replicate sites in four habitat types in two management areas were undertaken to estimate coral trout sizes and abundances (Figure 2.1). Habitat types were assigned based on the classification of Hatcher *et al.* (1988 in Webster *et al.* 2002) and on the habitats in which coral trout were likely to occur. Coral trout were estimated to the nearest centimeter (total length) for each of three 100x5m replicate transects at each site, resulting in a total survey area of 36,000m^2

Figure 2.1 – Location of underwater visual census sites and their habitat associated habitat types within the Easter Group of the Abrolhos Islands. The area denoted by the pink line is the reef observation area.

Analysis

The length and age at which 50% of females change sex was estimated using: (1) all male and female fish sampled; (2) fish sampled from within the ROAs; and (3) fish from fished areas outside the ROAs. Length (L_F mm) or age (years) (denoted by X) were incorporated into a non-linear (logistic) regression (Equation 2.1) to estimate length and age of 50% (L_{50} and A_{50}, respectively) and 95% (L_{95} and A_{95}, respectively) sex change.

$$p = 1 \big/ \left(1 + \exp(\ln(19) * ((X - L_{50}) / (L_{95} - L_{50})))\right) \qquad \text{Equation 2.1}$$

NB: L_{50} was replaced by $A_{50;}$ and L_{95} replaced by A_{95} in Eqn. 3.3 for estimates of age at sex change

Likelihood ratio tests were used to determine if there were significant differences between the logistic relationship for the size/age at sex change for fish from inside and outside of the ROAs. Data were analysed using the R© statistical software (R Core Development Team 2009).

2.4 Results

Size and Age at Sex Change

Sampling at the Abrolhos Islands resulted in the collection of 860 leopard coralgrouper, ranging in size from 157 to 704 mm fork length (L_F) (Table 2.2). All samples from inside the ROAs were project specific samples, as were the majority of those from outside the ROAs, though there were a few recreational samples also obtained. Those fish from ‘unknown’ locations were almost exclusively commercial samples, though there were a few recreational samples where a definitve location could also not be determined.

Table 2.2 – Numbers of coral trout sampled from inside and outside the ROAs and unknown locations (commercial catches) in each year from 2005 to 2009

	2005	2006	2007	2008	2009	Total
Unknown	62	50	5	25	3	145
In	3	52	57	0	14	156
Out	0	110	198	230	21	559

The smaller size classes (<300 mm L_F) were exclusively female, while the larger size classes (>550 mm L_F) were dominated by males (Figure 2.2). One male was 298 mm in length.

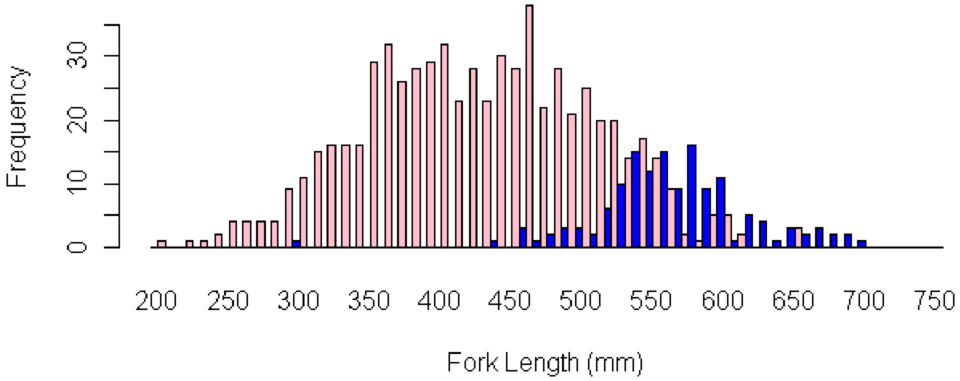


Figure 2.2 Length frequency of female and male *P. leopardus* from the Abrolhos Islands.

Sex ratios also differed between inside and outside the ROAs. Of the 150 fish from inside the ROA, 34 were male resulting in a 3.4:1 ratio of females to males. By contrast, despite 502 fish being sampled from outside the ROAs, only 42 were male leading to a sex ratio of 11 females to every male. Due to significant spawning omission (Chapter 3), an operational sex ratio could not be calculated. However, when the sex ratio was examined for fish greater than the size at maturity (L_{50} 403 mm LF; Chapter 4), the ratios were 2.2 females per male inside the ROAs (n=110) compared with 5.8 females per male outside the ROAs (n=280).

An examination of the length frequencies of coral trout recorded during UVCs inside and outside of ROAs showed that there was a clear lack of fish that were likely to be male in the fished areas (Figure 2.3 ab). There were 13 fish greater than the L_{50} for sex change inside the ROAs compared to two fish from outside. This resulted in an estimated sex ratio of 9.2 females per male inside and 65.5 females per male outside of the ROA.

Biological sampling of the commercial catches showed parity with a 1:1 ratio of males and females (n=86). Additional commercial samples where only length and weight measures were possible, combined with the biological samples, showed the clear targeting of larger (male) fish, as the males are being removed at a rate greater than their representation within the population (Figure 2.3 c).

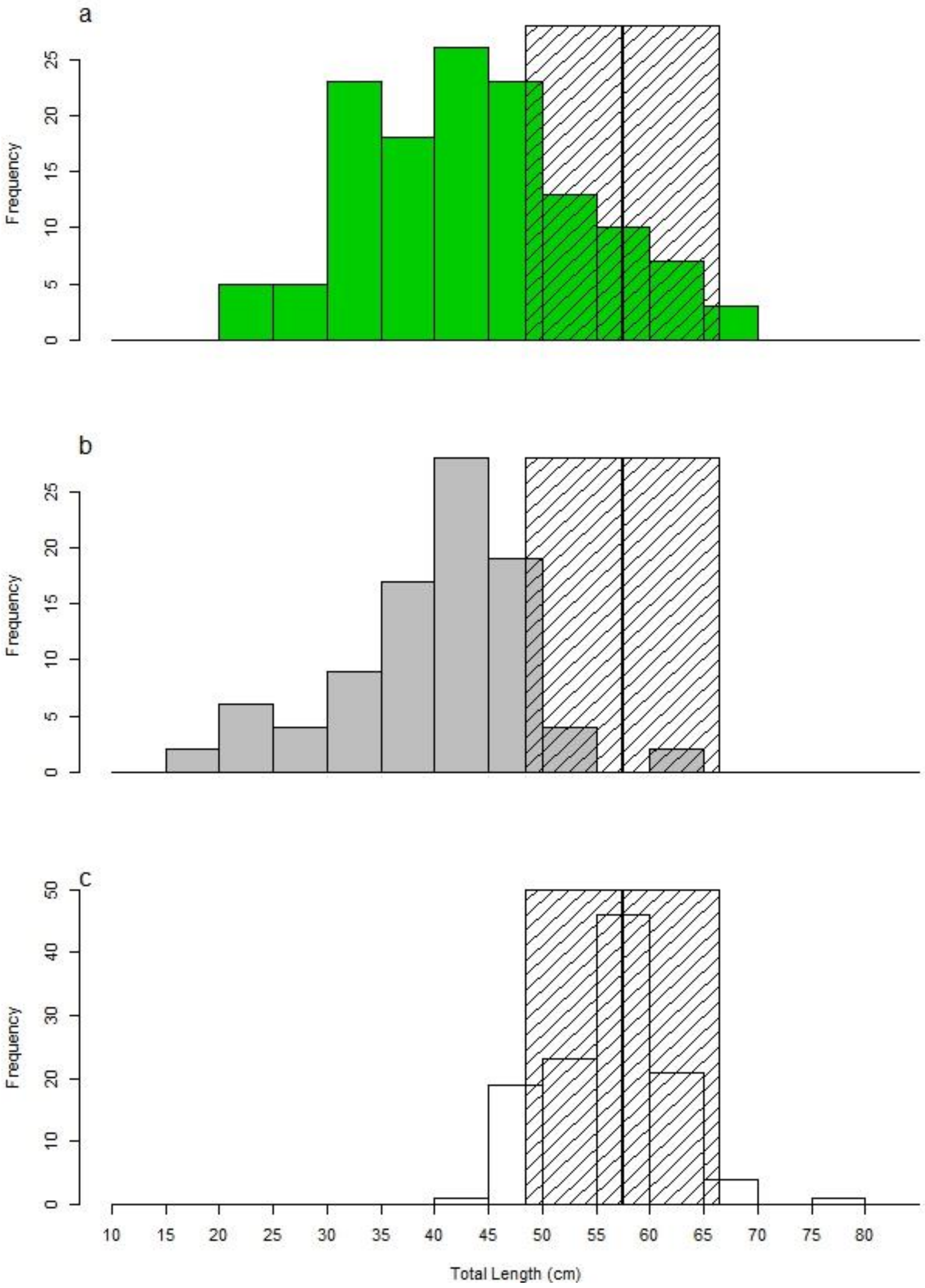


Figure 2.3 – Length (L_T cm) frequency of coral trout recorded a) inside and b) outside Reef Observation Areas from underwater visual census. c) The length frequency of the commercial catch. Dark line indicates the L_{50} of sex change with the hashed box the 90% confidence region of sex change

Logistic regressions of the proportion of males in the samples against length (L_F) and age resulted in respective L_{50} and A_{50} at sex change of 549 mm L_F and 9.9 years

(Table 2.3; Figure 2.4). In comparison, when data were restricted to those fish collected from either inside or outside the ROAs, the values were, similar (Table 2.3; Figure 2.4). The error associated with all estimates, L_F in particular, was very small, with 90% of all females changing sex over a 17cm L_F size range. There was no significant difference (p=1.0) between the models of length/age at sex change for fish inside or outside of the ROAs.

Table 2.3 Estimates of 50% (L_{50}; A_{50}) and 95% (L_{95}; A_{95}) sex change by length and age, respectively, for *P. leopardus* collected across the entire Abrolhos Islands (All) and sites inside (In) and outside (Out) of ROAs.

Sites	L_{50}(±SE)	L_{95}(±SE)	A_{50}(±SE)	A_{95}(±SE)A
All	548.96(± 2.54)	633.69 (± 8.23)	9.91 (± 0.19)	13.82 (±0.57)
Out	560.54(± 3.64)	647.77(±11.174)	10.17(±0.31)	13.65(±0.77)
In	564.38 (± 5.863)	640.99 (±19.24)	10.26 (± 0.23)	12.50(±0.72)

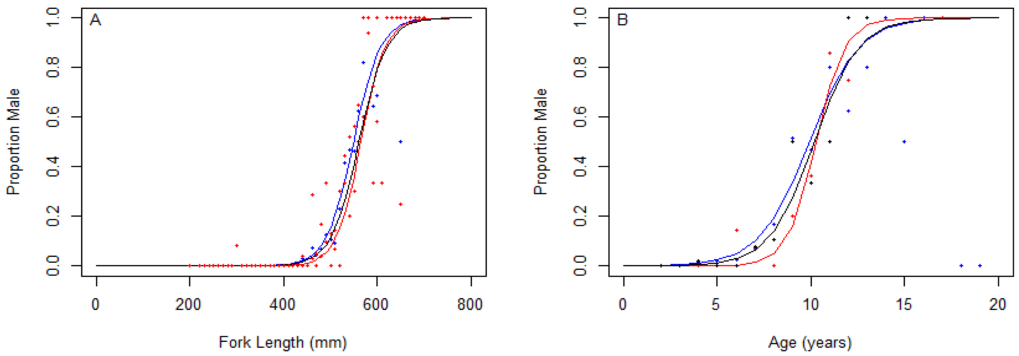


Figure 2.4 Logistic regressions for, (A) the length and (B) age, at sex change for *P. leopardus* collected across the entire Abrolhos Islands (blue) and sites inside (red) and outside (black) of ROAs. Points represent proportion of male fish by 10mm size or 1 year age class.

Histological measures of gonad wall thickness showed three separate groupings of mean ovarian wall thickness (Figure 2.5). The two major groups are those above 0.5mm and those between 0.2 and 0.4mm. There are a smaller number of females where their mean gonad wall thickness is 0.1mm or less.

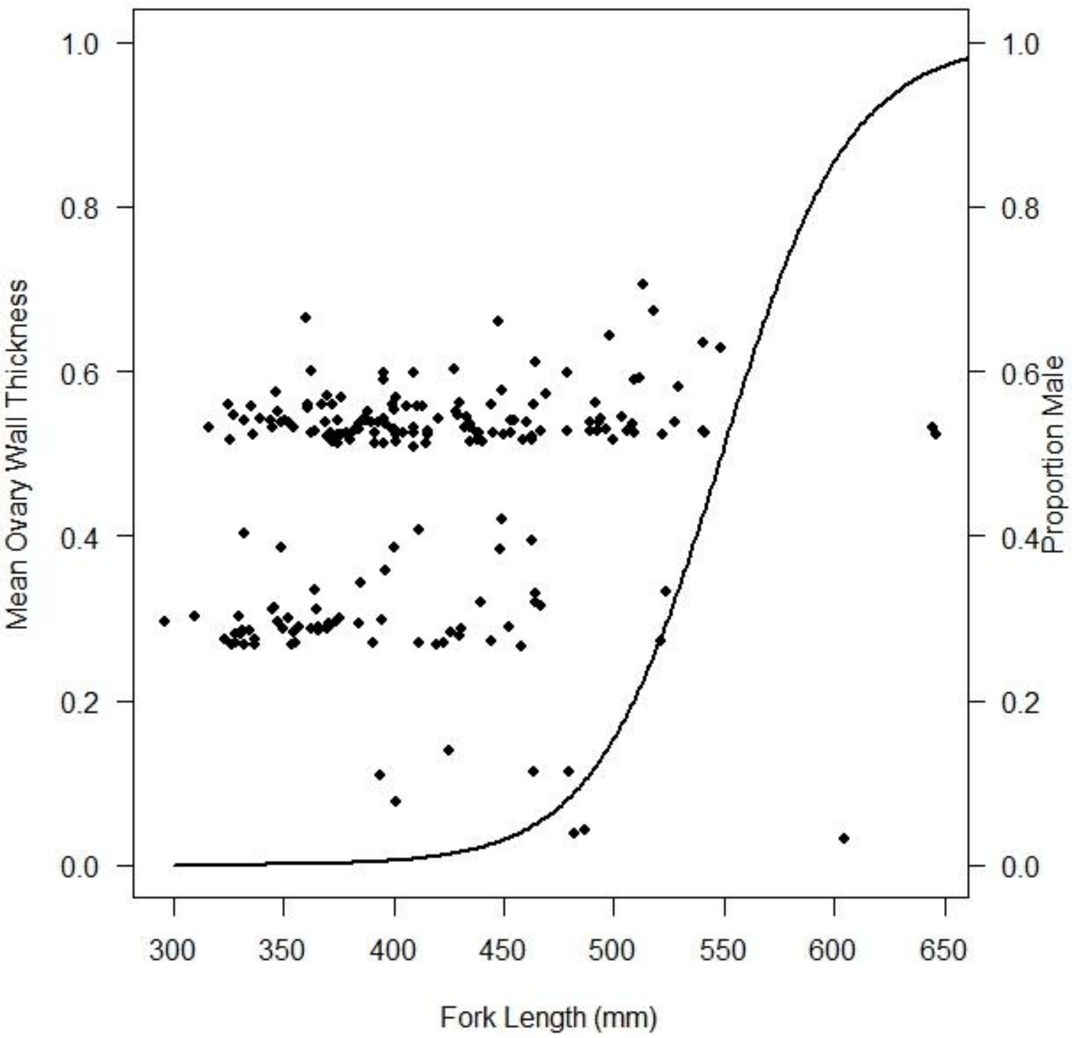


Figure 2.5 Mean ovarian wall thickness (dots) for 197 *P. leopardus* in a pre-vitellogenic state above the size at first maturity. Line indicates the logistic size at sex change relationship

Histological examination showed all fish <300 mm L_F to be immature females (Figure 2.6), i.e. no previous signs of reproductive development (F1-F3, Table 2.1). Ovaries from these fish were dominated by perinuclear stage oocytes, often tightly packed within a thin gonad wall (Plate 2.1a). Fish above this size and up to 550 mm were increasingly represented by mature females, i.e. those having shown reproductive development (F4-F6, Table 2.1), with concomitant declines in the proportion of immature females (Figure 2.6). Spermatic (male) tissue was observed in ovaries of immature (Plate 2.1b) and mature (Plate 2.1c) females from 300 mm L_F, with the proportion of fish containing male tissue increasing as they approached the size at sex change (500-549 mm L_F size class) (Figure 2.6). This male tissue was usually found in crypts around the dorsal blood vessel (Plate 2.1b). For fish greater than this size class, males dominated the samples and were the only sex present in the largest size class. However, there were some larger females well above the size of sex change that were either mature resting or immature (Figure 2.6). Immature fish were determined by the ovaries containing only pre-vitellogenic oocytes with no brown bodies evident and a thin ovary wall thickness (Figure 2.5).

Both primary and secondary males were present in samples from the Abrolhos Islands. Primary males contained pre-vitellogenic oocytes and developed spermatazoa (Plate 2.2a), including spermatazoa within the dorsal sperm sinus. In the case of secondary males, previous female function was confirmed by male gonads containing vitellogenic oocytes as well as developed spermatozoa (Plate 2.2b). Of the 74 fish examined from the spawning period that contained male tissue, 29 also contained immature ovaries and spermatic tissue (primary males), while 18 also contained mature ovaries (secondary males). However, some males did not contain female tissue (Plate 2.2c), indicating a complete and transition to a male.

Two males sampled well below the size at sex change were confirmed through histological examination (Plate 2.2d,). One of these males was 105 mm below the size at maturity (L_{50} 403 mm L_F; Chapter 4), and had a gonad containing pre-vitellogenic oocytes (Plate 2.2d), providing clear evidence of transition to a primary male.

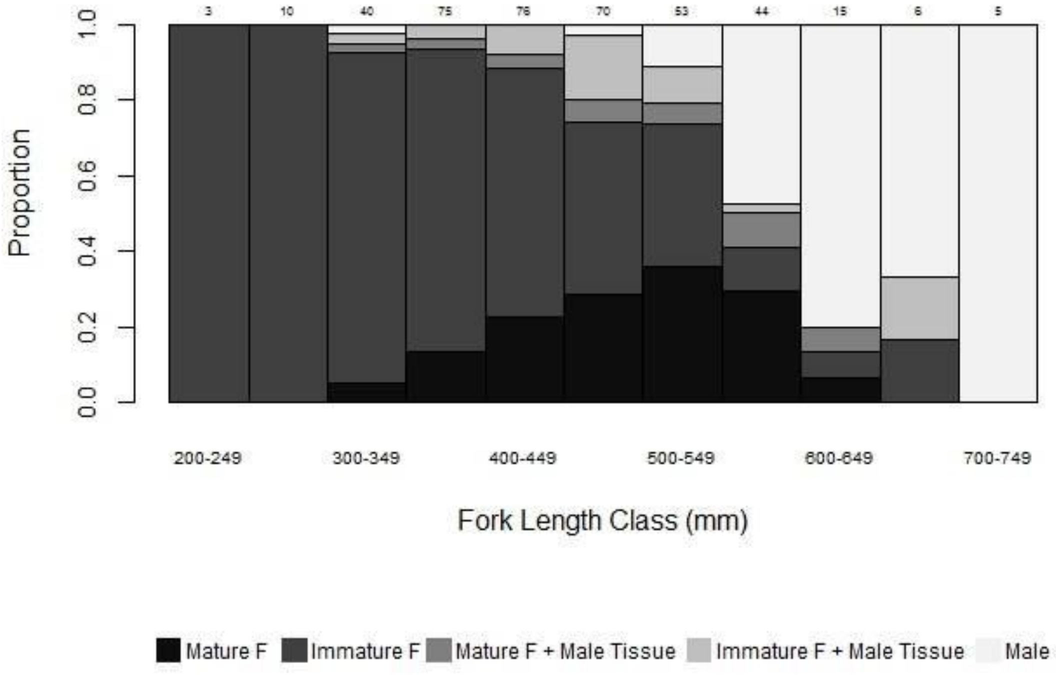
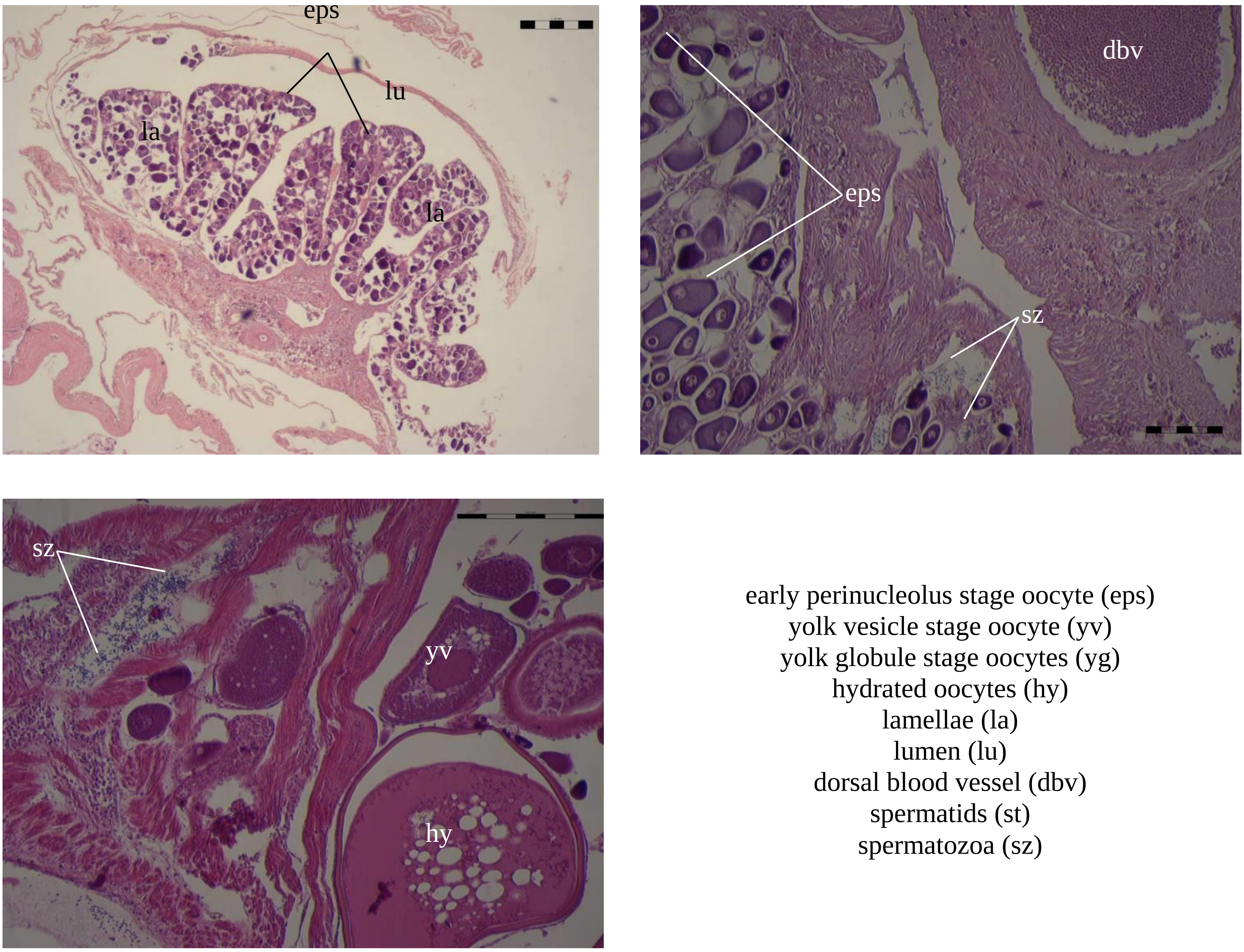


Figure 2.6 Proportion of immature females, mature females, immature females with male tissue, mature females with male tissue and males determined by histological from spawning and non-spawning sites



early perinucleolus stage oocyte (eps)
yolk vesicle stage oocyte (yv)
yolk globule stage oocytes (yg)
hydrated oocytes (hy)
lamellae (la)
lumen (lu)
dorsal blood vessel (dbv)
spermatids (st)
spermatozoa (sz)

Plate 2.1 Histological section of a) an immature female (scale bar 0.25 mm); b) a non-spawning female with spermatic tissue (scale bar 0.1mm); c) spawning female (0.2 mm)

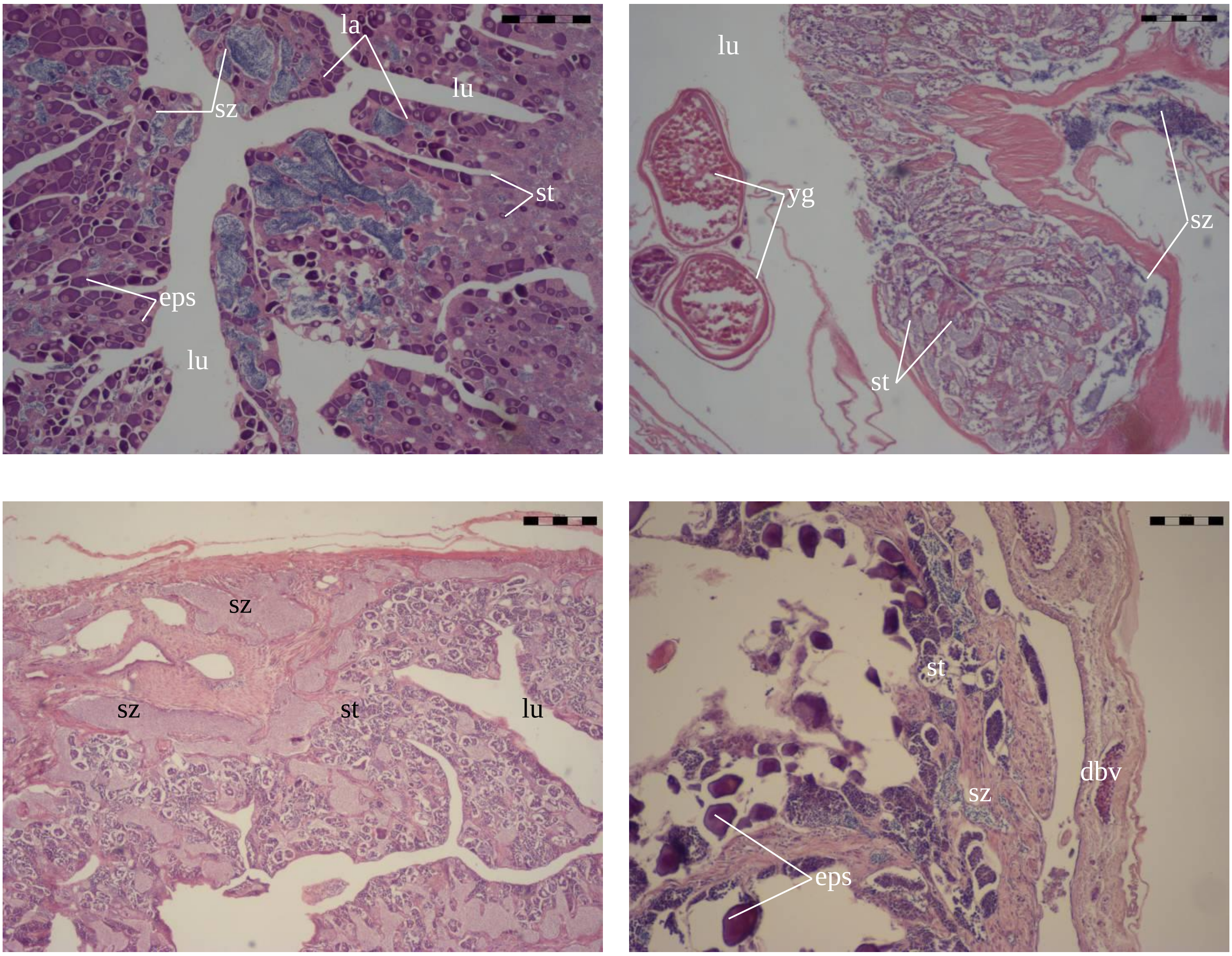


Plate 2.2 Histological sections of the testis of a) a primary male (scale bar 0.3 mm), b) a secondary male (scale bar 0.25mm), c) a male containing only male tissue (scale bar 0.25mm) d) a small male (L_F=298 mm) (scale bar 0.1 mm). Labels as per Plate 2.1

2.5 Discussion

Endogenous sexual transition

Variation in size/age at sex change in protogynous species has been postulated to be a result of social interactions (Shapiro 1987, Warner and Swearer 1991, Platten *et al.* 2002). A socially mediated (exogenous) mode of sex change is thought to ensure appropriate sex ratios in a population (Shapiro 1980, Mackie 2003). The alterations of sex ratios are usually through the differential removal of males as a result of fishing pressure (Jackson *et al.*2001).

There is a clear difference in fishing pressure between areas inside and outside of the ROAs at the Abrolhos Islands, as would be expected as the ROA serves as a marine reserve for fish. Fishing pressure on *P. leopoardus* was evident through the size distribution of fish sampled through the UVCs. Sites outside the ROA had a truncated size distribution, which lacked larger (male) fish compared to sites inside the ROAs. These larger males were however evident in the size distribution of *P.leopardus* from the commercial catch. This fishing pressure also resulted in a more female-dominated sex ratio in the fished areas, which is indicative of a non-compensatory response to fishing pressure (Bannerot *et al.* 1987).

There is the potential that such a skewed ratio where males are largely absent from outside the ROAs could occur through differential movement of large fish into the MPA. While larger fish (males) do tend to move greater distances (Chapter 5), inter-reef movements are uncommon (Davies 1996) and are not likely to be of the distance or magnitude required to explain these variations in sex ratios. Also they do not account for the male dominance in the commercial catches.

Fish from inside or outside the ROAs at the Abrolhos Islands had a L_{50} of sex change that varied by only 4mm, despite the aforementioned considerable difference in fishing pressure between inside and outside of the ROAs. The population of *P. leopardus* at the Abrolhos Islands therefore appears to have a more dominant endogenous (length) as opposed to a socially mediated (exogenous) cue for sexual transition.

There are few examples of endogenous mechanisms for sexual transition in protogynous fish species with either length (Bullough 1947) or age (McErlean and Smith 1964) related cues for sex change. The latter study was based on very limited sample sizes, leading to generally weak conclusions of an endogenous control. This contrasts with its con-specific on the GBR, where no clear exogenous or endogenous cue for sexual transition has been reported (Ferreira 1995, Adams *et al.* 2000). Also both of these studies appeared to have a greater overlap of males and females in their length frequency distributions than shown for the Abrolhos Islands population. The population of *P. leopardus* in the eastern Indian Ocean is a notable exception to the prevailing belief that sex change in most protogynous hermaphrodites follow a compensatory socially mediated mechanism (see Levin and Grimes 2002).

An endogenous (invariant) process of sexual transition is thought to occur across a range of taxa including teleosts (Allsop and West 2003ab). Sexual transition is thought to occur at 79% of their maximum size and at 2.5 times their age at maturity (Allsop and West 2003ab). For *P. leopardus*, the size at sex change ranges from 75.7-77.8% of its asymtotic length ($L_{\infty =}$ 725 mm L_F; Chapter 4), which relates well to the 79% suggested by Allsop and West (2003ab). However, age at maturity, which was 1.65-1.71 times its age at sexual maturity ($L_{50 =}$ 6.0 yearsChapter 4), does not appear to fit the proportion proposed by Allsop and West (2003ab). While this theory has been debated extensively in the literature (Allsop and West 2003ab, Buston *et al.* 2004, Allsop and

West 2004, Munday *et al.* 2006b), Munday *et al.* (2006b) noted that a more appropriate comparison for an invariant size at sex change should be made between groups within a population rather than at a population or species level. Thus, when those parameters for *P. leopardus* were compared between unfished (ROA) and fished parts of the population, reflecting the population's degree of exposure to fishing pressure, there was a very similar and strong size-related cue for sexual transition. However, it should be noted that a very small proportion (5 of 787) of *P. leopardus* at the Abrolhos Islands, did not fit the progression from an immature female to either a primary or mature female, which in turn became a secondary male associated with a strong length-related cue.

Diandric Protogyny

This study has shown that *P. leopardus* at the Abrolhos Islands is a functional diandric protogynous hermaphrodite. The presence of secondary males confirms functional hermaphrodism (Sadovy de Mitcheson and Lui 2008), while the histological confirmation of primary males with no indication of prior female maturation provides evidence of the alternative route of male development, and hence diandry (Sadovy de Mitcheson and Liu 2008). Since large numbers of immature females, as illustrated by their thin ovarian walls, were present close to the size at sex change during the spawning period at the Abrolhos Islands, it is unlikely that these immature females would mature prior to sexual transition. It would, therefore, appear that large numbers of immature females progress to males as primary males. However, there were also transitional fish sampled, showing a direct progression from a functional female to a male. Thus, both pathways for male transition, as either primary or secondary males, appear important for *P. leopardus* at the Abrolhos Islands. While this species has previously been classed as monandric (Ferreira 1995) on the GBR, a later study confirmed diandry (Adams 2003), which concurs with the mode of reproduction demonstrated for *P. leopardus* at the Abrolhos Islands.

Regardless of the pathway for transitioning to a male, all males had a testicular morphology similar to secondary males, retaining the remnant female lumen. Primary male testes from hermaphroditic species have been found to be similar in morphology to males from gonochoristic species (Robertson and Warner 1978, Warner and Robertson 1978, Munday *et al.* 2006a). However, this doesn’t appear to be the case for *P. leopardus* at the Abrolhos Islands, with all males passing through an initial immature female form. The remnant lumen of the ovary is a common feature of most males of protogynous epinephelids (Sadovy de Mitcheson and Lui 2008).

Management implications

Most protogynous hermaphrodites demonstrate a compensatory socially mediated mechanism (see Levin and Grimes 2002). Regardless of the degree of exposure to fishing pressure, *P. leopardus* at the Abrolhos Islands in the eastern Indian Ocean is one of a few reported protogynous species to demonstrate a strong endogenous (length) cue for sexual transition. This is contrary to the social (exogenous) mechanism that is common for this family (Shapiro 1987, Warner and Swearer 1991, Platten *et al.* 2002). The pathway for sex change for *P. leopardus* on the GBR was found to be the same as the population at the Abrolhos Islands, which is a functional diandric protogynous hermaphrodite, with both primary and secondary males developing from immature and mature females, respectively.

A large proportion of mature females with spermatic tissue sampled from within MPAs contrasts with the immature females with spermatic tissue that were predominantly sampled from outside MPAs. This is in keeping with the pattern of spawning omission that exists for *P. leopardus* at the Abrolhos Islands (Chapter 3). If *P. leopardus* was monandric, there would be a drastic reduction in the number of females transitioning to males. The dominance of mature females with spermatic tissue coming from MPAs suggests that these areas would be very important for the development of mature females. The large number of mature females with spermatic tissue in MPAs also concurs with the prevalence of spawning sites within the MPAs (Chapter 3). Also, with the strong size-related cue for sexual transition, the protection afforded by MPAs would permit time for these mature females to grow and develop into males.

Given that *P. leopardus* at the Abrolhos Islands is diandric, there is a pathway by which immature fish (predominantly from outside the MPAs) can develop into males providing they can reach the size at sex change. However, through restricting fishing activities, MPAs enhance the chance of fish to attain the size at which they can become male, regardless of the pathway. Therefore, it is likely that the MPAs may provide an important role in the sexual demography of *P. leopardus*, at least at the Abrolhos Islands.

A leopard coralgrouper from a non-spawning site showing the large fat deposits and the resting female gonads against the swim bladder

Chapter III

Fine-scale spatial patterns of spawning omission in a protogynous epinephelid (*Plectropomus leopardus*): potential effects of fishing pressure

3.1 Abstract

Spawning omission, when sexually mature fish of an iteroparous species skip spawning during a spawning season, has rarely been documented in wild populations. Where it has been described, it has usually been shown to occur over large (100s km) spatial scales. This study aimed to document persistent, fine-scale (km) spatial patterns of spawning activity and omission in a common and economically important tropical epinephelid, the leopard coralgrouper (*Plectropomus leopardus*). Leopard coralgrouper were sampled over peak spawning in four spawning seasons at a fine spatial scale across a range of coral-reef habitats inside and outside marine protected areas (MPAs) at the Abrolhos Islands, off the mid-west coast of Australia. A high proportion (~49%) of females were reproductively inactive over the spawning periods, and males were absent from a large proportion (69%) of sites and were almost exclusively absent from those sites where spawning omission was occurring. Spawning omission was shown to occur mainly outside MPAs where fishing pressure was relatively high. Differences in environmental conditions, such as water temperature and water movement, that form cues for reproductive development appear unlikely to explain the spatial patterns in spawning omission. Rather, generalised linear model showed a significant effect of both

male presence ($p<0.0001$) and length ($p<0.0001$) on female spawning activity. The co-occurrence of males and females at spawning sites, coupled with the impacts of fishing on a protogynous hermaphrodite such as leopard coralgrouper, lead to the conclusion that differential spatial patterns in fishing pressure may have resulted in the fine-scale spatial pattern of spawning omission. Such fishing-induced spawning omission has large implications on the effective management of the fishery, with MPAs being the most likely management tool to maintain egg production.

3.2 Introduction

Patterns in reproductive activity within a fish species can vary over large spatial scales, often reflecting differences in environmental and biological conditions (Sadovy 1996). For example, sexually mature fish within iteroparous species can skip spawning over a spawning period (Rideout *et al.* 2005), a phenomenon termed "spawning omission" or 'skipped spawning". Causes of spawning omission include limited food supply (Burton and Idler 1997) and low temperature (Pawson *et al.* 2000), and high energetic costs associated with reproductive development and spawning migration (Pollock 1984; Bell *et al.* 1992). Non-participation in spawning migrations often results in a spatial pattern in spawning omission, though spawning omission has been identified at a regional scale in a tropical emperor, *Lethrinus miniatus* (Williams *et al.* 2006), and a tropical epinephelid *Epinephilus andersoni* possibly due to individuals being at the edge of their geographic range (Fennesy and Sadovy 2002) and the possible resultant threshold temperature for spawning development not being attained (Wakefield *et al.* 2013). However, the cause of spawning omission in wild populations is often difficult to identify due to a lack of spatial and temporal data (Rideout *et al.* 2005).

Spawning aggregations are a common reproductive strategy for coral-reef fish (Thresher 1984), with spawning sites often occurring near reef passes or promontories and spurs off reefs (Russell 2001). The use of such locations is thought to be an adaptation to aid in the dispersal of fertilised eggs away from the spawning site (Barlow 1981), and thus reduce the risk of predation from ovigerous fish (Johannes 1978). As such, water movement at a location may be important in the formation of spawning aggregations and hence the occurrence of spawning fish. Species that form spawning aggregations are also susceptible to fishing pressure (Domeier and Colin 1997), with fishing generally disproportionately targeting larger fish in the population (Zeller and Russ 2000, Jackson *et al.* 2001). Spawning aggregations often present an opportunity for socially mediated protogynous hermaphrodites to assess sex ratios and undergo sexual transition if necessary to maintain appropriate ratios (Shapiro 1980). This can result in a considerable overlap in female and male size distributions (Fennessy and Sadovy 2002).

The potential removal of males can influence reproductive activity of females. For example, insufficient males, resulting in female biased sex ratios in the white sucker (*Catostomus commersoni*) lead to almost half of all females showing ovarian atresia leading to spawning omission (Trippel and Harvey 1990). Manipulation of this ratio by active male removal increased the incidence of spawning omission to around 75%. A lack of sufficient males has also been highlighted as a possible cause behind the loss of spawning potential in a population of the epinephelid *Mycteroperca microlepis* (Coleman *et al.* 1996).

Leopard coralgrouper, *Plectropomus leopardus*, is a common reef fish throughout the Indo-Pacific region and is a major target species by both commercial and recreational fishers (Mapstone *et al.* 1996). It is a protogynous hermaphrodite (Ferreira 1995; Adams 2003, Chapter 2) with a distinct size related endogenous cue for sex change in some populations resulting in all larger fish being predominantly male (Chapter 2). The species forms spawning aggregations during its spawning season (Samoilys and Squire

1994, Samolys 1997b, Chapter 5), though high proportions of inactive females and incomplete males have been noted during the spawning period on the GBR (Adams 1996). This study, therefore, aims to examine whether any spatially explicit and persistent patterns occur in spawning activity, and if spawning omission is apparent in the leopard coralgrouper at the Abrolhos Islands. To achieve this, I adopted a comprehensive sampling design focused on fine-scale sampling across a range of coral-reef habitats inside and outside marine protected areas over the spawning period of this species. Spatial patterns in spawning activity and omission are explained in relation to the potential environmental and anthropogenic factors that have been shown to influence the onset of reproductive development of fishes.

3.3 Methods

Study site and sample collection

The study was carried out at the Abrolhos Islands on the mid-west coast of Australia (Figure 3.1), and re-examines data collected for the analysis of sexual transition (Chapter 2). In this region, female *P. leopardus* attain first maturity at 338 mm L_T during a spawning season from December to March (Chapter 4). Details of spawning aggregation dynamics revealed that female spawning is associated with both new and full moons (Chapter 5). Therefore, sampling of spawning activity of *P. leopardus* during the spawning season was focused on lunar peaks of spawning in December-March across a range of coral-reef habitats known to be occupied by this species. Sites at the Abrolhos Islands were selected so as to provide an adequate spatial coverage to assess different combinations of environmental and anthropogenic effects of spawning omission. Fine-scale sampling resulted in leopard coralgrouper being collected from a total of 55 sites within and outside the MPAs in two island groups (Easter and Wallabi Groups; Figure 3.1), representing sites with relatively low and high fishing pressure.

Since some of the sites outside of the MPAs were large distances from fishers settlements on the islands (i.e., dark grey islands Figures 3.2 and 3.3), they also represented sites with relatively low fishing pressure. The majority of sampling occurred in the Easter Group (37 sites) due to its proximity to research facilities and availability of long-term environmental data sets.

