Spatial variation in female reproduction for an exploited coral reef fish, *Plectropomus leopardus*.

Thesis submitted by

Alex B Carter

B.Sc., Grad.Dip. James Cook University

in August 2015

for the degree of Doctor of Philosophy

Centre for Sustainable Tropical Fisheries and Aquaculture

and College of Marine and Environmental Sciences

James Cook University

Townsville, Australia

STATEMENT OF ACCESS

I, the undersigned author of this work, understand that James Cook University will make this thesis available within the University Library, and via the Australian Digital Theses network, for use elsewhere. I declare that the electronic copy of this thesis provided to the James Cook University Library is an accurate copy of the print thesis submitted, within the limits of the technology available.

I understand that, as an unpublished work, a thesis has significant protection under the Copyright Act and;

All users consulting this thesis must agree not to copy or closely paraphrase it in whole or in part without the written consent of the author; and to make proper public written acknowledgement for any assistance which they obtain from it. They must also agree to obtain prior written consent from the author before use or distribution of all or part of this thesis within 12 months of its award by James Cook University.

Beyond this, I do not wish to place any restrictions on access to this thesis.

10 August, 2015

Alex B. Carter

STATEMENT OF SOURCES

DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

10 August, 2015

Alex B. Carter

STATEMENT ON THE CONTRIBUTION OF OTHERS

Research funding

James Cook University Postgraduate Scholarship (stipend for 2.5 years)	$50 000
GBRMPA Science for Management Award	$3160
Graduate Research School, James Cook University	$4500
College of Marine and Environmental Sciences, James Cook University	$5400
PADI Project Aware	$2000

In-kind support

Centre for Sustainable Tropical Fisheries and Aquaculture, James Cook University

Centre for Tropical Water and Aquatic Ecosystem Research, James Cook University

Research support

The Effects of Line Fishing (ELF) Experiment was funded by the Cooperative Research Centre for the Great Barrier Reef Word Heritage Area from 1995 – 2005. Gonad samples and length and age data collected as part of the ELF Experiment used in Chapters 2, 3, 4 and 6 were provided by Bruce Mapstone and Campbell Davies. Dong Chun Lou processed the otoliths and estimated age for most of the individual fish in Chapters 2, 4 and 5. Sue Reilly made histological sections of *P. leopardus* gonads for Chapter 5. Field assistance was provided by Elio Parenti and Andrew Tobin (collecting samples); Guy Carton, Leanne Currey, Ann Penny, Margaret Carter and Shane Brown (processing samples); and Kylie Dodds and Rob Dodds (logistical support on Orpheus Island Research Station). Mike Emslie (Australian Institute of Marine Science) provided data on *P. leopardus* density and length along the Great Barrier Reef used in Chapter 6.

Use of infrastructure external to James Cook University

Egg lipid analysis (Chapter 5) was conducted by Heidi Pethybridge at CSIRO Hobart laboratories, Tasmania, Australia.

Supervisory committee

Dr. Ashley J Williams, Secretariat of the Pacific Community, Noumea, New Caledonia
Dr. Andrew J Tobin, Centre for Sustainable Tropical Fisheries and Aquaculture, and College of Marine and Environmental Sciences, James Cook University
Professor Garry R Russ, ARC Centre of Excellence for Coral Reef Studies, and College of Marine and Environmental Sciences, James Cook University
Dr. Alexander G Carton, Centre for Sustainable Tropical Fisheries and Aquaculture, and College of Marine and Environmental Sciences, James Cook University
Professor Mark I McCormick, ARC Centre of Excellence for Coral Reef Studies, and College of Marine and Environmental Sciences, James Cook University

Statistical and analytical support

Dr. Ashley Williams, Dr. Bruce Mapstone (CSIRO), Dr. Carole Wright (Queensland Department of Agriculture and Fisheries), Prof. Rhondda Jones (James Cook University), Dr. Susan Jacups (James Cook University), Dr. Mike Emslie (Australian Institute of Marine Science).

Editorial support

Dr. Ashley Williams, Dr. Andrew Tobin, Professor Garry Russ, Dr. Guy Carton, Professor Mark McCormick, Dr. Campbell Davies, Dr. Bruce Mapstone, Dr. Mike Emslie.

Provision of spatial data

Chapters 1, 2, 5 and 6 incorporate spatial data that are the copyright Commonwealth of Australia (Great Barrier Reef Marine Park Authority, 2009).

PERMITS AND ETHICS

Field sampling for Chapter 5 was conducted under Great Barrier Reef Marine Park Authority Permits (no. G08/27773.1; G09/31877.1) and Queensland Department of Agriculture and Fisheries Permits (no. 100986; 129165).

The collection and use of all samples from the Effects of Line Fishing Experiment (Chapters 2 - 4 and 6) were conducted under permits granted to James Cook University by the Great Barrier Reef Marine Park Authority (Permits G97/092, G98/292, and G00/346) and Queensland Department of Agriculture and Fisheries (formerly Department of Primary Industries and Fisheries; Permit no. PRM00083C, PRM00399I, PRM018751, PRM04950F, and PRM38291D).

The research presented and reported in this thesis was conducted in compliance with the National Health and Medical Research Council Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 7th Edition, 2004 and the Qld Animal Care and Protection Act, 2001. The proposed research study received animal ethics approval from the James Cook University Animal Ethics Committee Approval (no. A625_00, A1214, and A1292).

PUBLICATIONS ARISING FROM THIS THESIS

Carter AB, Carton AG, McCormick MI, Tobin AJ and Williams AJ (2015). Maternal size, not age, influences egg quality of a wild, protogynous coral reef fish, *Plectropomus leopardus.* Marine Ecology Progress Series **529**:249 – 263. doi:10.3354/meps11277.

Carter AB, Russ GR, Tobin AJ, Williams AJ, Davies CR and Mapstone BD (2014) Spatial variation in the effects of size and age on reproductive dynamics of common coral trout *Plectropomus leopardus*. Journal of Fish Biology **84**:1074 – 1098. doi: 10.1111/jfb.12346

Carter AB, Davies CR, Mapstone BD, Russ GR, Tobin AJ and Williams AJ (2014) Effects of region, demography, and protection from fishing on batch fecundity of common coral trout (*Plectropomus leopardus*). Coral Reefs **33**:751 – 763. doi: 10.1007/s00338-014-1164-z

Carter AB, Williams AJ and Russ GR (2009). Increased accuracy of batch fecundity estimates using oocyte stage ratios in *Plectropomus leopardus.* Journal of Fish Biology **75**:716 – 722. doi:10.1111/j.1095-8649.2009.02313.x

Carter AB, Davies CR, Emslie M, Mapstone BD, Russ GR, Tobin AJ and Williams AJ (*in prep*) Reproductive benefits of marine reserves vary with region for an exploited coral reef fish.

CONFERENCE AND WORKSHOP PRESENTATIONS

Carter AB, Russ GR, Tobin AJ and Williams AJ (2013) Spatial variation in reproductive dynamics of common coral trout on the Great Barrier Reef. *Plectropomus* Workshop, Townsville QLD, Australia. June 2013.

Carter AB, Mapstone BD, Russ GR, Tobin AJ and Williams AJ (2012) Reproductive benefits of no-take reserves vary latitudinally for *Plectropomus leopardus*. International Coral Reef Symposium, Cairns QLD, Australia. July 2012.

Carter AB, Carton AG, Russ GR and Tobin AJ (2011) Significant variation in spawning frequency of common coral trout, *Plectropomus leopardus*, on the Great Barrier Reef. Australian Marine Sciences Association annual conference, Fremantle WA, Australia. July 2011.

Carter AB, Mapstone BD, Russ GR, Tobin AJ and Williams AJ (2009) Effects of no-take zones and region on reproductive output of the common coral trout, *Plectropomus leopardus*. Australian National Network in Marine Science inaugural conference, Hobart TAS, Australia. December 2009.

Carter AB, Williams AJ, Russ GR and Tobin AJ (2009) The effect of no-take zoning, region and year on reproductive output of the common coral trout, *Plectropomus leopardus*. International Marine Conservation Congress/2nd International Marine Protected Areas Congress, Washington D.C, USA. May 2009.

ACKNOWLEDGEMENTS

I start by thanking my wonderful supervisors. Ashley Williams, for his unwavering support, patience and kindness throughout this journey, terrifyingly prompt replies to emails even though he was often god-knows-where in the middle of the Pacific, and for pushing me into the world of statistics. Andrew Tobin, for always challenging me with alternative arguments, his unique and valuable perspective as an academic and as a commercial fisherman, and his skills in catching coral trout. Garry Russ, for his support, excitement at every thesis installment, and vast knowledge on fisheries and marine protected areas. Guy Carton and Mark McCormick, for their formidable knowledge on all things egg quality.

This thesis would not have been possible without access to samples collected during the epic Effects of Line Fishing (ELF) Experiment (1995 – 2004). Financial support for the ELF Experiment was supplied by the CRC Reef Research Centre, the Fisheries Research and Development Corporation, and Great Barrier Reef Marine Park Authority. A research program of this scale is unlikely to be repeated on the Great Barrier Reef and I am indebted to Bruce Mapstone and Campbell Davies for instigating the program, allowing me to use the samples collected, and providing valuable comments on ELF-related thesis chapters.

Thanks go to the many organizations and individuals who offered their time and expertise to make this thesis possible. To the folk at the Centre for Sustainable Tropical Fisheries and Aquaculture for ongoing support. To the Long Term Monitoring Project crew at the Northern Fisheries Centre for giving me firsthand experience of the coral trout fishery on the line fishing surveys. To Mike Emslie from the Australian Institute of Marine Science who provided the underwater visual survey data essential for egg production calculations. To everyone who helped with fieldwork: Kylie and Rob Dodds at Orpheus Island Research Station; Guy Carton; my fishermen Elio Parenti and Andrew Tobin; Allison Paley, Ashley Frisch, Adam Reynolds, and Lyle Squire for valuable advice; and to the family and friends who volunteered their time to help out: Leanne Currey, Ann Penny, Margaret Carter, Shane Brown, Renae Tobin and Kara

Dew. Thanks to those who provided access to their labs, facilities and expertise: Sue Reilly, Dr Dongchun Lou, Jamie Seymour, John Morrison, Heidi Pethybridge, and Peter Nichols. Funding was provided through a James Cook University Postgraduate Scholarship, and grants from the Great Barrier Reef Marine Park Authority, PADI Project Aware, and James Cook University's Graduate Research School and College of Marine and Environmental Sciences.