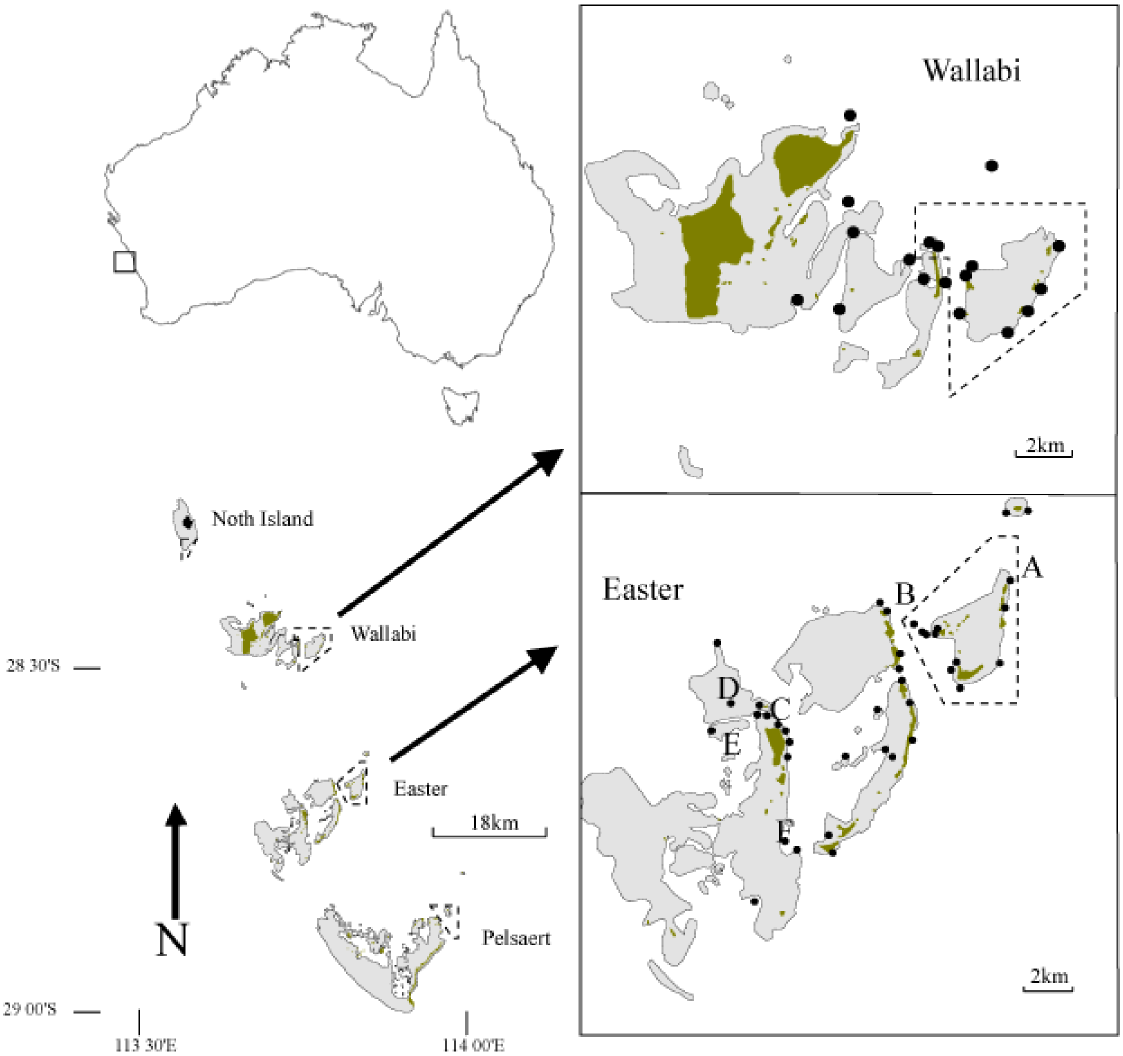


Figure 3.1 – Location of sampling sites (black filled circles), MPAs (dashed lines), islands (brown) and intertidal reefs (grey) at the Abrolhos Islands on the mid-west coast of Australia. Letters in the Easter Group inset denote locations of temperature or water loggers.

Samples were predominantly obtained through spearfishing to reduce size related biases associated with fishing (Jackson *et al.* 2001). To reduce sampling impacts particularly within the MPAs, a random sample of up to five fish was sampled at each site, and subsequently, with an additional two large fish targeted to provide information on the occurrence of males at a particular site. This was possible due to the strong size related relationship with sex change (Chapter 2). The number of samples collected during the study was kept to a minimum due to the sensitivity of the area and the vulnerability of leopard coralgrouper to over-fishing.

Total (L_T) and fork (L_F) length of each fish were measured to the nearest millimetre, and a total weight (W_T) was measured to the nearest 0.01 kg. Gonads were removed from each of the 791 fish collected, and then sexed and staged macroscopically (Chapter 2), weighed to the nearest 0.1g and fixed in formalin. As fat deposition can be indicative of nutritional state and a likely source of energy for gonad production (Pollock 1984; Rajasilta 1992), the digestive tract was removed from each fish and any fat deposited along it removed and weighed to the nearest 0.1g.

Four hundred gonads were sectioned, mounted and stained with hematoxylin and eosin for histological examination (Chapter 2). Histological sections were used to confirm sex and macroscopic stage, and to assess reproductive development staging, or determine potential prior spawning for resting fish. Prior spawning was identified by the occurrence of post-ovulatory follicles, atretic vitellogenic oocytes, or a thicker ovarian wall (Figure 3.2). Brown bodies were not a dominant feature of ovaries at the Abrolhos Islands, with only a few identified in histological sections.

The ovarian wall of fish thins as it stretches with the increasing volume associated with vitellogenesis oocytes. Post spawning, ovaries contract resulting in a thicker ovarian wall than immature females (Burton *et al.* 1997, Rideout *et al.* 2000, Adams 2003). Therefore, ovarian wall thickness provides another means by which to assess maturity in fish. As such, four replicate wall thickness measures were made for each of 197 ovaries from fish collected at both spawning and non-spawning sites and that were in a pre-vitellogenic state and greater than the size at first maturity, captured throughout the year.

Each site was designated as a “spawning” or “non-spawning” site, based on the macroscopic and histological stage of the ovaries from fish caught at the site. A site was defined as a “spawning site” if one or more female from that site had ovaries containing vitellogenic oocytes at any time over the study period. This biased sites to being classed as a spawning site, as a single fish was sufficient for a site to be deemed a spawning site. Consequently, it reduces the likelihood of a site inaccurately being classed a non-spawning site, as non-spawning require none of the females collected at that site to show signs of reproductive activity.

Historical biological data were available for a sample of leopard coralgrouper at the Abrolhos Islands. Data from this sample, containing 16 fish (13♀;3♂), is also presented to contrast patterns in spawning omission from this study.

Environmental data

A long-term monitoring station set up by the Western Australian Department of Fisheries for rock lobster settlement provided current and historical temperature recordings (site D; Figure 3.1 – inset) using Titbit™ temperature loggers. Water temperatures at all other sites were recorded from February 2008 to February 2009 using HOBO ™ temperature and light loggers. These loggers were deployed along with a series of water motion recorders, equipped with a HOBO ™ accelerometer, throughout the Easter Group (sites A, B, C, E, F; Figure 3.1 – inset) as part of a coral health monitoring study (Evans and Abdo 2010). The data collected from these water motion recorders provided a measure of relative water movement: see Evans and Abdo (2010) for details.

Analysis

The mean of the four ovarian wall thickness measures (ln transformed) was compared between spawning and non-spawning sites with L_T (cm) as a co-variate, through ANOVA. This was based on females greater than the size at first maturity that did not have vitellogenic oocytes, indicating that they were either immature or mature but resting. As the transformation did not result in a significant difference in variances between groups (Barlett's test; p=0.01), a higher p-value for significance was accepted (Underwood 1981**)**.

Relative fat content (fat-somatic index; FSI) was calculated for each female as a percentage of fat per unit body weight [FSI= $(W_F / W_T)*100$], where W_F is weight of fat in grams and W_T is the whole weight of fish in grams. Differences in FSI (square root transformed) were analysed using a two factor ANOVA for spawning site and reproductive activity. Data were tested for homogeneity of variance and normality using the Shapiro test and Bartlett test, respectively.

The Kolmogorov-Smirnoff (KS) test was used in pair-wise comparisons of the size composition data of: (i) reproductively active fish at spawning sites; (ii) inactive fish from spawning sites; and (iii) inactive fish from non-spawning sites. Bonferroni corrections were applied to the significance levels ($p<0.017$). Length-frequency distribution data were pooled from all years due to sampling restrictions within MPAs.

Generalised linear models (GLM: binomial; logit link) were used to assess the effect of a site's location, or the presence of a male at a site on spawning activity with fish size (L_T) as a covariate in both models. Locations was defined as low or high fishing pressure, based on being located either inside or outside of the MPA, or in locations distant to fishing settlements that experience light fishing pressure. All sites within the MPAs, as well as all sites outside of the Easter and Wallabi lagoons, were defined as "light" fishing pressure. This was supported by recreational fishing effort data (Sumner pers. comm.). All data was analysed using R (R Development Core Team 2009).

3.4 Results

Spawning Omission

Of the 390 fish larger than size at first maturity collected during the four spawning seasons, 351 (90%) were females based on macroscopic and histological examination (Table 3.1). Histological examination of a selected sub-sample of the ovaries from females at spawning and non-spawning sites validated the macroscopic staging (Chapter 2). Spawning females contained vitellogenic oocytes often with hydrated oocytes and occasionally post-ovulatory follicles. Non-spawning females showed no sign of vitellogenesis, major atresia that may be associated with the resorption of previously vitellogenic oocytes, and had a thin ovarian wall.

Fish 34-36cm L_T had a similar ovarian wall thickness in both spawning and non-spawning sites (Figure 3.2). However, pre-vittelogenic fish >38cm L_T at spawning sites had a noticeably thicker wall than those from non-spawning sites. This resulted in ovarian walls of pre-vittelogenic fish from spawning sites significantly thicker than pre-vittelogenic fish from non-spawning sites ($p<0.001$) (Figure 3.2). L_T was also a significant co-variate ($p<0.001$), with a non-significant interaction ($p>0.1$).

Table 3.1 Number of females sampled by month for the spawning seasons 2005/06 – 2008/09 from sites deemed "spawning sites" or "non-spawning sites" based on the macroscopic stage of the ovaries from fish caught at the site.

	2005/06		2006/07			2007/08				2008/09		Total
	Feb	Mar	Dec	Jan	Feb	Dec	Jan	Feb	Mar	Dec	Feb	
Non-spawning site	7	31	7	22	36	18	0	35	1	2	12	171
Spawning site	32	2	23	9	75	0	7	13	0	6	13	180

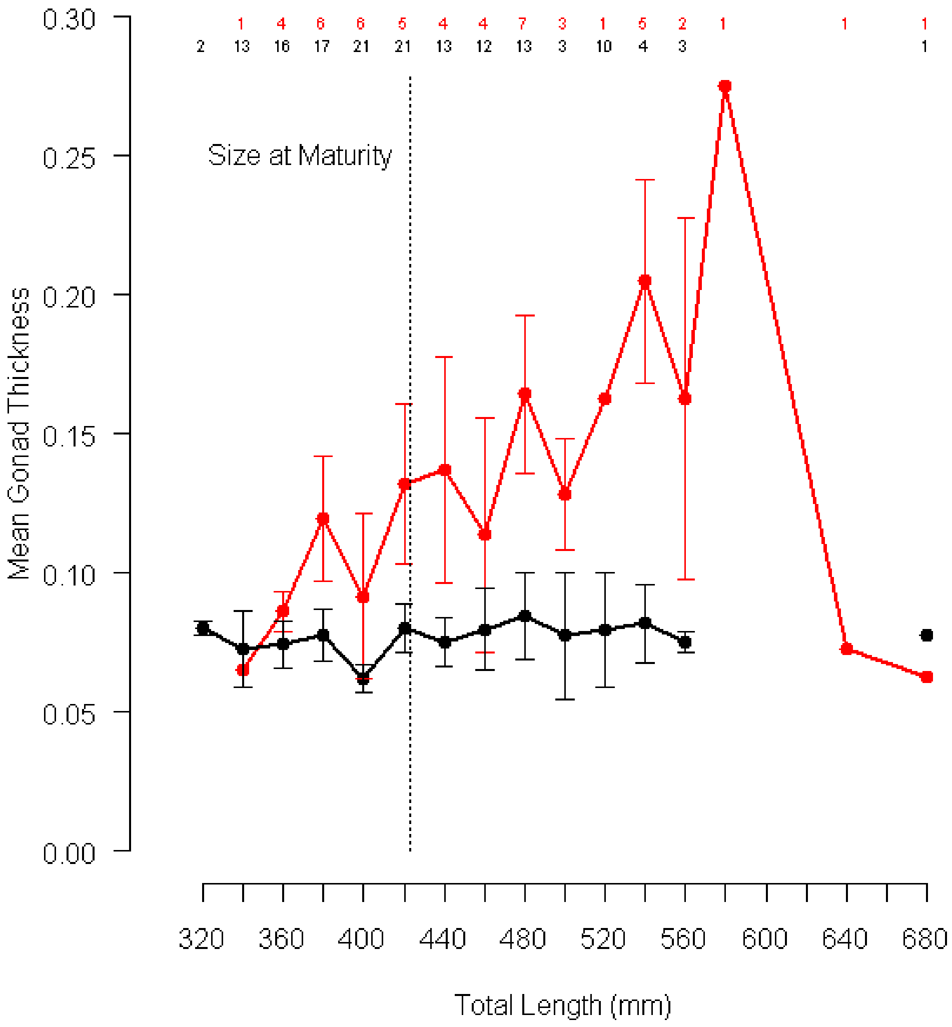


Figure 3.2 Mean (±SE) of the mean ovarian wall thickness for mature-sized females with ovaries in a pre-vitellogenic state from spawning (red) and non-spawning (black) sites for 1cm L_T length classes. Vertical dotted line indicates the L_{50} for maturity. Numbers represent sample size for spawning (red) and non-spawning (black) sites.

Spatial patterns in reproductive activity of females

Spatial separation of spawning and non-spawning areas was evident in both the Easter and Wallabi groups (Figures 3.3 and 3.4). Almost all sites (16 of the 18; 89%) in the MPAs at both island groups were classified as spawning sites. In contrast, only 11 of 37 (30%) sites outside the MPAs were classified as spawning sites. Spatial demarcation of spawning activity was clear in the Easter Group with spawning sites limited to outside the central lagoonal area and predominantly in the MPA (Figure 3.3). This demarcation was less clear in the Wallabi Group, as it lacked the same central lagoonal structure as the Easter Group. However, spawning occurred at several sites inside the MPA, and at a few sites outside the MPA in channels or reef promontories (Figure 3.4).

In predicting spawning activity, generalised linear models (GLM) showed a significant effect of relative fishing pressure (heavy or light) ($p<<0.0001$), and length ($p<0.001$). The effect of a 1cm increase in length increased the probability of spawning by 1.1 times (1.0-1.1: 95%CI), with the location effect (heavy or light fishing pressure) resulting in a 152.8 times (33.0-271.8: 95%CI) increase in the probability of spawning activity when moving from a heavy to light fishing location.

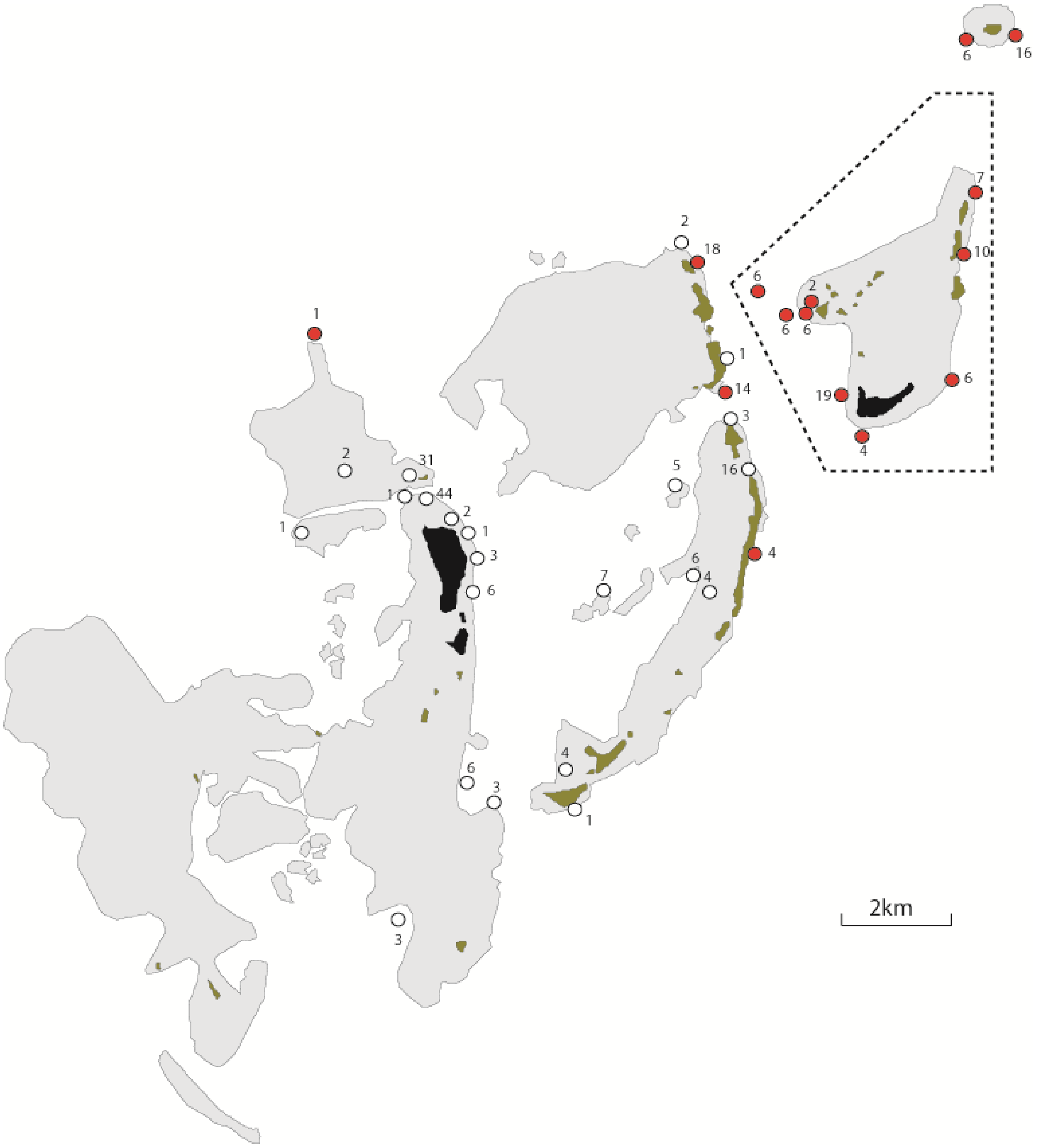


Figure 3.3 Location of spawning sites (black circles) and non-spawning sites (open circle) in the Easter Group with number of females above size at first maturity sampled over all seasons. Map shows “inhabited “islands (black); uninhabited islands (brown); intertidal reef (light grey); and the boundary of the MPA (dashed line).

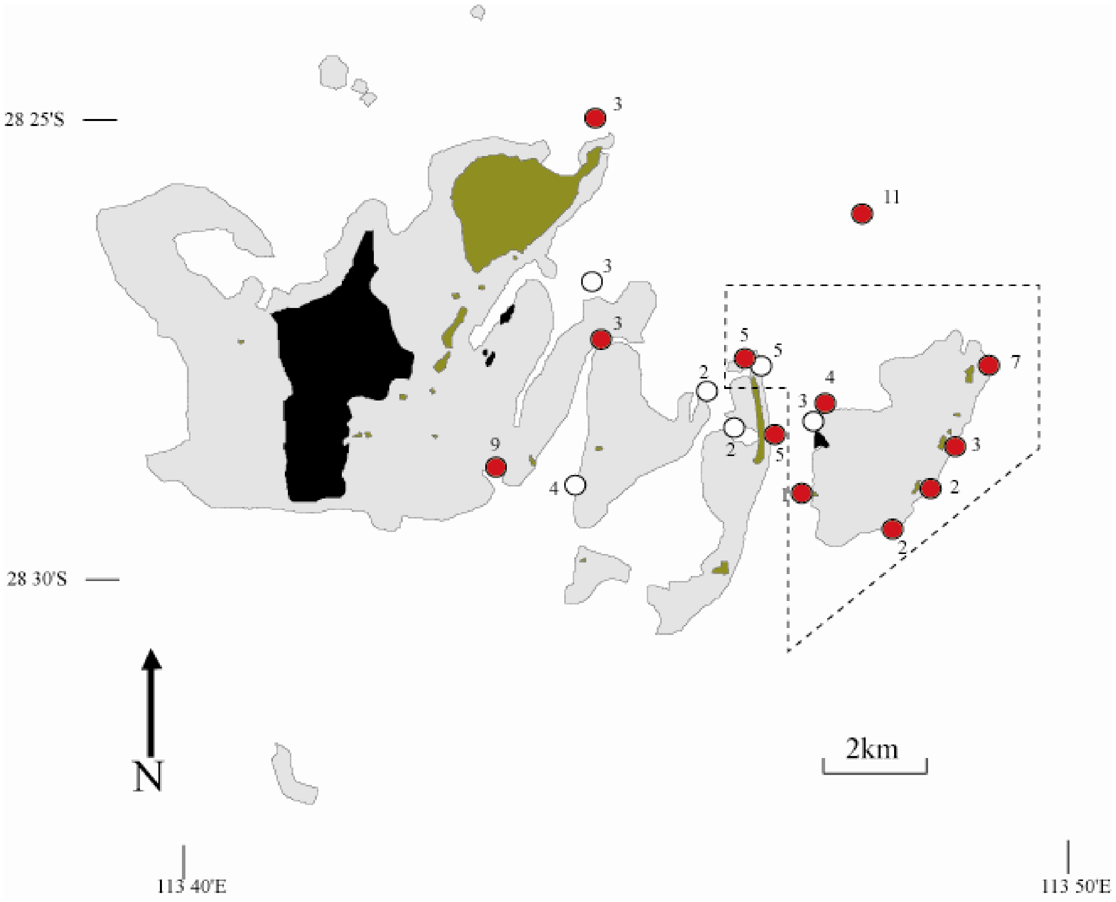


Figure 3.4 Location of spawning sites (red circles) and non- spawning sites (open circle) in the Wallabi Group with number of females above size at first maturity sampled over all seasons Map shows “inhabited “islands (black); uninhabited islands (brown); intertidal reef (light grey); and the boundary of the MPA (dashed line).

Fat deposition

Weight of fat per unit of body mass (FSI) was significantly lower in reproductively active than inactive fish ($p<0.0001$; Table 3.2). Reproductively inactive fish (mean FSI =1.12 ± 0.05 SE) had over two and a half times the mesenteric fat deposits per unit body weight than reproductively active fish (mean FSI =0.42 ± 0.05 SE).

Table 3.2 Results for ANOVA of fat somatic index (square root transformed) for fish activity (active / inactive) and site type (spawning / non-spawning).

	df	Sum Sq	Mean Sq	F value	Pr(>F)
Site Type	1	10.949	10.9493	60.972	7.343e-14
Activity	1	6.834	6.8336	38.054	1.966e-09
Residuals	338	60.697	0.1796		

Size distribution of females

A total of 351 females, ranging in size from 338 (size at first maturity) to 632 mm L_T, were collected from the two island groups. The length frequency distribution of reproductively active fish was not significantly different from inactive fish at spawning sites ($p>0.03$ based on Bonferroni adjusted alpha) and where hence plotted together (Figure 3.5a). However, there was a significant difference in the size structure of females between active fish at spawning sites (Figure 3.5a; red) and inactive fish at non-spawning sites ($p<0.0001$; Figure 3.5b). Overall, there were higher proportions of large reproductively active females at the spawning sites compared to fish at non-spawning sites (Figure 3.5). Despite the significant difference in size frequencies between active fish at spawning sites and inactive fish at non-spawning sites, there was still 75 fish from 24 sites greater than the size of maturity (L_{50}- 42.2 cm L_T; Chapter 4) at non-spawning sites. Given the ratio of mature fish in each size class at spawning sites (Figure 3.5a) a similar proportional distribution at non-spawning sites would have resulted in 94 fish that should have been mature at non-spawning sites.

Eleven of the 13 females collected in 1990 at Rat Island were greater than the size at first maturity (Figure 3.5c). Rat Island (near logger C; Figure 3.1-inset) was sampled at a number of locations with all sites designated as a non-spawning site in this study. There were 57 fish collected from around Rat Island ranging in size from 34.1-55.4 cm L_T. A similar size range was sampled in 11 fish above the size at maturity in 1990 at the same location with size ranging from 37-50 cm L_T (Figure 3.5c). However, eight of these fish were mature compared 57 immature fish collected as part of this study.

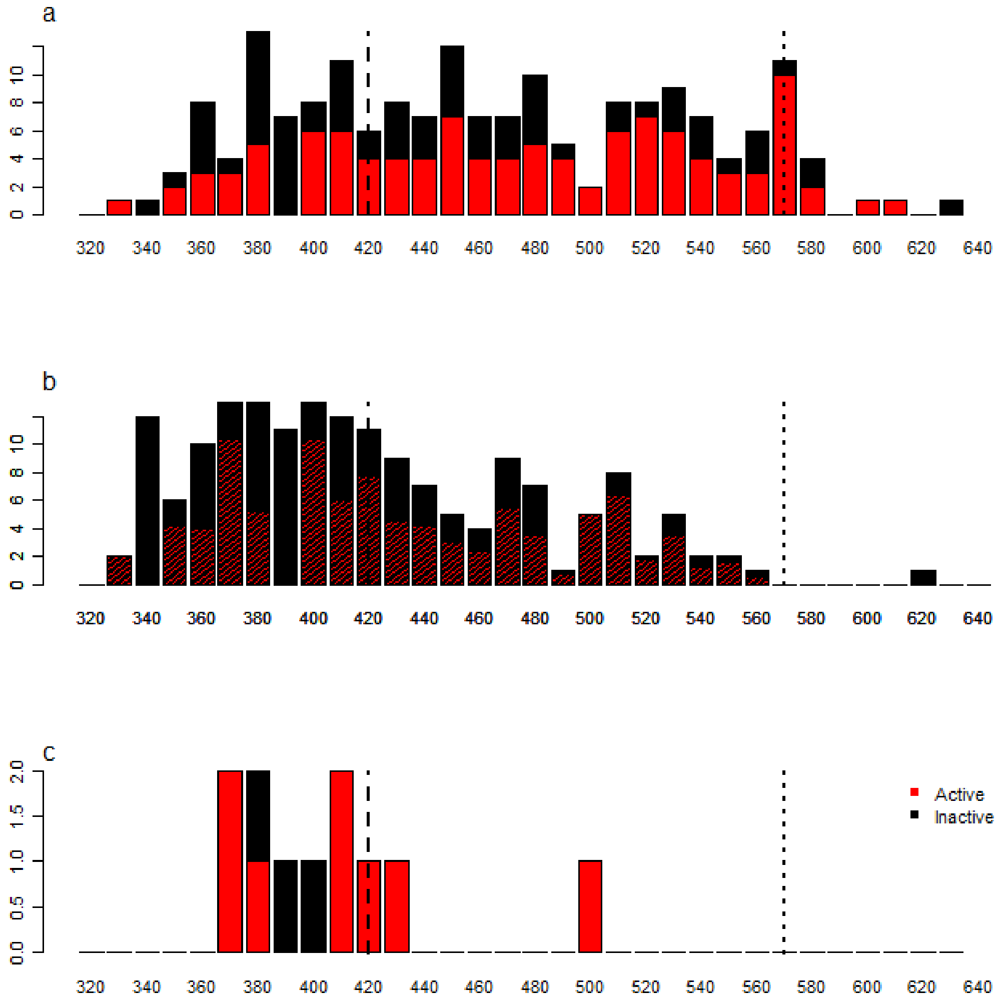


Figure 3.5 Female length frequency (L_T) distributions of active (red) and inactive (black) fish from a) spawning sites b) non-spawning sites; and c) 1990 sample of a current non-spawning sites. Red shading (b) indicates the number of fish that should be mature given the proportion mature from the spawning sites (a). The dashed line is the size at maturity and dotted line size at sex change for *P. leopardus* at the Abrolhos Islands

Spatial patterns in the presence of males

Thirty nine males were collected during this study and, similar to reproductively active females, males were predominantly caught in the eastern regions of the archipelagos and mainly in the MPAs of the two island groups (Figures 3.6 and 3.7). Males were caught at 55% of sites within the MPAs but at only 24% of the sites outside the MPAs. A GLM examining female spawning activity based on the presence of males collected at the same site, and length (L_T) as a covariate, showed a significant effect of both male presence ($p<<0.0001$) and length ($p<<0.0001$). At those sites where males were present, the odds of females being reproductively active increased 4.1 (2.5-6.8: 95%CI) times.

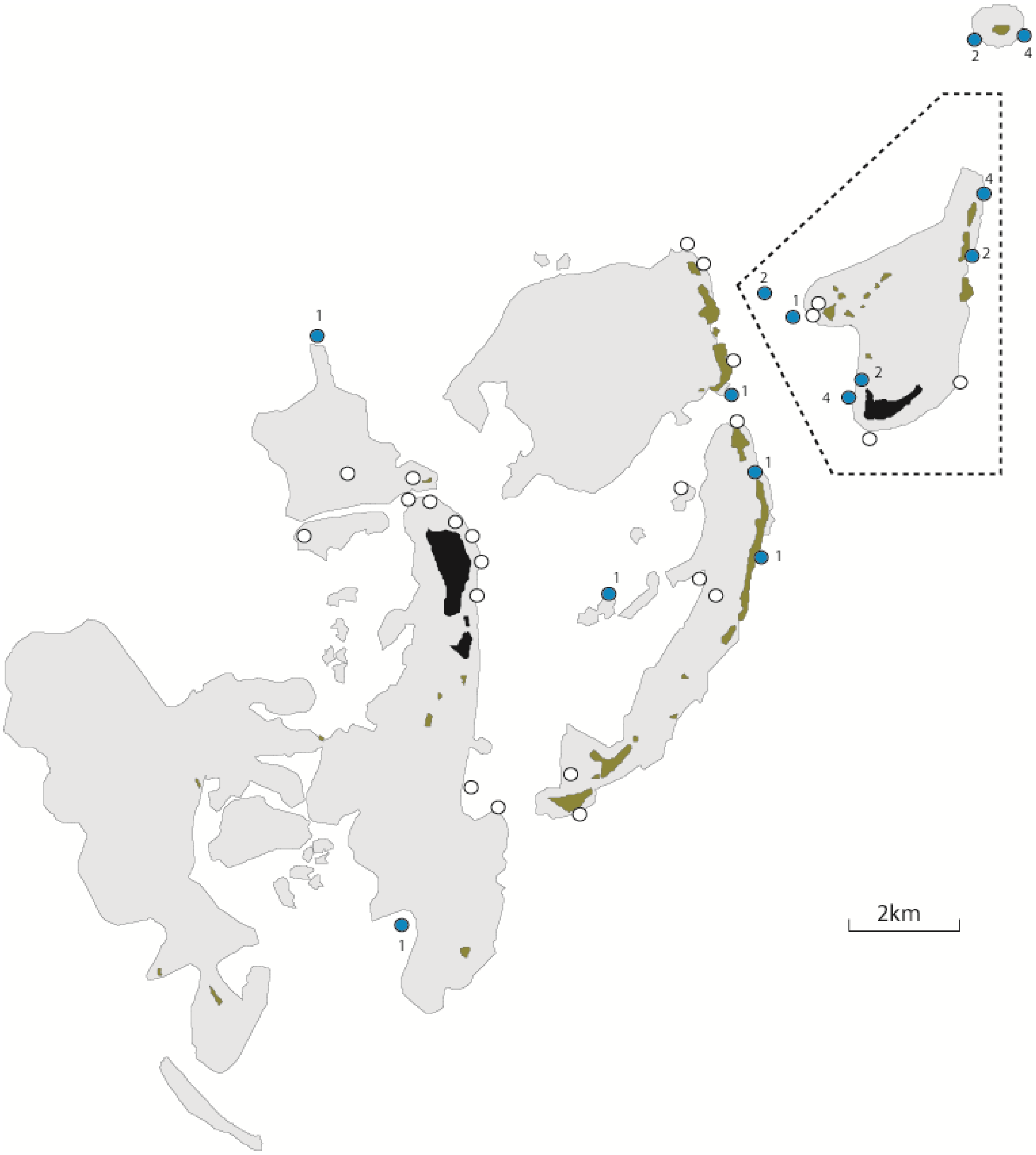


Figure 3.6 Location of all sites (open circle) and those where males were sampled (blue) in the Easter Group (text indicates number of males sampled over all seasons). Map shows “inhabited “islands (black); uninhabited islands (dark grey); intertidal reef (light grey); and the boundary of the MPA (dashed line).

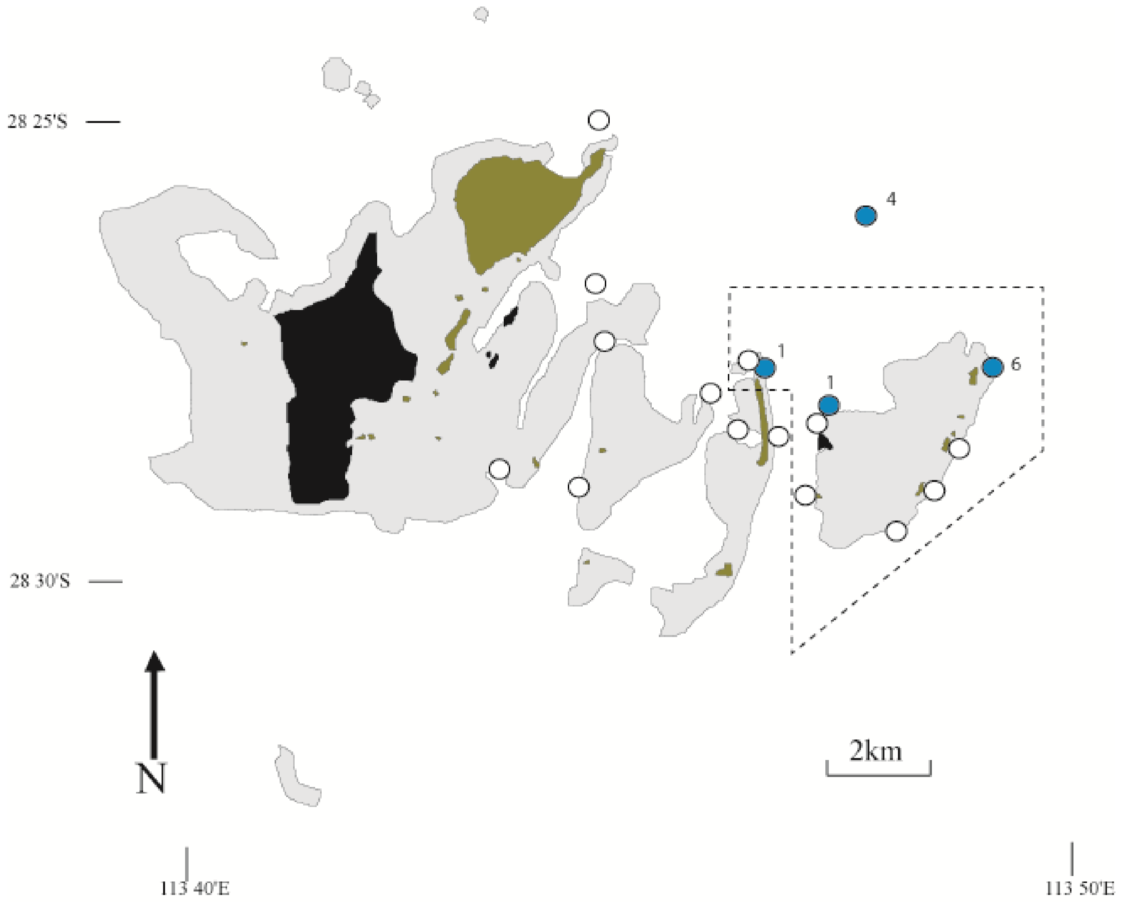


Figure 3.7 Location of all sites (open circle) and those where males were sampled (blue) in the Wallabi Group (text indicates number of males sampled over all seasons). Map shows “inhabited “islands (black); uninhabited islands (dark grey); intertidal reef (light grey); and the boundary of the MPA (dashed line).

Environmental variables

The temperature regimes varied slightly (<3^{o}C) between sites in central lagoonal areas and the eastern sites of the Easter Group (Figure 3.8). Temperature regimes were very similar (0.5 – 1.7^{o} C) prior to, and during, the spawning season (from October to March) at spawning and non-spawning sites.

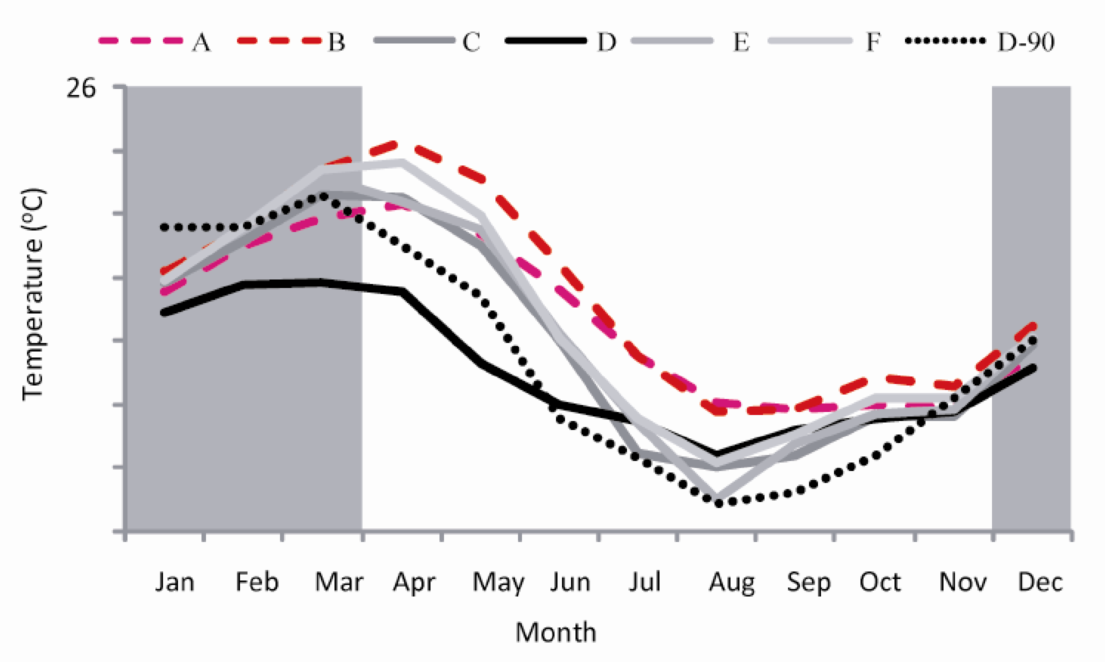


Figure 3.8 Average monthly temperatures for six sites in the Easter group for spawning sites (A and B) and non-spawning sites (C,D,E and F) from February 2008 until February 2009. Spawning season (grey bars). 1990 data also shown for site D as a dotted line.

Water motion in the Easter Group was often higher at spawning sites than non-spawning sites (Figure 3.9). The spawning site that logged water motion data for the duration of deployment (site B) generally showed a higher average daily water motion than any of the non-spawning sites (sites C, E and F; Figure 3.8). Water motion was highest at site A, but data were only available for the first half of the deployment (Feb – Jun 2008) period due to failure of the logger.

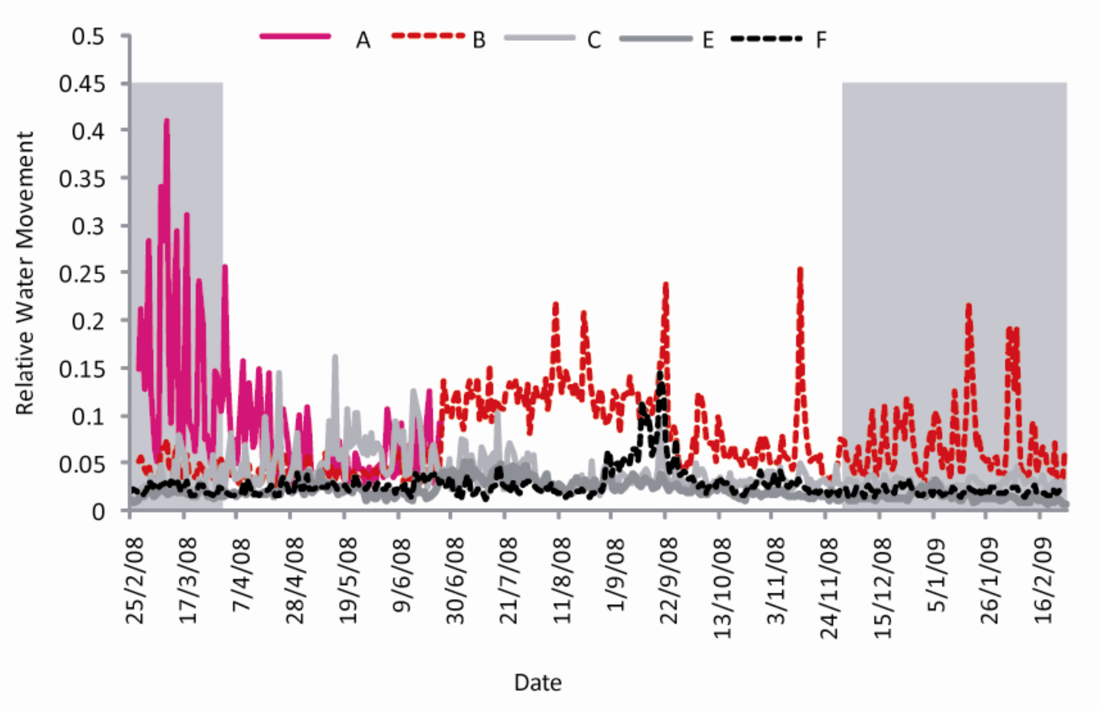


Figure 3.9 Average daily relative water motion for five sites in the Easter group for spawning sites (A and B) and non-spawning sites (C, E and F). Spawning season (grey shading)

3.5 Discussion

This study has shown that leopard coralgrouper spawn in spatially discrete areas of the Abrolhos Islands on the mid-west coast of Australia, and these areas are predominantly within the MPAs where fishing is prohibited, or in areas where fishing pressure is low. Despite the focus of sampling on peak lunar phases during the spawning season to maximise the potential of identifying developing and spawning fish, or those recovering from spawning, there was a considerable number of fish that were shown to not be spawning. A broader examination of the spawning season (Chapter 4) found that spawning was restricted to the austral summer (Dec-Mar) at the Abrolhos Islands, and there was no secondary peak in spawning activity in any areas of the archipelago. Furthermore, the designation of a site as a non-spawning site was more difficult than a spawning site, as a single reproductively developed fish at any time from that site would see it being classified as a spawning site. This study, therefore, provides strong circumstantial evidence that spawning omission occurs in the species at a range of locations in the coral-reef system.

It is not possible to repeat sample individuals and examine their gonads either macroscopically or histologically. However, no spawning activity was detected at non-spawning sites over four years, suggesting persistent spawning omission. Large areas of the two regions examined in the study showed no sign of reproductively active females, and even if spawning was to occur every two to four years, it would be expected that some would be detected in these areas given the longevity of the study.

Of the 171 fish above the size at first maturity from non-spawning sites, 94 would have been expected to be mature given the size at maturity relationship for *P. leopardus* at the Abrolhos Islands (Chapter 4). However, none of these or the 75 of those that were greater than size at maturity L_{50} showed signs of spawning activity. One site, currently ascribed as a non-spawning site, had 57 fish collected from it over the four years with no signs of spawning activity. However, a sample collected in 1990 at the same site, and encompassing fish across a similar size range, had eight of 11 females being reproductively active.

The lack of reproductive development was confirmed at non-spawning sites in this study through no evidence of gonadal development provided by macroscopic gonad staging or gonadosomatic indices, or through histological examination of the ovaries. No signs of vitellogenesis were found in fish at non-spawning sites. Evidence of spawning omission was further provided through the examination of ovarian wall thickness, which showed significantly thicker walls at spawning sites compared to non-spawning site. A thicker ovarian wall has been used to differentiate resting mature fish, from immature fish (Burton *et al.* 1997, Rideout *et al.* 2000, Adams 2003). In this context, 34-36 cm L_T fish from both spawning and non-spawning sites had similarly thin ovarian walls, but just prior to the size at maturity, there were noticeably thicker ovarian walls in fish at spawning sites compared to non-spawning sites. This suggests that no prior spawning had occurred at those sites, either during the year or in previous years, indicating a persistent spawning omission.

This conclusion of wide-spread spawning omission for leopard coralgrouper was supported by the large numbers of fish collected from each spawning month showing no indication of present or recent spawning, and the lack of histological features indicating that spawning was unlikely to have occurred during the spawning season, or indeed in previous years. Spawning omission typically refers to mature fish omitting from or skipping spawning (Rideout *et al.* 2005). Despite being all above the size at first maturity and a considerable number greater than the L_{50} for maturity, there was a lack of evidence of past and present maturation of these non-spawning fish, Therefore, there is good evidence to suggest that there currently is persistent and spatially explicit spawning omission occurring at the Abrolhos Islands.

Inactive females during the spawning season have been recorded for *P. leopardus* on the Great Barrier Reef (GBR), though there was no defined spatial pattern. Rather, it was thought to be the result of fine scale temporal variations in spawning (Adams 1996). While sampling at the Abrolhos Islands was targeted during the spawning period, additional sampling was conducted throughout the year (Chapter 2). This revealed no other potential spawning months. Similarly, the immature state of the gonads, as indicated by the thin gonad wall, would suggest that the inactive fish from non-spawning sites had not spawning at another time during the year.

The only spatial examples of spawning omission prior to this study are over a regional scale of 100s of kilometres (Pollock 1984; Bell *et al.* 1992; Williams *et al.* 2006; Fennessy and Sadovy 2002). The fine scale segregation of spawning and non-spawning leopard coralgrouper at spatial scales of 100s m - km in the Abrolhos Islands was persistent over years and widespread within a coral-reef system. This spawning omission could reflect a range of factors, including movement of fish from non-spawning sites to spawning aggregations, edge of range effects, nutritional limitation, spatial variation in habitat / environmental conditions or removal of large fish by fishing, or combination of these factors. The likely influence of these to spawning omission by leopard coralgrouper will be discussed.

Leopard coralgrouper exhibit relatively small movements to spawning aggregation sites (Zeller 1998). While movements of > 5 km to spawning aggregations do occur for *P. leopardus* (Zeller 1998) and its congeneric *P. areolatus* (Hutchinson and Rhodes 2010), the majority of movements to spawning aggregations were smaller (~500m) for both of these species. This is also the case for catchment distances for *P. leopardus* at the Abrolhos Islands where mean catchment distances were 569 m (Chapter 5). Since long term tagging studies have shown limited inter-reef movement of this species (Davies 1996), the spatial segregation of spawning and non-spawning sites indicates that these sites reflect the reproductive condition of the localised fish populations and do not represent a broad-scale process driven by migration, as has been documented in the yellowfin bream *Acanthopagrus australis* and the orange roughy *Holostethus atlanticus* (Pollock 1984; Bell *et al.* 1992).

An edge-of-range-effect hypothesis for spawning omission, as postulated for *Epinephelus andersoni* (Fennessy and Sadovy 2002; Wakefield *et al.* 2013), is unlikely to explain spawning omission in leopard coralgrouper at the Abrolhos Islands. While at the southern edge of its range, the population of leopard coralgrouper at the Abrolhos Islands has demonstrated defined cycles of reproductive activity at particular locations over several years (Chapter 4). Furthermore, edge-of-range-effects are unlikely to produce the consistent spatial patterns in reproductive activity documented in our study.

Poor nutritional state is considered to be the most common cause for spawning omission (Rideout *et al.* 2005). Reproductively inactive leopard coralgrouper at the Abrolhos Islands had a higher visceral fat content on average than reproductively active leopard coralgrouper. Visceral fat deposits are likely to be utilised in the development of reproductive tissue, as suggested for Baltic herring (*Clupea harenguis mambras*) in the Baltic Sea (Rajasitta 1992) and leopard coralgrouper (*P. leopardus*) on the GBR (Ferreira 1995). Higher fat content of reproductively inactive leopard coralgrouper strongly implies that poor nutrition is an unlikely cause for spawning omission at the Abrolhos Islands. However, it has been noted that a fish’s condition at the time of spawning may not reflect its condition at the time when development of reproductive tissue would have commenced (Rideout *et al.* 2000). Also, these unutilised energy reserves have not been transferred into increased growth (Chapter 4), as has been shown previously in fish which skip spawning (Holmgren 2003). Rather, fish from non-spawning sites appear to grow slower than those from the spawning areas (Chapter 4). In our study, spawning omission was recorded over several consecutive spawning seasons at the same locations within the coral-reef system, providing strong support that spawning at these sites was unlikely. Thus, the recorded spawning omission is likely to reflect longer term spawning omission at those sites and nutrition is unlikely to play a role in spawning omission patterns.

Environmental factors such as water temperature (Hilder and Pankhurst 2003) and water motion (Johannes 1978; Barlow 1981) are considered to influence spawning in fishes, but are unlikely to explain spawning omission in leopard coralgrouper. Low temperatures have been thought to result in the cessation of oocyte development

(Pawson *et al.* 2000) or result in spawning omission (Rideout *et al.* 2000). However, there was no apparent difference between temperatures at spawning and non-spawning sites in the Abrolhos Islands before and during the spawning season, suggesting that water temperature is not the likely cause for spawning omission in leopard coralgrouper. Increased tidal water movement associated with lunar cycles at the time of spawning is considered to reduce egg mortality (Johannes 1978) and/or increase egg dispersal (Barlow 1981) from spawning aggregations. The relative water movements were greater at two spawning sites than at non-spawning sites at the Abrolhos Islands, particularly during the spawning periods. However, spawning of leopard coralgrouper at the Abrolhos Islands occurs at a period of relatively low water movement compared to other times of the year. This concurs with more recent studies suggesting that high water movement may not be an important factor at spawning aggregation sites (Colin 1992; Nemeth *et al.* 2007). While local differences in water movement cannot be discounted as a possible cause for the spatial patterns in reproductive development among sites, it does not appear to be a major factor.

Spatial patterns in fishing pressure may account for spawning omission in particular locations in the Abrolhos Islands, with protogynous species, such as leopard coralgrouper, highly susceptible to fishing pressure. Fishing effort often targets larger individuals (Zeller and Russ 2000, Jackson *et al.* 2001), which can disproportionately remove males and cause a portion of females to be reproductively inactive (Coleman *et al.* 1996).

The paucity of males at non-spawning sites outside the MPAs was corroborated with data from a large underwater visual census program (UVC; Chapter 2). The largest leopard coralgrouper seen at non-spawning sites from this UVC program was 45cm L_T, considerably smaller than the size of sex change of leopard coralgrouper at the Abrolhos

Islands (57cm L_T; Chapter 2). While larger fish (>45cm) were collected from non-spawning sites during the current study, there was only one individual sampled that was greater than the size of sex change (Figure 3.4b). Spawning sites and male leopard coralgrouper were predominantly located within the MPAs where the catch of finfish is prohibited, although some illegal fishing does occur (How pers obs.). The relationship between spawning activity and “light” fishing pressure documented in the MPAs is supported further by the presence of males at spawning sites in areas outside the MPAs that also experience relatively “light” fishing pressure due to their distance from islands containing commercial lobster fishing camps (occupied for 3.5 months a year), and weather conditions restricting access by recreational fishers at certain times of the year. These conclusions are supported by the significant effect of both fishing pressure and the presence of males using GLM analyses.

The absence of males, or lack of sufficient reproductive cues from males, has been associated with the retention of vitellogenic oocytes in some fish species (Trippel and Harvey 1990; Coleman *et al.* 1996), and may therefore lead to reproductive failure though possibly effecting reproductive behaviour (Rhodes and Tupper 2008). However, in this instance, the removal of male leopard coralgrouper through fishing may diminish the potential cues for female reproductive development. Where males were absent, female leopard coralgrouper were reproductively inactive (previtellogenic oocytes) and immature (thin gonad walls), suggesting that a lack of males may not provide the cues for female maturity through vitellogenesis. This conclusion is further supported by historical data of the species in the region. The sample from Rat Island prior to the gazettal of the MPAs contained reproductively active females as discussed previously. This sample also contained three males which contrast with the absence of males at that location in the present study. Although this historical sample was from only one year, any reduction in spawning activity over the decade cannot be explained by differences in habitat or environmental conditions at the Abrolhos Islands.

The MPAs, where most of the current spawning occurs, were originally established in areas that were representative of ‘good quality reef habitat’ (Anon. 1998), and not located based on any biological information on this or any other species. While there are general habitat differences between spawning and non-spawning areas (Webster *et al.* 2002), these do not appear to contribute to the current pattern of spawning omission. Similar habitat differences between spawning and non-spawning areas persist from the time of the historical samples (Webster *et al.* 2002) to this current study (How pers. obs.). Decadal comparisons of water temperate at site D, showed that water temperatures at this site prior to the spawning season in 1990 were very similar to all other thermal recordings from November and December, including those from the same site 25-30 years later (Figure 3.8). Also, water movements were unlikely to differ markedly within the archipelago from 1990 to 2009 (A. Pearce pers. comm.).

Fishing effort increased at the Abrolhos Islands throughout the 1990s, with commercial fishing effort increasing from around 800 fishing days in 1992/93 to a peak of over 3000 days in 2003/04, before falling slightly to over 2000 days in 2005/06 (Wise *et al.* 2007). Additionally, the gazetting of the MPAs in the Abrolhos Islands in 1994 would likely to have redirected a high proportion of the fishing effort to accessible areas outside these MPAs, such as reef areas around Rat Island. The increase, and spatial shift, in fishing effort throughout the 1990s at the Abrolhos Islands is likely to have disproportionally removed larger, predominantly male leopard coralgrouper from areas outside the MPAs, which may have led to the change in the spawning condition of females in those areas.

While there are several examples of spawning omission in fishes reviewed by Rideout *et al.* (2005), none to our knowledge have been documented at such a fine spatial scale. Leopard coralgrouper, a common and economically important tropical epinephelid, exhibited clear and persistent spatial patterns in spawning omission over a scale of 100s m to kms within the Abrolhos Islands. Of the various factors that can influence reproductive development, fishing appears to be the most plausible explanation for the spawning omission at the Abrolhos Islands. High fishing pressure disproportionately removes males. Due to the strong endogenous size related cue over sex change at the Abrolhos Islands (Chapter 2), there isn't a compensatory mechanism by which females can become male. This has resulted in females not developing sexually over a number of years in areas where males are absent, possibly due to the lack of cues from males for reproductive development.

The small-scale spatial patterns in spawning omission and spawning activity have important implications for fisheries management. Modelling reproductive output assumes that all sexually mature individuals spawn annually for iteroparous fish (Rideout *et al.* 2005). Clearly, the lack of development of a high proportion of females in a population, as shown at the Abrolhos Islands, would have a large influence on the reproductive output of the species. Any modelling of egg production in this population of leopard coralgrouper would be substantially overestimated, having considerable ramifications on yield estimates. It appears that current egg production of leopard coralgrouper is facilitated through the use of MPAs at the Abrolhos Islands, which offer protection to the spawning biomass by reducing fishing effort and maintaining males at spawning aggregation sites. Currently, there is no protection of male leopard coralgrouper in fished areas at the Abrolhos Islands through a maximum legal size. Based on our results, the implementation of this management strategy has the potential to increase the spatial distribution of males, which would appear to have the potential to increase reproductive activity of females. However, the efficacy of a maximum size may be tempered due to the high post-release mortality of leopard coralgrouper (J. St John pers obs.). Furthermore, the genetically distinctness of the Abrolhos Islands population, suggests that the population requires a large degree of self-recruitment (van Herwerden *et al.* 2009). This would imply that those lightly fished areas in and adjacent to the MPAs may provide larval supply to replenish non-spawning areas. Thus, spatial management through MPAs is important to minimise fishing-induced spawning omission in leopard coralgrouper, and protect the likely primary source of recruits for the archipelago.

Collection of leopard coralgrouper at the Abrolhos Islands

Chapter IV

Growth and reproduction of a genetically isolated population of *Plectropomus leopardus:* implications of biological variation and spawning omission on management

4.1 Abstract

Leopard coralgrouper, *Plectropomus leopardus,* is a common coral-reef fish throughout the Indo-Pacific. Its importance as a fisheries species in the western Pacific Ocean has seen it the focus of considerable research on the Great Barrier Reef (GBR), but research on genetically isolated populations in the Indian Ocean is limited. This chapter examines the biological parameters of *P. leopardus* in the eastern Indian Ocean at the Abrolhos Islands, a high latitude coral reef system (28°S). There were numerous morphological (length/length and length/weight) and growth (L_∞ and t_0) parameters that differed from the GBR population with the Abrolhos Island being more comparable to southern GBR samples despite them being at a lower latitude (23^{o}S) than the Abrolhos Islands. There were also differences in reproductive parameters including a later spawning season (Dec-Mar) and a greater size at maturity (404 mm L_F). Failure to recognise and account for spawning omission could lead to misleading conclusions regarding the actual biology of a species and hence appropriate management by over estimating size at maturity (spawning 404 mm L_F and 6 years vs. non-spawning sites 608 mm L_F and 11.8 years). This chapter highlights the need for local studies of biology to be conducted rigorously throughout a population to ensure appropriate fisheries management measures are employed.

4.2 Introduction

Accurate information on life history characteristics of fishery species is critical for effective management strategies and regulations for exploited species, as they are often based on estimates of biological parameters that govern fisheries regulations, such as the size at maturity determining minimum legal sizes (McPhee 2008). However, significant variations in growth and reproductive parameters of teleost species occur throughout their geographic range, including growth rates, size and age at maturity and sex change (Williams *et al.* 2006, Caselle *et al.* 2011). These can complicate the implementation of appropriate management regulations across a broad spatial range (Melville-Smith and de Lestang 2006).

One such example of variable reproductive parameters is the leopard coralgrouper, *Plectropomus leopardus* (Epinephelidae), which is a common coral-reef fish found throughout the Indo-Pacific (Heemstra and Randle 1993). Throughout the Great Barrier Reef (GBR) it has shown variability in operational sex ratio and sex specific size/age structure (Adams *et al.* 2000). Similarly, there have been differences in morphometric relationships such as length-weight relationships throughout the GBR (Brown *et al.* 1994). Such a detailed study on species variability throughout its geographic range is testament to its importance as a part of commercial and recreational reef-fish fisheries on the GBR (Mapstone *et al.* 2001).

There is considerable genetic subdivision throughout the range of *P. leopardus*, with six genetically distinct populations occurring across the Indo-Pacific (van Herwerden *et al.* 2009). The population at the Abrolhos Islands on the west coast of Australia is genetically distinct from other populations (van Herwerden *et al.* 2009) and is targeted by both commercial and recreational fisheries. The few studies on this species in the eastern Indian Ocean have been restricted to examining its response to fisheries protection by examining abundance and biomass changes (Nardi *et al.* 2004, Watson *et al.* 2007, 2009, McLean *et al.* 2011). No studies have examined its biology, on which to base fishing regulations, and the relevance of the biological parameters derived from the well-studied GBR population is uncertain.

Given the variation already demonstrated within the GBR for a range of life history parameters, there is a need to understand the extent of any potential divergence in the biology of *P. leopardus* from the genetic isolated population at the Abrolhos Islands. However, given the documented spatial spawning omission at the Abrolhos Islands (Chapter 3), there is also a requirement to account for spawning omission which may lead to overestimates of spawning parameters (Rideout *et al.* 2005). This study therefore aims to estimate important life history parameters, including growth rates, size and age at maturity and spawning time for the population of *P. leopardus* at Abrolhos Islands in the eastern Indian Ocean. The study also examines the effects of any failure to account for spawning omission on reproductive parameter estimates of the species, and how these adjusted estimates compared with those from the GBR. It serves to highlight the importance of locally derived biological studies, in the application of appropriate fisheries measures.

4.3 Methods

Study Site

The Abrolhos Islands is approximately 60 km off the mid west coast of Australia near the edge of the continental shelf. The influence of the warm tropical water of the Leeuwin Current (Pearce 1997) makes the Abrolhos Islands the southernmost major coral reef in the eastern Indian Ocean (Wells 1997). Their location and influence by the Leeuwin Current, provide them with a unique blend of tropical and temperate species, as exemplified by the co-occurrence of kelp and corals (Wells 1997). However, there is a general spatial segregation of these two habitats, with kelp dominating the limestone windward western reefs, while corals dominate the leeward side of reefs (Wells 1997). This tropical/temperate interface is also evident in the fish fauna, which is dominated by tropical species (66%), but also contains sub-tropical (13%) and warm temperate (19%) species (Hutchins 1997).

Samples and Processing

Samples of *P. leopardus* were obtained from the commercial and recreational sectors throughout the year from 2005-2009, and augmented with fisheries-independent sampling mainly during the spawning season. Annual coverage of reproductive patterns was attained by pooling samples from each month across all years (2005-2009). Fisheries-independent sampling also allowed the capture of sub-legal (45cm L_T) fish permitting sampling of the full size range.

Biological measures such as length (total (L_T) and fork (L_F)), weight (total, gonad and fat) and sex were established for fish. Sex was determined macroscopically for all fish, with a subset of 400 fish examined histologically. For full details see (Chapter 2). Otoliths were also removed, washed and dried for subsequent age determination. Each otolith was embedded in epoxy resin and sectioned through its nucleus using a low speed (Buehler Isomet) saw following Jenke (2002), with sections mounted on slides using a casting resin and cover slip. Using a combination of reflected and transmitted light, with transmitted being the dominant light source (Plate 4.1), a double blind approach to counts of opaque zones was undertaken. Two independent readers counted opaque zones without knowledge of prior counts, fish size, sex or collection date. Counts were made along both sides of the sulcal groove (Plate 4.1). Where disagreement between readers occurred, they were re-read by one reader. If there was still no agreement, the counts from that fish were removed from further analysis.

Otoliths were marked with oxytetracycline (Alamycin LA 300) as part of an acoustic tracking study (Chapter 5). However, changes to Department of Fisheries policy during the project prevented the recapture of these individuals.

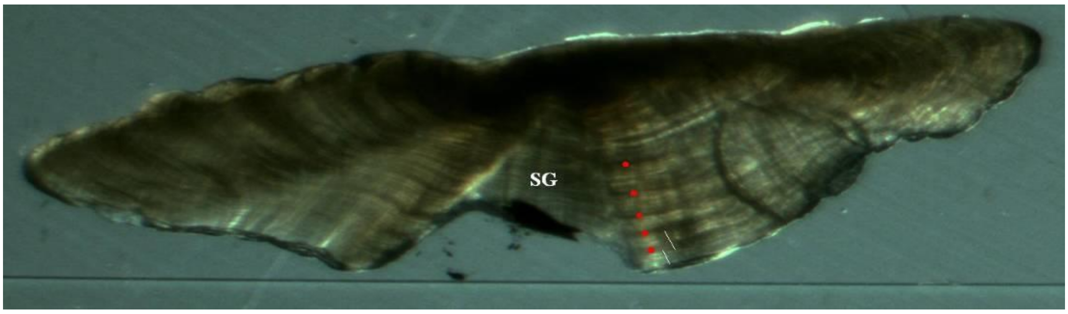


Plate 4.1 Image of a typical sectioned otolith of *P. leopardus* (♀ 30.9 cm LF) showing the sulcal groove (SG) and five annuli (red dots).

While oxytetracycline marked otoliths could not be used for validation of the annual formation of otolith opaque zones in *P. leopardus* in the current study, they have been used successfully previously for the species (Ferreira and Russ 1994). Therefore, the opaque zones in *P. leopardus* in the current study were assumed to represent an annuli in the Abrolhos Islands population.

Analysis

Morphological Relationships

Linear regressions were undertaken between length measures (total L_T and fork length L_F) and also on $\log_e$ transformed length (L_F) and total weight (W_T).

Age and Growth

Counts of opaque zones was used to calculate the fish’s age. Where an opaque zone was on the edge of the otolith but not delineated, that zone was considered to represent an annulus because another zone was about to be deposited which represents another year of growth. Fish were assigned a “birthday” of 1 February which represents the middle of the spawning period (see below). The year of each fish’s “birth” was determined by subtracting the number of annuli from the year of its capture. An age was then determined by subtracting the date of capture from its “birthday”.

A von Bertalanffy growth model was fitted to length (L_F) at age (years) data for all fish, and those fish ≥ 2 years as well as for fish from spawning and non-spawning sites (see Chapter 3) (Equation 4.1). Establishing a growth model for fish ≥ 2 years enabled comparisons to length at age data for this species on the GBR (Brown *et al.*1994, Ferreira and Russ 1994).

$$L_F = L_\infty(1 - \exp(-k(t - t_0))) \qquad \text{Equation 4.1}$$

where: L_F is the length at age (*t*); L_∞ = asymptotic length; t_0 = age at length 0; k = growth coefficient and *t* is the age in years.

Reproduction

Reproductive parameters were calculated for all samples collected, and separately for samples from: (1) ‘spawning sites’; and (2) ‘non-spawning sites’ (see Chapter 3) and sites of an unknown status due to a lack of definitive site location (GPS), which were from commercial and recreational sectors. Fish from spawning sites are likely to reflect reproductive estimates which are unaffected by fishing pressure. Parameter estimates utilizing only samples from unknown and ‘non-spawning’ sites are reflective of a sampling regime that only utilized samples that were easily attainable as they came from easily accessible high fishing areas. ‘Non-spawning sites’ are likely to reflect high fishing pressure areas (Chapter 3), as were unknown sites given that they were obtained by commercial or recreational sectors.

Timing and Duration of Spawning Season

The weight of the gonad (W_G) of each fish was standardised by W_T to calculate its gonadosomatic index (GSI) (Equation 4.2)

$$GSI = \left({}^{W_G}\!\!\diagup\!{}_{W_T - W_G}\right)*100 \qquad \text{Equation 4.2}$$

When there was no weight measurement available for samples, an estimated weight was generated using a length-weight ($\log_e$ transformed; $\ln(L_F)$ and $\ln(W_T)$) relationship (see below). Monthly mean (±SE) GSIs were calculated using fish above the size at maturity.

Length and Age at Maturity

The length and age at which 50 % of females mature (F4 or greater; Table 4.1) was calculated using the data for females captured during the spawning season (see below). Length (L_F mm) or age (years) (denoted by *X*) were incorporated into a non-linear (logistic) regression (Equation 4.3) to estimate length and age at 50% (L_{50};A_{50}) and 95% (L_{95};A_{95}) maturity.

$$p = {}^{1}\!\!\diagup\!\left(1+\exp(\ln(19)*((X - \mathrm{L}_{50})/(\mathrm{L}_{95} - \mathrm{L}_{50})))\right) \qquad \text{Equation 4.3}$$

NB: L_{50} was replaced by $A_{50;}$ and L_{95} replaced by A_{95} in Eqn. 4.3 for estimates of age at maturity/sex change

Data were analysed using the R© statistical software (R Core Development Team 2009).

4.4 Results

Morphological Relationships

The linear regression of L_F and L_T provided a strong relationship (r^2=0.9985; n=790) by which to convert L_T to L_F (Equation 4.4).

$L_F = 0.957304(L_T) -0.780390$ Equation 4.4

The log-log relationship between L_F (cm) and W_T (g) was also was a strong relationship (r^2=0.9863) (Equation 4.5), allowing estimates of W_T from L_F measures.

$\ln(L_F) = 3.14282 (\ln(W_T)) -4.69319$ Equation 4.5

Age and Growth

The growth analysis of fish ≥ 2 years old showed *P. leopardus* at the Abrolhos Islands to have a L_∞ of 724mm L_F,k of 0.113yr^{-1}, and length at age 0 (t_0) of -1.86 yr (Equation 4.6, Figure 4.1). The growth analysis of all fish, including the four fish < 2 years (1.01-1.97 years), resulted in a L_∞ of 718 mm L_F,k of 0.117yr^{-1}, and length at age 0 (t_0) of -1.75 yr.(Equation 4.7, Figure 4.1).

$L_F=724.9*(1-\exp(-0.113(t-(-1.86)))$ Equation 4.6

$L_F=718.3*(1-\exp(-0.117(t-(-1.75)))$ Equation 4.7

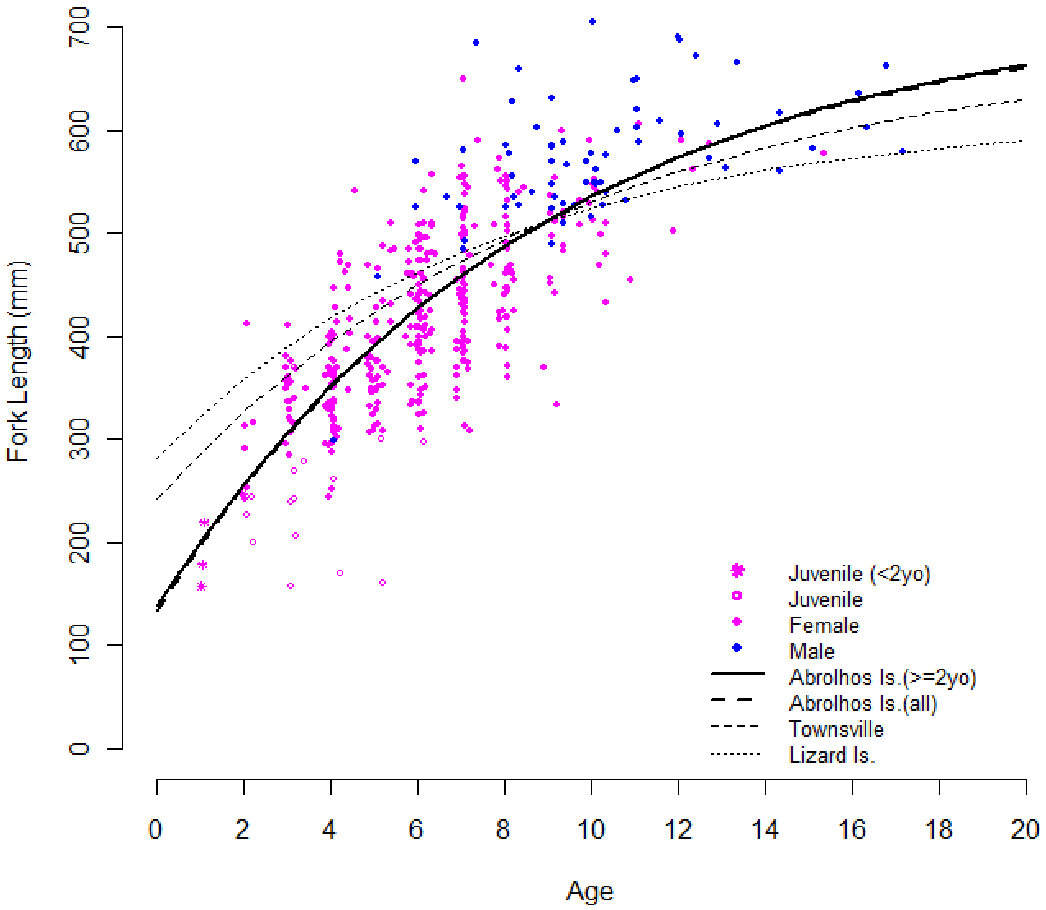


Figure 4.1 Age and associated lengths for leopard coralgrouper at the Abrolhos Islands with a fitted von Bertalanffy growth curve for fish≥ 2 years old and all fish. Superimposed are published growth curves for leopard coralgrouper at Townsville (Brown *et al.* 1994) and Lizard Island (Ferreira and Russ 1994).

The von Bertalanffy growth analysis of *P. leopardus* from spawning sites at the Abrolhos Islands produced an almost linear relationship with an L_∞ of 6012 mm $L_{F,}$k of 0.005yr^{-1}, and length at age 0 (t_0) of -6.76 yr (Figure 4.2). The growth analysis of *P. leopardus* from spawning sites, resulted in a L_∞ of 730 mm $L_{F,}$k of 0.09yr^{-1}, and length at age 0 (t_0) of -2.41 yr (Figure 4.2).

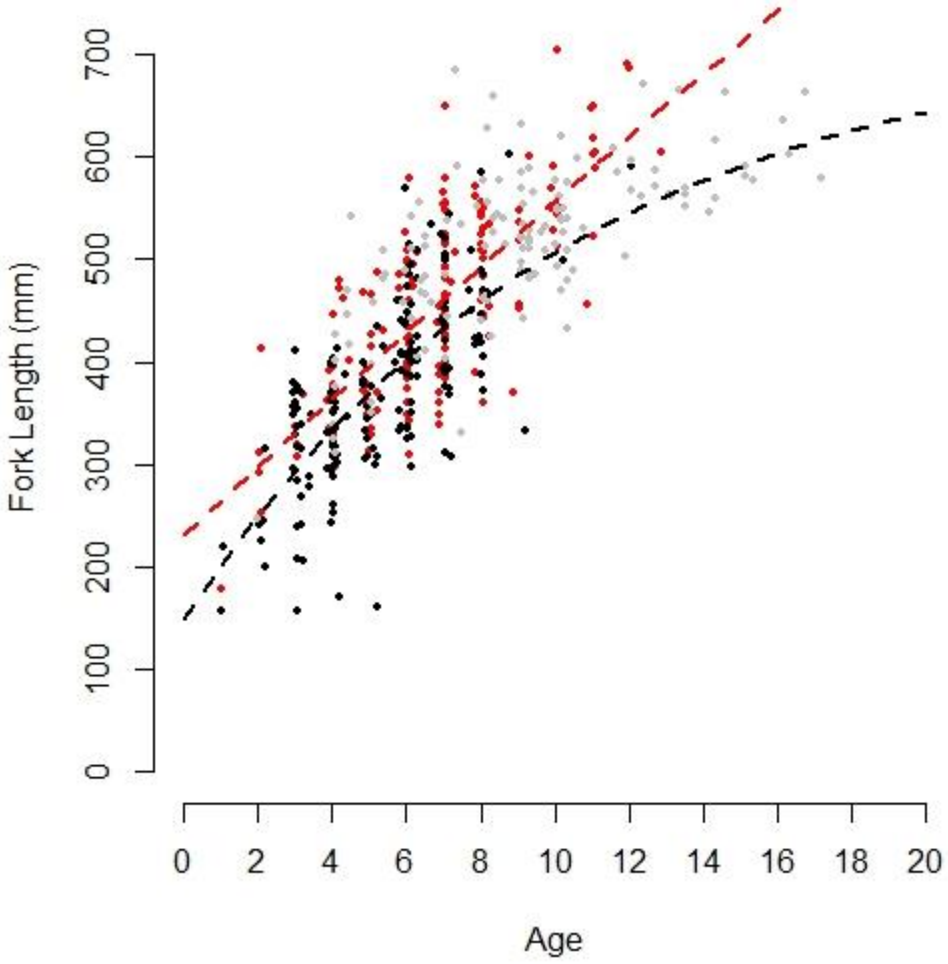


Figure 4.2 Age and associated lengths for leopard coralgrouper at the Abrolhos Islands with a fitted von Bertalanffy growth curve for fish from spawning areas (red), non-spawning areas (black) and ‘unknown’ sites (grey)

Spawning Season

Females collected from all sites and above the L_{50} displayed a relatively low proportion (0.4-0.5) of ovaries in developing or spawning condition between December and February and this declined to about 0.3 in March (Figure 4.3a). Females with recovering ovaries were also present in February and March, but in small proportions, and the proportion fish in this condition subsequently increased to 0.15 in April before declining to 0.06 by June. The overall proportion of fish in developing, spawning or recovering condition was low, with over half of all fish sampled from all locations during the main spawning period (December to March) possessing gonads in resting condition. This proportion increased to 0.8-1.0 during the remaining months (Figure 4. 3a).

Fish collected from spawning sites possessed a very high proportion of ovaries in developing or spawning condition between December and March (0.6-1.0, Figure 4.3b). By April and May, fish were either in resting or recovering states, and from June to November, fish showed no signs of reproductively active or recovering fish, though sample sizes were low (Figure 4.3b).

At non-spawning and unknown sites, there was a very high proportion (>0.7) of females in a resting condition throughout the year (Figure 4.4c). A small proportion (0.04-0.28) of spawning females, which were sampled from “unknown” sites (see Chapter 3), were found during December-April).

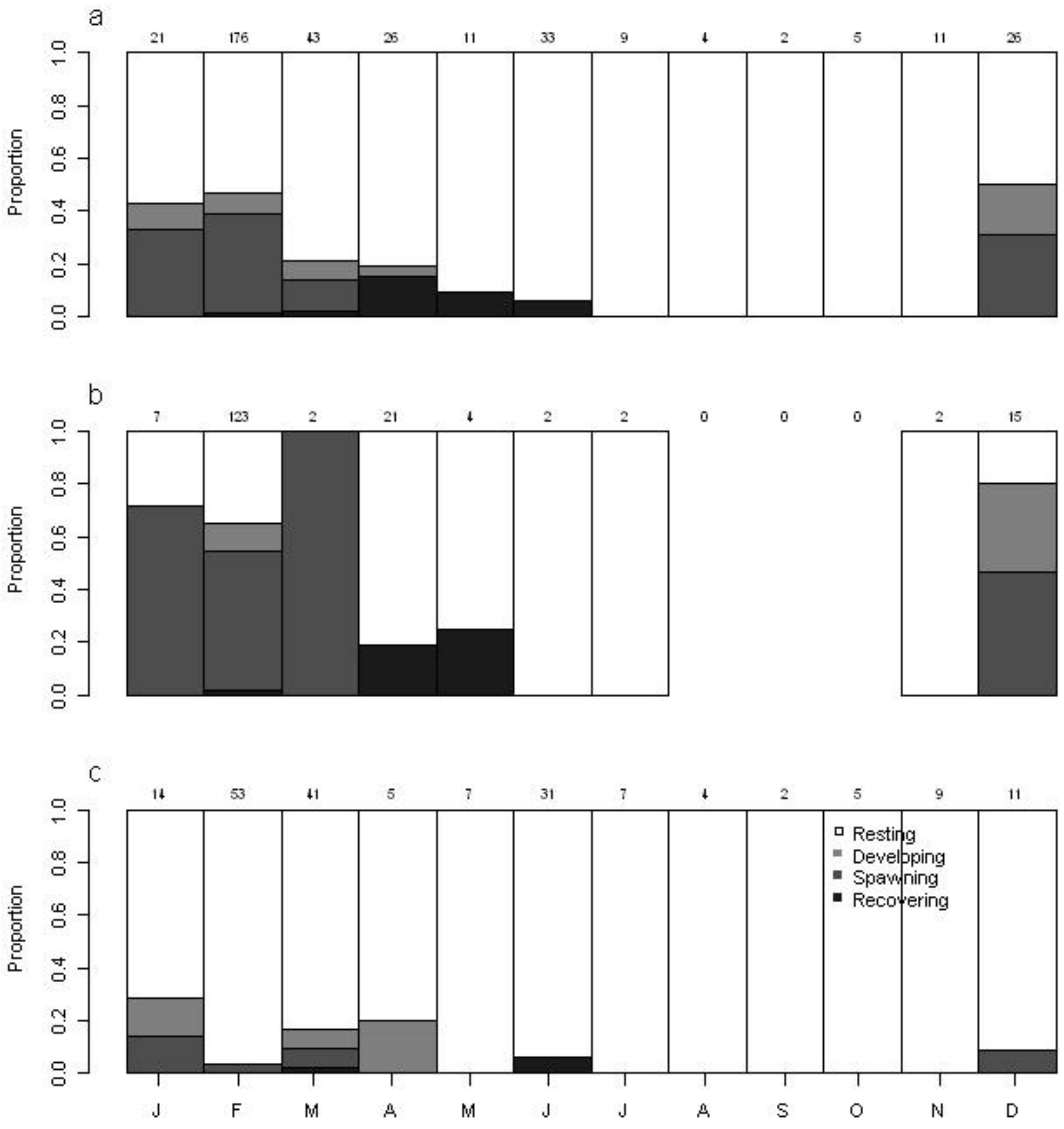


Figure 4.3 Monthly proportions of different stages of ovarian development in female *P. leopardus* above the size at maturity at a) all sites; b) spawning sites; and c) unknown and non spawning sites at the Abrolhos Islands. Numbers above indicate sample size

The mean GSI for female *P. leopardus* above L_{50} collected from all sites was *ca*2.6 in both January and February, after which it declined markedly to 0.8 by March. Mean GSIs remained around 0.5 until November when there was a rapid increase to 2.2 in December (Figure 4.4a). Mean GSIs for males collected from all sites followed a similar pattern to those of females, though with a markedly lower mean GSI in each month (Figure 4.4a). Elevated male GSIs occurred in December through to February, with consistently lower values for the rest of the year.

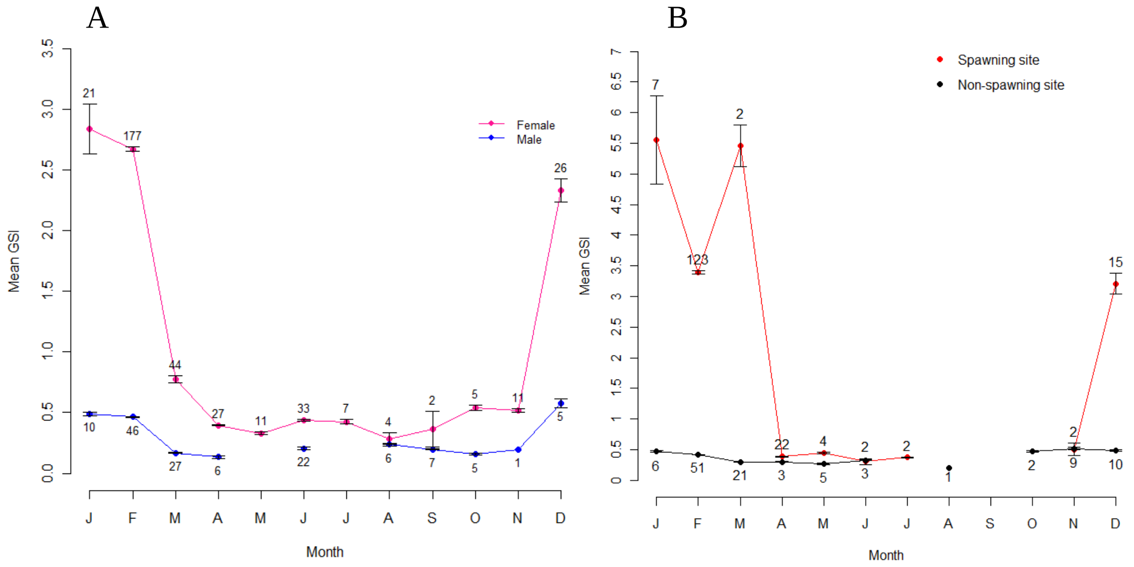


Figure 4.4 Mean (±SE) monthly gonadosomatic index of leopard coralgrouper above the size at female maturity for: A) females (pink) and males (blue); and B) females from spawning (red) and non-spawning (black) sites at the Abrolhos Islands. Numbers above points represent sample size.