It would be impossible to endure the mental marathon of a PhD without the welcome distraction of good people. Lauren Beim, thank you for the sisterly love and FaceTime study breaks from the other side of the world. Cameron Carter went beyond the call of brotherly duty to fix my (many) computer meltdowns. A special shout out to Dusty and Jules Steer for weekend distractions, and Leanne Currey for keeping me motivated on the final slog. Thanks to all of the TropWATER seagrass crew, particularly Jessie Jarvis for having as unhealthy an appreciation for a beautiful residual plot as I do, Rob Coles for valuable thesis chats, and Skye McKenna for putting up with my non-seagrass-related rants. Thanks also to the wonderful Townsville folk - Darren Coker, Allison Paley, Ann Penny, Owen Li, Robin Palmer, Jill and Ian McAllister - for your friendship, meals, and a place to sleep. It was a truly ridiculous decision to have two babies during the course of my PhD studies, and I am indebted to Margaret Carter, David Carter and Diane Gilks for all their help with the kids.

Finally, the hugest thank you to Shane, Harry, Jack and Ari Safari, for their love, support and (sometimes begrudging) patience; this thesis is dedicated to you.

Commercial line fisherman holding his *Plectropomus leopardus* catch on the Great Barrier Reef. Photo: A. Carter.

ABSTRACT

No–take marine reserves have the potential to benefit fished species via the net export of adult fish (spillover) and/or eggs and larvae (recruitment subsidy) of target species from protected to fished areas. Recruitment subsidy from reserves is expected because fisheries generally remove the Big Old Fat Fecund Females (BOFFFs) selectively from a population. BOFFFs are expected to contribute disproportionately to reproductive output of populations by producing relatively better quality eggs, in greater quantities, compared with smaller and younger females. Quantifying the relative contribution of reserves to egg production is a crucial step in evaluating the potential for recruitment subsidy to occur.

It is important when assessing any effect of reserves that appropriate spatial scales are used whereby, ideally, multiple reserves within the same ecosystem are assessed. This is important in egg production per unit area (EPUA) calculations because the reproductive dynamics of species that occur across broad spatial ranges often vary at a regional scale due to variation in environmental conditions. Such variation may complicate fisheries management if reserves created with the objective of enhancing EPUA of the target species are placed in areas where reproductive output is naturally limited, or where high adult fish densities represent larval sinks, not larval sources. Furthermore, comparisons of EPUA between fished and reserve reefs may be confounded where reproductive data are collected only from fished areas, thereby ignoring potential fishing-related effects such as density-dependent reproduction, Allee-type depensation, altered spawning behaviour, and skewed sex ratios for hermaphroditic species.

In this thesis, I present extensive information on the reproductive biology of the common coral trout *Plectropomus leopardus* [Lacépède 1802], on Australia's Great Barrier Reef (GBR). *P. leopardus* is a valuable food fish in the tropics and the major target species of both recreational and commercial line fisheries on the GBR. The species occurs throughout the Great Barrier Reef Marine Park (GBRMP) across 14° of latitude and is a protogynous hermaphrodite. The objectives of this thesis were to: determine the relationships between maternal traits of *P. leopardus* and egg quantity and quality; compare these relationships at an appropriate spatial scale; test for the existence of BOFFFs; and estimate EPUA for *P. leopardus* along the GBR. The GBRMP is an excellent study site as the extensive network of no-take reserves offers access to comparable fished and reserve (protected) reefs. The vast area also allows environmental and fishing pressure influences to be tested on a regional scale.

In Chapter 2, I quantify the effects of length and age on the sex ratio, proportion of vitellogenic females, and spawning frequency of *P. leopardus* on fished and reserve reefs in the northern, central and southern GBR. In Chapter 3, I outline a new method that uses the ratio of the number of migratory nuclei to the number of hydrated oocytes to estimate batch fecundity of *P. leopardus.* Previous estimates of batch fecundity were biased due to a small sample size, reliant on sampling the few hours prior to dusk spawning when the maximum number of hydrated oocytes is present in the ovary. My new method increases the time during the day over which samples can be collected and, therefore, increases the sample size available and reduces biases in batch fecundity estimates. This method was used in Chapter 4 to quantify the relationships between length, weight and age with batch fecundity of female *P. leopardus* on fished and reserve reefs in the northern, central and southern GBR. Maternal effects also are widely known to influence egg quality, and

therefore the early life history traits of offspring. In Chapter 5, I examined the maternal effects of length, weight, age, and hepatosomatic index with indicators of egg quality (egg size, oil droplet size, total lipid content and lipid classes) for *P. leopardus*. Chapter 6 combined a 10-year length-density data set with reproductive data from Chapters 2 – 4 of this thesis to quantify EPUA of *P. leopardus* in fished and reserve reefs in the northern, central and southern GBR.

Despite the broad distribution of *P. leopardus* along the GBR, reproductive potential of females was maximized in the central section, particularly in the absence of fishing. Length, weight, and age had positive effects on batch fecundity of females from northern and central reefs but negligible effects on spawners from southern reefs. The proportion of vitellogenic females increased with length and age, as did the proportion of males in the population (as expected for a protogyne). However, female-male sex change occurred at smaller sizes and younger ages in the southern GBR, particularly on fished reefs. *P. leopardus* spawned most frequently on central reserve reefs (every 2.3 days during the spawning season) and as infrequently as once every 2 – 3 months in the southern GBR regardless of reserve status. No effect of length on spawning frequency was detected. Maternal length and/or weight also had positive effects on indicators of egg quality, including egg size, oil droplet size, and the proportion of long-term storage lipid wax esters within the egg. Large female *P. leopardus* in the central GBR, therefore, generally produce more eggs, and eggs with enhanced provisioning, providing a potential advantage for larvae during the critical transition to successful exogenous feeding.

Male biased sex ratios and low individual female fecundity in the southern GBR limited the reproductive benefits expected from higher population densities or larger fish in reserves. Egg production per unit area was greatest on reserve reefs in the central GBR where on average an estimated ~800 000 oocytes 250 m^{-2} $year^{-1}$ were produced, and lowest on southern GBR reefs where average EPUA was approximately ~30 000 oocytes 250 m^{-2} $year^{-1}$. The effect of reef protection on EPUA was inconsistent among regions: EPUA was 180% greater on reserve than fished reefs at Townsville and Cairns in the central GBR and 16% greater on reserve than fished reefs in the southern GBR, but 88% greater on fished reefs in the northern GBR. Individual fecundity had a consistent, positive effect on reef EPUA and was greatest on reserve reefs in the central GBR (>1.7 million oocytes $female^{-1}$ $year^{-1}$), and much reduced in the southern GBR (~9 000 oocytes $female^{-1}$ $year^{-1}$). I hypothesize that regional variation in reproduction is most likely driven by water temperature. The presence of BOFFFs in the northern and central GBR meant that EPUA increased with density at a much steeper rate than in the southern GBR, despite greater densities of *P. leopardus* in the south. Fork length had a positive effect on EPUA until fish reached ~400 mm *FL*, at which size EPUA became asymptotic (northern and central GBR) or decreased slightly (southern GBR).

The spatial variation in reproductive biology of *P. leopardus* identified in this thesis demonstrates the varied outcomes that reserve reefs can have on EPUA when the distribution of a target species spans a broad geographic range. The presence of BOFFFs on central GBR reserve reefs may have important implications for recruitment subsidy at a regional scale (north-to-south recruitment subsidy) as well as the local scale (reserve-to-fished reef recruitment subsidy). Male bias and lack of spawning activity on southern GBR reefs, where densities of adult *P. leopardus* are highest,

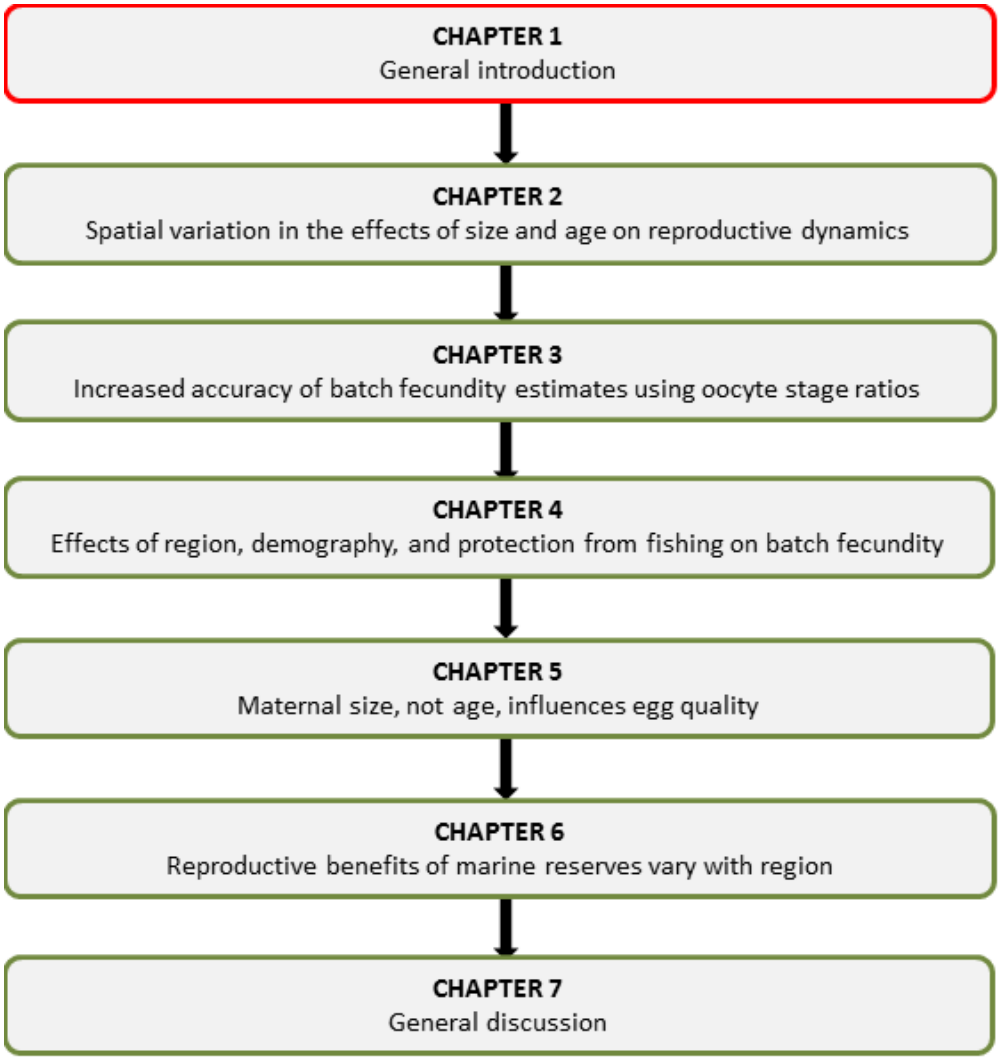
suggests recruits may be supplied from the central GBR. This thesis highlights the need for further research on reproductive responses of target species to fishing at appropriate spatial scales, particularly hermaphroditic species, and careful consideration of the suitability of single conservation or fishery management strategies for species distributed across large and diverse spatial scales.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1 General Introduction



1.1 Marine reserves and Big Old Fat Fecund Females

Marine fisheries are under pressure from over-harvest by an increasing human population and improved fishing technology, which has seen the scale and intensity of fishing grow exponentially in the last century (Jackson et al. 2001, Pauly et al. 2002, Pauly 2009, Froese et al. 2012, Watson et al. 2013). Overfishing has led to a number of fisheries collapses worldwide, particularly where large, long-lived, predatory fishes have been targeted (Hughes 1994, Jackson et al. 2001, Pauly et al. 2002). Fishery collapses have mainly been attributed to recruitment overfishing, where fishing reduces the spawning stock biomass to the extent that recruitment to the stock also is significantly reduced (Beverton 1983, Myers et al. 1994). Not surprisingly, a major objective of fisheries management is the prevention of recruitment overfishing by maintaining the size of the spawning stock to ensure replacement occurs (Myers and Mertz 1998). This is mostly done through establishment of minimum legal sizes, corresponding to size of maturation or above.

No-take marine reserves, referred to simply as reserves throughout this thesis, have become a popular tool worldwide for the conservation of biodiversity and, potentially, fisheries management (Russ 2002, Edgar et al. 2014). Reserves theoretically could benefit fished species via net export of adult fish (spillover) or eggs and larvae (recruitment subsidy), provided reserves result in significantly higher biomass of target species in reserves than fished areas (Russ 2002, Sale et al. 2005) (Figure 1.1). Debate continues about the extent and circumstances under which such export may compensate for fish catch lost due to reduction of available fishing area (Hilborn et al. 2004, Buxton et al. 2014, Fletcher et al. 2015). Spillover has been demonstrated where reserves contain

higher densities of target species than adjacent fished areas (Abesamis and Russ 2005, Stobart et al. 2009, Halpern et al. 2010). This causes density-dependent (from space limitation or territorial interactions) and/or density-independent (diffusion from high to low density areas, or random/seasonal/daily/ontogenetic movements) export of fish from within to outside the reserve (Russ 2002, Abesamis and Russ 2005). Spillover benefits tend to be localized and detectable only on scales of tens of metres to a few kilometres beyond reserve boundaries (Halpern et al. 2010). Fisheries-related benefits from spillover also are particularly limited for species that are either sedentary (Little et al. 2009) or highly mobile post-settlement (Le Quesne and Codling 2009).

Recruitment subsidy has a greater potential effect on fish catch well beyond reserve boundaries because pelagic eggs and larvae can disperse tens to hundreds of kilometres (Cudney-Bueno et al. 2009, Jones et al. 2009, Harrison et al. 2012). Contemporary research has demonstrated that reserves can contribute recruits to fished areas (Harrison et al. 2012, Almany et al. 2013), filling a critical knowledge gap in the science of reserves (Sale et al. 2005, Graham et al. 2011). Reserves contribute more recruits per unit area to the fishery than fished areas because egg production per unit area (EPUA) is generally greater inside reserves, often by one or two orders of magnitude (Branch and Odendaal 2003, Willis et al. 2003, Babcock et al. 2007, Evans et al. 2008).This is driven by higher densities of target species and the preservation of Big Old Fat Fecund Females (BOFFFs) (Hixon et al. 2014).

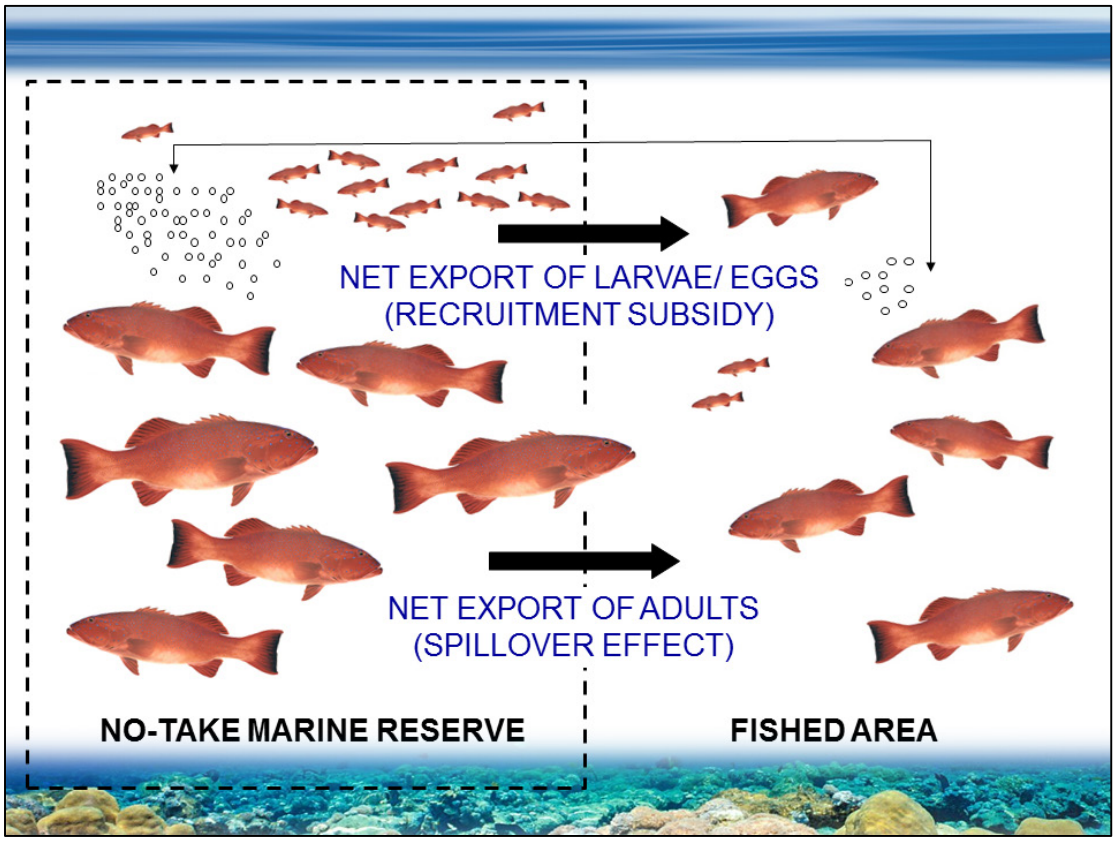


Figure 1.1 No-take marine reserves as a fisheries management tool where reserves act as net exporters of adult fishes via the spillover effect, and eggs and larvae via recruitment subsidy. Recruitment subsidy due to greater densities of Big Old Fat Fecund Females (BOFFFs) leads to greater egg production per unit area of target species in reserves relative to fished areas (figure adapted from Russ 2002).

Central to the BOFFF hypothesis is the assumption that the largest and oldest females produce more eggs and better quality eggs compared with smaller and younger conspecifics (Berkeley et al. 2004a, Green 2008, Hixon et al. 2014). There are a number of pathways for BOFFFs to achieve this, including spawning larger batches (Sadovy 1996, Lambert 2008), spawning more frequently (Lowerre-Barbieri et al. 2009, Farley et al. 2013), spawning over a longer time period (Claramunt et al. 2007), or spawning high quality eggs with a greater chance of larval survival (Berkeley et al. 2004a, Green 2008). BOFFFs generally don't occur, or occur in lower proportions, in fished areas because fishing frequently causes reductions in the mean size of individuals (Hawkins and Roberts 2004). Fishing may also reduce size at sex change (Hamilton et al. 2007), and lead to dramatically skewed sex ratios of hermaphroditic species (Heppell et al. 2006). Fishing-induced age or size truncation of a population can therefore result in a far greater reduction in that population's reproductive potential than just a reduction in mature female biomass (Berkeley et al. 2004b, Venturelli et al. 2009, Hixon et al. 2014). The majority of studies on maternal effects on egg quantity and quality focus on temperate fishes, many of which are gonochores (see reviews by Wiegand 1996, Green 2008, Venturelli et al. 2009). Where protogyny (female-male transition) is common in tropical fisheries (Jennings and Kaiser 1998) the applicability of BOFFF theory to such fisheries is uncertain. In these fisheries, older, larger and potentially more fecund females are "removed" by fishing, and by fishing-induced female-male sex change at younger sizes and ages as a compensatory response following the loss of large males (Hawkins and Roberts 2004, Hamilton et al. 2007, Götz et al. 2008, Chan et al. 2012). These changes ensure that females in targeted protogynous populations remain at the lowest levels of fecundity (Sadovy 1996, Jennings and Kaiser 1998).