When mean GSIs were re-examined separately for fish collected from spawning and non-spawning sites, mean monthly GSIs of females were 6.9-19.5 times higher at spawning sites compared to those from non-spawning sites from December – February (Figure 4.4b). Mean GSIs for females at non-spawning sites remained at approximately 0.5 throughout the year, which was similar to those fish from spawning sites during the non-spawning months (Figure 4.4b).

Size and Age at Maturity

The smallest mature female recorded was 322 mm $L_{F.}$ Logistic regressions of length (L_F) and age (A) against the proportion of mature females collected from all sites provided estimated L_{50} and A_{50} of 506 mm L_F and 8.1 years, respectively (Table 4.1 Figure 4.5). In comparison, L_{50} of 404 mm L_F andA_{50} of 6.0 years were 20 and 26% lower when the regressions were restricted to fish from spawning sites (Table 4.2; Figure 4.5). Estimates of maturity were larger at non-spawning and ‘unknown’ sites, where L_{50} was 608 mm L_F and A_{50} was 11.85 years resulting in 50 and 97% larger estimates, respectively, compared to those derived from spawning sites (Table 4.2; Figure 4.5).

Table 4.1 Estimates of 50% (L_{50}; A_{50}) and 95% maturity (L_{95}; A_{95}) for female *P. leopardus* by length and age, respectively, for the whole fish collected from all sites, spawning sites, and non-spawning and unknown sites.

Sites	L_{50}(±SE)	L_{95}(±SE)	A_{50}(±SE)	A_{95}(±SE)
All	506.73 (±11.52)	771.95 (±45.64)	8.12 (±0.36)	14.62 (±1.48)
Non Spawning & Unknown Sites	607.94 (±26.38)	788.58 (±65.92)	11.85 (±1.06)	18.05 (±2.58)
Spawning Sites	404.30 (±15.01)	705.00 (±62.38)	6.01 (±0.38)	12.18 (±1.54)

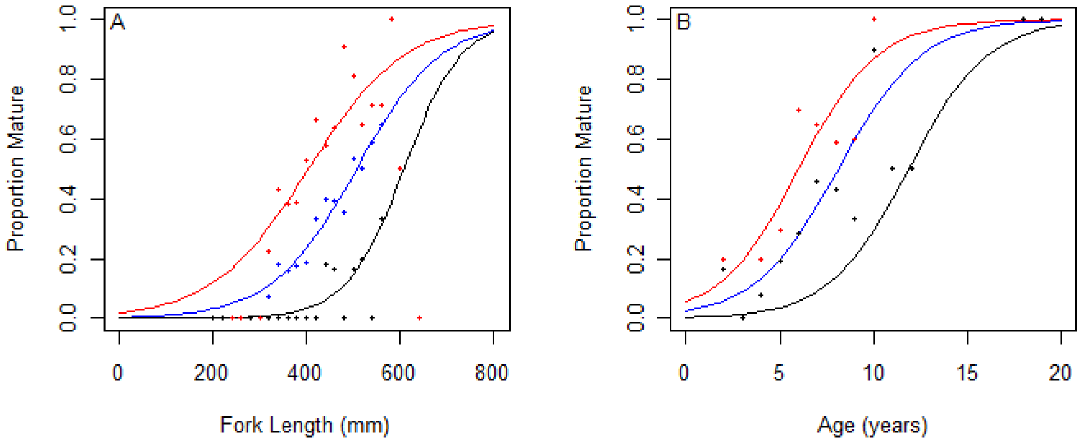


Figure 4.5 Logistic regressions for: (A) the length; and (B) age at maturity, for female *P. leopardus* collected from all sites (blue), spawning sites (red), and non-spawning and unknown sites (black) at the Abrolhos Islands. Points represent proportion of mature fish by 20mm length or 1 year age classes for fish collected from all sites (blue),spawning sites (red),and non-spawning and unknown sites (black).

4.5 Discussion

Given the plasticity of life history parameters already seen for *P. leopardus* (Adams *et al.* 2000) and other targeted fisheries species within the GBR (Williams *et al.* 2006), variations in reproductive parameters between the genetically isolated populations of the GBR and Abrolhos Islands (van Herwerden *et al.* 2009) are expected. The current study has shown that morphological and growth parameters, along with key reproductive parameters, varied between the populations at the Abrolhos Islands and GBR. More importantly, the spawning omission observed in Chapter 3 resulted in large variation in a range of key reproductive parameters, which has large implications for the management of the species and the need for localised, detailed assessment of species biology for fisheries management even within a region.

Morphological and Growth Relationships

There were several notable differences in morphological relationships and growth patterns between the populations of *P. leopardus* at the Abrolhos Islands and GBR. The morphological relationships of the higher latitude Abrolhos Island population appear to be comparable with lower latitude samples on the GBR. Basic variation in morphology was highlighted by the difference even in the simple morphometric relationship of L_F and $L_{T,}$ which was different between the Abrolhos Islands and the GBR ($L_F = 0.957(L_T)-0.780$ vs $L_F = 1.048(L_T)-0.485$, respectively) (Brown *et al.* 1994). This is likely to reflect the more pronounced indentation in the tail for fish from the Abrolhos Islands. In terms of the L_F - W_T relationship, fish from the Abrolhos Islands (28°S) were comparable to lower latitude populations of the GBR such as those from the Swains and Capricorn Bunker Groups (23.5°S) (Brown *et al.* 1994). However, the L_F - W_T relationship of the GBR population varies among areas, with fish from the southern Swains region being heavier for a given length compared to fish from the Cairns region

(16.9°S) (Brown *et al.* 1994). Thus, the Abrolhos Island population had a heavier L_F - W_T relationship compared GBR population in lower latitudes at Lizard Island (14.5°S) and Townsville (19°S) (Brown *et al.* 1994). Similarly, the Abrolhos Island population had a larger L_∞ (725 mm) and smaller t_0 (-1.86 years) than those for the GBR population at Lizard Island (L_∞ 612.9mm; t_0 -4.66 years; Ferreira and Russ 1994) and Townsville (L_∞ 682.7; t_0 -4.13years; Brown *et al.* 1994), regardless of whether fish <2 years old were included or excluded from the analysis. However, there doesn't appear to be a difference in the growth parameter estimates of fish from the Abrolhos Islands (k=0.133) and Lizard Island (k=0.132; Ferreira and Russ 1994) though both are higher than fish from Townsville (k=0.106; Brown *et al.* 1994).

The differences exhibited in the L_F - W_T relationships coupled with the larger L_∞ than the northern GBR studies, follow the pattern of larger fish and heavier fish for a given size occurring in cooler climates (Kozlowski *et al.* 2004, Caselles *et al.* 2011). However, the concomitant slower growth rate expected of higher latitude populations does not appear to occur, with similar growth rates observed at the high latitude Abrolhos Islands and the lower latitude of Townsville (Ferreira and Russ 1994) and Lizard Island (Brown *et al.* 1994) on the GBR. The lack of a consistent slower growth may be due to the influence of the Leeuwin Current on the west coast. This southward flowing current provides more elevated water temperatures than would otherwise be expected for the latitude of the Abrolhos Islands (Caputi *et al.* 1996), potentially minimizing some of the expected temperature differences for a 10° latitudinal change between Lizard Island (mean=26.6°C: http://data.aims.gov.au/aimsrtds/latestreadings.xhtml) and the Abrolhos Islands (mean=23.4°C). The variation in growth and morphological relationships between the populations at the Abrolhos Islands and GBR, coupled with the unique oceanographic conditions within which the Abrolhos Islands reside, highlight the need for a local understanding of basic growth parameters.

The lack of a representative growth curve for fish from spawning sites was due to a lack of younger fish being sampled from spawning sites. Therefore, it was not possible to make a realistic statistical comparison of the effects of spawning omission and possible compensatory growth in this species as has been found previously (Holmgren 2003). It should be noted, however, compensatory growth does not appear to be occurring since non-spawning fish had a similar and potentially older age at length relationship to those from spawning sites. Greater sampling of smaller fish from spawning sites would be necessary to provide a more robust assessment.

Spawning omission and maturity estimates

While the pattern and potential cause of spawning omission at the Abrolhos Islands is explored in Chapter 3, recognition of this phenomenon and accounting for it in estimation of reproductive parameters is likely to be critical for effective management. Spawning omission can have ramifications for the calculation of GSIs (Bell *et al.* 1992), as was clearly identified at the Abrolhos Islands, with mean GSIs differing between spawning sites and the whole population. It also affected size/age at maturity estimates (L_{50};A_{50}), where L_{50} and A_{50} varied by 507 mm and 8.1 years based on analyses of the entire dataset versus those from spawning sites. Similarly, when females from unknown sites and non-spawning sites were used, estimates of size/age at maturity were respectively 203 mm L_F or 5.8 years greater than those from spawning sites. Estimates from spawning sites (404 mm L_F or 6 years, respectively) are likely to be reflective of the ‘true’ size/age at maturity, because they are based on females sampled from locations where spawning is known to occur. Therefore, not accounting for spawning omission resulted in considerably larger estimates of size/age at maturity, which can have ramifications for management, with size/age at maturity a common method for assigning legal minimum size (McPhee 2008).

Coincidently, the legal minimum size (LMS) in Western Australia for *P. leopardus* is 450mm L_T (423 mm L_F), which was not based on any specific biological information but is larger than the estimated 404 mm L_F from spawning sites in this study. Thus, the LMS in Western Australia provides *P. leopardus* at the Abrolhos Islands the opportunity to spawn prior to being exposed to fishing pressure. However, L_{50} estimates based on fish from all locations (507 mm) would have indicated a larger LMS would be needed, highlighting the need to gain accurate reproductive data. In comparison to the Abrolhos Islands, the legal size on the GBR is 380 mm L_T, which corresponds to 360 mm L_F (Adams *et al.* 2000). This is the upper size range given for the size at maturity for *P. leopardus* (320-360 mm L_F) by Ferreira (1995). The application of a size at maturity estimates from the GBR would clearly expose the Abrolhos Islands population to exploitation before they obtained their size at maturity. Furthermore, if spawning omission occurs in the GBR population and maturity estimates have not accounted for this, those values may be overestimates of size at maturity.

Spawning season and temporal closures

Life history parameters such as spawning season have often been used to set management strategies such as temporal closures, and this has been the basis for management measures on the GBR (DEEDI 2010), which to date, were not available for the Abrolhos Islands. The population of *P. leopardus* at the Abrolhos Islands spawns over a four month period from December and March, which differs markedly from the September to December spawning in the GBR (Ferreira 1995). This information, along with data on spawning aggregations forming on the new moon (Samolys 1997b), was used to establish two nine day closures centered on the new moon in October and November (DEEDI 2010) to protect the highly vulnerable spawning aggregations throughout the GBR. The application of the GBR temporal closures would have completely exposed the spawning aggregations of *P. leopardus* to exploitation due to the later spawning season, and their aggregation formation on both full and new moons (Chapter 5).

In Western Australia, there is a temporal closure for temperate demersal species within the west coast bioregion, which encompasses the Abrolhos Islands from 15 October – 15 December (Fairclough *et al.* 2011). While the implementation of the closure was to reduce overall levels of effort, it unfortunately encompasses only approximately 15 days of the four month spawning season of *P. leopardus*. A species-specific temporal closure does exist at the Abrolhos Islands, where the capture of baldchin grouper (*Choerodon rubescens*; Labridae) is not permitted from 1 December to 31 January (Fairclough *et al.* 2011). While this closure does cover half of the spawning season of *P. leopardus*, this species is not protected and may actually be subjected to greater fishing pressure with a shift of effort from *C. rubescens*.

Conclusions

The *P. leopardus* population at the Abrolhos Islands differs in both its morphological, growth and reproductive parameters from its eastern Australian con-specific, highlighting the importance for a local assessment of a species biology. Two of the common fisheries management methods based on species biology (legal minimum size and spawning closures) would result in no protection for the Abrolhos Island population if estimates from the GBR were to be used. This study also highlights the need for comprehensive surveying of the population, including fish within closed areas. A reliance on samples obtained from commercial or recreational samples (‘unknown’ sites), or other exploited sites (non-spawning sites) in this study at the Abrolhos Islands, would have resulted in an overestimated size at maturity by 200 mm L_F. This inflated estimate, if used for fisheries management, would afford the species greater protection with a higher legal minimum size. However, the use of samples from all locations in the present study suggests very little spawning activity at the Abrolhos Islands, leading to the conclusion that local recruitment may not be an important driver in population levels. While some gene flow is considered to come from Scott Reef to the north, a large degree of local recruitment is likely, based on genetic analysis (van Herwerden *et al.* 2009). If a full sampling regime including closed areas was not undertaken, the conclusion on limited local spawning may lead to a greater level of exploitation. This would be due to the belief that the ‘spawning biomass’ is not being fished. Therefore, to ensure appropriate measures are in place for management of exploited fish stocks, detailed, extensive local sampling is necessary to gain an accurate assessment of the necessary parameters for management.

Leopard coralgrouper at the spawning aggregation, with a gravid female near the coral and numerous fish mid-water in the background

Chapter V

Movement patterns and spawning aggregation dynamics of the leopard coralgrouper *Plectropomus leopardus* in the Indian Ocean

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5.1 Abstract

The spawning aggregation dynamics of *Plectropomus leopardus*, a common and commercially important tropical reef fish, was assessed at the Abrolhos Islands on Australia's mid-west coast. Spawning aggregations of fish are highly susceptible to fishing pressure due to their predictable temporal and spatial nature. This was achieved by using automated acoustic telemetry, underwater visual census (UVC), and histological examination of post-ovulatory follicles in the ovaries. Acoustic telemetry of eight fish demonstrated that larger (male) fish displayed frequent attendances at spawning aggregations, while smaller (female) fish attended less frequently. Spawning of *P. leopardus* occurred from mid-morning to mid-afternoon around both the new and full moons throughout the spawning season with fish, representing a significant deviation from the typical dusk / night spawning period around new moons for *P. leopardus* in the Great Barrier Reef (GBR) and many other fish species that form spawning aggregations. *P. leopardus* was generally very sedentary, with a single acoustic receiver dominating the detections of each fish, and accounting for almost all nocturnal detections. Leopard coralgrouper moved on average 569m from this dominant receiver location to their nearest spawning aggregation. There were a number of

similarities of this population with its con-specifics in the Great Barrier Reef (GBR) in relation to its relatively sedentary nature and attendances at spawning aggregations around the new moon. However, differences in the spawning aggregation dynamics of *P. leopardus* between regions emphasises the need to understand specific aggregation dynamics to ensure adequate protection of these highly vulnerable yet important spawning aggregations.

5.2 Introduction

Spawning aggregations occur when individuals of a species group at particular locations in significantly greater abundances than during non-reproductive periods for the purpose of spawning (Domeier and Colin 1997; Domeier 2012). This is a common reproductive strategy for a range of coral-reef fish (Thresher 1984; Choat 2012) and often occurs at times and locations that are predictable and persistent through time (Colin 1996; Johannes *et al.* 1999, Domeier 2012). However, this predictability makes them susceptible to fishing and over-exploitation (Domier and Colin 1997), and has lead to the local depletion of a number of spawning aggregations in the Caribbean and Indo-Pacific regions (Colin 1992; Claydon 2004; Sadovy and Domeier 2005). Such depletions can have a negative impact on fisheries, resulting in declines in spawning biomass and potentially leading to an overfished stock (King 1995; Sadovy and Domeier 2005). Consequently, understanding the dynamics of spawning aggregations is vital for effective fisheries management for all species with this reproductive strategy.

Marine protected areas (MPAs) are one means by which spawning aggregations have been protected. Their spatial nature requires identification of the spawning aggregation site/s, but also movements or catchment distances to the aggregations. Where these movements occur along known migratory corridors, they are targeted by fishers (Rhodes and Tupper 2008). These spawning aggregation migrations often represent the maximum movements exhibited by a species. This is particularly the case for species in the family Epinephilidae, whose movements are generally localised (Zeller 1997, Hutchinson and Rhodes 2010), thereby further enhancing their protection by MPAs.

Typically, spawning aggregations have been surveyed using underwater visual census (UVC), providing estimates of abundance and how that varies temporally (Colin 1996; Colin and Clavijo 1998; Eklund *et al.* 2000; Pet *et al.* 2005). However, while providing valuable abundance estimates, the use of UVC is limited by the amount of time that can be spent observing one or more aggregations, and is complicated further with logistics as many aggregations spawn at dusk or at night (Colin 1992; Rhodes and Sadovy 2002). Biological samples from commercially exploited spawning aggregations have also been utilised to, not only confirm the location, but also determine the timing of aggregation formation by sex and size (Shapiro *et al.* 1993; White *et al.* 2002). Fish tracking approaches, particularly those using automated acoustic systems, provide a means to simultaneously study multiple aggregations over extended periods without the logistical limitations of UVC approaches, or destructive sampling from fishing for biological data. Such non-destructive techniques permit studies of aggregations within MPAs. However, the combination of these three techniques provides multiple and independent measures of spawning aggregation dynamics.

Leopard coralgrouper, *Plectropomus leopardus,* is a common coral-reef fish in the Indo-Pacific, forming a major component of commercial and recreational reef-fish fisheries in some areas (Mapstone *et al.* 2001) and the live reef trade in south east Asia (Sadovy and Domeier 2005). This species has been shown to form spawning aggregations on the Great Barrier Reef (GBR) (Samoilys and Squire 1994; Samoilys 1997b; Zeller 1998) and in Papua New Guinea (Hamilton 2003). Aggregations of *P. leopardus,* studied through UVC or manual acoustic tracking, have been recorded on new moons during the spawning season between August and December, with individuals moving around a kilometre from their home ranges to spawning aggregation sites (Samoilys and Squire 1994; Samoilys 1997b; Zeller 1998). However, not all individuals participate in spawning aggregations with 14% (Samoilys 2000 in Samoilys 2012) and 31% (Zeller 1998) of mature *P. leopardus* thought to form spawning aggregations, with pair spawning thought to occur outside of spawning aggregations (Samoilys 2000 in Samoilys 2012).

In this study, I aimed to use a multi-method approach based on acoustic telemetry in combination with UVC and gonad histological data, to examine the movement patterns and spawning aggregation dynamics of *P. leopardus*. The study focused on acoustic telemetry of *P. leopardus* at the Abrolhos Islands, located on the mid-west coast of Australia and eastern boundary of the Indian Ocean before, during and after the spawning season, which occurs at the Abrolhos Islands from December to March (Chapter 2). This will highlight variations in participation rates and aggregation dynamics from its con-specifics on the GBR and other teleost species that form spawning aggregations (Thresher 1984; Samoilys 1997b). As management of spawning aggregations usually involves spatial or temporal closures based on knowledge of the location or dynamics of spawning aggregations, respectively (Johannes *et al.* 1999; Burton *et al.* 2005), a detailed understanding of movement into and away from spawning aggregation sites provides information critical to the consideration of spatial and temporal closures, and other management approaches to maintain sustainability of a fish stock.

5.3 Methods

Acoustic array

An acoustic array was established within and adjacent to the ROA in the Easter Group of the Abrolhos Islands (Figure 5.1) to determine the movement of *P. leopardus* to and from spawning aggregations along the reef slope. The array was placed along fringing reefs consisting of a gently sloping reef down to ~8m before descending steeply to a sandy bottom at 40 m. The area was characterised by high cover of mainly tabular forms of coral, particularly in the north-eastern and eastern part of the array. The acoustic array, comprising 21 VR2 (Vemco™) receiver stations, was deployed in two stages (Figure 5.1). Receivers (hydrophone pointing up) were moored off the reef slope on the deep sand (40m) to allow maximum reef slope coverage, with minimal acoustic “holes” in the array. An initial range test was carried out to the south of the array area prior to deployment to determine the optimal spacing of stations in the array. This range test indicated that stations needed to be deployed no more than 600 m apart along the reef slope to allow 100% detection zones (Appendix A).

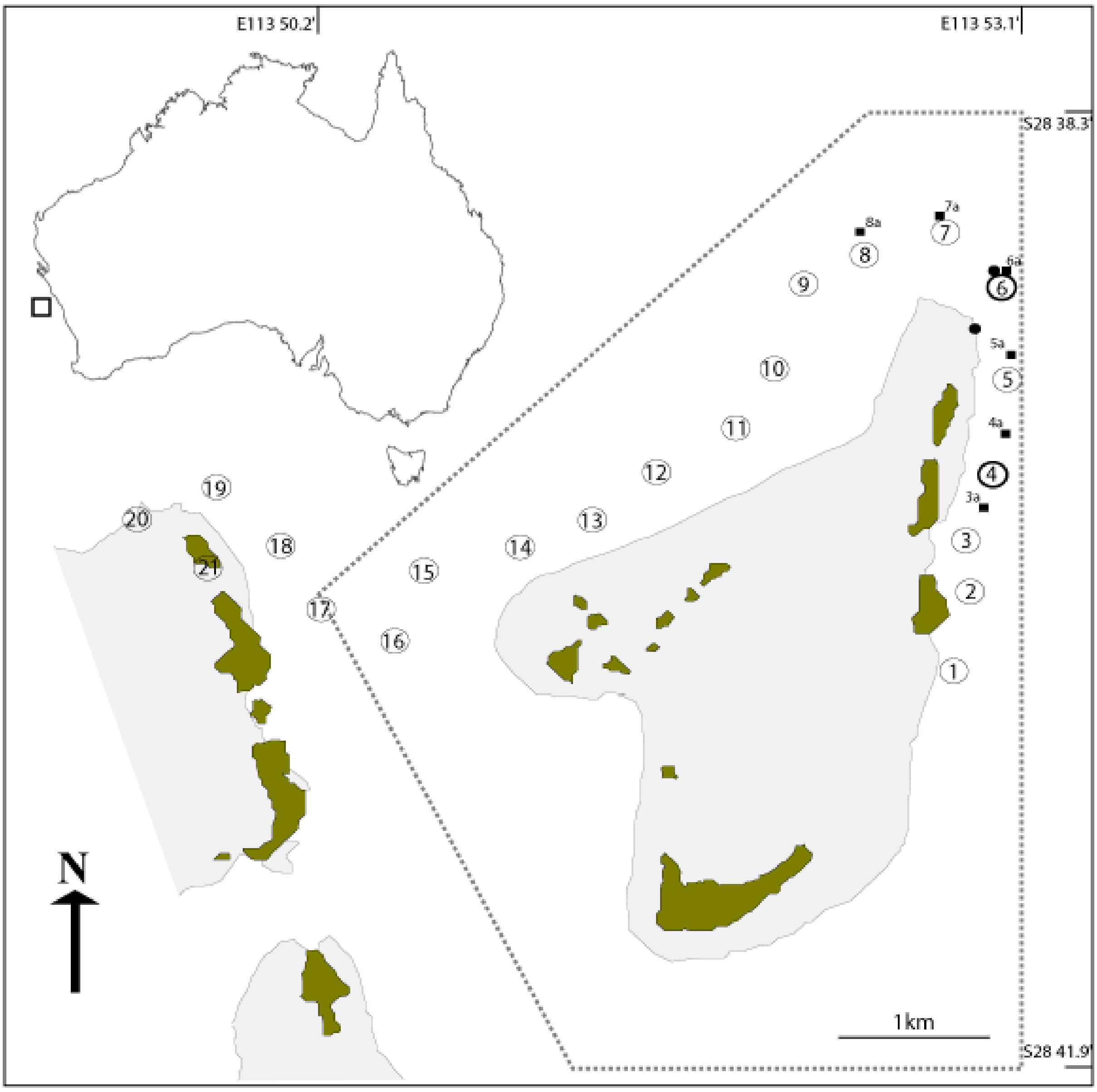

Figure 5.1 Position of the acoustic array at the Easter group of the Abrolhos Islands, with stations (numbered circles; spawning stations heavy circle), original array stations prior to redeployment (filled square), and reference transmitter locations (closed circle). Reef Observation Area (ROA) boundary (dotted line) with associated islands (brown) and approximate intertidal zone (light grey).

Stations 1-16 were deployed on the 16th October 2007 around the northern part of the ROA and prior to the commencement of the spawning season (Chapter 4). The array was then augmented with a further five stations (17–21) in the channel and along another reef group to the west of the original array (Figure 5.1) on the 4th–6th December 2007. This allowed detection of fish movements across deep channels (40m) between reefs, and outside the ROA. Six of the stations deployed in stage one (3a–8a) were also relocated to increase acoustic coverage along the reef slope, such that receivers were approximately 100m from the reef slope. The whole array was retrieved on the 15th–16th April 2008 after the spawning season had ceased, providing movement data of *P. leopardus* over a six-month period before during and after the spawning season.

Spawning aggregation sites were identified during a pilot UVC study, with stations 4 and 6 being placed adjacent to (150m offshore) two spawning aggregation sites (Figure 5.1). These enabled movements to and from the aggregation sites as well as the temporal dynamics of these aggregations to be examined.

Spawning aggregations for *P. leopardus* were confirmed at the Abrolhos Islands through multiple techniques, including observations of intra and inter-sexual behavioural interactions (How pers. obs.). A spawning aggregation site, as defined by Domeier and Colin (1997), was confirmed for Station 6, where densities at spawning times (96 per 1000m^2) were 96 times greater than those in non-spawning times (1 per 1000m^2; Shedwadi pers. comm.). Histological analysis confirmed that hydrated oocytes were present in ovaries of females at the periphery of the aggregation during spawning times. In the case of Station 4, a pilot UVC study found spawning season densities of 19.33 per 1000m^2 (How, unpubl. data). This station could not be “confirmed” as a spawning aggregation site, since non-spawning densities were not available for this specific site. Previous surveys of *P. leopardus* in the Easter Group ROA, had densities of approximately 14-16 per 1000m^2 in 2002, which equated to a 10 fold higher density of “non-crytic sizes” in protected compared with fished areas (Nardi *et al.* 2004). Subsequent surveys using baited underwater video of *P. leopardus* abundances in and out of the ROAs have shown a decline in abundances since 2004. Abundances in ROAs progressively declined from 2004 such that, there was to no difference between the ROA and fished areas by 2009 (Watson *et al.* 2007, McLean *et al.* 2011). Therefore, current densities within the ROA were likely to be $^1/_{10}$th those reported by Nardi *et al.*

(2004) (i.e. ~1.5 fish per 1000m^2).This is very similar to that reported above for non-spawning season densities for station six (1 per 1000m^2; Shedwadi pers. comm.). Therefore the density at station four would exceed the 3 times abundance criteria used by Domeier and Colin (1997).

Fish capture and surgery

Eight *P. leopardus* (385–615 mm total length, L_T) were captured on hook and line within the array on 16th and 17th October 2007 and implanted with either V13-1L (R04k; 30–90sec) or V9-2H (R04k; 20–40sec) (Vemco™) transmitters (Table 5.1) with an expected life of 623 and approximately 70 days, respectively (www.vemco.com). Capture and surgical procedures were adapted from Zeller (1999) and Semmens (pers. comm.). Prior to fish capture, the cradle, sling and smooth material for covering the fish's head were sprayed with fungicide (Nycex; 15g /5l) and Vidalife™. Dissecting gear, transmitters, dart-tags, tag applicator and measuring board were sterilised using 100% Betadine, and the acoustic transmitter was placed in a solution of 10% Betadine.

Table 5.1 Fish number, transmitter type and size of acoustically tracked fish with date and depth of capture and estimated sex

Fish number.	Transmitter type	Date of capture	Depth (m)	Total length (mm)	Estimate Sex
1	V9-2H	17/10/2007	6.5	385	Female
2	V13-1H	17/10/2007	4.3	427	Female
3	V13-1H	17/10/2007	4.5	430	Female
4	V13-1H	16/10/2007	8.0	440	Female
5	V13-1H	17/10/2007	6.1	558	Female
6	V13-1H	17/10/2007	8.0	570	Female
7	V13-1H	17/10/2007	6.1	575	Unknown
8	V13-1H	17/10/2007	3.0	615	Male

Each fish caught using a hook and line was brought on board with the use of a sling, and after the hook had been removed, its eyes were covered with a smooth material and its total length measured to provide an estimate of weight (Chapter 4) and an appropriate dosage of oxytetracycline (Alamycin LA 300) as a post operative antibiotic. The fish was then placed in an on-board 160L tank with oxygenated seawater where it was left to recover while surgical preparations were made. For surgery, each fish was placed in a plastic covered foam v-shaped cradle with its ventral side up, which limited the fish's ability to move. A 2-3cm long incision was made approximately 1-2 cm from the anus, and 1 cm laterally from the mid ventral line (Zeller 1999). After the incision area was flushed with Betadine, the transmitter was inserted. Gloves were worn and covered with Vidalife™ to prevent skin irritation. The incision was then closed using surgical adhesive (3M Vetbond™). Oxytetracycline (Alamycin LA 300) was administered at 25mg/kg (McFarelane and Beamish 1987) before the fish was returned to the 160L tank to recover and be monitored (up to 10 minutes) prior to release. The tank was dark and had pure oxygen constantly applied to reduce stress and improve recovery of the fish. The surgical component took approximately 5 minutes for each fish.

To return fish to the site of capture, each fish was removed from the recovery tank in a sling, and two numbered dart tags were inserted either side of the dorsal fin before being lowered back into the water. Tagging enabled later identification of the fish. A diver observed the initial swimming motion of the fish after release and its behaviour for up to 5 minutes, or until the fish swam out of sight, or it positioned itself under a ledge. Only one fish could not be observed on release (fish 4), but all the other fish showed normal swimming behaviour with all but one (fish 8) sheltering under plate corals. Fish 8, which had been observed prior to capture, returned to a normal slow swimming speed and natural behaviour around the capture site on release and then off the reef slope.

Underwater visual census of a spawning aggregation

Fish abundances were estimated using underwater visual census (UVC) on SCUBA during a spawning aggregation (adjacent to Station 6; Figure 1) over the new moon in

December 2006. The survey times were focused around dusk on the new moon as these were the previously published times of spawning for *P. leopardus* on the GBR (Samoilys and Squire 1994; Samoilys 1997b), with the December being the start of the spawning season at the Abrolhos Islands (Chapter 4). A comparable dawn survey was not possible due to diving regulations, with 08:00 the earliest survey possible time. This resulted in surveys being conducted at 20:00 hrs on 18th December and then three times a day (08:00, 12:00 and 20:00 hrs) until 0800 hrs on 22nd December. Total length (L_T) of each fish in the census was estimated to the nearest 5 cm length class within 30x10m belt transects along the depth contour on the reef slope. The first four surveys consisted of two transects, with an additional transect down the reef slope for the final seven surveys. This resulted in s survey area of 900m^2. All surveys were conducted by the same diver (J. How) enabling consistency of observer counts, size estimation and transect width.

Histological assessment of post-ovulatory follicles

Histological analyses were conducted on gonads from 400 fish captured through hook and line and spear fishing throughout the Abrolhos Islands between 2006 and 2009. None of these samples were obtained from the spawning aggregation sites being studied to preserve the integrity of these aggregations. Gonads were immediately placed in formalin after capture and subsequently, medial sections were cut, mounted and stained with hematoxylin and eosin before being staged and examined for recent spawning based on the presence of post-ovulatory follicles (POFs). This yielded 27 mature females with POFs, whose collection times were recorded. These were then staged according to Samoilys and Roelofs (2000), and in cases where sections contained POFs of different stages, fish were categorised by the most recent stage observed.

Analysis

The longitudinal design of the acoustic array and steep reef slopes meant that area estimates of home range could not be calculated. A surrogate for home range was linear distance, which was the distance around the reef slope between the outermost stations in which a fish was detected (reef slope distance). This technique of using linear distances has previously been used to describe home range attributes (Zeller 1997).

Fish movements were primarily analysed through amalgamating detections into one hour time periods (time bins). Attendance times at a spawning aggregation were calculated using only time bins where a fish was recorded solely at a spawning aggregation station. The first and last time they were recorded during these time bins were used to determine their arrival and departure times. Spawning aggregation stations (stations 4 and 6, Figure 5.1), were located adjacent to the two spawning aggregation sites identified in the ROA of the Easter Group. The distance of detection from a receiver can change with a range of environmental conditions. In order to ensure that a fish was detected near the receiver, and not at the edge of a potentially large acoustic range (Appendix A), the attendance of a fish at a spawning site was defined by meeting all of the following criteria: (1) the fish was only detected on a spawning aggregation station within a particular time bin; (2) the fish was present for more than a single time bin; and (3) the fish had more than 5 detections at the spawning aggregation station for the time bins it was detected at that station. As a result, singular or small numbers of detections at spawning aggregation stations were not deemed a spawning aggregation attendance. Such low detection rates are likely to reflect a fish being at the limit of the receiver’s detection rather than being at the station (aggregation) itself.

Catchment distance (i.e. the distance a fish moves from its home range to the spawning aggregation) was classed as the distance from the fish’s preferred nocturnal station

(Figure 5.2) to the spawning aggregation site. Where the nocturnal site was located between two stations (due to the alteration to array design), the distance from the spawning aggregation site to the midpoint between these two stations was classed as the catchment distance. Due to a lack of detections for fish 1 and 2, a robust estimate of the preferred nocturnal station, and hence an estimate of catchment distance, was not possible for these two fish.

For visual census data, differences in relative abundance (square root transformed) between time of day and date were performed using successive Kruskal-Wallis tests, with associated pair-wise comparisons (R package “asbio”; at http://cran.r-project.org/). Size data (ln transformed) were analysed through two factor ANOVA, with a Tukey HSD post hoc analysis. Validity of size estimates was assessed as part of a concurrent biological sampling program (Chapter 4). Estimates of fish L_T (to the nearest centimetre) were made prior to sampling the fish through spearfishing with each fish’s actual L_T subsequently measured to the nearest centimetre. Total length observation estimates were not significantly different ($p=0.18$) to actual measures.

Leopard coralgrouper is a protogynous hermaphrodite (Ferreira 1995; Adams 2003; Chapter 2). The sex of each fish was estimated by utilising the size to sex relationship at the Abrolhos Islands (Chapter 2). The probability of being a male was determined using the logistic relationship between L_T and sex, and where the probability of being male was ≥ 0.7 ($\geq$ 60.6 cm L_T), the fish was deemed to be male, while those ≤ 0.3 ($\leq$ 57.1 cm L_T) were classed as female. The remaining fish (57.1-60.6 cm L_T) were classed as unknown. Males could also be identified by intra and inter-sexual spawning related behaviours and different colour morphs or features (Plate 5.1). All data were analysed using R (R Development Core Team 2009).

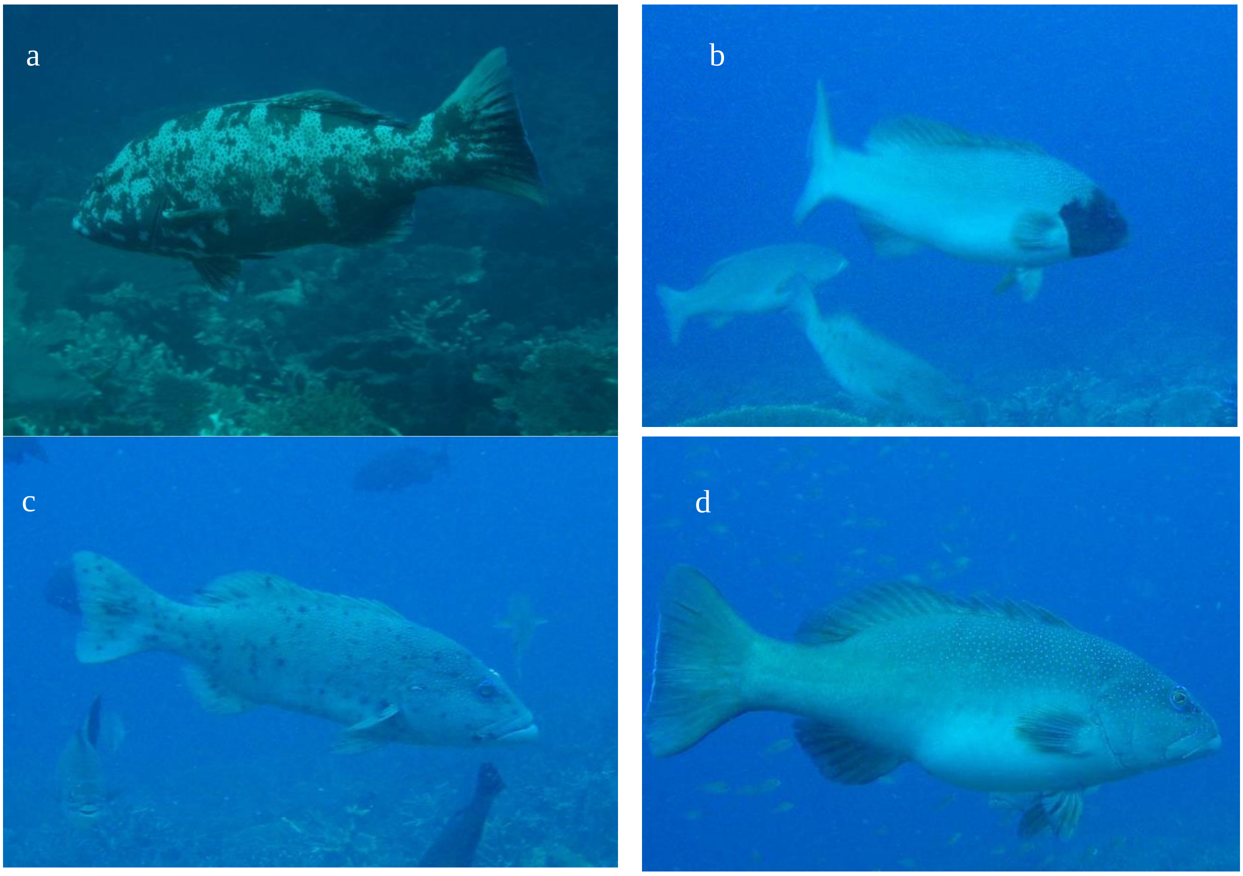

Plate 5.1 Images *P. leopardus* at a spawning aggregation illustrating the distinctive markings and range of colour morphs of males (a-c), and gravid female (d)

5.4 Results

Movements patterns

On average, each fish was detected for 168 days, with over half of the fish detected within the first 24 hours after release, and frequently during the following 183 days that the array was deployed (Table 5.2). The detections for each fish were dominated by a single "preferred" station (Figure 5.2), with this station accounting for almost all detections during each night for a fish.

Table 5.2 Release, detection and time at liberty for acoustically tracked *P. leopardus*

Fish	Release date	Time at liberty (days)	Release to first detection (days)	No. 1hr bins detected	% of potential total 1 hr bins detected
1	17/10/07	181	30	233	5.4
2	17/10/07	168	56	65	1.5
3	17/10/07	94	<1	488	11.2
4	16/10/07	182	<1	1678	38.4
5	17/10/07	181	3	502	11.6
6	17/10/07	181	<1	2341	53.9
7	17/10/07	181	1	1437	33.1
8	17/10/07	175	<1	983	22.6

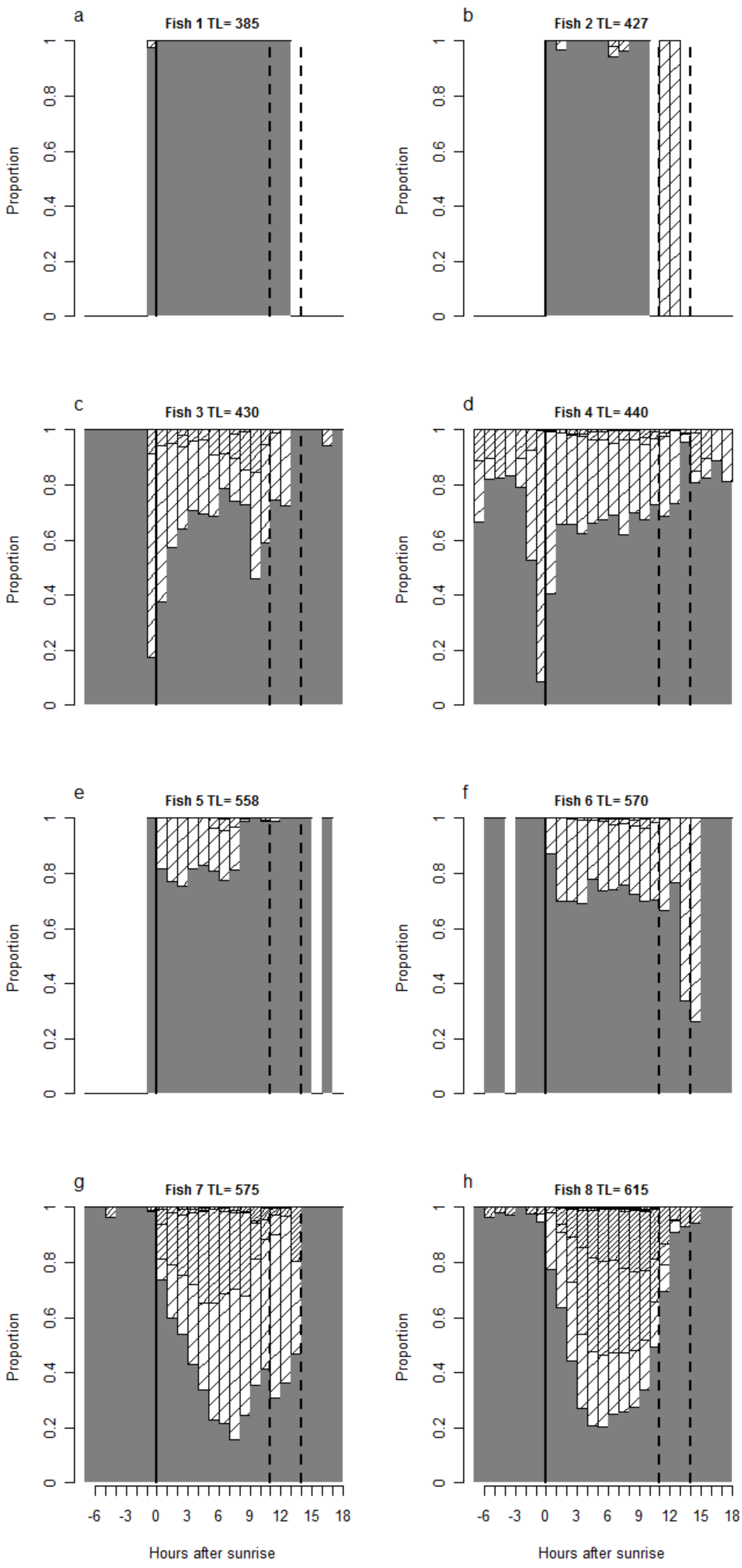

Figure 5.2 Proportion of detections per hour (standardised to hours after sunrise) of each tagged *P. leopardus* for the dominant receiver (grey) and other receivers (diagonal lines). Solid line denotes sunrise. The two dashed lines indicate the minimum and maximum number of hours after sunrise that sunset occured during the study

All fish were detected at either one or the other spawning aggregation site (stations 4 or 6) for varying periods throughout the study (Figure 5.3). Four of the eight fish (fish 3, 4, 5 and 8) first arrived at either spawning aggregation site at the start of the spawning season in early December (before the new moon), while fish 1 and 7, which had previously been detected at these stations, also re-appeared at the site in early December. Fish 6, which had a spawning aggregation site (Station 6) as its preferred nocturnal position (Figure 5.2), also had a series of "stays" (days where it did not move from the spawning aggregation site) in early December (Figure 5.3). Fish 2 was the only fish not detected at either spawning aggregation site in early December, but it was detected at one of these sites later in the study (Figure 5.3).