Empirical studies comparing EPUA between reserves and fished areas are rare for tropical fishes (although see Sluka et al., Evans et al. 2008). The majority of EPUA studies have focused on invertebrates (Babcock et al. 2007, Díaz et al. 2011) or temperate fishes (Paddack and Estes 2000, Willis et al. 2003). Calculations of EPUA often combine density data (commonly size frequency distributions of target species collected within and outside of a reserve) with a size-fecundity relationship. However, a limiting concern is that the size-fecundity relationship often is developed from individuals collected only in fished areas (Willis et al. 2003, Denny et al. 2004, Evans et al. 2008). This methodology is often necessary because of the difficulty in obtaining permits for destructive sampling within reserves. Inaccurate estimates of EPUA are likely if fishing-induced changes in reproductive traits occur. These changes potentially include skewed sex ratios for protogynous populations (Hawkins and Roberts 2004), reproductive compensation (Koslow et al. 1995), Allee-type depensation (Sadovy 2001), and altered spawning behaviour (Muñoz et al. 2010).

Assessing the effects of reserves at appropriate spatial scales often requires evaluating multiple reserves within the same ecosystem (Graham et al. 2011, Smith et al. 2014). Regional variation in reproductive dynamics of fishes that have a broad geographic range are rarely considered in EPUA calculations, despite correlations among reproductive dynamics and environmental conditions or fishing pressure (McIntyre and Hutchings 2003, Kokita 2004, Wakefield et al. 2013). Spatial variation in reproductive dynamics could potentially confound fisheries management if reserves created to enhance target species EPUA are in areas where reproductive output is naturally limited, or where high adult fish densities represent larval sinks and not larval sources.

Accurate comparisons of EPUA between fished and reserve areas require reproductive data collected within and outside of reserve areas, and at an appropriate spatial scale to encompass potential regional variation.

1.2 The common coral trout, *Plectropomus leopardus*

Plectropomus leopardus [Lacépède 1802] are distributed throughout Indo-Pacific coral reefs and are a valuable food fish in the tropics (Heemstra and Randall 1993). *P. leopardus* also hold a significant trophic role as a major predator of reef fish (Goeden 1978, Graham et al. 2003). *P. leopardus* are found along Australia's Great Barrier Reef (GBR) and are the major species targeted by recreational, charter, and commercial line fishers in the State of Queensland's Coral Reef Finfish Fishery (CRFF) (Mapstone et al. 2008). Commercial fishers in the GBR and in Southeast Asia, particularly the Philippines, target *P. leopardus* for the lucrative live fish export market, with live fish sent predominantly to Hong Kong and mainland China (Sadovy de Mitcheson et al. 2013). The IUCN listed *P. leopardus* as "near threatened" in 2004 due to population declines in various countries, primarily from overfishing (Cornish and Kiwi 2004).

The Queensland *P. leopardus* fishery is subjected to two regimes of management. The fishery is indirectly managed at a federal level because it occurs within the Great Barrier Reef Marine Park (GBRMP, proclaimed under the *Great Barrier Reef Marine Park Act* 1975) and the Great Barrier Reef World Heritage Area (GBRWHA, established in 1981). The GBRMP is managed for conservation of biological diversity under a multiple use zoning plan most recently amended in 2004 when no-take marine reserves were increased from ~5% to ~33% of the GBRMP (Fernandes et al. 2005) (Figure 1.2). Within areas of the GBRMP zoned open to fishing, the fishery is managed

at a state level by Queensland's Department of Agriculture and Fisheries by a number of mechanisms including bag limits for recreational and charter fishers, a minimum retention size limit of 380 mm (total length), limited commercial entry to the fishery, and Individual Transferrable Quotas (ITQs) operating under a Total Allowable Commercial Catch (TACC). Two 5-day closures to all fishing during peak *P. leopardus* spawning times around new moons in the Austral spring are also implemented.

The stock status of coral trout on the GBR was downgraded in 2012 from "sustainably fished" to "uncertain" following several consecutive years of declines in catch, with catch in 2011 – 12 the lowest level since 1988 – 89 (State of Queensland 2013). A recent stock assessment found that there was a poor correlation between catch rates and population size (as determined by underwater visual surveys). It concluded recent catch declines were due to a significant effect of tropical cyclones resulting in depressed *P. leopardus* catch rates, and not from overfishing (Leigh et al. 2014). Fishing pressure for *P. leopardus* varies regionally on the GBR, with the bulk of commercial effort occurring south of approximately 18º S where densities of *P. leopardus* are greatest (Mapstone et al. 2004, Miller et al. 2012, Tobin et al. 2013). Recreational fishing concentrates in the central GBR (~16 – 20º S) where reefs are most accessible and closer to major population centres. Little commercial or recreational fishing pressure occurs north of approximately 16º S due to the northern region's isolation from major population centres and relative inaccessibility (Mapstone et al. 2004), although there is a dedicated commercial fishery further north in Torres Strait (Williams et al. 2008).

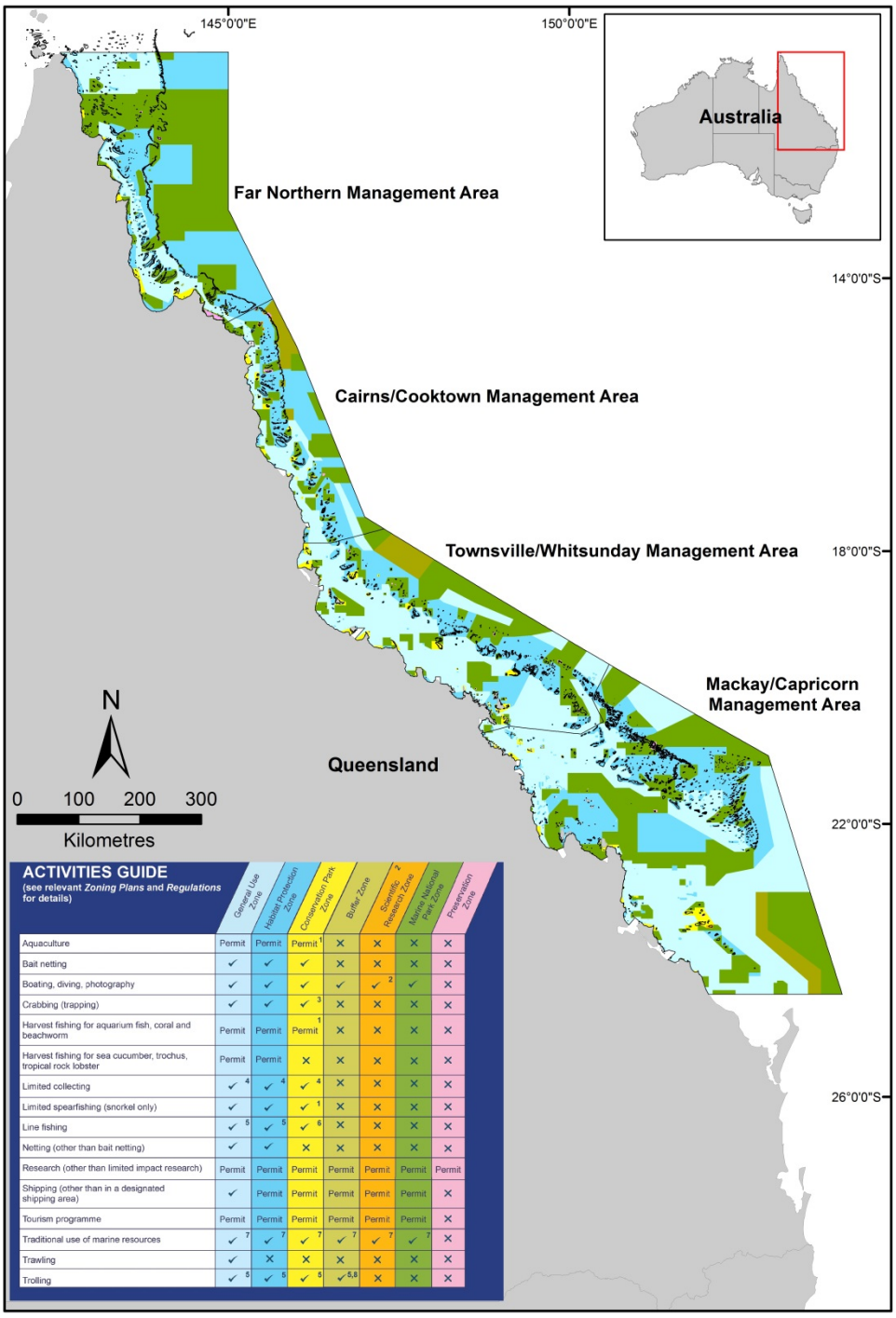


Figure 1.2 The Great Barrier Reef Marine Park (GBRMP) Zoning Plan and activities guide. Black line: GBRMP Management Area. Zoning and reef spatial layers courtesy of Great Barrier Reef Marine Park Authority © Commonwealth of Australia 2009. Green zones are the major no-take reserves.

Like most Epinepheline serranids, *P. leopardus* have several characteristics which make them vulnerable to overfishing, including protogynous hermaphroditism (Heemstra and Randall 1993) and the propensity to aggregate for spawning (Samoilys and Squire 1994, Zeller 1998, Cornish and Kiwi 2004). Underwater visual survey data from inshore and offshore reefs along a 1000 km north-south gradient covering the central and southern GBR regions demonstrated that density of coral trout (*Plectropomus* spp.) is greater on reserve reefs than on fished reefs (Miller et al. 2012, Emslie et al. 2015) though other data indicate that population densities were similar on reserve and fished reefs in the northern GBR (Mapstone et al. 2004). Fishing pressure is greatest in the central and southern GBR, corresponding to the areas where reserve-fished reef differences are most evident, where reserves also are characterized by larger adults (Mapstone et al. 2004). Like many serranids, *P. leopardus* rarely move between reefs post-settlement (Davies 1995, Zeller et al. 2003, Matley et al. 2015). Any potential reproductive benefit provided by reserves into fished areas is, therefore, more likely to result from recruitment subsidy than spillover of adults.

Since Goeden's seminal *A Monograph of the Coral Trout* (Goeden 1978), much research has been conducted on *P. leopardus*, a testament to the species' importance and iconic status on the GBR. This was particularly so from 1995 to 2004 when the Effects of Line Fishing (ELF) Experiment was conducted on the GBR. The ELF Experiment was a broad-scale, manipulative experiment which assessed the distribution and intensity of fishing catch and effort, patterns in relative abundance of fish, responses of target species to fishing, and evaluated the efficacy of reserves for several important target species including *P. leopardus* (Mapstone et al. 2004). A recurring theme that emerges from *P. leopardus* research is significant regional variation in the biology of the species

irrespective of reserve status. For example, southern reefs have higher densities of under- and legal-sized *P. leopardus* (Mapstone et al. 2004), smaller average size (Mapstone et al. 2004), male-biased sex ratios (Adams et al. 2000, Davies et al. 2006), and significantly different otolith chemistry (Bergenius et al. 2005) compared with *P. leopardus* from reefs in the central and northern GBR. Despite this, *P. leopardus* currently is managed as a single biological stock on the GBR (Leigh et al. 2014). As recently as 2014 when the first stock assessment of *P. leopardus* was undertaken, no region-specific biological information was incorporated into the assessment despite the recognition by the authors that regional variation in life history traits for this species occurs (Leigh et al. 2014). Spatial variation in reproductive dynamics among regions and between fished and reserve reefs, particularly dynamics that influence the quantity and quality of eggs produced, have not been evaluated for *P. leopardus.* This information will be highly relevant to future stock assessments, including the appropriateness of managing *P. leopardus* as a single stock on the GBR, and the role reserves may play in recruitment subsidy for this species.

1.3 Thesis objectives

The goal of this thesis is to contribute the critical information on the reproductive biology of *P. leopardus* required to effectively manage the fishery at the GBRMP scale. This goal includes determining the relationships between maternal traits and egg quantity and quality, comparing these relationships at an appropriate spatial scale (incorporating fished and reserve reefs among the northern, central and southern GBR), testing for the existence of *P. leopardus* BOFFFs, and estimating the EPUA for *P. leopardus* along the GBR. To achieve this, my specific objectives were to:

1. Quantify the effects of size and age on sex ratios and female sexual maturity of *P. leopardus* among regions (northern, central and southern GBR), and between management zones (reefs open to fishing and reserves) on the GBR (Chapter 2);
2. Quantify the maternal effects of size and age on the quantity of eggs produced by *P. leopardus* (spawning frequency, batch fecundity) among regions and between management zones on the GBR (Chapters 2, 3, 4);
3. Quantify the maternal effects of size, age and body condition on measures of *P. leopardus* egg quality (Chapter 5);
4. Estimate and compare the EPUA for *P. leopardus* among regions and between management zones on the GBR (Chapter 6).

1.4 Thesis outline

This thesis has been written in a format to enable publication of each data chapter (Chapters 2 – 6) in a peer reviewed journal. Figure 1.3 outlines the structure of the thesis. This diagram will be repeated at the start of each chapter to orientate the reader to where in the framework of the thesis they are located. I was lead author on all publications arising from this thesis. Varying members of my thesis committee (Andrew J Tobin, Ashley J Williams, Garry R Russ, Mark I McCormick and A Guy Carton) were co-authors on Chapters 2 – 6. Co-authorship of Chapters 2, 4 and 6 included two researchers instrumental in the development and success of the Effects of Line Fishing Experiment and custodians of the *P. leopardus* samples used in these chapters (Bruce D Mapstone and Campbell R Davies). Co-authorship of Chapter 6 also included Mike J Emslie from the Australian Institute of Marine Science (AIMS) who provided the *P. leopardus* density and size data collected as part of the AIMS long term monitoring project. I created all of the figures and tables included in this thesis. The Great Barrier Reef Marine Park Authority

(GBRMPA) provided spatial information as GIS layers that are included in several maps and that were used in the analysis in Chapter 6. GBRMPA have been acknowledged in the figure captions of the relevant maps.

In CHAPTER 1, I provide a general introduction to the concept of managing fisheries for reproductive output, the expected benefits of reserves including recruitment subsidy, the role of BOFFFs, the study species and study region. I also present the key research objectives and thesis outline. I wrote the chapter and AJT, AJW and GRR assisted with editing.

In CHAPTER 2, I quantify the effects of size and age on the sex ratio, proportion of mature females, and spawning frequency of populations of *P. leopardus* on reserve and fished reefs in the northern, central, and southern GBR. Achieving this required a large number of fish. These were collected as part of the much larger and broader Effects of Line Fishing (ELF) Experiment. The methods section in Chapter 2 outlines the sampling design and techniques used during the ELF Experiment which also apply to Chapters 3, 4 and 6. Chapter 2 also outlines general laboratory methods used throughout this thesis including histological processing, reproductive staging, and otolith processing and age determination. This chapter was published in *Journal of Fish Biology* in 2014 with Andrew J Tobin, Ashley J Williams, Garry R Russ, Bruce D Mapstone and Campbell R Davies as co-authors. BDM and CRD conceived and designed the ELF Experiment from which samples were sourced. I analyzed the histological sections and collated the data. I analyzed the data and interpreted the models with assistance from AJW. I wrote the chapter. AJT, AJW, GRR, BDM and CRD assisted with editing.

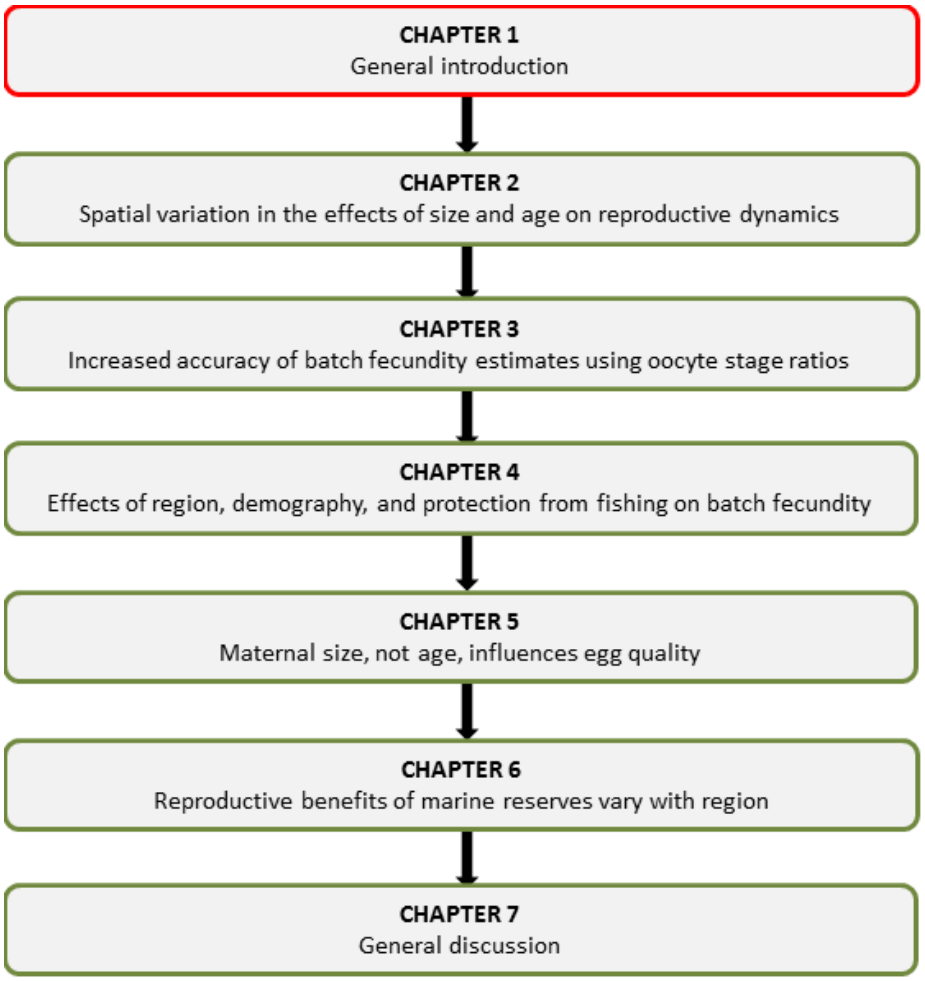


Figure 1.3 Diagram of the thesis structure. This diagram will be repeated at the beginning of each chapter to indicate to the reader where in the thesis they are located. The position of the chapter within the thesis is indicated by a red outline.

In CHAPTER 3, I outline a method that I developed to more accurately estimate batch fecundity of *P. leopardus*. This method uses the ratio of migratory nucleus: hydrated stage oocytes and was applied to batch fecundity estimates that form the basis of the analysis in Chapter 4. This chapter was published in *Journal of Fish Biology* in 2009 with Ashley J Williams and Garry R Russ as co-authors. I conceived and designed the experiment, analyzed the data and wrote the chapter. AJW and GRR assisted with editing.