Movements from "preferred" nocturnal sites to spawning aggregation sites (catchment distance) ranged from 0 m (e.g. fish 6 and 8 whose nocturnal station was a spawning aggregation site) to an average of 569 m for those fish whose nocturnal station was not at a spawning aggregation site (n=4). The maximum catchment distance was 795 m (fish 7). Invariably, all fish moved to the spawning aggregation site closest to their 'preferred' station.

Prior to the spawning season, the linear distance for movement ranged between 0 and 1,835 m, with an average of 991 m. There was a significant positive relationship (r^2=0.771; p=0.002) between fish size and linear distance travelled around the reef slope (reef slope distance) in the pre-spawning period. However, for the duration of the study, including the spawning season and the proceeding 2 weeks, the maximum linear distance moved along fringing reef slopes was 2,961m.

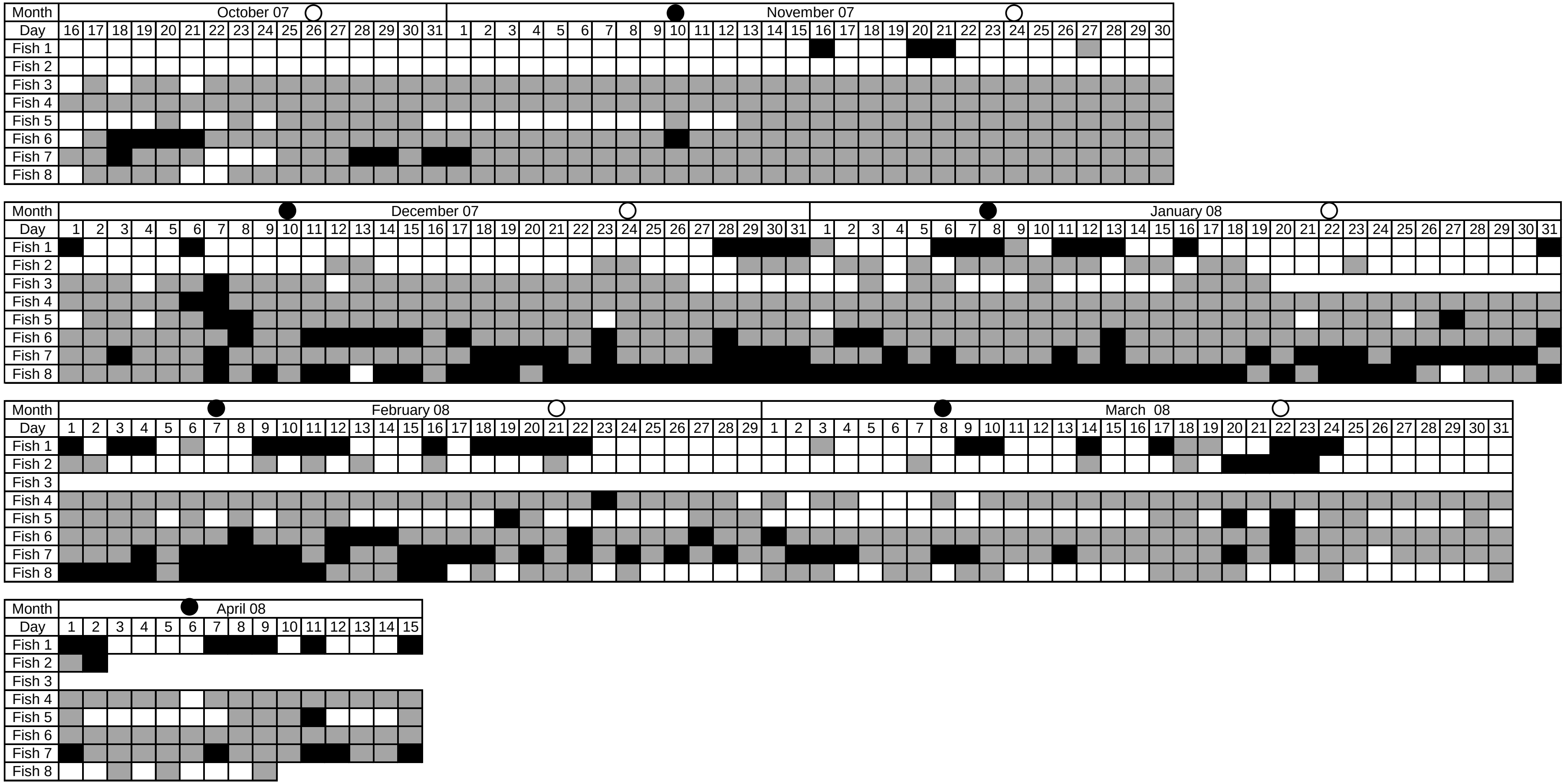

Figure 5.3 Tracking period of eight P. leopardus at the Abrolhos Islands, with full (open circles) and new (filled circles) moons indicated. Grey squares indicate days in which each fish was detected within the array, but not at the spawning aggregation sites; black squares indicate days when each fish was detected at spawning aggregation sites. For fish 6 black squares indicate days where it remained at the spawning aggregation site as the spawning aggregation site was also its preferred nocturnal location.

During the spawning season, there was considerable variation in the number of days fish were detected at spawning aggregation sites, with attendances ranging from 1 (fish 3) to 59 (fish 7) days (Table 5.3). The frequency for the number of spawning aggregation attendances was bimodal, with 4 fish having less than 10 attendances, and 2 fish having 51-60 attendances. Of the remaining two fish, the ‘preferred’ nocturnal station for fish 6 was its spawning aggregation site (Figure 5.2f), while fish 1 was only detected at that station (barring one detection; Figure 5.2a). These data may not truly reflect spawning-related attendances of those 2 fish at these spawning aggregation sites, as the spawning aggregation stations were their ‘preferred’ nocturnal station. For this reason, they were removed from further analyses relating to arrival and departure times from the spawning aggregations.

Table 5.3 The number of days recorded on a spawning site receiver for each acoustically telemetered fish. Average arrival time after sunrise (± SE) and average time duration detected by the spawning receiver (±SE).

Fish	No days @ aggregation Station	Arrival time post sunrise (hours)	Average stay duration (hours)
1	43	4:55 (±0:23)	4:25 (±0:23)
2	5	2:16 (±0:06)	5:23 (±1:05)
3	1	3:20	5:47
4	3	5:08 (±2:41)	6:15 (±0:39)
5	7	3:48 (±0:33)	2:27 (±0:38)
6	26	0:07 (±0:33)	14:59 (±0:43)
7	59	3:31 (±0:19)	6:12 (±0:26)
8	56	5:13 (±0:25)	7:22 (±0:26)

The four fish (fish 2–5) that had a low frequency of spawning aggregation attendances were all presumed to be females (Table 5.1). They had a strong lunar association exhibiting bimodal attendance peaks around the full moon and just prior to the new moon (Figure 5.4a). Fish 7 and 8 (unknown sex and presumed male) each displayed a relatively high frequency of spawning aggregation attendances, and were generally persistent at spawning aggregation sites irrespective of lunar phase (Figure 5.4b). This is exemplified by fish 8 which was recorded at the spawning aggregation daily for 29 consecutive days (Figure 5.3).

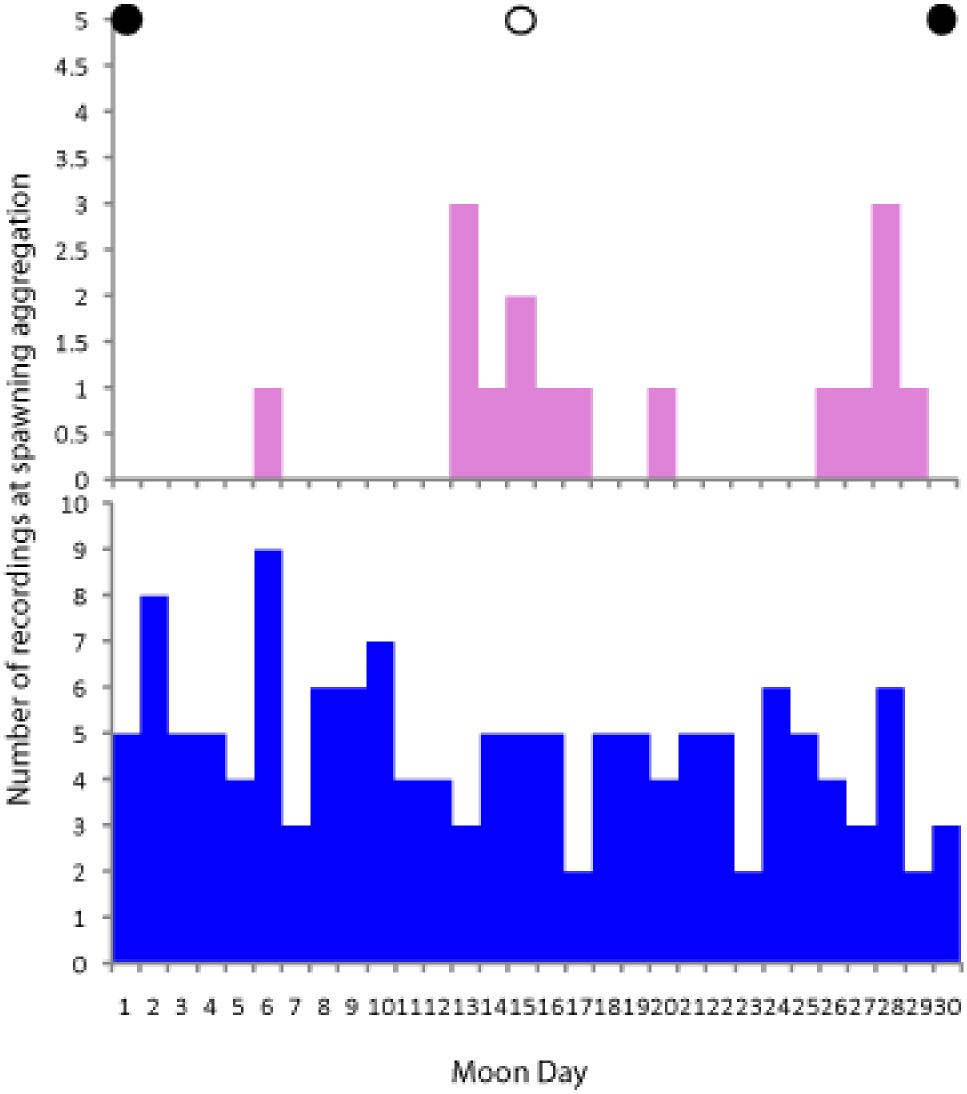

Figure 5.4 Spawning attendances (aggregated across fish and months) of *P. leopardus* over the spawning period by lunar day for a) fish (2-5) with a lower frequency of spawning aggregation attendances; b) fish (7 and 8) with a high frequency of spawning aggregation attendances. Moon phase shown (filled circle – new moon; open circle – full moon).

All fish which did not have a spawning aggregation station as its “preferred” station, moved to the aggregation in the mornings. Half of all arrivals occurred within the first two hours after sunrise, and 80% within five hours from sunrise (Figure 5.5a). However, fish that regularly frequented the spawning aggregation sites (fish 7 and 8) had a uni-modal departure time 13 hours after sunrise. In contrast the four female fish had bimodal departure times, firstly with a small mode at 4 hours after sunrise and then again with the major mode 9 hours after sunrise (Figure 5.5b).

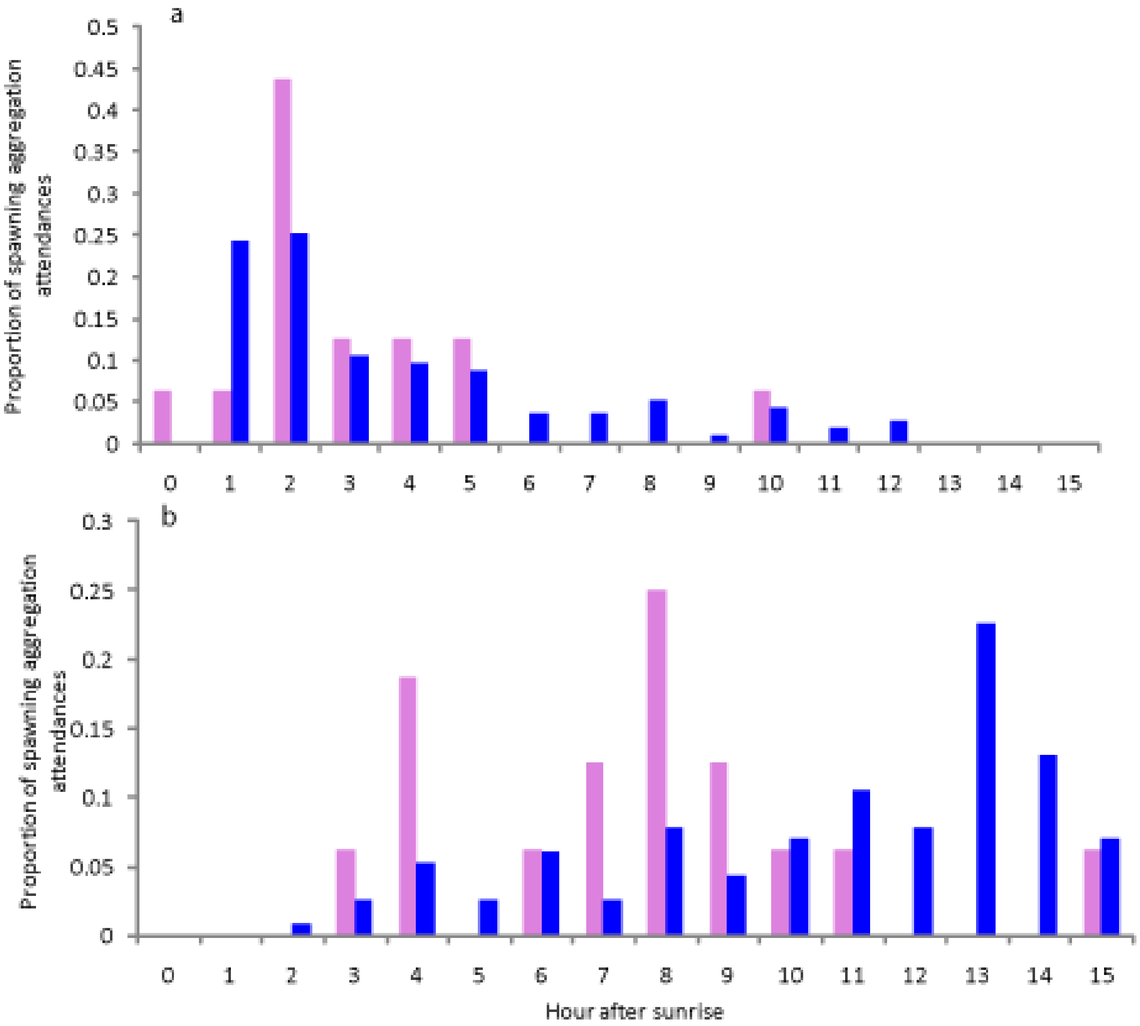

Figure 5.5 Proportion of a) total arrival and b) total departure times for P. leopardus at spawning aggregation sites standardised to hours after sunrise for fish (combined across all spawning months) with few (light grey) and many (dark grey) spawning attendances.

Abundance patterns at spawning aggregations

Based on UVC data, Kruskal-Wallis analysis showed that relative abundances of *P. leopardus* in a spawning aggregation differed among days (p=0.005) and times (p=0.016) over a 4-day period prior to and after a new moon (Figure 5.6). Abundances were significantly higher on 19th December than the 21st or 22nd December. Post hoc analysis showed no significant differences among times of the day. However, abundances peaked in the morning (08:00 hrs), during both the day before and the day of the new moon. The peak in the morning persisted throughout the survey, though the number of fish observed each morning was less than the previous morning (Figure 5.6). The peak mean abundance during the survey was 14.5 fish per $300m^2$, which equates to 96 per $1000m^2$. This pattern in abundances was primarily driven by changes in female abundance, with male and unknown sex individuals usually present throughout the survey in lower numbers (Figure 5.6).

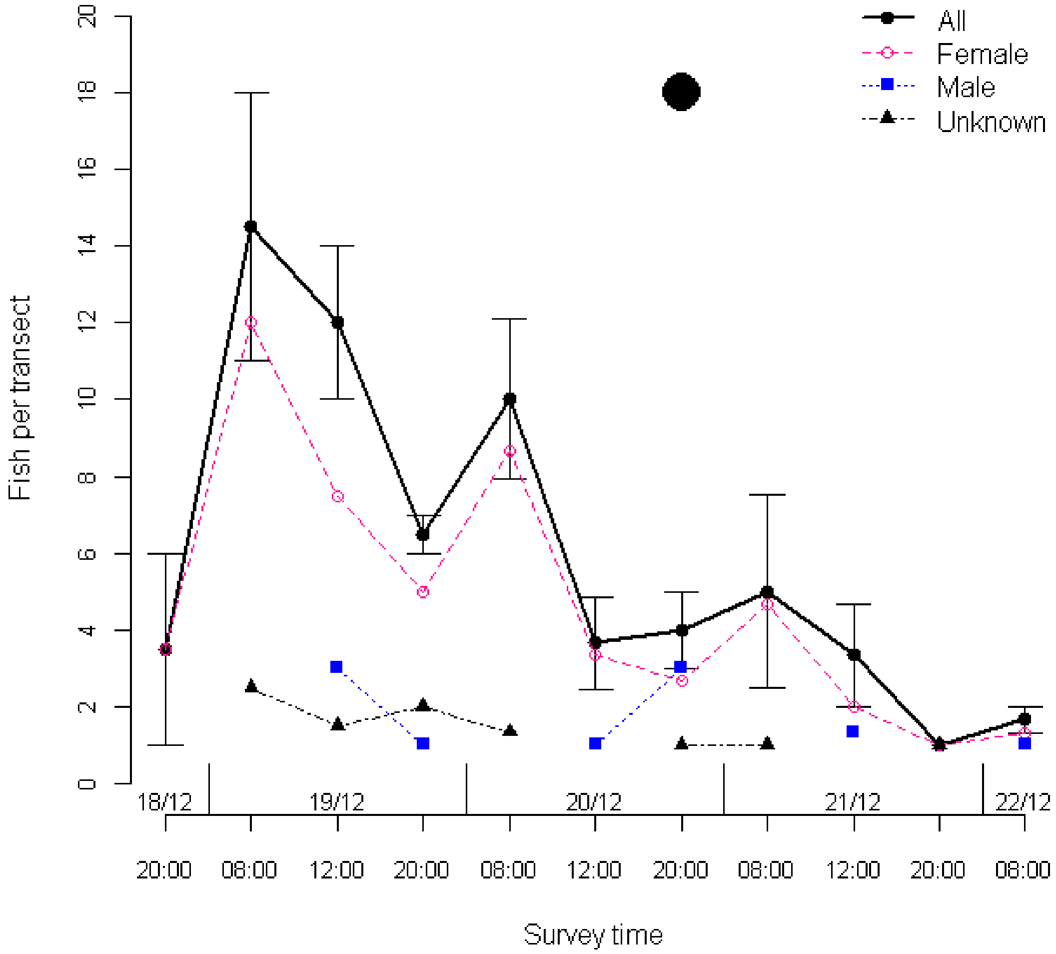

Figure 5.6 Mean relative abundance of all fish (± 1 SE) of *P. leopardus* for the 11 surveys conducted on a spawning aggregation site at the Abrolhos Islands (heavy line), as well as mean relative abundances by sex. New moon (large filled circle).

Fish lengths did not differ significantly among days (p=0.848), but did differ significantly among times (p=0.002), and there was a significant interaction between these factors (p=0.04). An examination of the length frequency distributions of each survey period demonstrated that this was due to a reduction in the numbers of smaller fish throughout the day, as opposed to an increase in the abundance of larger fish, which tended to be present at most survey times (Figure 5.7).

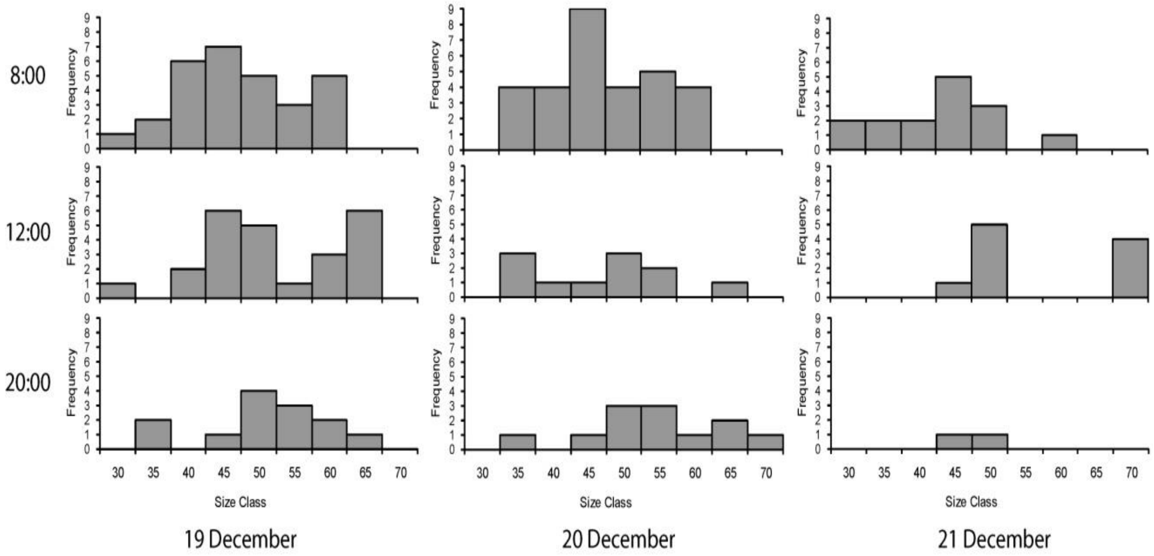

Figure 5.7 Size frequency classes of *P. leopardus* surveyed through the three full days of the study 19 – 21 December 2006.

Histological timing of spawning

Of the 27 histological sections of ovaries containing post-ovulatory follicles (POFs) that could be ascribed a collection time, 10 were classified as containing either very early (stage 1) or early (stage 2) POFs. They were collected in the afternoon, predominantly around 16:00 hrs (Figure 5.8). All remaining samples from these collection times or those collected in the morning had older stage POFs (predominantly stage 4). These samples came primarily from sites within or adjacent to the ROA at the Easter Group (Figure 5.1) at distances ranging from 1.3 to 6km from the focal (station 6) spawning aggregation site. POFs were also examined from sites from the other two major island groups which are up to 30km away from the focal spawning aggregation.

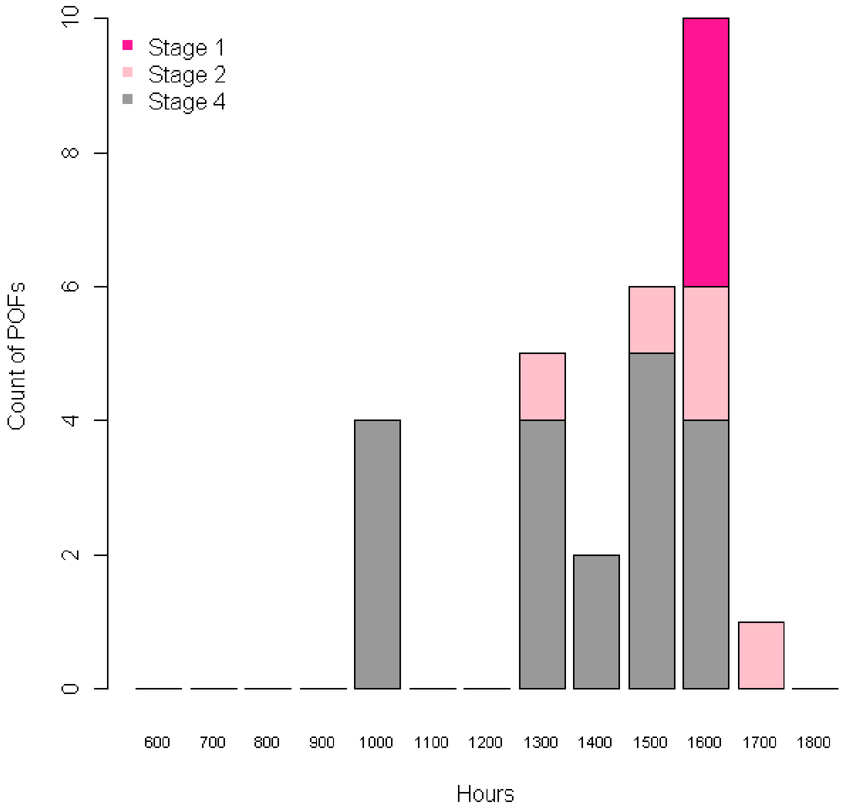

Figure 5.8 Frequency of post ovulatory follicles (POFs) of different stages (1,2 and4) collected throughout the day (years 2005-2009 combined).

5.5 Discussion

Despite the social and economic importance of *P. leopardus* throughout tropical Indo-Pacific region (Mapstone *et al.* 1996), our understanding of the spawning aggregation dynamics and movement patterns of this species is based solely on studies from the Great Barrier Reef (GBR) in the Pacific Ocean (Samoilys and Squire 1994; Samoilys 1997b; Zeller 1998). Through a range of approaches, the current study of *P. leopardus* in the Abrolhos Islands in the eastern Indian Ocean has demonstrated some noticeable differences, as well as similarities, in spawning aggregation dynamics to this species on the GBR (Samoilys 1997b), suggesting a need to gain data on the particular population being managed rather than relying on generalisations based on other populations.

Aggregation dynamics

Acoustic telemetry showed that all tagged fish visited one of the two spawning aggregation sites within the array at some stage during the spawning season, with some individuals regularly visiting these sites during the spawning season. Furthermore, UVC of spawning aggregations showed densities during the spawning season peaking at 96 1000m^2, which was larger than or similar to the 75 and 18 fish per 1000m^2 recorded by Samoilys (1997b) at two spawning aggregations on the GBR.

Spawning aggregations often form at certain lunar phases, generally at full or new moons (Rhodes and Sadovy 2002; Pet *et al.* 2005). Unlike *P. leopardus* in the GBR, where aggregations form only on the new moon (Samoilys 1997b), spawning aggregations at the Abrolhos Islands do not appear to be confined to a single lunar phase. This is similar to its con-generic *P. areolatus*, which also formed aggregation on the new and full moon phases at the Komodo Islands (Pet *et al.* 2005).

The two larger fish in the current study (fish 7 and 8), frequently attended the spawning aggregation being detected at either aggregation site on all days over a lunar cycle. In comparison, the four female fish (fish 2-5) were strongly associated with attendance at spawning aggregations during both the full, and just prior to new moon. The decline in the UVC abundances of fish, driven by a decline in female fish prior to the new moon is mirrored by the acoustic telemetry data, which showed the formation of the aggregation prior to the new moon, with no females detected at aggregations on the new moon. These results, coupled with the only direct observation of *P. leopardus* spawning rush at the Abrolhos Islands (D. Abdo pers. comm.), provides strong evidence that spawning aggregations of *P. leopardus* at the Abrolhos Islands form in the morning and dissipate before dusk. These findings contrast with other aggregation forming epinephelids (Colin 1992; Rhodes and Sadovy 2002) and many other species (review by Thresher 1984), where spawning occurs around dusk. Acoustic telemetry showed that there was no difference in the arrival time of fish presumed to be either female or male. Half of all fish were present at the aggregation within three hours of sunrise, and 80% within six hours of sunrise. UVC densities peaked in the mornings, with significantly more fish at 08:00 hrs (three hours after sunrise) than at 20:00 hrs (15 hours after sunrise and 1 hour after sunset). Telemetry detections revealed that females had few attendances at the spawning aggregation and their duration at the aggregation varied, but most left the spawning aggregation stations by early to mid-afternoon. A similar pattern was observed in the UVC data, where female fish were less abundant in evening surveys. The apparent early to mid-afternoon spawning is further supported by the histological data. Early (and very early) stage POFs were found exclusively in fish that were captured in early to late afternoon (1300 – 1730 hrs; mode= 1600 hrs). These early stage POFs were similar in appearance to POF stages in other fish which have been aged at between 0-6 hours (Hunter *et al.* 1986; Hesp *et al.* 2004), and they do not typically show major inter-species or degenerative differences (Hunter and Macewicz 1985). Thus, based on an average age of 3 hours for those POFs, spawning times would have occurred between 10:00 to 14:30 hrs. This concurs with peak density periods from UVC and greater detections using acoustical telemetry at the spawning aggregation. Similarly, the only direct observation of a *P. leopardus* spawning rush, which occurred at another spawning aggregation site, occurred in the early afternoon (D. Abdo pers. comm.). Since females are considered to be the main limiting factor for spawning output (Trippel 2003), our results suggest that egg output was greatest from mid-morning to mid-afternoon, rather than at dusk. This does not appear to vary throughout the Abrolhos Islands, with detailed UVC and acoustic data at the primary aggregation sites being mirrored by histological samples of females collected from a range of sites across the Abrolhos Islands. Histological samples from sites up to 30 km from the aggregation revealed similar patterns of midday spawning. The observation of a spawning rush also occurring in the early afternoon at an aggregation site 22.7 km away (D. Abdo pers. obs.) adds further weight that midday spawning may be ubiquitous throughout the archipelago.

The relatively infrequent attendances of females at the spawning aggregations concur with findings of other studies on epinephelid aggregations, where females make fewer visits to a spawning aggregation than males (Zeller 1998; Nemeth *et al.* 2007; Starr *et al.* 2007). The greater attendances of the larger (male) fish at spawning aggregations and their longer duration at the aggregation, lends further weight to the hypothesis that *P. leopardus* exhibits a lek type mating structure at spawning aggregations (Samoilys and Squire 1994). The greater time at the aggregation enables males to maintain territories, with observations confirming larger male fish patrolling territories with confrontations between males at territorial boundaries (How pers obs.).

While female attendances at spawning aggregations were relatively infrequent, both males and females were detected on the receiver at one of the two spawning aggregation sites. While acoustic data showed that some fish had a preferred nocturnal site that was adjacent to the spawning aggregation site, all tagged *P. leopardus* at the Abrolhos Islands moved to the spawning aggregation site nearest to their pre-spawning activity centre, indicating strong aggregation fidelity. This contrasts with other studies of leopard coralgrouper on the GBR, where spawning at aggregations were formed from 14-31% of the fish in the area (Zeller 1998, Samoilys 2000 in Samoilys 2012). The maximum number of attendances at a spawning aggregation by a female at the Abrolhos Islands, where the spawning receiver was not its preferred nocturnal receiver, was seven. This is considerably less than the 28 spawning events reported for females elsewhere (Samoilys 2000 in Samoilys 2012). This may indicate spawning occurs outside of the spawning aggregations as has been suggested previously for this species (Zeller 1998, Samoilys 2000 in Samoilys 2012). Though the number of spawning events for *P. leopardus* at the Abrolhos Islands is unknown, it is difficult to make definitive statements as to the degree of spawning outside the aggregations. Regardless, with all fish moving to an aggregation at some stage during the spawning season, it does make the management of spawning aggregation sites of great importance.

Movement Patterns

Plectropomus leopardus at the Abrolhos Islands demonstrated limited nocturnal activity and strong affinity for nocturnal shelter sites, based on a single station dominating the acoustic detections during the night for each of the eight fish detected acoustically during the 6-month study period. This concurs with results for the species in the GBR (Zeller 1997). Furthermore, *P. leopardus* at the Abrolhos Islands is relatively sedentary, with movements along fringing reef slopes limited to distances <3,000m during the study. These reef slope distances are considerably larger than those linear dimensions of home ranges estimated for *P. leopardus* on the GBR, which averaged 223m, with a maximum of 377m (Zeller 1997). While reef slope distances utilised in this study can be curved along the reef slope, for half of the fish detected at the Abrolhos Islands, the reef slope distance would equate to the maximum linear dimension as defined by Zeller (1997). While home range estimates were not possible in this study (see Methods: Analysis), *P. leopardus* are relatively sedentary, remaining within the minimum 50 x 30m block size (Zeller *et al.* 2003) or 2–2.5km long section of fringing reef (Davies 1996). The larger linear distances in the current study suggest that the home range of *P. leopardus* is larger at the Abrolhos Islands. The significant relationship between fish size (equating to a shift from female to male) and reef-slope distance moved in the pre-spawning period, suggests that males exhibit greater movements, which concurs with provisional observations by Samoilys (1997a).

Movements to spawning aggregations (catchment distance) from their nocturnal shelter sites were generally smaller for *P. leopardus* at the Abrolhos Islands than in the GBR. Some serranids have shown considerable migration (100s km) to spawning aggregations (Colin 1992; Luckhurst 1998; Bolden 2000; Nemeth *et al.* 2007), principally because some individuals move to more distant spawning aggregations despite other aggregations being closer to their pre-spawning home ranges (Zeller 1998; Bolden 2000). The average one-way movement (aggregation “catchment” distance) of *P. leopardus* was 569 m at the Abrolhos Islands. This corresponds to around two thirds the catchment distances recorded by Zeller (1998) on the GBR. The largest catchment distance recorded by Zeller (1998) was 5.21 km. If this extreme value is removed, the mean catchment distance of *P. leopardus* on the GBR is within 16m of that at the Abrolhos Islands. Similarly while catchment distances of *P. areolatus* in Pohnpei, Micronesia averaged 5.28 km for six acoustically tracked fish (Hutchinson and Rhodes 2010), the majority of catchment distances (n=4) in that study were 0.02-1.44 km, which was similar to *P. leopardus* at the Abrolhos Islands. Therefore, while large movements of >5 km to spawning aggregations do occur, most of the movements of *P. leopardus* and *P. areolatus* are about 500m, indicating a generally small catchment distance, a view shared by Rhodes and Tupper (2008).

Conclusions

The spawning aggregation dynamics and movement of *P. leopardus* at the Abrolhos Islands differed in a number of ways to its con-specifics on the GBR and other teleosts that aggregate to spawn. Spawning aggregations of *P. leopardus* at the Abrolhos Islands appear to form in the morning, with smaller female fish departing earlier than larger male fish. While no spawning rushes were observed at the studied spawning aggregation sites, the evidence from three independent techniques, UVC, histology and acoustic telemetry, supports mid-morning to mid-afternoon spawning of this population. This contrasts markedly with the general trend of dusk spawning for *P. leopardus* on the GBR (Samoilys and Squire 1994; Samoilys 1997b) and a number of other aggregate spawners (Thresher 1984). While the cause of such a deviation from the paradigm is unknown, it does highlight the need to understand region specific aggregation dynamics of a species.

The concentration of spawning stock at predictable locations, and their increased susceptibility to exploitation, makes the protection of spawning aggregations a potentially powerful tool for the sustainable management of the species. This has been utilised by both traditional (Johannes *et al.* 1999) and government managers (DEEDI 2010), where closures have been implemented during the spawning period to reduce exploitation of fish in aggregations. An understanding of the aggregation dynamics, catchment distances and location of spawning aggregations is vital information for temporal and spatial management of fish stocks. The approach in this study has shown that the small localised movements of *P. leopardus* to spawning aggregations in the Abrolhos Islands makes marine protected areas a powerful tool for the protection of spawning stocks for this species, as well as other species displaying similar movements.

Acoustically tagged Coronation Trout released at Ningaloo

Chapter VI

Movement patterns of six epinephelid species at Ningaloo Reef: implications for spatial closure management

6.1 Abstract

Movement estimates of exploited fish species are a critical consideration in the design or evaluation of marine protected areas (MPAs). However, these assessments are rarely conducted, and when undertaken, are typically for a single species, despite many reef fisheries targeting multiple species. This chapter examined the movement patterns of six species of the Epinephelidae, a globally exploited reef-fish family, to determine if there are species-specific differences in movement patterns and home ranges. Twenty fish were tracked simultaneously using a passive acoustic array for up to two years in or near to a sanctuary (no-take) zone in Ningaloo Marine Park in north-western Australia, providing a robust assessment of movement patterns. All species tracked were sedentary, with home ranges of approximately <1.7km^2 and demonstrating limited movement between different habitats, Most species, with the exception of *Epinephelus rivulatus*, were primarily associated with the reef slope or deeper offshore waters. By contrast, *E. rivulatus* was solely found within the lagoon. The reef slope was broken by a channel, and despite individuals being tagged on either side of this channel, no fish was observed crossing it.

Such information is vital to effectively design MPAs for the adequate protection of reef fishes by providing home range estimates and habitat utilisation to establish appropriate MPA size and natural barriers to determine the location of boundaries.

6.2 Introduction

Marine protected areas (MPAs) are often used as a management tool for the protection of exploited fish species (Halpern 2003). Significant increases in abundance and biomass of fish species in marine reserves compared to adjacent fished areas have been shown in numerous studies (see review Halpern 2003) and provide support for MPAs as a fisheries management tool. Effective management using MPAs requires an understanding of the spatial dynamics of the species it is protecting, including the area required, the habitats to be included, and the location of marine reserve boundaries, to utilise potential natural barriers to movement such as open un-vegetated channels (Sheaves 1993, Lowe *et al.* 2003, Popple and Hunte 2005). Previous studies have demonstrated significant differences in home range estimates of the same species at the same location occupying different habitats, which is driven primarily by the shape of the home range (Zeller 1997). Whilst showing similar home range sizes, *Kyphosus sectatrix* demonstrated markedly different home range shapes, depending on available reef shape resulting in differential movement rates across a marine reserve boundary, located along contiguous reef habitat (Eristhee and Oxenford 2001). Movements across natural boundaries can also have major influences on the effectiveness of the marine reserve (Topping *et al.* 2005), with reef fish generally showing sedentary movement behaviour, and reluctance to cross sand channels to adjacent reefs (Barrett 1995, Chapman and Kramer 2000).

The degree of protection afforded by a marine reserve is dependent on the proportion of time spent by an individual within the reserve (Kramer and Chapman 1999). Therefore, ultimately the area of the reserve in relation to the area of the exploited species' home range will largely dictate the reserve's effectiveness for that given species. This has been postulated to be an order of magnitude greater than the daily movements of the widest ranging individual tracked (Barrett 1995). This home range information is critical to ensure the size and shape of MPAs are adequate for fisheries management.

Finfish fisheries in coral-reef systems are often multi-species fisheries, targeting groups of species as opposed a single species (eg. Reef Line fishery on the Great Barrier Reef: Welch *et al.* 2008). As a result, marine reserves designed as a tool to manage such fisheries require information on the suite of species that are being targeted. One such highly exploited fin-fish group is the family Epinephelidae, which has worldwide fisheries importance (Morris *et al.* 2000). The iconic status of many species within this family has resulted in considerable focus on a number of aspects of its biology and ecology (Colin 1992; Coleman *et al.* 1996, Zeller 1997, Fennessy and Sadovy 2002, Pear *et al.* 2007). There have been numerous studies on the movement patterns of members of this family, however, they have typically been single species assessments with tracking occurring generally for less than one year (Table 6.1; Zeller 1997, Lembo *et al.* 2002, Kaunda-Arara and Rose 2004b, March *et al.* 2010). The conclusions on the family's mobility have generally shown them to be sedentary, however, some species can make considerable migrations. The Nassua grouper (*Epinephelus striatus*) has a home range of around 0.02 km^2 (Bolden 2001), though during the spawning season they may undertake a migration over 100s of kilometres to spawning aggregation sites (Colin 1992, Bolden

2000). Therefore, to adequately understand the spatial utilisation of a species encompassing all of it movements, a long-term tracking study is necessary.

There have been few, if any, long term tracking studies on multiple species (Table 6.1), with none examining a suite of species from a family simultaneously. In this study, the movement patterns of six Epinephelidae species have been assessed over two years inside and adjacent to a closed area in a coral-reef system in north-western Australia, with the aim of establishing generalised movement patterns, home ranges, habitat use and natural barriers to movement of this exploited teleost family.