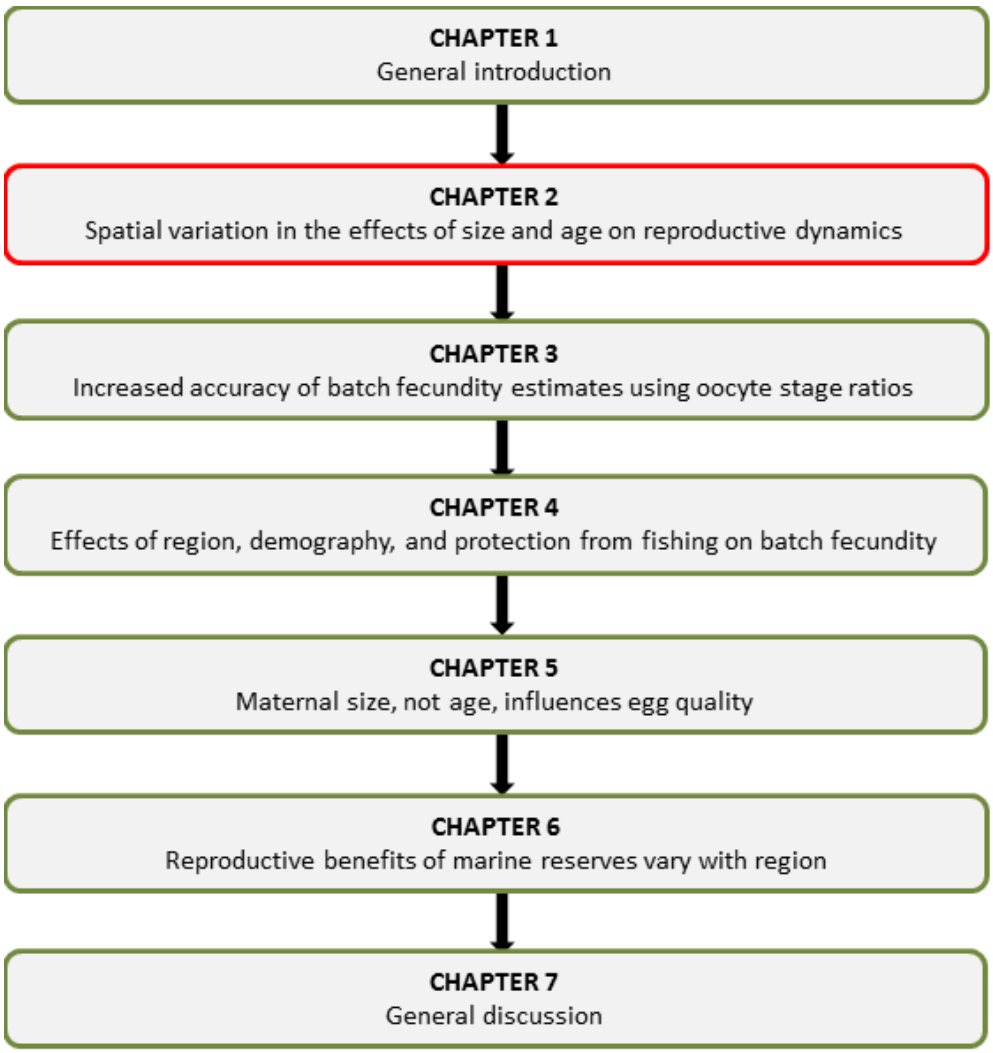
In CHAPTER 4, I quantify the effects of *P. leopardus* size and age on batch fecundity of populations on reserve and fished reefs in the northern, central and southern GBR. This chapter was published in *Coral Reefs* in 2014 with Andrew J Tobin, Ashley J Williams, Garry R Russ, Bruce D Mapstone and Campbell R Davies as co-authors. BDM and CRD conceived and designed the ELF Experiment from which samples were sourced. I analyzed the samples and collated the data. I analyzed the data and interpreted the models with assistance from AJW. I wrote the chapter. AJT, AJW, GRR, BDM and CRD assisted with editing.

In CHAPTER 5, I quantify the effects of *P. leopardus* size, age and hepatosomatic index on indicators of egg quality (egg size, oil droplet size, total lipid content and lipid classes) of populations on reefs in the central GBR. This chapter was published in *Marine Ecology Progress Series* with Andrew J Tobin, Ashley J Williams, Mark I McCormick and A Guy Carton as co-authors. I conceived and designed the experiment with assistance from AGC. I collected the *P. leopardus* and egg samples with assistance from AGC and AJT. I collected the egg size and oil droplet size data and CSIRO (Hobart, Tasmania) conducted the lipid analysis. I collated and analyzed the data. AJW assisted with model interpretation. I wrote the chapter. AJT, AJW, MIM and AGC assisted with editing.

In CHAPTER 6, I quantify the EPUA for *P. leopardus* on reserve and fished reefs in the northern, central and southern GBR. I used data detailed in Chapters 2 and 4 and *P. leopardus* density and size data collected during the AIMS long term monitoring program. This chapter will be submitted for publication in a peer reviewed journal with Andrew J Tobin, Ashley J Williams, Garry R Russ, Bruce D Mapstone, Campbell R Davies and Mike J Emslie as co-authors. BDM and CRD conceived and designed the ELF Experiment from which reproductive samples were sourced. MJE provided the *P. leopardus* density and size data used in the analysis. I collated and analyzed the data. BDM, MJE and AJW assisted with interpretation of the models. I wrote the chapter. AJT, AJW, GRR, BDM, CRD and MJE assisted with editing.

In CHAPTER 7, I summarize Chapters 1 – 6 and discuss the implications of my findings on BOFFF theory, reproductive benefits of reserves, potential recruitment subsidy, and management of the *P. leopardus* fishery. I also evaluate the approach taken in my thesis and outline future research directions. I wrote the chapter and AJW, GRR and AJT assisted with editing.

CHAPTER 2 Spatial variation in the effects of size and age on reproductive dynamics



Chapter 2 has been published as: Carter AB, Russ GR, Tobin AJ, Williams AJ, Davies CR and Mapstone BD (2014) Spatial variation in the effects of size and age on reproductive dynamics of common coral trout *Plectropomus leopardus*. Journal of Fish Biology **84**:1074 – 1098. doi: 10.1111/jfb.12346

2.1 Introduction

Sustainably managed fisheries require the maintenance of sufficient reproductive adults and egg production (Sadovy 1996). The importance of incorporating assessment of reproductive biology into the management of fish stocks is now recognized widely (Jakobsen et al. 2009, Murua et al. 2010, Bernal et al. 2012), requiring increased study of the reproductive biology of fish (Lowerre-Barbieri et al. 2011). A proliferation of studies has emerged in the past decade focused on reproductive biology topics such as maturity schedules, sex ratios, fecundity, and spawning frequency to reveal species-specific variation driven, for example, by female size and age, variation in climate and region (Adams et al. 2000, Portner et al. 2001, Fennessy and Sadovy 2002, Williams et al. 2006), disturbance from fishing (Muñoz et al. 2010), and food availability and body condition (Ganias 2009, Somarakis et al. 2012). Understanding the factors that influence the reproductive output of a population and their interaction with fishing and management measures is important for the design of fisheries management tools, such as seasonal fishing closures, reserves and minimum sizes of retention.

Size and age influence reproductive traits of fishes in a fairly predictable way. Sex ratios of hermaphroditic fishes vary predictably with size and age as individuals change from one sex to the other (Williams et al. 2006). Maturity schedules also vary with size and age, with the proportion of females that are reproductively active often increasing with female size and age as energy balances shift from somatic growth to reproduction (Fennessy and Sadovy 2002, Shuter et al. 2005). Larger and older females also generally spawn more frequently than smaller, younger females (Claramunt et al. 2007, Lowerre-Barbieri et al. 2009). Less predictable is the way reproductive traits of

individual species vary across geographic scales and respond to fishing pressure. Sex ratios and maturity schedules have been demonstrated to vary spatially (Adams et al. 2000, Williams et al. 2006) and will determine the number of mature females and spawning frequency at the local or regional scale. Spawning frequency of tropical fishes has become increasingly well quantified (Dadzie and Abou-Seedo 2008, Maki Jenkins and McBride 2009, van der Velde et al. 2010, Ganias 2012) but documented cases of spatial variation in spawning frequency for tropical species remains limited (although see Brown-Peterson et al.). Spatial variation in spawning frequency of temperate marine fishes has received particular attention and has been attributed to variations in water temperature and fish health (Korta et al. 2010, Somarakis et al. 2012).

As discussed in Chapter 1, regional variation in several life history and population traits have been documented for *Plectropomus leopardus*, with the southern GBR characterised by male-biased sex ratios (Adams et al. 2000), smaller mean length and age at sex change (Davies et al. 2006), smaller mean size (Mapstone et al. 2004), and higher densities (Mapstone et al. 2004) than in the northern GBR. As protogynous hermaphrodites, *P. leopardus* can exhibit diandric male development (Adams 2002), which is particularly prevalent in the southern GBR where the largest proportions of primary males are found (Adams et al. 2000). As also discussed in Chapter 1, *P. leopardus* on reserve reefs generally are older and larger, change sex when older and larger, and have higher densities than on reefs open to fishing (Ferreira and Russ 1995, Adams et al. 2000, Adams 2002, Mapstone et al. 2004, Begg et al. 2005). The maintenance of larger and older female *P. leopardus* within reserves may result in enhanced reproductive output, assuming there is a positive effect of female size and age on spawning frequency. Two previous studies estimated that *P. leopardus* spawn every

2 – 3 days during the Austral spring spawning season (Brown et al. 1994, Samoilys 2000) and determined that there was no relationship between maternal size and spawning frequency (Brown et al. 1994). These estimates were based on fish collected from one small area (two adjacent reefs near Cairns, ~17° S), however, and did not examine the relationship between age and spawning frequency, nor whether reserves affect spawning frequency. Evaluation of the effects of regional variation and reserves on the reproductive dynamics of *P. leopardus* is particularly important considering *P. leopardus* is currently managed as a single stock on the GBR, with the implicit assumption that reproductive productivity does not vary spatially throughout the GBR or that the stock is well-mixed during reproduction and does not result in regional variation in productivity.