Table 6.1 Details of reviewed passive acoustic tracking papers with number of species, individuals, receivers in the array and duration of the study. * denotes duration of longest tracked individual where study duration couldn't be determined. + denotes Invertebrate families

Species Name	Family	No. Spp	No. Individs	No. Rec	Duration days	Authors
Tautoga onitis	Labridae	1	27	8	186*	Arendt *et al.* 2001
Gadus morhua	Gadidae	1	126	69	365	Comeau *et al.* 2002
Epinephelus marginatus	Epinephelidae	1	7	5	40	Lembo *et al.* 2002
Paralabrax clathratus	Serranidae	1	4	6	156*	Lowe *et al.* 2003
Pagrus auratus	Sparidae	1	18	9	365	Egli & Babcock 2004
Carcharhinus limbatus	Carcharhinidae	1	33	74	480	Heupel *et al.* 2004
Salmo salar & Salmo trutta	Salmonidae	2	40	12	24*	Finstad *et al.* 2005
Caranx melampygus	Carangidae	1	5	27	173	Meyer & Honebrink 2005
Ocyurus chrysurus & Mycteroperca bonaci	Lutjanidae & Epinephelidae	2	14	5	237*	Lindholm *et al.* 2005
Carcharhinus limbatus	Carcharhinidae	1	108	25	720	Heupel & Simpfendorfer 2005
Albula vulpes	Albulidae	1	11	8	61*	Humston *et al.* 2005
Semicossyphus pulcher	Labridae	1	16	6	840	Topping *et al.* 2006
Salmo salar	Salmonidae	1	273	103	365	Whoriskey *et al.* 2006
Sepioteuthis australis	Loliginidae +	1	46	83	129	Pecl *et al.* 2006
Sphyrna tiburo	Sphyrnidae	1	36	49	480	Heupel *et al.* 2006
Gadus morhua	Gadidae	1	10	22	500	Brooking *et al.* 2006
Pogonias cromis	Sciaenidae	1	34	12	365	George 2007
Cheilinus undulatus	Labridae	1	1	4	210	Chateau & Wantiez. 2007
Epinephelus striatus	Epinephelidae	1	45	12	1460	Starr *et al.* 2007
Choerodon schoenleinii	Labridae	1	9	9	120	Kawabata *et al.* 2007
Panulirus cygnus	Palinuridae +	1	34	20	180	MacArthur *et al.* 2008
Carcharhinus leucas	Carcharhinidae	1	56	20	460	Heupel & Simpfendorfer 2008
Epinephelus marginatus	Epinephelidae	1	6	5	365	Pastor *et al.* 2009
Pseudocaranx dentex	Carangidae	1	32	15	1167	Afonso *et al.* 2009
Serranus scriba	Serranidae	1	15	25	240	March *et al.* 2010
Pagrus auratus	Sparidae	1	39	30	150	Parsons *et al.* 2010
Zebrasoma flavescens	Acanthuridae	1	53	11	180	Claisse *et al.* 2011

6.3 Methods

Study site and array design

Ningaloo Reef is the largest fringing reef in Australia, located off the North West Cape of Western Australia, and declared a marine park in 1987 (Anon. 2005). In 2005, increased zoning resulted in 34% of the park being protected within sanctuary (no-take) zones (Anon. 2005). One of these sanctuary zones, Mangrove Bay, was the focus of this tracking study.

A total of 58 receiver stations, utilising Vemco ™ VR2W and VR3 receivers were located within and adjacent to the Mangrove Bay sanctuary zone (Figure 6.1). They were established to assess movement patterns of a range of teleost and elasmobranch taxa across reserve boundaries, reef channels and deep offshore areas. The array was established in February 2006, with additional receivers located on the reef slope and reef channel in May 2007. Data from the stations were downloaded approximately every six months, with battery replacement occurring annually. The final download occurred in May 2010.

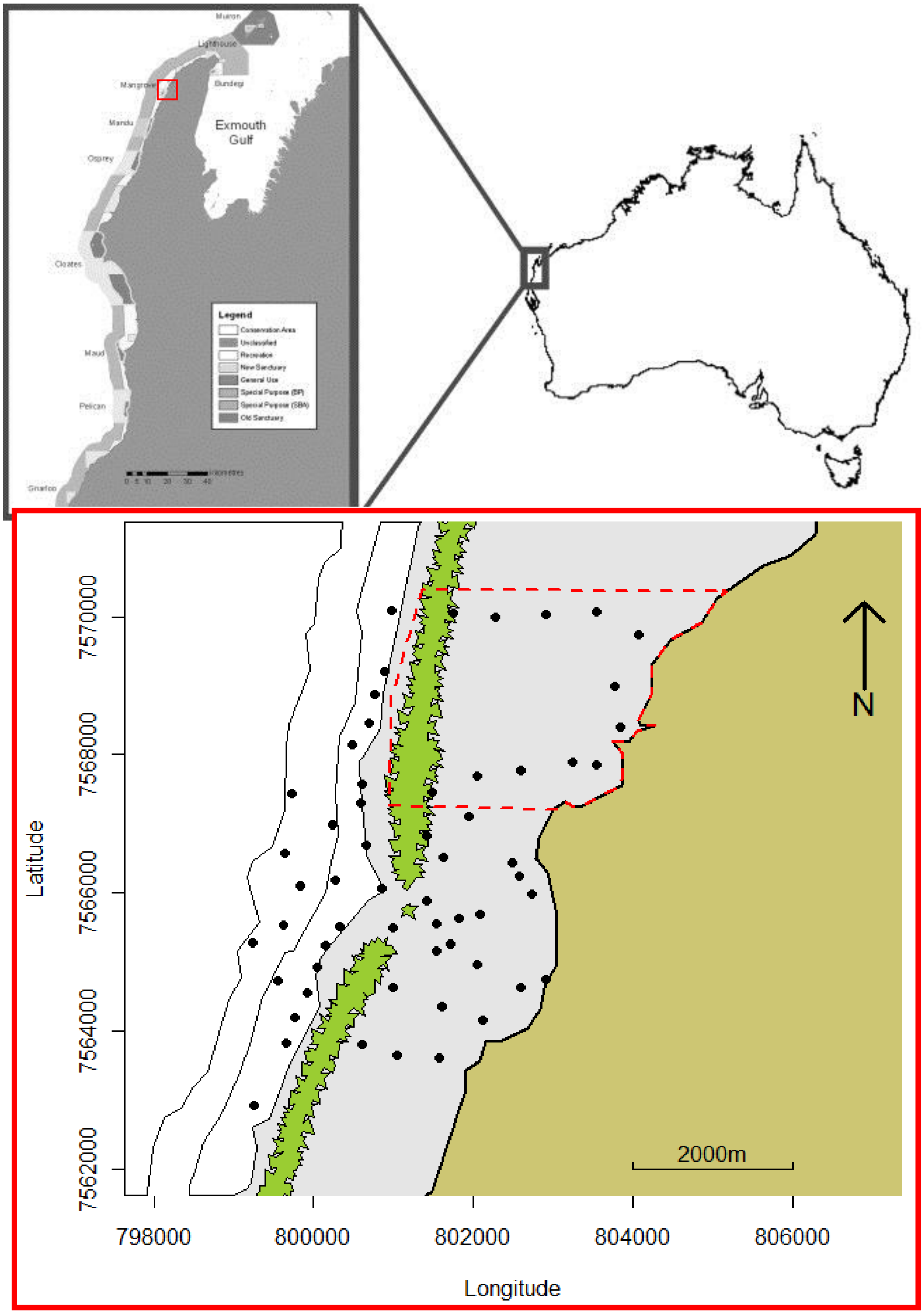

Figure 6.1 [above] Location of Ningaloo Marine Park (adapted from Bochetti *et al.* 2010) and showing location on Mangrove Bay (red square) [below] Array configuration showing receivers (closed circles), mainland (khaki), reef line (green), sanctuary zone boundary (red dashed line), 0-5m (light grey), 20m and 50m isobaths (lines)

Fish capture and surgery

Epinephelids were captured predominantly on the reef slope and offshore areas, with the exception of *E. rivulatus*, which was captured within the lagoon. Fish were caught on hook and line, using heavy gear to reduce capture stress, and brought on-board a research vessel. When fishing in deep water (>20m), effort was made to bring fish to the surface slowly to reduce potential barotrauma related injury or mortality. Once on board, fish were anaesthetised with clove oil, and once sedated, placed upside down in a v-shaped cradle. Surgical techniques followed that of Chapter 5, however, wounds were closed with absorbable sutures. A total of 30 epinephelids including individuals from 6 species (Table 6.2) were captured and surgically implanted with either V16-4H, V16-5H or V16-6L transmitters depending on body size. Transmission rates permitted battery life to exceed at least 1 year, with projected transmitter life up to 3 years.

Analysis

All data were analysed using R (R Development Core Team 2009), with spatial analysis using the package adehabitat (Calenge 2006). All data presented are with “ghost” detections (n=29) removed. Ghost detections occur when detections are recorded for an individual within an array at a time or location where it is unlikely that the animal occurred, which could be due to multiple acoustic signals from either multiple acoustic transmitters or collisions of biologically derived acoustic pulses causing the detection of an otherwise invalid record. “Ghost” detections were identified as a single detection, outside the area of other detections or capture location for the particular individual, or at times considerably after the time of the previous valid detection.

The initial design of the array was based on a number of adjacent receivers having abutting detection zones, which provided a good coverage throughout the array. However, there is considerable temporal variation in the detection range of acoustic receivers due to a range of biotic and abiotic factors (Appendix A). This resulted in the occurrence of acoustic “holes” within the array of varying extent throughout the study. As such, position estimates of individuals were restricted to the location of the receiver on which they were detected for analysis purposes.

Utilisation distribution (UD) kernels with the “ad hoc” smoothing factor (Calenge 2006) generally resulted in a number of separate polygons around the receivers where an individual was detected. Biologically, this is unlikely and is an artefact of using the location of the receiver, when the fish may be up to 400m from the receiver. Adjustments were, therefore, made to the smoothing factor to ensure that the 95% UD kernel formed a single polygon. No additional adjustments were used when the smoothing factor generated by the ad hoc method fitted these criteria. This enables estimates of core areas (50% UD) and broader home ranges (95% UD).

Inter-species comparison of home range was done through a Kruskal-Wallis rank sum test due to a lack of normality. Relationships of home range and size (L_F) were assessed through linear regression.

Despite being detected 94 times on 13 receivers over a nine-day period (Table 6.2), ET6 (*E. tauvina*) was removed from all subsequent analyses. This movement pattern contrasted with that of other members of this species and family that were tracked. Furthermore, the short retention time is similar to that found for a fish which may have been acoustically tagged through voluntary gastric insertion (Winger *et al.* 2002, How unpublished data), where transmitters are retained for around 1-2 weeks. Therefore, the more vagile movement pattern and tracking period for this fish was similar to that of a transmitter inserted through gastric ingestion and suggests that this movement may relate to that of a predator, rather than the fish

6.4 Results

Seven fish were not detected after release, and another seven were detected for less than a week after release. The 51,041 detections recorded during the study came from the remaining 16 fish (Table 6.2). These fish were detected for up to 2 years (716 days; *E. multinotatus* EM2), with eight fish detected over a period in excess of a year, and further five detected for over three months after they were released (Table 6.2). The study has therefore provided good long-term movement information for epinephelid species.

Table 6.2 Size (fork length; L_F), release and detection details of tagged epinephelids released at Mangrove Bay, Ningaloo.

Spp	L_F	Release	Last Detect	Count	Receivers	Days detected	Liberty	Detect rate (%)
Epinephelus multinotatus								
EM1	70.5	3/06/2008		0	0	0	0	0
EM2	77	27/05/2008	12/05/2010	14032	9	666	716	93.02
EM3	57	28/05/2008	28/05/2008	2	1	1	1	100
EM4	47.5	28/05/2008	30/05/2008	6	1	3	3	100
EM5	64.5	26/05/2008	10/01/2010	358	8	21	595	3.53
EM6	35	21/10/2008	14/05/2010	11710	8	556	571	97.37
EM7	52.5	21/10/2008		0	0	0	0	0
Epinephelus rivulatus								
ER1	28.5	14/12/2007	12/06/2008	115	1	19	182	10.44
ER2	34	1/12/2007	2/01/2008	1	1	1	33	3.03
ER3	34	23/01/2009	24/02/2009	1689	2	33	33	100
ER4	37	24/01/2009	25/06/2009	2	1	2	153	1.31
ER5	28	24/01/2009	31/10/2009	88	1	47	281	16.73
Epinephelus tauvina								
ET1	56	25/05/2008		0	0	0	0	0
ET2	53	30/05/2008	15/06/2008	334	2	11	17	64.71
ET3	48	19/10/2008	8/05/2010	1255	1	250	567	44.09
ET4	49	17/10/2008	4/12/2008	107	5	6	49	12.24
ET5	51			0	0	0	0	0
ET6	52	31/05/2008	8/06/2008	94	13	8	9	88.89
ET7	48	28/05/2008	22/07/2008	215	4	19	56	33.93
Plectropomus leopardus								
PL1	72	28/05/2008	15/02/2009	10429	9	261	264	98.86
Plectropomus maculates								
PM1	60	29/05/2008		0	0	0	0	0
Variola louti								
VL1	36	28/05/2008	3/06/2008	12	3	5	7	71.43
VL2	48	30/05/2008	1/02/2010	11	2	10	613	1.63
VL3	61	30/05/2008	21/02/2009	310	3	154	268	57.46
VL4	38	29/05/2008	14/11/2009	1717	3	377	535	70.47
VL5	50	30/05/2008	3/06/2008	6	2	3	5	60
VL6	48	29/05/2008	1/02/2010	235	2	132	614	21.5
VL7	53	29/05/2008		0	0	0	0	0
VL8	49	2/06/2008	21/01/2010	8166	1	144	599	24.04
VL9	57	18/10/2008	24/10/2008	147	6	7	7	100

Chinaman Cod (*Epinephelus rivulatus*)

Three of the four *E. rivulatus* detected multiple times (ER1, ER4 and ER5) were detected on only one receiver (Table 6.2; Figure 6.2), with the dominant receiver for all four fish being the one closest to the point of capture and release. All four fish were detected within the lagoonal part of the array (Figure 6.2). Only ER3 was detected on two receivers, with the receiver further from the point of capture only having 2 detections at the end of its detection period.

The time at liberty for *E. rivulatus* was considerable, ranging from one to nine months (Table 6.2). Despite this, the number of detections for each fish was low, resulting in an intermittent detection profile throughout the tracking period. Two *E. rivulatus*, ER4 and ER5 had a significant period of time from capture to first detection (151 and 128 days, respectively). The notable exception to this was ER3, which was detected on every day for the 33 days it was at liberty. Estimates of home range were not possible as all *E. rivulatus* were detected on two or less receivers.

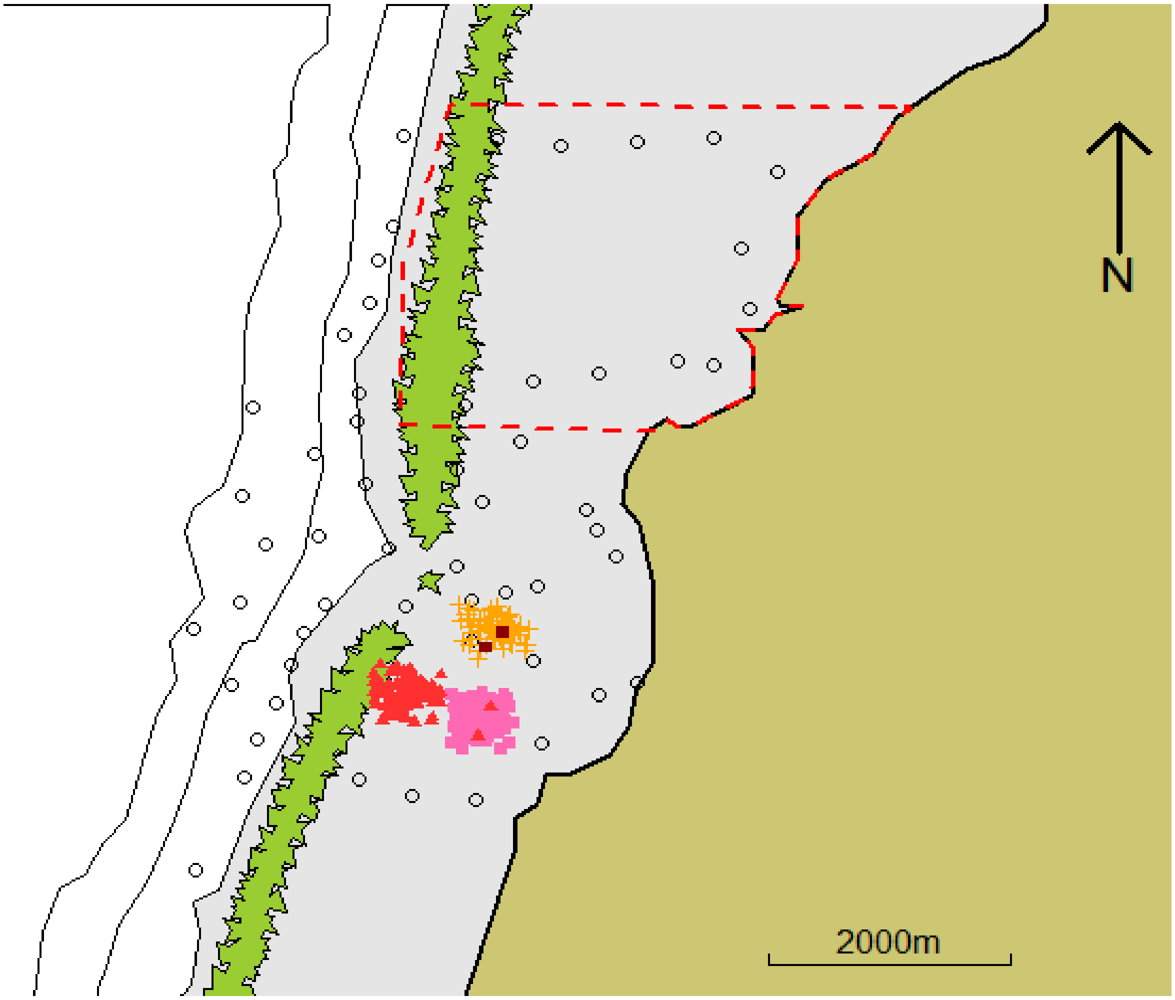

Figure 6.2 Location of detections for the four *E. rivulatus*: ER1 (pink square), ER5 (orange +), fish ER4 (brown square), ER3 (red triangle). Locations are for illustrative purposes only and generated by a randomised distance from the receiver. Other symbols as per Figure 6.1.

Rankin Cod (*Epinephelus multinotatus*)

Of the seven *E. multinotatus* that were tagged, two were not detected (EM1 and EM7), and a further two (EM3 and EM4) were detected only on one receiver and <10 times for no more than three days (Table 6.2). The remaining three *E. multinotatus* had over 350 detections on 8-9 receivers, with two detected over 14,000 and 11,700 times for EM2 and EM6, respectively, providing robust home range estimates. Despite their considerable size difference of 77 and 35 cm fork length (L_F), both fish displayed similar home range estimates of 0.19 and 0.23 km^2 for the core area (50% kernel) and 1.22 and 1.40 km^2 for the 95% kernel area, respectively (Figure 6.3). Both fish remained exclusively on the south side of the channel opening, moving around the reef slope and associated offshore areas. The smallest *E. multinotatus* (EM6) had a core movement area that was split over both deep and shallow water (Figure 6.3). Contrasting this, the largest *E. multinotatus* (EM2), had a core area focused in deeper water despite being detected on the reef slope (Figure 6.3).

The third *E. multinotatus* (EM5) showed a distinctly different detection and movement pattern. During an initial 10-day period in late May 2008, it made a number of movements to and from the reef slope and lagoon (Figure 6.3). This was reflected in its home range, which spanned both reef slope and lagoon, with core areas in both habitats. On each occasion, the fish moved to and from the southern reef slope and southern lagoon without crossing the channel. In May 2009, almost a year after it was last detected, there was a series of single detections on an offshore receiver, with more detections six months later in January 2010. These detections were at a receiver adjacent to the point of capture and release.

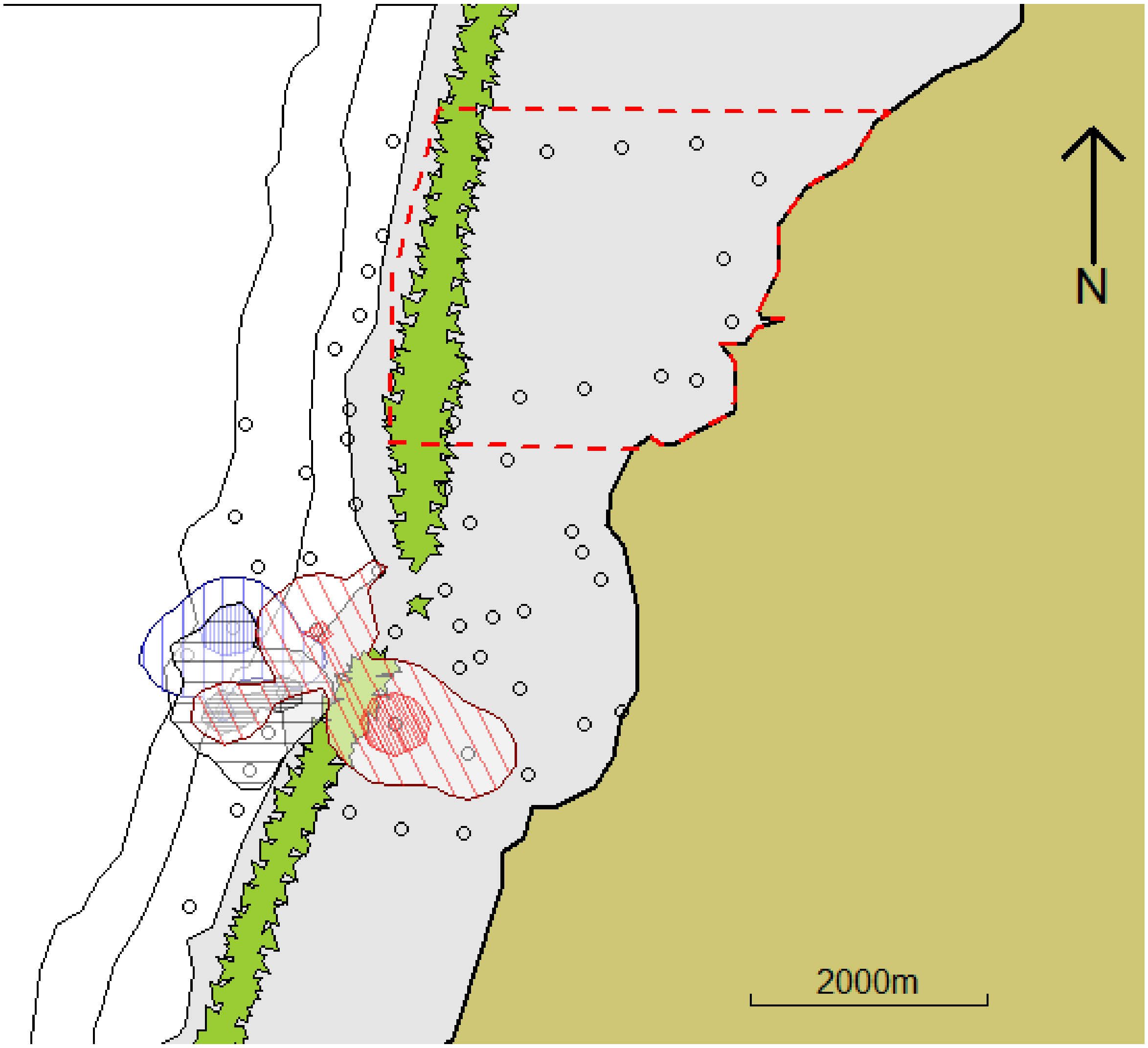

Figure 6.3 Core area (50% kernel; heavy shading) and home ranges (95% kernel; light shading) for three *E. multinotatus*, EM5 (red), EM6 (blue) and EM2 (black). Other symbols as per Figure 6.1.

Reef Cod (*Epinephelus tauvina*)

Five of the seven tagged *E. tauvina* (ET2, ET3, ET4, ET6, ET7) were detected 94-1,255 times, with period at liberty exceeding 18 months (Table 6.2). This species was detected in a diverse range of habitats encompassing the reef slope, offshore reefs and channel opening. Four of these five fish exhibited little movement. Two individuals (ET2; ET3) were detected on only one or two receivers, with the ET3 detected over 1,255 times over 567 days on one receiver and ET2 detected over 300 times on two receivers either side of its capture location (Figure 5.4).

Kernel estimates were possible for two fish (ET7 and ET4), which were detected on 4-5 receivers. These fish yielded similar core area estimates (50% kernel) of 0.38 and 0.20 km^2 and home ranges (95% kernel estimates) of 1.80 and 1.42 km^2, respectively (Figure 6.4). However, the habitats occupied by these two fish differed, with ET7 detected predominantly on two offshore receivers at around 30m depth, and with several detections on the reef slope. In contrast, ET4 was detected around the mouth of the channel opening, and a few surrounding receivers. There were a few detections on a receiver located on the reef slope and directly outside the channel opening, with no detected movement up either side of the channel (Figure 6.4).

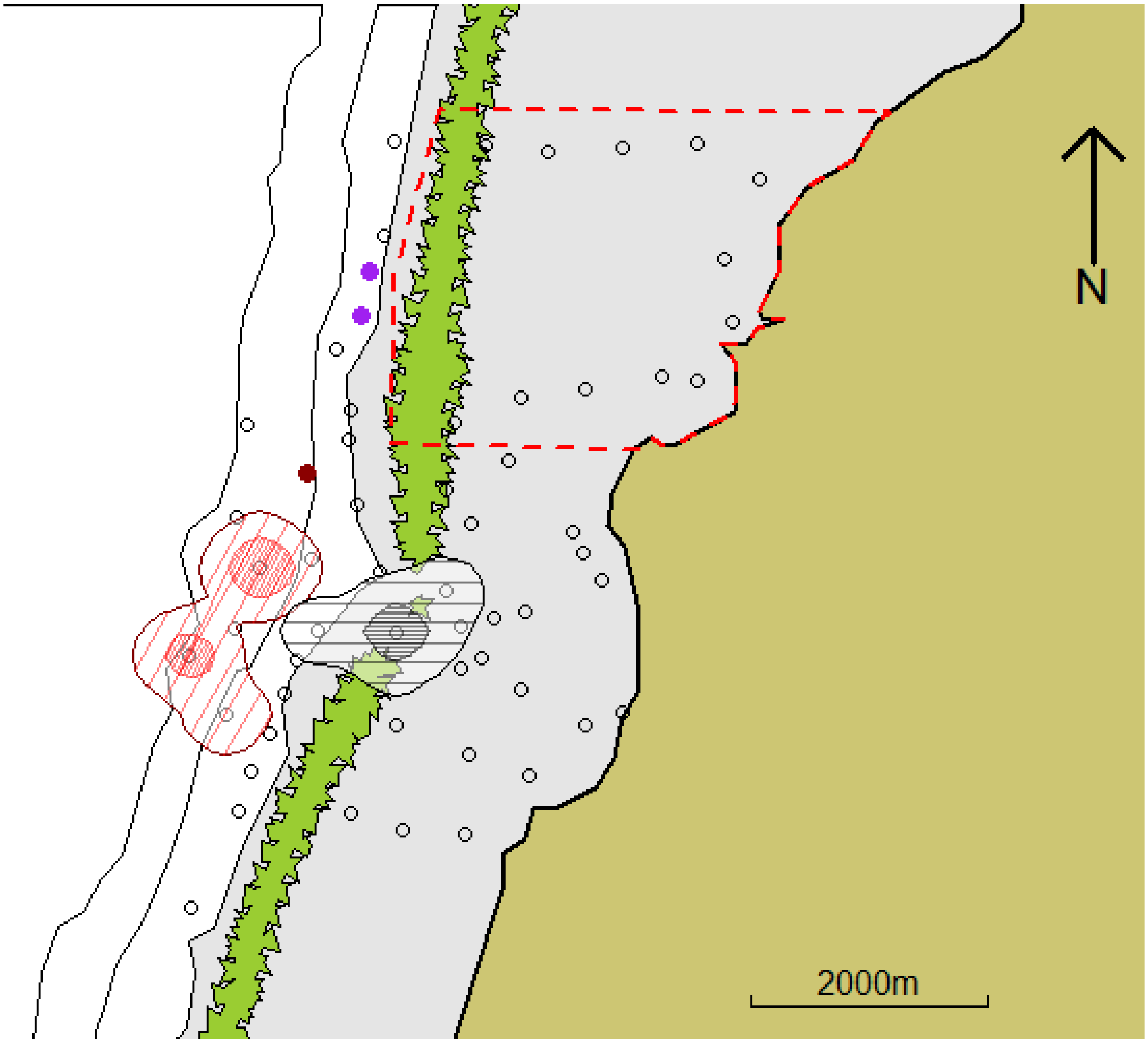

Figure 6.4 Core area (50% kernel; heavy shading) and home ranges (95% kernel; light shading) for two *E. tauvina* ET4 (black) and ET7 (red), detected on multiple receivers. Filled symbols (ET2-brown[1 receiver]; ET3-purple[two receivers]) for fish that were detected on less than 3 receivers. Other symbols as per Figure 6.1.

Leopard Coralgrouper (*Plectropomus leopardus*)

Only one *P. leopardus* (PL1) was tagged but was detected on all but three of the 264 days it was at liberty, yielding a total of 10,429 detections (Table 6.2). Detections were concentrated on the reef slope and around the mouth of the reef channel. Detections were mainly on two receivers within the 50% kernel (0.3 km^2), one in shallow water and the other in deeper offshore water (Figure 6.5). There was no obvious shift in the area being utilised within this region through the time at liberty. The total home range of the *P. leopardus* was 1.52 km^2.

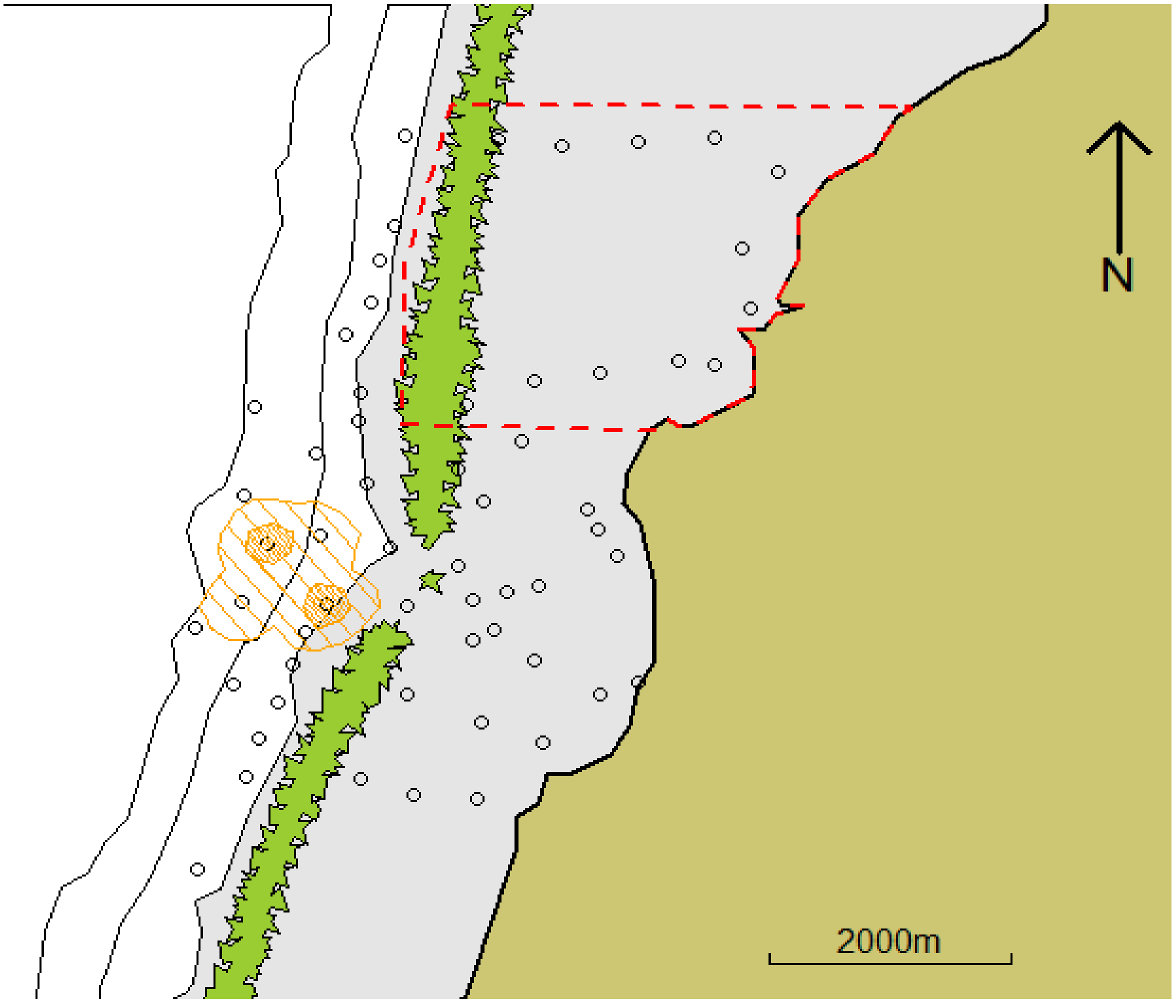

Figure 6.5 Core area (50% kernel; heavy shading) and home range (95% kernel; light shading) of tagged *P.leopardus*. Other symbols as per Figure 6.1.

Coronation Trout (*Variola louti*)

Of the nine *V. louti* tagged, all but VL7 were detected for at least three days, and four of these were detected for more than 100 days (Table 6.2). VL8 was detected more than 8,000 times whilst at liberty for 600 days on a single receiver (Table 6.2). The only epinephelid of the six species tracked that was detected by receivers on both sides of the reef channel was a VL9 (Figure 6.6). It was detected on a receiver on the northern edge of the channel, but the majority of detections occurred south of the channel on the reef slope (Figure 6.6). As there were no detections further along the northern reef slope, these detections were likely the result of VL9 being in the mouth of the channel as opposed to crossing it.

The size of the core areas and home ranges of this species were considerably more variable than the other members of the family. Core areas ranged from 0.17 to 0.64 km^2, with overall home ranges varying between 0.77 and 3.43 km^2. There was no significant relationship with size and the 95% kernel home range (p=0.46), with two small individuals, VL1 (36 cm L_F) and VL4 (38 cm L_F), having home ranges of 1.24 and 3.43 km^2, respectively. The smallest fish's (VL1) home range was similar to that of a 57cm TL fish (VL9; 1.67 km^2). Contrasting this was the largest *V. louti* tracked (VL3; 61cm L_F), which had the smallest home range (0.77 km^2) of the four fish.

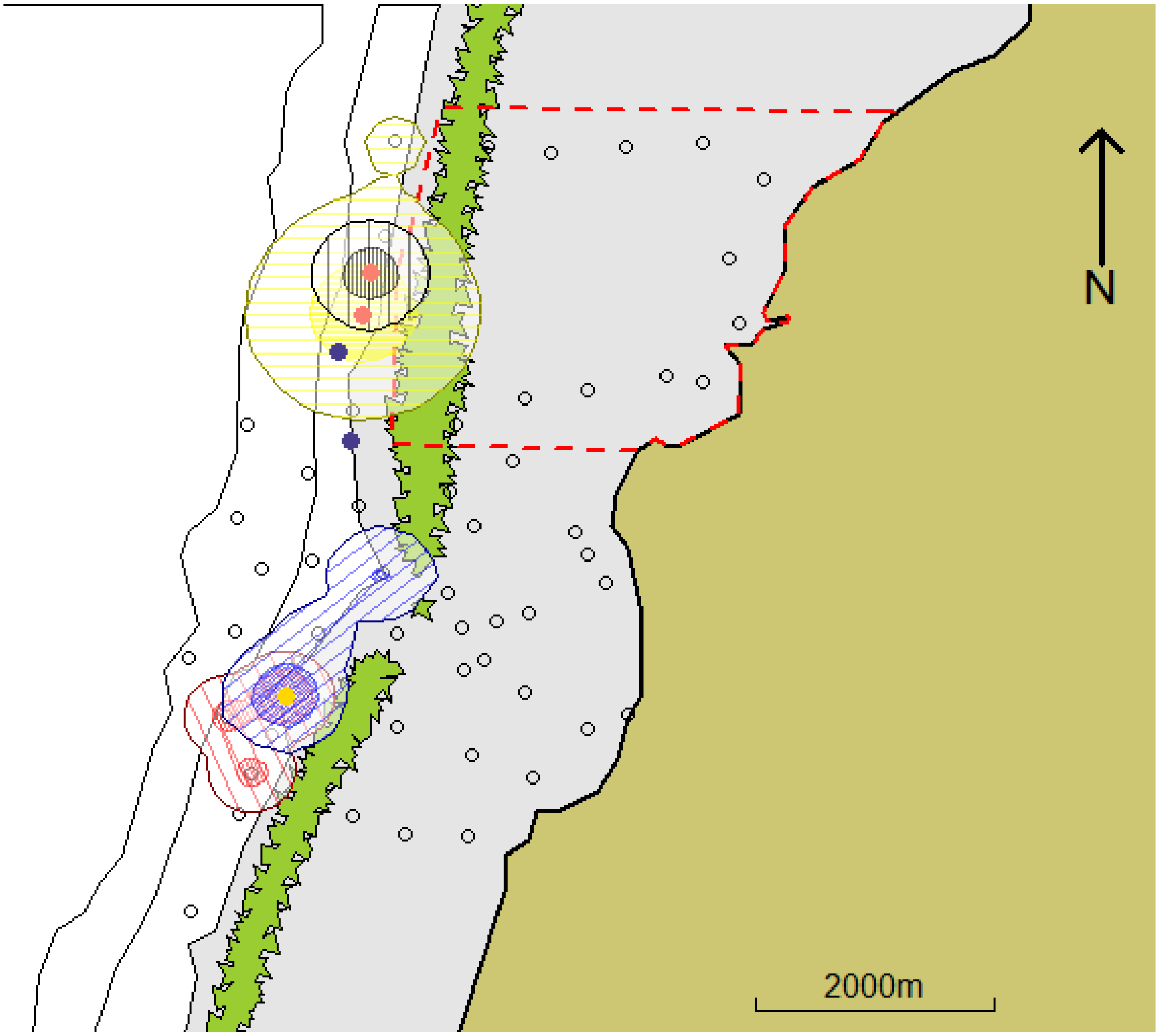

Figure 6.6 Core area (50% kernel; heavy shading) and home ranges (95% kernel; light shading) for four *V.louti*, (VL1-red; VL3-black; VL4-yellow; VL9-blue) detected on multiple receivers. Filled symbols for fish that were detected on less than 3 receivers (VL-purple; VL5-dark blue; VL6-salmon; VL8-gold). Other symbols as per Figure 6.1.

Species comparison

There was similarity in the home range estimates and core areas for all species (*E. mulitnotatus*, *E. tauvina*, *P. leopardus* and *V. louti*) that were represented by fish detected on more than two receivers (Figure 6.7). There was no significant difference in either core area (p>0.1) or home range area (p>0.1) among *E. mulitnotatus*, *E. tauvina*, and *V. louti*, which provided core and home range estimates for two or more fish.

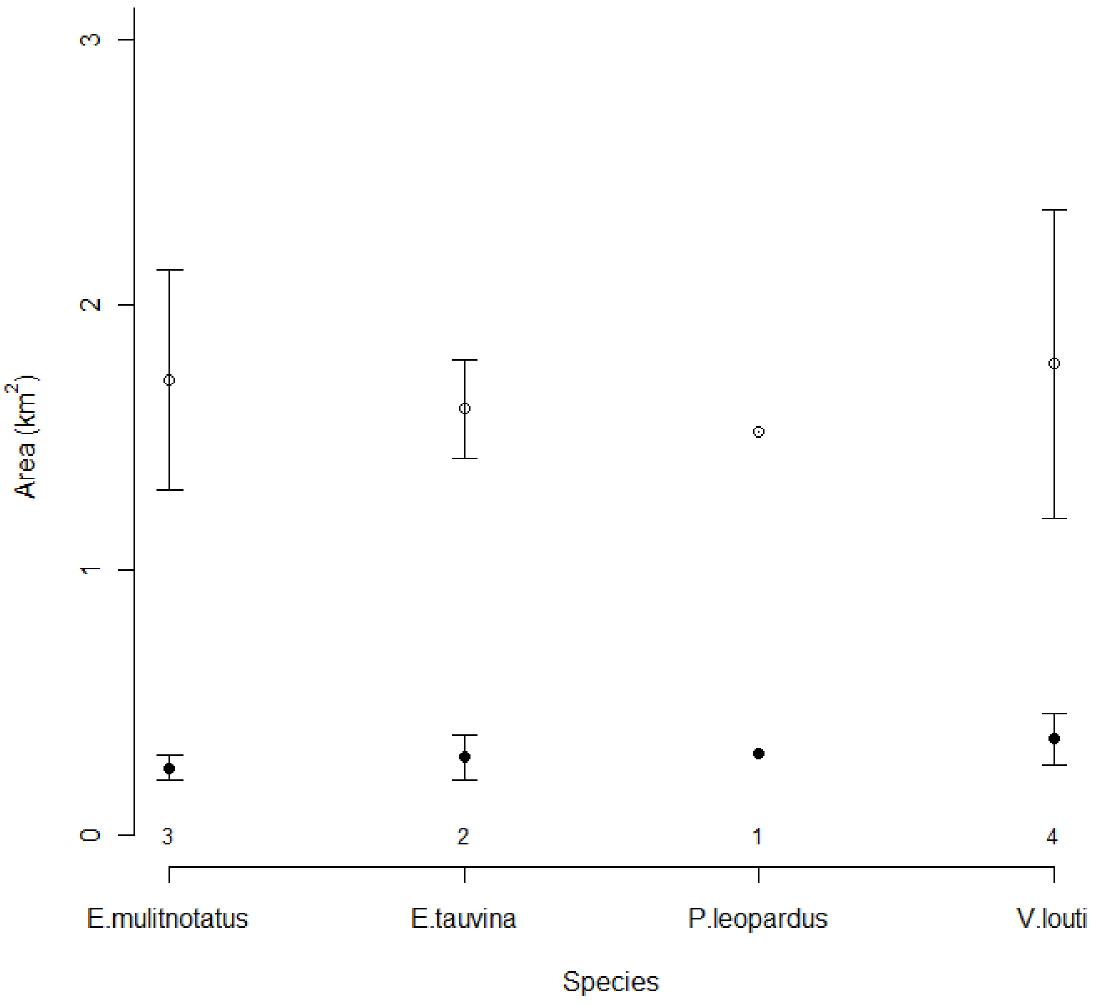

Figure 6.7 Mean (± SE) home range (open circle) and core areas (filled circle) for *Epinephelus multinotatus*, *E. tauvina*, *P. leopoardus*, and *Variola louti*. Numbers indicate sample size.