The objective of this chapter was to examine the reproductive dynamics of *P. leopardus* to determine whether sex ratio, female maturity, and spawning frequency varied by female size and age, region, and between reserve reefs and fished reefs on the GBR.

2.2 Methods

Several of the methods described in this section are common to many of the data chapters within this thesis (Table 2.1). Common methods are therefore described in detail in this section, while the methods sections of Chapters 3 – 6 refer back to this section.

Table 2.1 Methods common to multiple chapters in this thesis.

Chapter	Method
2, 3, 4, 6	Sample collection (ELF Experiment samples only)
2, 4, 5, 6	Otolith extraction and age estimation
2, 3, 4, 5, 6	Histology and reproductive staging
2, 6	Spawning frequency
3, 4, 6	Batch fecundity*

*Described in detail in Chapters 3 and 4.

2.2.1 Sample collection

P. leopardus were collected over a four year period during structured commercial line fishing surveys from 1998 to 2001 as part of the Effects of Line Fishing (ELF) Experiment (see Mapstone et al. 2004). As outlined in Chapter 1, the ELF Experiment was a large adaptive management program that quantified the effects of fishing on reef fish populations, including *P. leopardus* (Punt et al., Mapstone et al. 2004). Four regions were sampled along a latitudinal gradient during the ELF Experiment: Lizard Island (~14.5º S) in the northern GBR; Townsville (~18.5º S) in the central GBR; and Mackay (~20.5º S) and Storm Cay (~21.5º S) in the southern GBR (Figure 2.1). Four reefs were sampled within each region: Two reefs that were zoned Marine National Park (no-take reserve reefs) and protected from fishing for 12 – 15 years prior to sampling and remained closed to fishing for the duration of the years sampled; and two reefs that were zoned General Use (fished reefs) and historically open to commercial and recreational fishing, although one of these reefs was closed to fishing for the four years of sampling and the other was closed to fishing from March 2000 onwards (Figure 2.1). These historically fished reefs were considered to be fished reefs for the purposes of the analyses because the period of closure to fishing was very short (< 2 – 4

years) relative to the longevity of *P. leopardus* (up to 16 years) and it was assumed that any response of spawning frequency to changes in fishing intensity or population density would be relatively slow. The same effort, gear, and sampling design were used on each reef in each year (Mapstone et al. 2004).

Fish were caught during the first quarter – full moon or full moon – third quarter moon phases (Geoscience Australia 2011) of the Austral spring spawning season (September – December) in each of the four years. *P. leopardus* spawn throughout the complete lunar cycle, although spawning activity peaks during new moon periods when spawning aggregations averaging 44 individuals 1000 m^{-2} form at the reef edge (Samoilys and Squire 1994, Samoilys 1997, Samoilys 2000). Sampling during the new moon was avoided in the ELF Experiment to reduce potential bias in abundance indices if large spawning aggregations were encountered. The presumption was made that the effects on reproductive metrics examined were not influenced significantly by lunar phase, within the spawning season, and that any relative differences among regions or zones were consistent regardless of whether sampling occurred during or outside new moon periods.

For each fish caught, fork length (L_F) was measured (to nearest mm), time of capture was recorded, and whole gonads and otoliths were removed. Gonads were preserved in 10% phosphate buffered formalin until histological sections were made. Otoliths were used to estimate fish age by counting annuli in sectioned otoliths using the method described by Ferreira and Russ (1994).

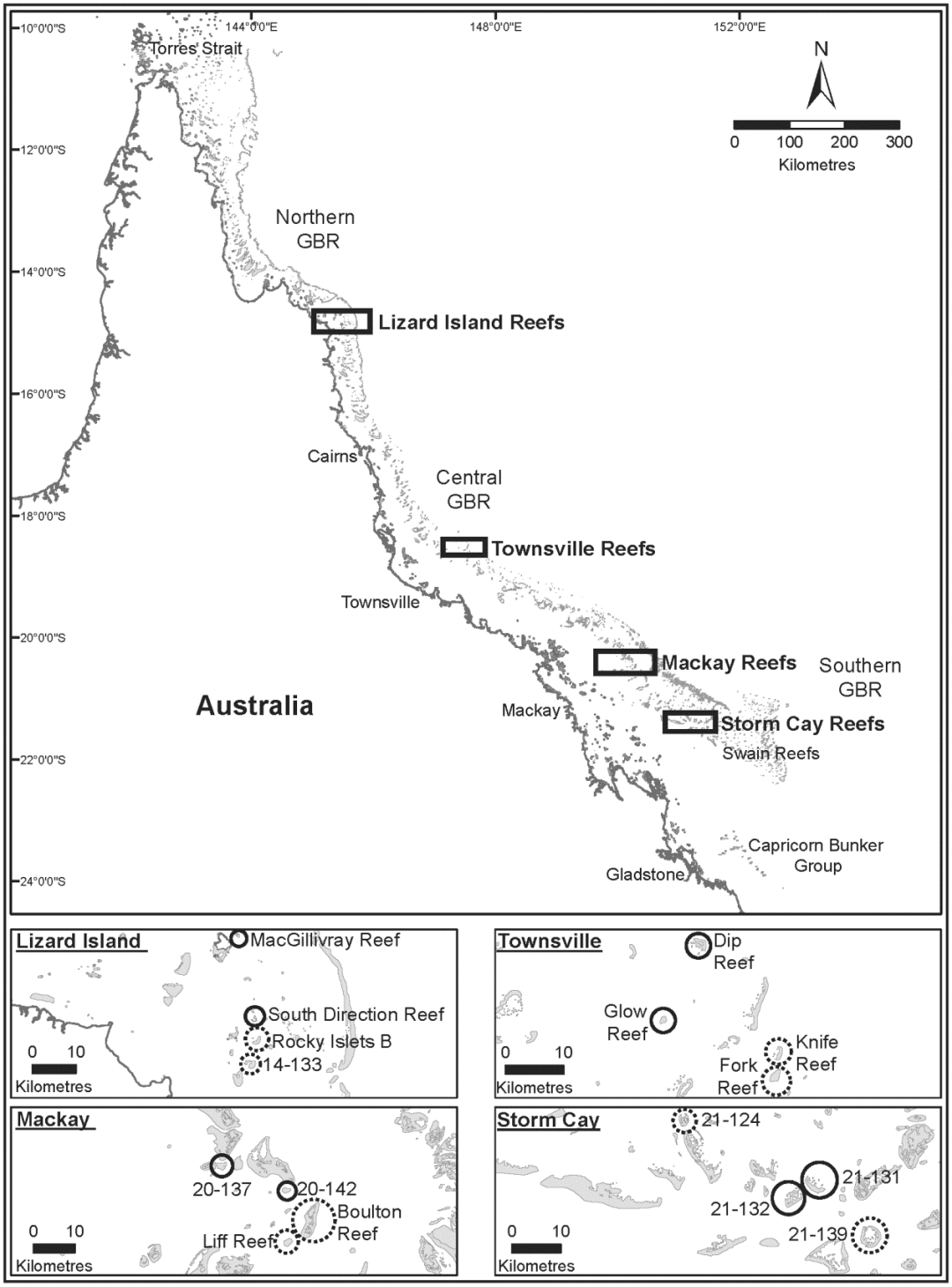


Figure 2.1 *Plectropomus leopardus* were sampled from four reef clusters on the Great Barrier Reef. Inset maps: reserve reefs sampled (O) and reefs historically open to fishing sampled (⁘). Reef spatial layer courtesy of Great Barrier Reef Marine Park Authority © Commonwealth of Australia 2009.

2.2.2 Histological processing

Sex was determined histologically from the preserved gonads of 5901 *P. leopardus*. Gonad sections were embedded in paraffin wax and sectioned at 5 μm then stained using Myer's haematoxylin and Young's eosin-erythrosin (Bean et al. 2003). Histological assessment was conducted on the medial section of one gonad lobe because in *P. leopardus* oocyte development does not differ between right and left lobes (Samoilys and Roelofs, 2000) and is uniform along the length of the lobe (Adams et al. 2000).

Females, males, transitionals (dorsal sperm sinus not fully formed, spermatic tissue present in a gonad that consists largely of pre-vitellogenic ovarian tissue, ovarian tissue sometimes degenerating) and bisexuals (male and female germinal tissue present with no evidence of either tissue degenerating - dorsal sperm sinus fully developed and filled with spermatozoa while gonad also resembles a running ripe female with vitellogenic and hydrated oocytes, indicates female and male cells are capable of spawning at the same time) were categorised according to the criteria in Samoilys and Roelofs (2000), Adams (2002) and Brown-Peterson et al. (2011). Transitionals made up a very small portion of the catch (< 0.5%), probably because sex change commonly occurs after the spring spawning season. Transitionals ranged from 1 – 7 years old, with the largest proportion of transitionals aged 3 – 4. This was consistent among all regions. Sex ratios (P_M, proportion male) were calculated from the number of mature males divided by the sum of females and males (n = 5288). Females were further classified histologically into reproductive phases (immature, developing, spawning capable, regressing, regenerating) according to Brown-Peterson et al. (2011). The proportion of vitellogenic, reproductively active females (developing + spawning capable +

regressing) (P_V) relative to the total mature female population (developing + spawning capable + regressing + regenerating) (n = 2162) was then calculated in each region and management zone (fished or reserve) combination.

2.2.3 Spawning frequency

Spawning frequency was determined histologically using the postovulatory follicle (POF) method (Hunter and Goldberg 1980, Hunter and Macewicz 1985). Postovulatory follicles encase developing eggs and remain in the ovary as ruptured follicles after hydrated eggs are ovulated (Figure 2.2). Postovulatory follicles degenerate quickly, lasting 24 hours in *P. leopardus* (Samoilys 2000), a common timeframe for POFs in the tropics (West 1990). The predictability of POF absorption means that they can be used reliably to back-calculate time of spawning (Ganias 2012).