6.5 Discussion

The suite of epinephelid species displayed a consistent sedentary nature based on acoustic tracking for periods up to two years. Over half of all fish tracked, including individuals from the four major species tracked, were detected on only one or two receivers, which were often adjacent to the point of capture and release of tagged fish. Given an approximate detection range of 300 m for these receivers, those fish would have remained mainly within an area of around 0.3 km^2. Furthermore, individuals of *E. mulitnotatus, E. tauvina, P. leopoardus* or *V. louti*, whose utilisation distributions could be calculated due to detections on three or more receivers all displayed 0.2 – 0.3 km^2 core (50%) and 1.5 - 2 km^2 home range (95% UD) areas. The similarly small core and home range areas among these species, and the fidelity of other fish to areas immediately around the point of capture and release, indicate a consistently limited movement pattern by epinephelid species.

The sedentary nature of Epinephelidae in this study is consistent with the findings for members of this family in a range of predominantly single-species studies. Estimates of home range (minimum convex polygon) for *P. leopardus* on the Great Barrier Reef was between 0.01-0.02 km^2 depending on the habitat type they were in (Zeller 1997). These were thought to be an underestimate at the time due to the limited number of detections (Zeller 1997), and that does appear to be the case, with the over 10,000 detections resulting in a home range (95% UD) of 1.5 km^2 for the single fish tracked at Ningaloo. However, such a conclusion needs to be treated with caution due to the tracking of only one fish for this species. While estimates of home range were not possible for the population at the Abrolhos Islands, metrics of movement were considerably larger than the GBR population (Chapter 5), and may be closer to those recorded at Ningaloo Reef. The home range estimates (95% UD) for *E. tauvina* at Ningaloo Reef (1.6 ± 0.18km^2) were again considerably larger than the estimates of home range (MCP) for *E. tauvina* in Kenya, which were 0.34 km^2 (± 0.23 km^2; Kaunda-Arara and Rose 2004b). The home range sizes of *E. rivulatus* from this study were small, though not as small (<0.001 km^2) as the mark–resighting estimate at Mangrove Bay home ranges (MCP), at the same location previously (Mackie 1998). Most *E. rivulatus* in this study were only detected on one receiver, which would equate to a home range of <0.1 km^2. Movements may have been smaller than this, but the acoustic array did not permit finer scale estimates.

While there is some variation in the home range estimates, they all demonstrate a sedentary nature, despite study location, home range estimate technique and tracking technique. Epinephelid movements have been addressed through tag recapture–resighting (Sheaves 1993, Chapman and Kramer 2000), release–resighting (freeze-branding) (Samoilys 1997a), manual acoustic tracking (Zeller 1997, Kaunda-Arara and Rose 2004b, Hutchinson and Rhodes 2010), passive acoustic tracking (Lembo *et al.* 2002, Starr *et al.* 2007, Chapter 4) or a combination of these techniques (Zeller and Russ 1998).

While the home ranges were generally small, any differences are likely to be due to a number of factors relating to tracking technique, habitat or population differences, or even the smoothing factor that was employed, as it can have a considerable influence on home range estimates (Worton 1989). Tracking duration may also be a major factor in the discrepancy between this and previous published studies. Based on manual acoustic tracking, movement data for *P. leopardus* on the GBR and *E. tauvina* in Kenya were limited logistically to approximately 52 and 63 tracking days, respectively (Zeller 1997, Kaunda-Arara and Rose 2004b). Long-term tracking through passive acoustic techniques is likely to provide more accurate estimates of home ranges as it will account for shorter-term, seasonal and annual movements. This is especially valuable for highly sedentary species, allowing for greater duration in which to detect transmissions. Of the 28 passive acoustic tracking studies examined (Table 6.1), half lasted less than eight months, with very few (~10%) extending to 2 years or beyond. Long term tracking provided sufficient data for a robust estimate of home range with each of several fish detected over 8,000 times for periods up to two years. This provided a good estimate of their home range, allowing for any potential deviation in the home range over time to be assessed. Movements to spawning aggregations usually involve movements from established home ranges (Zeller 1998). While some of these movements may be considerable (Luckhurst 1998, Nemeth *et al.* 2007), a number of studies have shown that these movements are often relatively small (<1.5km), particularly for members of the genus *Plectropomus* (Zeller 1998, Hutchinson and Rhodes 2010, Chapter 5). The longevity of the current study, thus, provided a robust assessment of movement patterns of Epinephelidae, and this was enhanced by the extensive coverage of the array, and the simultaneous assessment of multiple species within the same location.

While there were considerable detections of fish over extended periods of time, resulting in high detection rates, this was not universal. One possible explanation, which has implications for home range estimates, is that some of the fish may be more vagile than previously described, moving out of the detection area of the acoustic array. While possible, this is unlikely with all fish tagged well inside the array, generally either in the lagoon (*E. rivulatus*) or on the reef slope or offshore reefs (remaining species). Similarly, there were few detections on receivers at the extremity of the array. So while these reported home ranges may be an under-representation of all the movements, it is unlikely.

Epinephelids are important commercial and recreational species in a range of coral-reef systems throughout the Indo-Pacific region (Morris *et al.* 2000). Reef-fish fisheries, such as those based on epinephelids, are often multi-species fisheries, and often managed through marine reserves (Welch *et al.* 2008). Such a management tool requires an understanding of the movement patterns of the suite of species to increase the likelihood that a marine reserve will protect those targeted species. The lower the movements and the less time targeted species spend outside the reserve, the greater the protection afforded by a marine reserve (Kramer and Chapman 1999). Thus, the relatively small home ranges in epinephelids, suggests that the protection of areas of several square kilometres would provide protection for the majority (95%) of detections, for a number of species simultaneously.

In addition to size, the habitat(s) encompassed within a marine reserve, and the location of its boundaries can alter its effectiveness in protecting species. The sedentary nature of the epinephelids tracked at Ningaloo Reef indicated little movement among habitats within the array. With the exception of *E. rivulatus*, all epinephelid species tracked remained outside the lagoon. The strong utilisation of the reef slope by a number of species is consistent with underwater visual surveys of the fish fauna in Ningaloo Reef, which demonstrated reef slope communities being most distinct from other habitats, with species such as *V. louti* not being recorded anywhere but on the reef slope (Babcock *et al.* 2008). While not common, some fish on the reef slopes showed movements across habitats, but these were mainly based on movements to and from the reef slope and deeper offshore areas. In the case of the Mangrove Bay sanctuary zone, the boundaries were mainly restricted to the lagoon habitats and did not encompass the reef slope, and therefore, provide little protection for epinephelid species, and perhaps other taxa that occur mainly on the reef slope. A review of the recent rezoning process of Ningaloo Reef, showed that the areas ‘in deeper waters seaward of the reef’ were under-represented in terms of achieving the 34% no take target for the marine park (Beckley and Lombard 2011). Given the relative importance of this habitat for the epinephelids, the lack of reef slope or deeper offshore areas within the Mangrove Bay sanctuary zone or under-representation of those habitats in the park’s sanctuary zones as a whole, reduces the degree of protection afforded to this highly exploited group of fish.

The permeability of a marine reserve boundary to fish movement can be altered based on the habitat in which the boundary occurs. Previous studies have shown that expanses of rubble or sand may provide a natural barrier to fish movement (Barrett 1995, Lowe *et al.* 2003, Popple and Hunte 2005), with the restriction of movements between river banks possibly caused by the channel associated with the river creating a barrier (Sheaves 1993). However, a few studies have shown epinephelid species such as *E. striatus* move 100s of kilometres during migrations to spawning aggregation sites, requiring movements between reefs that are often separated by deep channels (Colin 1992, Bolden 2000). Re-homing experiments have also demonstrated that *E. tauvina* does move across sand channels to return to their original home range (Kaunda-Arara and Rose 2004b). In contrast, there was no evidence of epinephelids in this study crossing the reef channels, despite some individuals utilising the reef channel area. This suggests that, although a number of coral bombies were present within the channel (pers. observation), the reef channel may provide a natural barrier to movement of epinephelids. Such barriers can result in a reduced movement across a marine reserve boundary (Kaunda-Arara and Rose 2004a), which directly contrasts to those reserves where boundaries are along contiguous habitats (Eristhee and Oxenford 2001). Thus, the placement of marine reserve boundaries that incorporate such natural barriers may greatly increase the success of the reserve, by utilising the natural movement patterns of the fish to create an effective boundary edge as opposed to a boundary edge along contiguous habitats, which can cause greater cross-boundary movement (Barrett 1995).

The long-term tracking employed in this study, coupled with simultaneous tracking of several species from an exploited teleosts family, enabled a robust assessment of the movement patterns and habitat use of these epinephelids. It also provided valuable information on natural barriers to fish movements, whose incorporation into marine reserve boundaries may naturally reduce flux rates across reserve boundaries. With strong parallels to other epinephelids, movement data in the current study adds strength to the existing knowledge of epinephelid movements and how these may be considered in spatial management.

Sunset at the Abrolhos Islands

Chapter VII

General Discussion

This thesis highlights the importance for a local assessment of species biology and ecology in ascribing appropriate management measures, particularly those associated with spatial and temporal closures. Through the detailed assessment of *P. leopardus* at an isolated high latitude coral reef, I have demonstrated the variation from traditional paradigms for this species, and other members of the Epinephilidae. Notably, my study has shown the: (1) strong endogenous size-related sexual transition; (2) spatially explicit and temporally persistent spawning omission associated with higher fishing pressure; and (3) temporally distinct spawning aggregations and season compared with the GBR population, spawning on both the full and new moon and the spawning occurring during the middle of the day. Furthermore, the study has confirmed the limited movement of *P. leopardus* and Epinephilidae. Without such a localised assessment and accounting for any variation in aspects of the biology and ecology of this widely distributed species, management measures would be ineffective, yet such variation is unlikely to be unique. The temporal and spatial patterns, particularly in the reproductive biology and movement of *P. leopardus* at Abrolhos Islands, and the movement patterns of other epinephilids at Ningaloo Reef will be discussed in relation to spatial and temporal closures and more traditional management strategies, such as legal size limits.

6.1 Spatial closures

The primary intent behind the implementation of spatial closures is often the protection of biodiversity with associated conservation benefits (IUCN 1994). Implementation of spatial closures is predominantly a political one, with stakeholders and government largely influencing the location and size or indeed duration of a closure, as opposed to a scientifically based assessment (see Halpern and Warner 2003). However, the suitability of spatial closures as a management tool for one or more species needs to consider the size of the closure, its location in regard to habitats contained within the zone, and potential natural barriers to fish movement (Roberts 2005). This influences the degree of protection offered to exploited species by MPAs through the amount of time an individual is exposed to fishing through movements out of the MPA (Kramer and Chapman 1999). Variation in the time exposed to fishing pressure has been provided as an explanation between some of the mixed results in terms of fish abundance between species inside and outside MPAs (Nardi *et al.* 2004). The benefits of the no-take zone at the Abrolhos Islands (termed “Reef Observation Area” or “ROA”) to *P. leopardus* stocks (albeit initially; Nardi *et al.* 2004) were due to the compatibility of the ROA’s design to the movement patterns of *P. leopardus*.

The current study showed that the no-take zone (termed “sanctuary zone”) at Mangrove Bay in the Ningaloo Marine Park provided little protection for the suite of epinephelid species that were tracked in the region (Chapter 6). Tracking of epinephelids in and adjacent to the Mangrove Bay sanctuary zone occurred in the lagoon, reef flat, reef slope and deeper offshore reefs, as well as patch reefs associated with a reef channel. There were marked differences in habitat use of those species, with only the Chinaman cod

(*Epinephelus rivulatus*) solely occupying the lagoon, which was the only habitat protected by the sanctuary zone. However, the vast majority of detections of epinephelids occurred on the reef slope or deep offshore reef areas outside the zone (Chapter 6). Furthermore, since these habitats are under-represented in the broader Ningaloo Marine Park (Beckley and Lombard 2011), the protection of several species of Epinephelidae to fishing are likely to be limited. My results suggest that, at least for epinephelids, the inclusion of a range of coral-reef habitats within the sanctuary zones is likely to increase their benefit for protection against fishing pressure to those species which exhibit limited movements (Hilborn *et al.* 2003).

In contrast to Ningaloo Reef, the ROAs at the Abrolhos Islands afforded good protection for *P. leopardus*, which was shown to be a relatively sedentary species in the eastern Indian Ocean (Chapters 5 and 6) and elsewhere (Davies 1996, Zeller 1997, Zeller *et al.* 2003), even during spawning migrations (Chapter 5). This study has shown that the ROAs at the Abrolhos Islands provided benefits for *P. leopardus* that extended beyond the normal accumulation of larger and more fish that have been shown for a range of fish species including epinephilids (Gell and Roberts 2003, Halpern 2003, Nardi *et al.* 2004). Sites within and directly adjacent to these no-take zones possessed breeding females and males during the spawning season, which contrasted with more distant areas that were exposed to greater fishing pressure.

The association of spawning omission with areas of increased fishing pressure highlights the importance of MPAs to provide areas of reduced fishing pressure (Chapter 3). The ROAs at the Abrolhos Islands, and adjacent areas where fishing pressure is “light”, possessed spawning females during the spawning season. Males were also associated with these spawning sites. As there is a strong endogenous size-related cue for sexual transition for *P. leopardus* at the Abrolhos Islands, females must attain a certain size before their transition to males (Chapter 2). In areas where fishing pressure is ‘light’, females are more likely to attain that transitional size. Those areas that lacked males were also those where there was no reproductive development of females, suggesting that the absence of males may have disrupted reproductive behaviour leading to spawning omission. This was further highlighted by some historical data showing reproductively active females collected with males from a site where there was no evidence of spawning females or males in the current study. These fish were collected prior to an increase in fishing effort at the Abrolhos Islands (Wise *et al.* 2007), further re-iterating the association of fishing pressure, with a lack of males and spawning females. Therefore, the ROAs at the Abrolhos Islands provide an area where fishing pressure is removed and normal reproductive interactions between males and females can still occur, resulting in the maintenance of reproductive output. This reaffirms the importance of MPAs in the reduction of fishing pressure, which can only be attained if the MPA provides sufficient protection using an appropriate design.

The benefits of the ROAs to the breeding stock (Chapter 3) and abundance (albeit initially; Nardi *et al.* 2004) of *P. leopardus* at the Abrolhos Islands, likely reflects some of the criteria used to identify the location of those ROAs during the planning process. Selection criteria for the ROAs were: “an identifiable area, a whole reef platform, representative of good quality reef habitat, popular use area, suitable for research; and safety aspects of an area” (Anon. 1998). Selection of the whole reef area resulted in the surrounding “buffer” zone of deep sand channels being placed around the ROA, which aided in the retention of *P. leopardus* within the ROA. Both *P. leopardus* at the Abrolhos Islands and other epinephilids studied at Ningaloo Reef did not show evidence of crossing channels (Chapter 6), suggesting that these channels provide an effective natural barrier to movement.

In comparison to the objectives of the ROAs at the Abrolhos Islands, those at Mangrove Bay sanctuary zone at Ningaloo Reef did not consider fish movement or habitat utilisation. This may account for the differential results in the effectiveness of these no-take reserves for epinephelids. The examination of movement patterns, habitat preferences and the identification of possible natural barriers to movement in the studies of exploited teleost taxa at the Abrolhos Islands (Chapter 5) and Ningaloo Reef (Chapter 6) highlight the importance for the inclusion of biological or ecological information in area closure planning. The design of the ROA unwittingly incorporated important features for the protection of *P. leopardus*, but alteration to the design of the Mangrove Bay sanctuary zone would be required to adequately protect epinephelids. This would include a seaward extension of the sanctuary zone to incorporate the reef slope and deeper offshore reefs, and would also benefit from using reef passes as boundaries for the sanctuary zone, to limit cross boundary movements of exploited epinephelids.

6.2 Temporal closures

Temporal closures generally benefit those species that aggregate to spawn, making them less susceptible to localised depletions through targeted fishing pressure on aggregations (Colin 1992; Claydon 2004; Sadovy and Domeier 2005). They can have different temporal resolutions with some closures encompassing the spawning season (Johannes *et* *al.* 1999, Wakefield 2010, Fairclough *et al.* 2011), or refined to just cover specific lunar periods when aggregations form (DEEDI 2010). Finer temporal resolutions of closures require robust data on the spawning timing and period of the primary targeted species to ensure protection over the most susceptible (aggregating) period.

Comparison of spawning aggregation dynamics of *P. leopadus* demonstrated significant differences between the populations at the Abrolhos Islands and the GBR (Chapters 4 and 5). This has implications for temporal closures at a number of temporal resolutions. The spawning period at the Abrolhos Islands, confirmed through general reproductive information (Chapter 4), acoustic tracking and underwater visual census information (Chapter 5), is December to March. This contrasts with the GBR, where spawning occurs from September to December (Ferreira 1995). Since spawning has been shown to be strongly temperature related (Colin 1992), and epinephilid species spawn at different times of the year in tropical versus temperate areas (Thresher 1984), a different annual spawning season of the higher latitude population at the Abrolhos Islands compared to its more tropical GBR counterparts is not surprising. Water temperatures at the Abrolhos Islands at spawning time are lower (Figure 3.8) than in the GBR (threshold of 24^{o}C Samoilys 1997b). While differences were not surprising, it does re-affirm the need to assess the biology, particularly reproductive biology, of the local exploited population when planning broad seasonal spatial closures.

Unlike *P. leopardus* in the GBR (Samolys 1997), spawning aggregations of the species at the Abrolhos Islands are not confined to a single lunar phase around the new moon. At the Abrolhos Islands, acoustically tracked females attended spawning aggregation sites on both full and new moons and males were almost daily attendees at the aggregation site during the spawning season (Chapter 5). While the formation of spawning aggregations of fish species generally occurs on the full or new moons (Samolys 1997, Rhodes and Sadovy 2002; Pet *et al.* 2005), the current study indicates that the frequency of those aggregations can differ within species. Aggregations around the new moon on the GBR were used to tailor two new moon closures (Oct and Nov) during the spawning season for the species in that region (DEEDI 2010). However, the use of such data from the GBR for the species at the Abrolhos Islands would expose the females to exploitation of spawning aggregations on the full moon in this region. In addition, males would be subject to additional exploitation between any lunar closures. Males attend the aggregation site throughout the spawning season (Chapter 5), which concurs with dynamics of other aggregating species where males attend aggregation sites more than females (Zeller 1998; Nemeth *et al.* 2007; Starr *et al.* 2007). The vulnerability of males through increased attendances at spawning aggregation sites and disproportionate targeting by fishing has a dramatic effect on reproductive output (Chapter 3, Coleman *et al.* 1996). Therefore, the research conducted on the dynamics of the spawning aggregations of *P. leopardus* at the Abrolhos Islands has highlighted the need for local assessments of species' biology in the planning and application of appropriate temporal closures.

6.3 Traditional Management Measures

There are several "traditional" management measures that are in place for *P. leopardus* at the Abrolhos Islands, specifically a minimum legal size of 450 mm total length and a recreational bag limit of one fish per day. The commercial sector (West Coast Demersal (Interim) Managed Scalefish Fishery) is a multi-species fishery, which is a limited entry fishery and effort is limited in each management area to a maximum number of hours fished (Fairclough *et al.* 2011). The “Mid-West Area”, which contains the Abrolhos Islands, has a maximum effort of 24,398 hours annually (Anon. 2007).

The current minimum legal size at 450 mm L_T is greater than the 423 mm L_T (404.3 mm L_F; Table 4.2) for the size at maturity for fish from spawning sites. This allows females from spawning sites to reproduce at least once before being exposed to fishing pressure, a common criterion used for this strategy (McPhee 2008). As males appear to be important in the onset of female reproductive development (Chapter 3), and females transition to males based on a size-related cue (Chapter 2), there is a need to protect larger individuals. Fishing generally targets larger individuals (Jackson *et al.* 2001), which in the case of protogynous hermaphrodites are males. Therefore, a maximum size limit may serve to allow larger individuals to escape fishing pressure once they attain the maximum size limit. This would allow males to return to fished areas and potentially the resumption of reproductive activity in those areas. A similar rationale was used for the proposal of a maximum size limit for *P. aereolatus* in the Torres Strait (Williams *et al.* 2008). *P. aereolatus* was found to have a strong female dominated sex ratio, which was postulated to be a result of ‘a less flexible size and age at sex change’ and hence male protection would be afforded best by a maximum size limit. Maximum size limits have been used in protandrous species to afford protection to large females (Griffin 2007), but they also may provide benefits to protogynous species, particularly those with relatively inflexible size/age at sexual transition.

Fishing pressure for *P. leopardus* at the Abrolhos Islands appears to be high. The size and age structure was shown to be truncated at non-spawning sites, with these locations having few large fish (Figure 3.5). The lack of large fish was also demonstrated by the largest fish (45 cm L_T) seen during UVCs at non-spawning sites (How, unpubl. data). All of this leads to the conclusion that the total catch of *P. leopardus* is likely to be too high. High fishing pressure has also been demonstrated for baldchin groper (*Choerodon rubescens*) in the Mid-west zone, which incorporates the Abrolhos Islands (Wise *et al.* 2007). This highlights that fishing pressure is high for several species and not specific to *P. leopardus*, which is expected because of the multi-species nature of the fishery. The recreational bag limit of one fish is the lowest bag limit possible for a species before moving to a specific catch reduction system for this species such as quota tags which are used to limit catch of *Pagrus auratus* in Freycinet Estuary, Shark Bay (Department of Fisheries 2010). Given the association of fishing pressure with reduced male abundance and the persistent spawning omission at the Abrolhos Islands outside the ROAs (Chapter 3), the total catch (recreational and commercial), needs to be reduced.

6.4 Suggested Management Measures

For *P. leopardus* at the Abrolhos Islands, there are several management measures in place, though there would be benefits from additional management measures. Good protection is afforded to *P. leopardus* through the ROAs, though their location is restricted to the eastern part of each island group (Figure 3.1). The eastern parts of the island groups within and surrounding the ROAs have been shown to be virtually the only sources of egg supply (Chapter 3). The sinks for the resultant larval recruits are as yet unknown. Through conducting dispersal modelling using oceanographic current information (see below), these sinks may be identified. This would then allow areas that are not being replenished by the current spawning sites to be identified and protected.

Spatial closures have been shown to be effective at the Abrolhos Islands in protecting spawning biomass, so additional closures may be of benefit to ensure adequate larval dispersal throughout the archipelago.

Fine-scale temporal closures would not be effective for *P. leopardus* at the Abrolhos Islands. As males are persistent at spawning aggregations throughout the spawning season (Chapter 5), they would still be exposed to fishing pressure between any lunar closures. Given the likely disproportionate importance of males in the maintenance of reproductive output, a maximum size limit may be a more appropriate measure for the protection of males. However, the level of exploitation of *P. leopardus* is such that even a maximum size limit of 549 mm L_F (L_{50} sexual transition; Chapter 2) would provide little benefit. Fishing pressure in non-spawning sites resulted in only one female (Figure 3.5) and two males (Figure 3.6) above this size being sampled, while no fish was seen above this size in UVCs (How, unpubl. data; Chapter 3). Thus, at current exploitation levels, few fish would progress through the exploitable size slot to be afforded protection above the proposed maximum size limit. Reducing fishing pressure is, therefore, likely to be the most appropriate management measure to permit greater male abundance throughout the Abrolhos Islands. This may be achieved either at localised areas through additional no-take zones, or a significant reduction in catch of *P. leopardus.* An increase in male abundance may then lead to the resumption of spawning in a greater number of areas at the Abrolhos Islands.

In terms of the Ningaloo Marine Park, modifications should be made to the boundaries of sanctuary zones to increase protection to several species of Epinephildae by incorporating reef slope and deeper offshore habitats into the zones, as well as natural barriers for movement such as deep channels at the boundaries. While the sanctuary zones in the park have been designed mainly for biodiversity purposes (Anon. 2005), modification of the zones would provide a greater level of protection for the Epinephildae of the area, which are important recreational species in the area (Sumner *et al.* 2002) but occurs in habitats that are not adequately protected.

6.5 Future Research

Future research should examine if similar differences occur at the edge of the distribution range of *P. leopardus*, which are found throughout the Indo-Pacific (Figure 1.1). With potentially 12 genetically isolated populations (van Herwerden *et al.* 2009), examining the biology and ecology of other isolated populations (e.g. Japan/Taiwan) would provide an excellent opportunity to determine the effects of genetic isolation and variations in environmental condition on such a ubiquitous reef predator. While *P. leopardus* does occur at lower latitudes in the eastern Indian Ocean, it is not commercially exploited. An understanding of the biology and ecology of other populations in the eastern Indian Ocean, such as those at Scott Reef, would provide a broader spatial understanding of the population in the region and whether the traits of the assemblage at the Abrolhos Islands reflect its high latitude or the population as a whole.

Thus, understanding local oceanographic features would be an important area of future research. Located within the southward flowing Leeuwin Current, the Abrolhos Islands is thought to cause a disruption to the current’s flow, potentially creating localised eddies (Feng *et al.* 2010). Given that spawning occurs at spatially discrete locations (Chapter 3), and at specific times (Chapter 5), understanding the influence that these eddies play on larval dispersal from spawning locations is of great importance. This will assist in understanding the degree to which protection needs to be afforded to different non-spawning locations within the archipelago, such that there are sufficient larval sources to replenish most areas within the Abrolhos Islands.

Findings on the timing of spawning and the aggregation dynamics of *P. leopardus* at the Abrolhos Islands came predominantly from 8 tagged coral trout (Chapter 5). While the data from these tagged fish concurred with UVC and histological data, an expanded tagging program would provide greater certainty regarding the movement patterns of the species and enable a definitive resolution to the potential for *P. leopardus* to move from non-spawning to spawning areas (Chapter 3).

The extent of spawning omission demonstrated in this thesis for *P. leopardus* at the Abrolhos Islands is unlikely to be demonstrated through a reliance on commercial and recreational samples. Reliance on fisheries catches would not have provided any indication as to the extent of omission and the potential causes of it. Future biological sampling regimes should also include comprehensive, fine-scale sampling where possible. Sampling at such a level, while logistically challenging, was the only way that spatially discrete and persistent spawning omission was identified at the Abrolhos Islands. Specifically, sampling in remote locations and within MPAs identified spawning individuals. Without such sampling effort, very few spawning individuals would have been sampled, potentially leading to the conclusion that there was limited spawning at the Abrolhos Islands and they were reliant on external recruits. Such a finding could lead to a reduction in protection measures with a perceived external breeding stock being more important for protection than those fish at the Abrolhos Islands. Spawning omission in wild-populations is difficult to assess due to the resolution of spatial and temporal data needed (Rideout *et al.* 2005). However, recently more examples of spawning omission have been identified in natural populations (e.g. Wakefield *et al.* 2013). Spawning omission is therefore likely to be more pervasive than previously thought, and thus fine-scale sampling should be included in research activities to provide a better opportunity to identify it in the future.

Finally, as highlighted by Willis *et al.* (2003b), conclusions from many studies on MPAs are often confounded due to the lack of a BACI design. This is indeed the case with this particular study as the protected areas were in place for many years prior to the initiation of this study. While there are some important findings that have resulted from this study, the role that protection has played in the ability of females to reach a size at which they can transition to males (Chapter 2) and the persistence of spawning in the protected areas (Chapter 3) can not be conclusively determined. Therefore, future work should examine these processes with sampling prior to the establishment of a protected area.

The tropical temperate mix at the Abrolhos Islands, with plate coral and kelp side by side

Chapter VIII

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Acoustic equipment on the back deck of the Fisheries boat PV Chalmers before deploying a range test at the Abrolhos Islands

Appendix A

Acoustic tracking: issues affecting design, analysis and interpretation of data from movement studies

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A.1 Abstract

Acoustic telemetry systems are an increasingly common way to examine the movement and behaviour of marine organisms. However, there has been little published on the methodological and analytical work associated with this technology. We tested transmitters of differing power outputs simultaneously in several trials, some lasting approximately 50 days, to examine the effects of power output and environmental factors (water movement, temperature, lunar cycle and time of day). There were considerable and volatile changes in detections throughout all trials. Increased water movement and temperature significantly reduced detection rates, while daytime and full moon periods had significantly higher detection rates. All ten transmitters (from seven transmitter types tested) showed a sigmoidal trend between detection frequency and distance. Higher-powered transmitters had a prolonged detection distance with near-maximal detections whereas lower-powered transmitters showed an almost immediate decline. Detection frequency variation, transmitter type and the modelled relationship between distance and detection frequency were incorporated into a positioning trial which resulted in markedly improved position estimates over previous techniques.

A.2 Introduction

Acoustic tracking or telemetry is becoming an increasingly popular tool for determining movement patterns, home ranges, habitat utilisation and behaviour of marine organisms (Heupel *et al.* 2006). The range of transmitter sizes and transmission rates, along with the advent of automated single-frequency acoustic receivers that can be remotely deployed, have enabled long-term tracking studies of large numbers of individuals from a range of taxa in a variety of environments (see Heupel *et al.* 2006). Despite the rapid growth in the use of this technology in animal tracking, few studies have dealt with the methodological and analytical issues around these tracking studies (e.g Clements *et al.* 2005; Simpfendorfer *et al.* 2008; Hedgar *et al.* 2008).

Tracking movements of organisms using automated acoustic receivers typically utilise two broad study designs: gated/curtain arrays and grid arrays (Heupel *et al.* 2006). Multiple lines or gates of receivers are often deployed to determine directional movement, such as along a migration path or through the entrance to an embayment (e.g. Finstad *et al.* 2005). For an individual, directional movement can be determined if its transmitter is detected at consecutive times as it moves within range of receivers in multiple gates. To ensure a transmitter “recapture”, detection ranges of receivers in a gate need to overlap to ensure that when individuals pass through a gate they are detected by one or more receivers. Therefore, it is critical to understand the acoustic detection range of the receivers at the site of the study and how the extent of that range varies during a study.

Grid arrays are often designed such that the likely zone of detection of a receiver overlaps that of one or more other receivers. This allows the position of an individual to be estimated within the array (Heupel *et al.* 2006). Simpfendorfer *et al.* (2002) provided a useful means of translating numbers of detections of an individual recorded in a grid of receivers into short-term activity centres. This method allows an average position to be determined during a specific time period (e.g. each hour) based on the location of receivers and the proportion of detections at each receiver. Appropriate receiver spacing based on the typical range or distance of transmission of sound from an acoustic transmitter is crucial to meeting the objectives of the study.

The distance of travel from its source (range) of an acoustic signal in water is dependent on a number of factors, including transmitter power output, water conditions (e.g. sea state, air entrapment (bubbles), currents, eddies, salinity, temperature, depth, suspended matter) and abiotic, biotic and/or anthropogenic noise (e.g. Voegeli and Pincock 1996; Winter 1996; Thorstad *et al.* 2000). As these factors vary among different locations and times or with the species being tracked (Lacroix and Voegeli 2000; Heupel *et al.* 2006), testing of transmitter performance at new sites over a range of such conditions is important (Voegeli *et al.* 1998; Lacroix and Voegeli 2000; Lacroix *et al.* 2005).

This study tests the influence of different transmitter power output as well as several environmental factors on the detection range of acoustic receivers. We examined the performance of seven acoustic transmitter types (Vemco Limited, Shad Bay, Canada) of differing power outputs commonly used in acoustic tracking studies to see if acoustic detection distance was greater for transmitters with higher power outputs. Although water conditions have been implicated in the reduced acoustic range of detections in other studies, this factor has not been empirically tested. Therefore, we were interested in the degree to which water movement may impair detection ranges for a range of transmitter powers. Biotic noise has been shown to vary on diel, lunar and seasonal scales (Radford *et al.* 2008), and with noise levels influencing acoustic detection rates (Heupel *et al.* 2006), we investigated time of day and lunar phase to see if potential increases in acoustic noise reduced acoustic detection rates. Similarly, changes in temperature can affect the activity of animals, particularly ectotherms. Thus, temperature was examined to see if elevated temperatures reduced detection rates.

Finally, using findings from this study, an alternate method of positioning transmitters within an acoustic array to that adopted by Simpfendorfer *et al.* (2002) is demonstrated, utilising detection profiles for appropriate transmitter selection while also accounting for variation in detection range throughout the study.

A.3 Methods

Study sites, design, and transmitters

Experiments were conducted at several locations throughout south-west Australia (FigureA.1). Trials were aimed to examine the acoustic range of the transmitters of different power, the most appropriate model to describe acoustic decline with distance, temporal variation in detection rates and what environmental factors may influence detection rates (Table A.1).

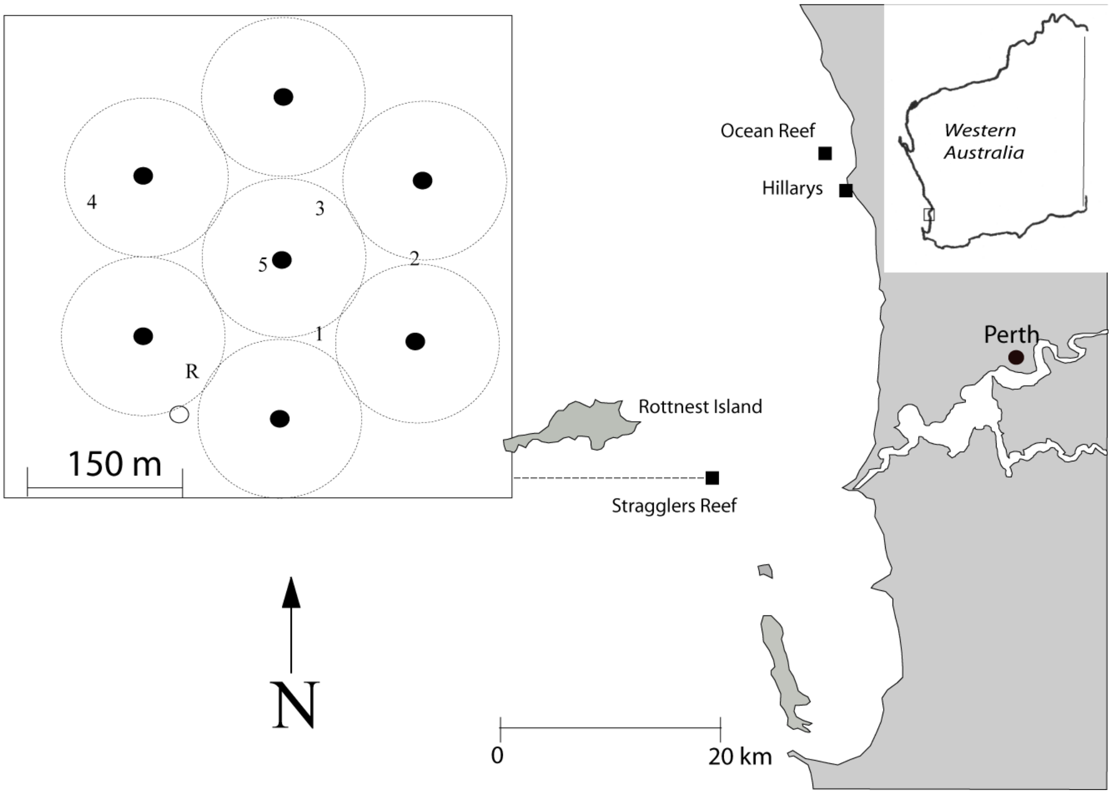

Figure A.1 Locations of acoustic testing experiments. Inset: Array at Stragglers Reef for positioning test with receivers (closed circles), reference receiver (open circle), deployment of transmitter for estimation (1 to 5) and reference transmitter (R).

Table A.1 Details of range testing experiments, with power output of transmitters from

Location	Depth (m)	Transmitters (power dB re 1uPa @ 1m)	Transmission Min Max (sec) (sec)	Test dates (duration)	Receiver distances (m)	Factors Examined
Jurien	40	V13-1L (147) V13-1H (150) V16-6L (153)	30 – 90 30 – 90 20 – 80	4-Nov-07 to 10 Nov 07 (7 days)	0; 100; 204; 306; 406; 604; 805	• Transmitter comparison • Detection variation • Model for signal detection and distance
Abrolhos Islands	8 – 40	V13-1L (147)	30 – 90	28-Feb-08 to 15-Apr-08 (48 days)	126	• Detection variation • Environmental effects
Perth (Ocean Reef)	12	V7-4L (136) V9-2L (142) V9-2H (147) * V13-1H (150)	60 – 60 60 – 60 60 – 60 20 – 60	25-Sep-08 to 20-Nov-08 (56 days)	0; 8; 32; 91; 132; 192; 240; 323; 390; 463	• Transmitter comparison • Detection variation • Model for signal detection and distance • Environmental effects
Perth (Stragglers Reef)	9	V7-4H (136) V16-4H (158)	60 – 60 120 – 120	24-Apr-09 to 30-Apr-09 (6 days)	0; 22; 53; 71; 106; 156; 206; 308; 408; 610; 808	• Transmitter comparison • Detection variation • Model for signal detection and distance

Vemco™ transmitter specification sheets; http://www.vemco.com/products/transmitters/index_coded.php

The range test at Jurien examined detection profiles of three large transmitters simultaneously for possible use in a deepwater lobster tracking program. Considerable variation in detection rates at Jurien led to the incorporation of a reference transmitter in an acoustic tracking program of leopard coralgrouper (*Plectropomus leopardus*) at the Abrolhos Islands. This long term deployment of a reference transmitter allowed environmental influences on detection rates to be examined. An expanded replicate experiment was conducted at Ocean Reef, Perth, where simultaneous range-testing of three small transmitters was examined against environmental conditions. Finally the smallest transmitter had its range test replicated at Stragglers Reef, Perth, as part of a positioning trial. This test also included a large transmitter to compare its detection profile to previously tested transmitters. This resulted in testing 10 separate transmitters from seven different transmitter types (defined by different size and power output) in four trials encompassing almost four months of data (Table A.1).

Range tests were conducted by mooring VR2 receivers (Vemco Limited, Shad Bay, Canada.) at set distances from transmitters (Table A.1). The mooring line with the transmitters also had a receiver attached 0.5 m below the first transmitter to provide data for 0 m from the transmitter. The transmitters were attached to a mooring line with cable-ties in a vertical orientation to minimise variation and increase detections (Clements *et al.* 2005). Moorings consisted of a 30-kg cement block attached to a series of floats by 8-mm rope. The receiver was cable tied to the rope 2.5 m above the mooring and 2 m below the first subsurface float, designed to maintain the receiver in a vertical orientation. A second float was located 5 m under the surface from the terminal end of the mooring rope to avoid damage or loss from shipping and to aid in receiver recovery. Receivers were deployed in a straight line moving away from the base receivers (with transmitters attached) at the pre-set distances for each range test (Table A.1).

Positioning Test

A positioning test was conducted at Straggler Reef by placing six receivers in a circular pattern around a central receiver. The seven receivers were spaced such that the distances of 50 % attenuation of the transmitter signal, calculated from the pre-trial range test at this location (D50 = 77 m), were adjoining. This resulted in an average spacing between receivers of 152.25 m (range 139 -162 m).

A V7-4L transmitter with a set period of time between transmissions (off time; 60 s) was placed at five consecutive locations (of known latitude and longitude; Garmin GPS Map 76Cx; accuracy <5m) within the circular array (FigureA.1). The transmitter was present at each location for at least 1 h, which was the time period over which the position was estimated. For position 1, the transmitter was left for over 2 h to provide a second position estimate to examine variation in position over time. This resulted in six position estimates for the five locations.

Detections of a reference transmitter (V7-4L; 300 second off time) with known distances to each receiver provided a simultaneous range test during the positioning trial. To provide a detection rate relatively close to the reference transmitter (36 m), an additional receiver was added to the array (Figure A.1)

Environmental Data

All environmental data were recorded during the trial at the Abrolhos Islands and the range test off Ocean Reef (Figure A.1), using an accelerometer (Onset Hobo, Massachusetts U.S.A.; UA-004-64), which logged angular displacement every 4 minutes, while water temperature (ºC) was recorded every 5 minutes using a Onset Hobo UA-002-64 meter. These were located on a logger unit situated at the same depth and adjacent to the transmitters at Ocean Reef (Table A.1). At the Abrolhos Islands, the transmitter was attached to the logger unit on the edge of a steep reef slope. The receiver was 126 m from the reef in 40 m. However, there was no physical obstruction between the transmitter and receiver due to the bathymetry of the area.

Data Analysis

For each transmitter, the numbers of transmissions received per hour were determined. Due to the random transmission of codes (Table A.1) and periods of code collision (see below), the detections in each hour period were standardised. Each transmitter for each study was standardised by expressing the detection frequency in each hour as a proportion of the maximum-recorded detection frequency of that transmitter on any receiver over the entire study.

When transmissions from two transmitters coincide (code collision), they can produce false detections (i.e. detections of transmitters that are not present in the study, Heupel *et al.* 2006). This occurred during the Ocean Reef trial. Despite a set off time for three of the transmitters (Table A.1), apparent ‘drift’ in these off times resulted in periods of significant code collisions between two transmitters. These periods of code collision were identified by the presence of false detections (indicating potential code collision) in conjunction with a drastically reduced detection frequency for two transmitters at “zero” m. Those detections were removed from all analyses, as the changes in detection frequency do not reflect changes in environmental conditions. One receiver (192 m) was removed from the data analysis for V7 and V9 transmitters in the Ocean Reef range test because the receiver was found to be incorrectly configured to detect these transmitters.

The relationship between standardised detections and distance was expressed using a number of linear and non-linear equations. A linear model (Eq. 1) was examined as it had previously been used to describe attenuation of acoustic transmitters (Lembo *et al.* 2002; Simpfendorfer *et al.* 2002). A logarithmic model (Eq. 2) was tested because sound pressure levels decline logarithmically due to spherical spreading (Urick 1983), while other non-linear models (sigmoidal and exponential – Eq. 3 (Melville-Smith and de Lestang 2006; Helidoniotis *et al.* 2011) and 4 (Leike 2002)) were also explored based on empirical observations. The sigmoidal model (Eq 3) is shown as a three-parameter model. This was also examined as a two-parameter model where pmax was fixed to 1. Each model was fitted to all hourly standardised detection data for each distance, and compared using Akaike's Information Criterion (AIC), with the most appropriate model being that with consistently the lowest AIC across the transmitters tested. Model residual plots were also examined for bias. The most appropriate model was then used to test for significant differences between transmitter attenuation using likelihood ratio tests (Mood *et al.* 1974; Cerrato, 1990).

$$R = aDist + b\,,$$

Eq. 1

$$R = c\ln\left(Dist + 0.00001\right) + d\,,$$ Eq. 2

$$R = {p_{\max}}\Big/\left(1 + \exp(\ln(19) * \left(\left(Dist - D_{50}\right)/\left(D_{95} - D_{50}\right)\right)\right)\,,$$ Eq. 3

$$R = g * \exp\left(-Dist \big/ h\right), \qquad \text{Eq. 4}$$

where R is the proportion of the maximum transmissions received, Dist is the distance of the transmitter from the receiver and a, b, c, d, g, h, pmax, D_{50} and D_{95} are parameters. For the sigmoidal model, the proportion pmax represents the theoretical maximum detections for the model, while D_{50} and D_{95} represent the distance where the proportions of detections have declined by 50% and 95% of pmax.

Relative water movement was averaged each hour to smooth any potential outlying records as advocated by Evans and Abdo (2010). The day of the lunar cycle, recorded as a proportion from 0 (new moon) to 1 (full moon), was ascribed to each hour time period for that day. Water temperature recordings were averaged for each hour time period and time of the day was categorised as: night (2000 – 0400), day (0800 – 1600) and crepuscular (0400 – 0800 and 1600 – 2000). Environmental factors were then standardised to have a mean of zero for each variable at each site (Abrolhos Islands and Jurien).

The effects of environmental factors on the detection rate of the reference transmitter at the Abrolhos Islands were assessed with a generalised linear model (GLM; family = binomial (link=logit). At Ocean Reef, a three-parameter sigmoidal model was used to assess variation in rates close to the transmitter (at or near pmax) as well as at the distance where the proportions of detections recorded had fallen by 50% and 95% of pmax. The effect of the environmental variables on the estimation of each of the three main parameters was investigated through an additional parameter for each of the four environmental variables (water movement (wave), water temperature (temp), lunar cycle (lunar), time of day (diel)). These were added to each of the three main parameters, coupled with the initial three parameters for the model as a whole, resulting in a 15-parameter model (Eq. 5).

$$\mathrm{Re}\,c = J \Big/ \left(1+\exp(\ln(19)*((Dist-K) \big/ (L-J))\right) \qquad \text{Eq. 5}$$

where $J = p_{\max} + p_{\max(1)} *(wave) + p_{\max(2)} *(temp) + p_{\max(3)} *(lunar) + p_{\max(4)} *(diel)$

$$K = D_{50} + D_{50(1)} *(wave) + D_{50(2)} *(temp) + D_{50(3)} *(lunar) + D_{50(4)} *(diel)$$

$$L = D_{95} + D_{95(1)} *(wave) + D_{95(2)} *(temp) + D_{95(3)} *(lunar) + D_{95(4)} *(diel)$$

Positioning Analysis

The detection frequency at receivers, and known distances from the reference transmitter, enabled the generation of an in-situ range test for each of the six time periods. As there were no receivers close to the transmitter, the model used to describe signal attenuation with distance (three-parameter sigmoidal) was modified slightly, with the parameter describing the maximum proportions of reception (pmax) fixed at 1. This in-situ attenuation information was used to triangulate the positions of the transmitters within the detection field. A second set of positions was estimated using signal attenuation information derived from the pre-trial range test of the V7-4L transmitter at Stragglers Reef (Figure A.1) to examine the suitability of a separate range test in position estimation.

Distance of a transmitter from a receiver was estimated by fitting the proportion of detections at each receiver in the array, following the general activity centre / time bin approach developed by Simpfendorfer *et al.* (2002), to the modelled signal attenuation parameters from pre-trial and in situ range tests. When no detections were recorded on a receiver, a distance was not determined and this receiver was not used in the position analysis.

Error around each distance estimate was calculated and used in the estimation of the transmitter position. Bootstrapping (resampling the proportion of detections with replacement 10 000 times) of both the pre-trial range test and the in situ range test data over the 7.5 h of the positioning test was used to generate upper and lower 95 % confidence intervals (CI) (Boos 2003). The detection frequency for each receiver was then fitted to the modelled upper and lower 95% CI (from each range test), to provide a 90% confidence region of distances from each receiver. The CI region was divided by four to produce a standard deviation estimate.

Log-likelihood densities of each transmitter position were produced from the known acoustic receiver positions, the distance estimate from each receiver and the associated standard deviation. Thus, for each receiver, a unique log-likelihood density was assigned to every 1 m2 throughout the tracking array based on that 1 m2's distance from a receiver and the estimated mean and 95% CI distance of the transmitter from that receiver. The likelihood density was based on the function $f(x) = 1/\left(\sqrt{2\pi} \times \sigma\right) \times e^{\left[(x-\bar{x})^2/\left(2\omega^2\right)\right]}$, where $\bar{x}$ is the mean of the distribution and σ the standard deviation. The sum of the natural logarithm of the likelihood density for all receivers in each 1 m2 was then considered the relative likelihood of the transmitter being located in that block. The block with the greatest log-likelihood density was considered the most plausible location of the transmitter and all blocks with log-likelihood densities within 1% of this maximum considered to comprise the 99% confidence region. All data were analysed using R (R Development Core Team 2009).

A.4 Results

Model for Acoustic Detection and Distance

For the range test conducted at Jurien, there was an immediate decline in detections for all transmitters regardless of their power output (Figure A.2a). The three-parameter sigmoidal and exponential models provided the best description of detection rate decline with distance, with residuals from both models being evenly distributed. The immediate decline also accounted for the considerably larger modelled p max values and negative D_{50} distances (Table A.2). The relationship between signal attenuation and distance was significantly different between the three transmitters (likelihood-ratio test: all $p < 0.05$; Figure A.2a).

Table A.2 – Estimated maximum proportion of detections (p_{max}), the distance where detections recorded had reduced to 50% (D_{50}) and 95% (D_{95}) of p_{max} using a three parameter model. $Distance_{50}$ and $Distance_{05}$ represent the distance at which the proportion of detections are 0.5 and 0.05 respectively

Location	Transmitter	p_{max}	D_{50}	$Distance_{50}$	D_{95}	$Distance_{05}$
Jurien	V13-1L	7.06	-244.98	65.46	110.11	351.16
	V13-1H	1.97	-36.66	88.32	297.12	375.68
	V16-6L	1.83	-23.65	58.85	224.94	277.89
Ocean Reef	V7-4L	1.1	94.85	111.46	286.21	295.48
	V9-2L	1.1	133.63	158.33	420.67	434.44
	V9-2H	0.99	144.79	143.42	344.13	343.41
	V13-1H	0.68	360.54	332.54	441.55	430.28
Stragglers Reef	V7-4L	1.1	78.30	88.55	183.95	186.81
	V16-4L	0.88	531.31	514.34	716.65	708.30

For the range tests conducted in Perth (Ocean Reef and Stragglers Reef), the two larger transmitters (V16 and V13) had markedly different detection profiles from the smaller (V7 and V9) transmitters (Figure A.2b-c), with the proportion of detections declining rapidly with increasing distance for the smaller transmitters. In contrast, the distances before a marked reduction in the proportion of detections occurred were considerably further in the high-powered V13 transmitter (ca 200 m) and the even higher-powered V16 (ca 350 m) (Figure A.2b-c).

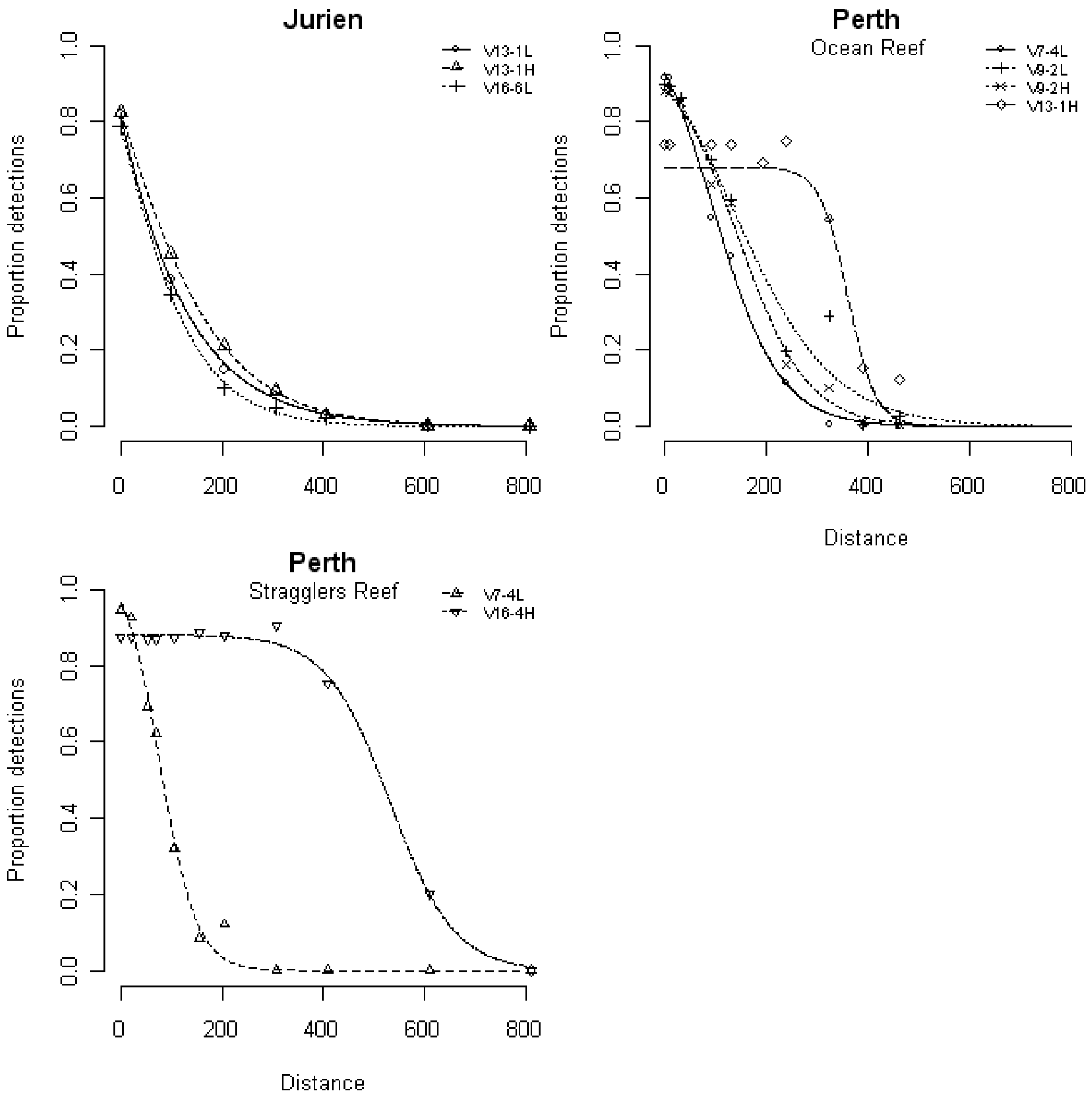

Figure A.2 Mean detection frequency (±SE) and three-parameter sigmoidal modelled attenuation curves for various transmitters used in a) deepwater off Jurien and b) Ocean Reef and c) Stragglers Reef.

Akaike's Information Criterion (AIC) indicated that in six of the nine range tests (68%), the three-parameter sigmoidal model was the most appropriate of all the models tested for describing the declining detection of acoustic signals with increasing distance (Table A.3). Of the other models tested, the two-parameter sigmoidal model was the most appropriate in one comparison and only marginally better (as was the exponential model) than the three-parameter sigmoidal model in another comparison (Table A.3). Given the consistently better performance of the three-parameter sigmoidal model and the fact that examination of residual plots indicated no marked bias, this model was chosen to conduct subsequent comparisons of signal attenuation between each of the various transmitters examined in this study.

Table A.3 AIC values for sigmoidal (2 and 3 parameters), linear, exponential and logarithmic models of acoustic attenuation for the seven tested transmitters type at the three locations. Bold figures indicate the lowest AIC for each transmitter

Location	Transmitter	Sigmoidal (2 parameter) (r)	Sigmoidal (3 parameter) (r)	Linear (r)	Exponential (r)	Logarithmic (r)
Jurien	V13-1L	-587.8	-618.2	38.3	**-619.9**	-309.0
	V13-1H	-547.5	**-567.2**	38.3	-563.1	-151.1
	V16-6L	-840.4	**-849.3**	-34.5	-847.0	-541.7
Ocean Reef	V7-4L	-9834.8	**-10000.5**	-5085.5	-8685.7	7312.2
	V9-2L	-4358.9	**-4461.8**	-3758.1	-3614.4	7803.7
	V9-2H	**-8369.1**	-8368.3	-6223.9	-6521.0	6151.8
	V13-1H	-11.8	**-544.1**	2.4	118.0	492.7
Stragglers Reef	V7-4L	-1851.8	**-1863.8**	420.4	-1541.0	761.9
	V16-4L	**-1801.5**	-988.5	-1187.9	-629.1	761.9

The relationship between of proportion of detections and distance for each of the five transmitters (V7-4L, V9-2L, V9-2H, V13-1H and V16-4H), as described by the three-parameter sigmoidal model, differed significantly from each other (likelihood-ratio test: all $p < 0.05$; Figure A.2b-c). Parameter estimates highlight the variations between transmitter detection profiles (Table A.2). For the Jurien trial, the pmax and D_{50} estimates from modelling do not reflect the performance of the transmitter in a practical sense

(Table A.2). As such, Distance_{50} and Distance_{05} were also calculated, representing distances where the proportions of detections were equal to 0.5 or 0.05, respectively. The greater D^{50} at the Perth location of the two larger (V13 and V16) transmitters demonstrates the greater distance (> 300 m) where there is little reduction in detection of their transmissions. This, however, was not evident in the Jurien trial, with these larger transmitters having an almost immediate decline in detections. The difference in Distance_{50} for the V13-1H trialled at both Jurien and Ocean Reef was 244m greater at Ocean Reef. The Jurien trial only ran for 7 days and may represent a period of poor environmental conditions. The smaller V7 and V9 transmitters contrasted the larger transmitters tested at the same Perth sites, with the D_{50} of 75 – 150 m. The considerably smaller pmax of the V13 reflects its random transmission schedule, where the number of transmissions emitted in an hour period can vary markedly.

Environmental Effects

Considerable variation in detection rates was evident for the reference transmitter at the Abrolhos Islands, and all transmitters in the Ocean Reef range test (Figure A.3). The detection rates for the Ocean Reef receivers were demonstrated for the receiver closest to the transmitter-specific D_{50} (Figure 3). During both the Abrolhos Islands and Ocean Reef trials, the proportion of maximum detections from each transmitter varied dramatically from 100 % signal detection (proportion = 1) to no detections (proportion = 0) with major changes occurring over periods of several hours (Figure A.3). Transmitters used in the Perth trials, with the exception of the V13-1H, all had a set off time (Table A.1) resulting in the same number of transmissions each hour Figure A.3e-g, i,j). Therefore, variation in delay schedules would not cause this variation. For the remaining transmitters where there was a zero distance (Figure A.3a-c,h), maximal differences in transmission schedules would result in a decline of about 0.4. As this is an extreme estimate, with the random delay schedule making this a rare occurrence, changes are likely to reflect environmental rather than signal transmission differences.

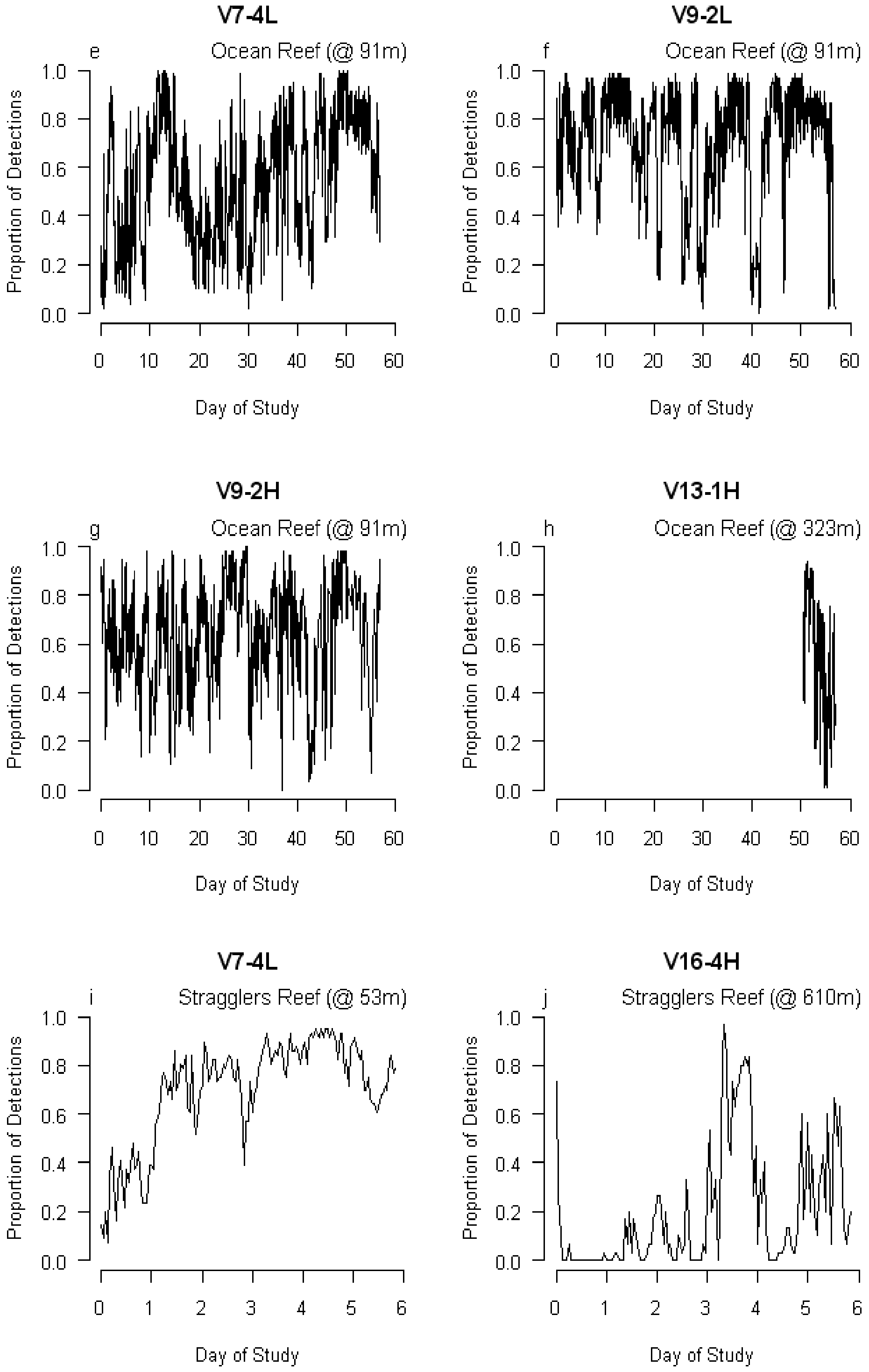

Figure A.3 Variability in hourly detection rates of receivers at specific distance from transmitters for all trials

All four environmental factors measured (i.e. water motion, temperature, lunar phase and time of day) were found to significantly alter signal attenuation at both the Abrolhos Islands and Ocean Reef. The GLM for detections at the Abrolhos Islands revealed highly significant ($p<0.001$) negative effects of increasing water movement and temperature on the proportions of detections (Figure A.4a,b). Time of day and lunar day exhibited a highly significant ($p<0.001$) positive effect, with an increasing proportion of detections with time of day (night to day) and lunar day (new to full moon) (Figure A.4c-d). These same parameters also significantly affected parameters in the three-parameter sigmoidal model for the proportion of detections with distance in the Ocean Reef range test (Table A.4). Relative water movement and temperature both displayed significant inverse relationships with the proportions of detections for all four transmitters (Figure A.4a-b). Conversely, signal detection was significantly positively related to lunar day (from new to full moon) and time of day (from night to day) (Figure A.4c-d). Of the four factors examined, water movement was correlated with the biggest change in signal detection (proportions ranging from about 0.6 to 0.02) at both the Abrolhos Islands and Ocean Reef trials, whereas the other three factors (except temperature at the Abrolhos Islands) were only associated with relatively small changes in the proportions of detections.

Table A.4 *p*-value and sign of the coefficient for the environmental parameters (when significant) in the sigmoidal model of acoustic range (+ = positive, - = inverse relationship with increasing variable value).

Variable	Parameter	V7-4L	V9-2L	V9-2H
Overall	p_{max}	< 0.001 (+)	< 0.001 (+)	< 0.001 (+)
	D_{50}	< 0.001 (+)	< 0.001 (+)	< 0.001 (+)
	D_{95}	< 0.001 (+)	< 0.001 (+)	< 0.001 (+)
Relative Water Movement	p_{max}	0.289	0.195	< 0.001 (+)
	D_{50}	< 0.001 (–)	< 0.001 (–)	< 0.001 (–)
	D_{95}	< 0.001 (–)	< 0.001 (–)	< 0.001 (–)
Water Temperature	p_{max}	0.001 (–)	0.360	0.017 (–)
	D_{50}	0.662	< 0.001 (–)	0.990
	D_{95}	< 0.001 (–)	< 0.001 (–)	< 0.001 (–)
Lunar Phase	p_{max}	0.676	< 0.001 (+)	0.007 (–)
	D_{50}	< 0.001 (+)	0.0826	< 0.001 (+)
	D_{95}	< 0.001 (+)	< 0.001 (+)	0.066
Time of Day	p_{max}	0.965	0.005 (+)	0.330
	D_{50}	< 0.001 (+)	0.272	< 0.001 (+)
	D_{95}	< 0.001 (+)	< 0.001 (+)	< 0.001 (+)

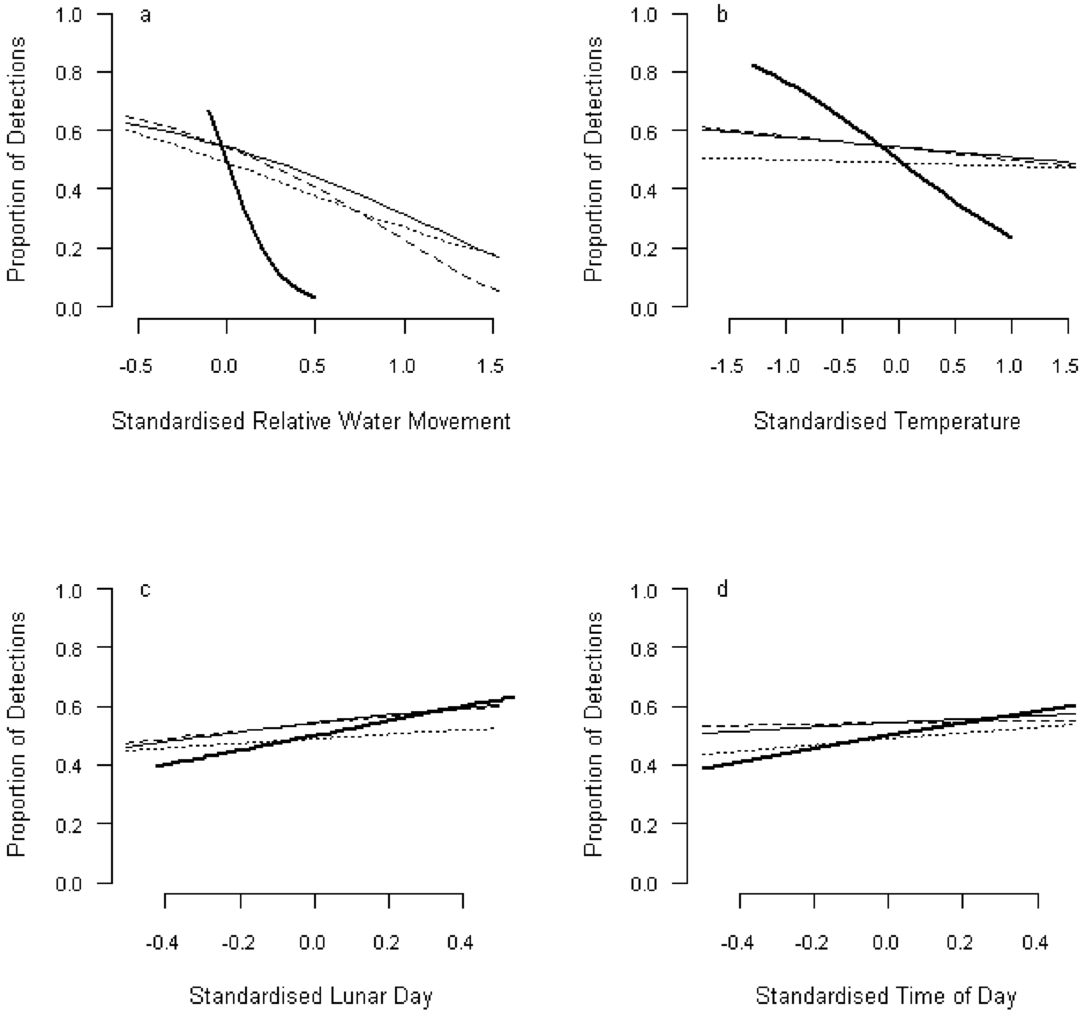

Figure A.4 – Proportion of detections recorded at the distance of 50 % attenuation for the V7-4L (solid line), V9-2L (long dashed line), V9-2H (short dashed line) and Abrolhos Island V13-1L (thick line) for range of standardised (a) relative water movement, (b) temperature, (c) lunar day and (d) time of day.

Positioning Analysis

The results of the positioning analysis provided good estimates of transmitter position, with the closest estimate being only 3 m from the actual transmitter location (Table A.5). However, when information from only one receiver was available, the position estimate could not be accurately estimated (Table A.5 - #5). In this case, eight positions were estimated to have an equal likelihood with the model choosing the first maximal value. Another one of the eight positions was estimated to be 20 m from the position of the transmitter. Due to the large number of potential locations for this time period, and utilising only one receiver, this position estimate was removed from all further analysis.

When range test data compiled prior to the positioning test were used to determine position estimates, the mean error (distance between estimated and actual transmitter position) was 44.0 m ± 8.7 m SE (Table A.5). This was an improvement on previous techniques that yielded a mean error of 56.8 m ± 14.4 m SE (Simpfendorfer *et al.* 2002). Mean error markedly declined when the real-time range test was used, with the mean distance between estimated and actual position more than halving to 20.3 m ± 5.5 m SE (Table A.5). All estimated positions produced using the in-situ range tests were within the 99% confidence regions of the actual position (FigureA.5). For the location that was estimated twice (position estimates 1 and 2), the distance between these two estimates was 17 m. The best position estimate was produced when a minimum of four receivers recorded signals (Table A.5).

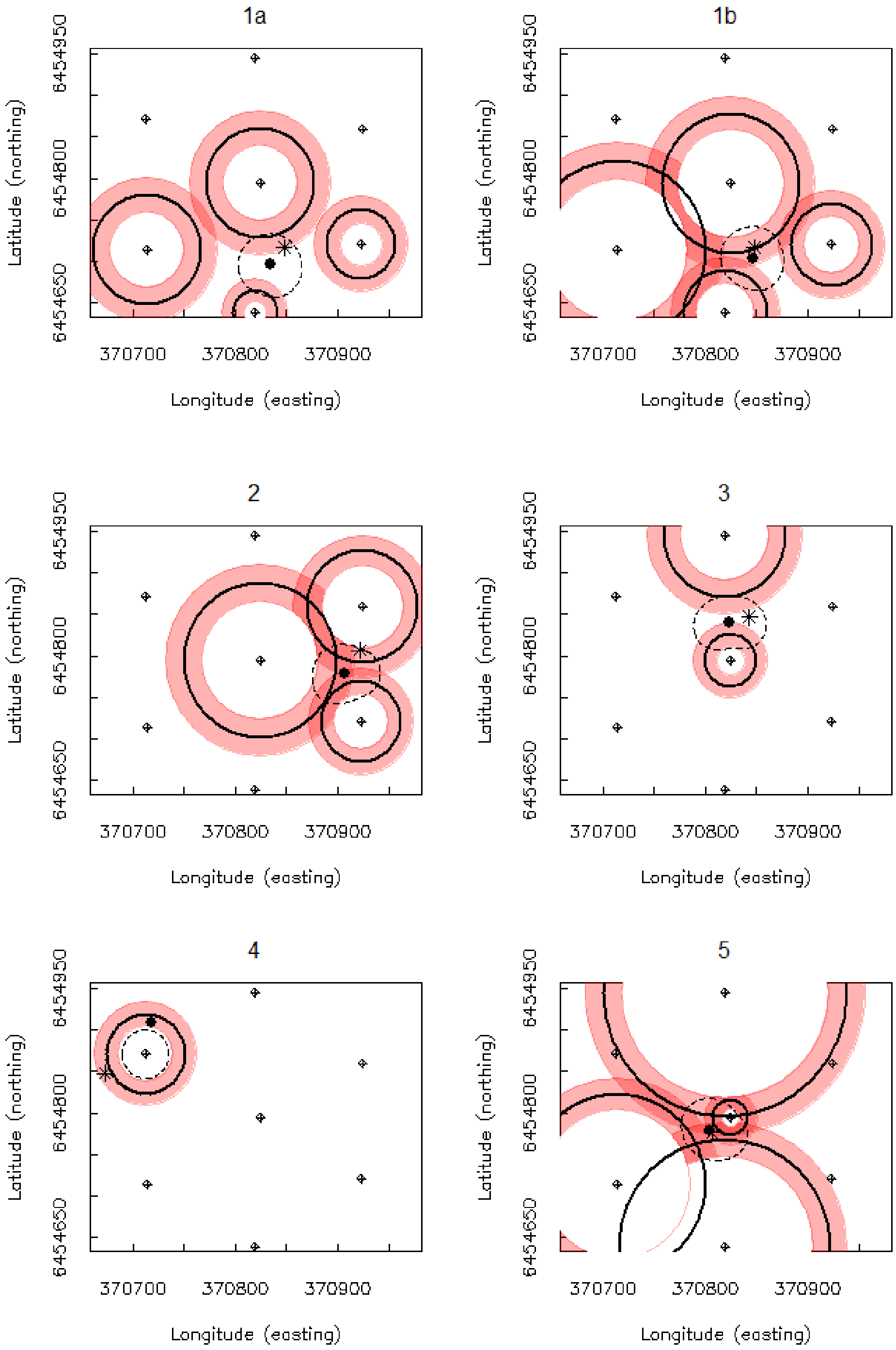

Figure A.5 Estimated location using *in situ* range data (large black dot) compared to the actual location (asterisk) of transmitters within the array of receivers (small black dots). Estimated distances from each receiver (black circle) ± standard deviation (grey circles), with the 99% contour (dotted line) for the six position estimates.

Table A.5 The distance of estimated positions from the actual location of the transmitters and the number of receivers used in the estimate. Estimates were performed using Simpfendorfer *et al.* (2002) position estimation, and estimations from this trial with range data from before and during the positioning trial.

Position estimate	No. receivers	Distance (m) Simpfendorfer *et al.* 2002	Pre test range data	Concurrent range data
1a	4	48.0	48.0	27.0
1b	4	18.0	40.0	13.2
2	3	26.0	70.3	33.8
3	2	113.0	45.8	24.7
4	1	79.0	101.0	83.5
5	4	66.0	16.1	2.8
Mean (±SE)		56.8 (±14.4)	44.0 (±8.7)	20.3 (±5.5)

A.5 Discussion

There were major changes in the detection frequency recorded from transmitters throughout this study. These changes were volatile, varying from the maximum possible detection frequency (prop=1) to no detections (prop=0), often over a short timeframe (hours). This clearly demonstrates that environmental factors affect the detection of acoustic signals and account for the volatility in detection rates experienced in tracking studies (Lembo *et al.* 2002)

Environmental effects

Environmental factors influence signal detection either through introducing noise into the system or by interfering with the signal strength (Urick 1983). While sea condition has been suggested as a potential influence on acoustic transmission range (Lacroix and Voegeli 2000; Lembo *et al.* 2002; Finstad *et al.* 2005), our study empirically confirmed that acoustic detection of a reference transmitter at the Abrolhos Islands and the acoustic range of the three low-powered transmitters at Ocean Reef significantly declined as water motion, such as that caused by swell and wind driven seas, increased. Increased water movement had the greatest effect of the four environmental factors examined. Water movement can have multiple detrimental effects, through air entrapment and suspension of particulate matter, physically reducing signal transmission. Increased water movement can also increase activity of animals (e.g. western rock lobster *Panulirus cygnus*, Srisurichan *et al.* 2005), which in turn can increase acoustic noise, further reducing successful acoustic detection.

Water temperature also affected acoustic detections. Reduced temperatures had higher detection rates at both the Abrolhos Islands and Ocean Reef. This is contrary to acoustic theory, where lower water temperatures reducing signal strength and detection rates through increased absorption at the signal frequency (Winter 1996). Temperature did increase by 2.3oC at the Abrolhos Islands and 3.3oC at Ocean Reef trials in a general linear fashion. The changes in temperature may reflect a trend through the study independent of temperature (e.g. fouling). However, when day of study was substituted in the analysis for temperature, it was not a significant factor. One possible cause is that periods of increased temperature over a shallow sandy bottom at Ocean Reef may have caused a thermocline to establish. Thermoclines can cause signals to bend or refract causing changes (Voegeli and Pincock 1996; Heupel *et al.* 2006) or reductions in acoustic range (Singh *et al.* 2009). However, this does not appear to account for the change at the Abrolhos Islands as this trail was in deep water with strong water movement reducing the possible formation of a thermocline. The increased temperature may also have increased faunal activity, and hence noise, by ectothermic organisms.

The reduced acoustic range at night and around new moons may again reflect increased biological noise in the marine environment at night and over new moon periods. While noise levels were not recorded in this study, Payne *et al.* (2010) found increased biological noise at night. When Radford *et al.* (2008) examined different bandwidths to the frequency of these acoustic transmitters, they found increased biological noise at night and over the new moon. This would explain the reduced acoustic range found for both the Abrolhos Islands and Ocean Reef at night and new moon periods, through increased noise reducing the receiver's ability to detect the signals of the transmitters. Influences of noise on acoustic detection rates are currently being examined.

Transmitter power and detection distance

There was a significant difference in the detection profiles of all transmitters examined in range tests at Jurien, Ocean Reef and Stragglers. Two general detection profiles were apparent, with the smaller transmitters, which declined immediately, contrasting with the larger transmitters that produced signals that covered greater distances before they declined. This contrast was not evident at Jurien where three high-powered transmitters all showed an immediate decline in detections. The marked deviation in detection profiles from similar transmitters tested at Ocean Reef and Stragglers Reef highlights the importance of local testing of acoustic transmitters. The placement of the Jurien range test on a moderate- to high-relief deep-water temperate reef contrasts dramatically with the shallow sand bottom of the two Perth range tests. It is likely that environmental and biological differences between these locations accounted for this major discrepancy in detection profiles. Similarly, while not as dramatic, the significant difference between the two V7-4L transmitters examined, given their same size and power output, is a likely reflection of the different study locations with varying environmental conditions.

As they were tested simultaneously, the variation in detection profiles of low- and high-powered transmitters used in the Perth range tests was a more direct comparison of the impact of power output. Hence, transmitters with different power outputs may be potentially more suitable for different study types. For studies using gates or curtain-designs, higher-powered transmitters enable detection over a greater range than the smaller lower-powered transmitters. They also have a considerable distance over which there is little reduction in the proportion of detections. This enables greater spacing between receivers to achieve a higher area of coverage than if lower-powered transmitters were used, but also a considerable distance over which almost all transmissions are likely to be detected, and hence greater certainty that individuals will be detected by the gate or curtain.

The precipitous decline in the proportion of detections after the considerable period of high detection rates reduces the ability to determine changes in detection rates as a function of distance. In comparison, the more gradual and almost immediate reduction in proportion of detections of the smaller transmitters allows for a greater contrast between distance and proportion of detections, thereby increasing the accuracy of position estimation within an array of receivers. The selection of transmitter type is often a function of the species being studied and is based on the transmitter’s weight not exceeding a percentage of the animal’s weight (i.e. ca 2 % Winter 1996). This often results in the selection of transmitters that are close to that limit to maximise either power output or battery life. Therefore, while a species may be capable of carrying a larger transmitter, if the tracking study’s objective is to provide estimates of movement within a grid acoustic array, a smaller lower-powered transmitter would provide a better position estimate using VR2 receivers. However, if using a VR2W in a Vemco Positioning System (VPS) array, a higher-powered transmitter would be more beneficial to ensure simultaneous detection of a single transmission on multiple receivers.

The variation in detection rates and transmitter type performance between locations highlights the need for longer-term range testing to be performed at the intended study site, encompassing the range of environmental conditions likely to be encountered during the study to ensure appropriate receiver placement (Voegeli *et al.* 1998; Lacroix and Voegeli 2000; Lacroix *et al.* 2005). The long-term recording of detection rates demonstrated considerable variation in the detection frequency recorded for a given distance. While some of the potential environmental impacts on this have been demonstrated, it is important to consider this when inferring animal behaviour based on the detection frequency present at particular periods of time, either lunar or diel. The significant lunar and diel effects shown can lead to reduced or elevated detection rates and hence may cause temporal changes in an individual’s detection rate that are a reflection of changes in detection range as opposed to any behavioural or movement pattern (Payne *et al.* 2010). The incorporation of a reference transmitter, which increased precision of position estimates within a grid array, has also been shown to be beneficial in a gate study. This allows interpretation of detections from tagged animals to be compared with those of the fixed transmitter, which provides data on the variation in detection range under different environmental conditions.

Model for acoustic detection

Lembo *et al.* (2002) and Simpfendorfer *et al.* (2002; 2008) have shown a decline in acoustic detections with increasing distance, but described the relationship as linear.

Despite variable power outputs and study locations, all transmitter detection profiles analysed during this study were best described by a sigmoidal model. A sigmoidal model was also considered the most parsimonious of five models examined by Melnychuk and Walter (2010) from estimated detection probabilities of migrating fish through fixed receiver stations. The direct testing of the range attributes of the seven transmitters types with different outputs empirically confirms the results of Melnychuk and Walter (2010).

Positioning test

The position estimate trial that was conducted utilised the detection properties of a low powered transmitter (V7-4L), which provides a good detection accuracy within a grid array. This was due to the greater resolution of the relationship between the proportion of detections and distance. This technique utilised the activity centre approach of Simpfendorfer *et al.* (2002), but incorporated the sigmoidal detection profile of the specific transmitters as opposed to the linear model used by Simpfendorfer *et al.* (2002), providing increased accuracy in position estimates. Due to variation in the detection range, the use of the initial detection profile generated over a five-day period did not accurately reflect the acoustic conditions at the time of the positioning test. However, a reference transmitter within the array enabled better predictions of the transmitter locations, as it represented the acoustic condition at the time of estimation.

The Vemco Positioning System (VPS) is a new technique utilising independent automated receivers to provide a fine-scale position estimate using differential times of arrival. VPS requires detection of the same transmission on three or more synchronised receivers to provide an instantaneous position estimate with conservative estimates of 95 % of positions within a 15-m error circle (Vemco 2008). Position estimates generated from the positioning analysis in this study were, on average, within 20 m of the actual location of the transmitter and all estimates were within the 99 % confidence bounds for position estimates with more than one receiver. The slightly higher error in the average position and the fact that a one-hour time period is used, as opposed to instantaneous position estimates using the VPS method, is mitigated by the technique used in this study as it does not require detections of the same transmission, and can provide good estimates of position with only two receivers. However, this study had only a small sample size of position estimates.

This study has demonstrated the detection profiles of a range of transmitters with different power outputs and their potential benefits to different tracking studies. It is the first to empirically demonstrate the effects of relative water motion (sea state) on the detection rate of acoustic signals. Similarly, the diel, lunar and thermal effects on transmission performance also highlight the need to consider detection rates before making any behavioural inferences based on relative numbers of detections for a given time period without the use of a reference transmitter. The long-term range test at Ocean Reef and reference transmitter tests at the Abrolhos Islands totalling almost four months were considerably longer than previous published acoustic detection trials and highlights volatility in detection rates throughout the study. It demonstrates the need for reference or sentinel transmitters in tracking studies to aid in quantifying detection probabilities, which is critical for gate-type studies (Melnychuk 2009) and, as shown in this project, position estimates.

Finally, the methodological issues highlighted in this study were combined to produce a modified activity centre approach (Simpfendorfer *et al.* 2002) for estimating an individual’s location within a grid array. Utilising a small transmitter and a sigmoidal model of detection decline with distance increased resolution of the position estimate while accounting for changes in environmental conditions using an in situ range tests. Initial results demonstrated an average accuracy and precision of around 20 m.

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