Postovulatory follicles were classified following the descriptions of Samoilys and Roelofs (2000). Presence or absence of POFs, and the stage of POF (early "0-Day", or late "1-Day"), was determined from 1676 randomly subsampled mature (vitellogenic) ovaries (Table 2.2). The spawning fraction (P_S, proportion of mature females that were spawning per day, hereafter proportion of spawners) was calculated as the total number of mature females with 1-Day POFs present divided by the total number of mature females (Picquelle and Stauffer 1985). These calculations only use the incidence of females with 1-Day POFs. Using the incidence of females with hydrated oocytes or 0-Day POFs is inappropriate because the presence of these structures within the ovary is dependent on time of sampling (Hunter and Macewicz 1980) and because females who are actively spawning may be more susceptible to fishing (Picquelle and Stauffer 1985). In this study, 1-Day POFs were found throughout the day (0730 – 1800). *P. leopardus*

spawn at dusk (Samoilys 2000) and sampling occurred during daylight hours, so the presence of 1-Day POFs in the ovary indicated spawning occurred approximately 12 to 24 hours prior to sampling. Spawning frequency for the season was calculated as the days elapsed between spawns, or 1/ P_S (Claramunt et al. 2007). The number of batches spawned per season by each mature female was calculated by dividing the length of the Austral spring spawning season for *P. leopardus*, commonly reported as lasting four months (122 days) on the GBR (Goeden 1978, Brown et al. 1994, Ferreira 1995, Samoilys 2000, Davies et al. 2006) by spawning frequency (days).

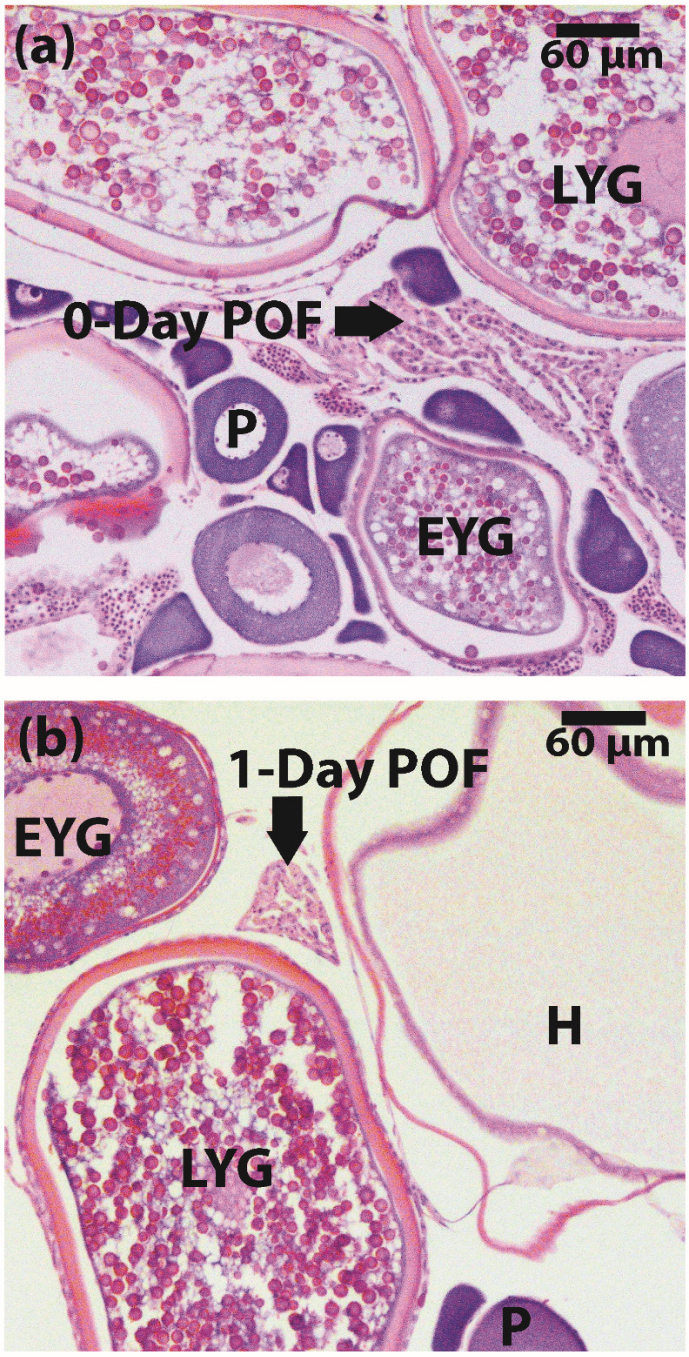


Figure 2.2 *Plectropomus leopardus* postovulatory follicle (POF) stages used to identify recent spawners. (a) 0-Day POF is large with no signs of degeneration. Follicle cell layers are cord-like and convoluted and form tight folds; (b) 1-Day POF. Photographs: A. Carter.

Table 2.2 Numbers of *Plectropomus leopardus* sampled by region and management zone (fished and reserve). Females collected from four regions comprising two management zones. Spawners (1-Day postovulatory follicles, POF, present); non-spawners (1-Day POFs absent), spawning frequency and number of batches spawned per season assuming a 4 month spawning season are given.

				Mature females				
Region	Zone	Month	Lunar quarter sampled	Spawners	Non-spawners	Total mature females	Spawning frequency (days)	Batches spawned per season
Lizard Island	Fished	October	First quarter - full moon	134	263	397	3.2	38.4
	Reserve	October	First quarter - full moon	22	209	231	12.0	10.1
Townsville	Fished	October – November	Full moon - last quarter	32	138	170	5.3	22.9
	Reserve	October – November	Full moon - last quarter	200	278	478	2.3	52.0
Mackay	Fished	November	First quarter - last quarter	0	72	72	na.	na.
	Reserve	October – November	First quarter	2	160	162	83.2	1.5
Storm Cay	Fished	November – December	Full moon - last quarter	6	88	94	17.2	7.1
	Reserve	November – December	Full moon - last quarter	3	92	95	28.8	4.2
Total			***N***	**399**	**1300**	**1699**	**4.3**	**23.0**

na., not applicable due to absence of spawners in fished reefs.

2.2.4 Data analysis

Generalized linear mixed-effects models (GLMM) were used to examine the fixed effects of fork length (L_F), age (A), region (R) and management zone (Z), and the random effect of reef, on sex ratio (P_M), proportion of vitellogenic females (P_V), and spawning fraction (P_S). Each factor was modelled as an additive term and as an interaction with other factors. Three-way interactions between R, Z, and L_F or A were considered in the analyses. The factor reef was modelled as a random effect term in all models to eliminate potential bias or psuedoreplication resulting from the non-independence of samples collected at the same time from a single location. The response variables P_M, P_V and P_S were all modelled with a binomial error distribution and logit link function. Akaike's Information Criterion (AICc) for small sample sizes (Burnham and Anderson 2002) was used to determine the best set of explanatory factors for adequately predicting each response variable, and to compare functional forms for the relationship between factors and response variables. The best-fit model was considered to be the simplest model within two of the lowest AICc (Burnham and Anderson 2002). The best-fit model was used as a basis to predict the expected values of response variables across the observed ranges in L_F and A. All GLMMs were done in R using the *lme4* package (Bates et al. 2012).

2.3 Results

2.3.1 Sex ratio

Sex ratios became increasingly male-biased with length and age, as expected for a protogynous hermaphrodite. Variation in sex ratio of *P. leopardus* was best described by a model that included an interaction between region, zone and L_F, and a model that

included an interaction between region, zone and age (Table 2.3). Populations at Mackay and Storm Cay had higher proportions of males across all lengths and ages compared with Townsville and Lizard Island in fished and reserve reefs (Figure 2.3). Fifty percent of fish were male by the time fish reached 455 mm L_F and 464 mm L_F at Townsville and Lizard Island reserve reefs, respectively, while over 80% of 453 mm L_F and 394 mm L_F fish were male at Mackay and Storm Cay reserve reefs. The size at which 50% of fish were male was much smaller at Mackay (359 mm L_F) and Storm Cay (303 mm L_F) reserve reefs than in other regions (Figure 2.3). The size at which 50% of fish were male was consistently smaller on fished reefs than reserve reefs, but the difference varied among regions. Fifty percent of the population were male at 455 mm L_F on Townsville reserve reefs and 406 mm L_F on Townsville fished reefs whilst 50% of the population at Storm Cay were male at 303 mm L_F on reserve reefs and 300 mm L_F on fished reefs (Figure 2.3).

Male bias was evident in the youngest of age classes (1 and 2 years) on Mackay and Storm Cay fished and reserve reefs compared with Lizard Island and Townsville. Fifty per cent of the population was male at age 6 on Lizard Island and Townsville reserve reefs, and age 1 on Mackay and Storm Cay reserve reefs (Figure 2.4). *P. leopardus* populations did not reach 80% male until 10 and 12 years on Townsville and Lizard Island reserve reefs respectively, while 80% of fish were male by age 7 and 4 years on Mackay and Storm Cay reefs respectively. Sex change occurred approximately 3 years younger on Townsville fished reefs compared with reserves, while 50% sex change did not differ between management zones at Lizard Island (6 years, reserve and fished reefs) or Mackay and Storm Cay (1 year, reserve and fished reefs) (Figure 2.4).

Table 2.3 Parameter estimates from generalized linear mixed effects models (GLMM) examining the effects of fork length (L_F), age (A), region (R) and zone (Z) on sex ratio (P_M, proportion male), proportion of vitellogenic females (P_V) and spawning fraction (P_S, probability of spawning) of *Plectropomus leopardus*. The four best-fitting models are shown, and the final model selected (in bold) was considered to be the simplest model within two of the lowest AICc. Models with interaction terms (*) also include main effects. Sex ratio, P_V and P_S were modelled with a binomial distribution and logit link function. β_{reef} is the random effect of reef, and ε is the error term. AIC_c is the small-sample bias-corrected form of Akaike's information criterion, Δ is the Akaike difference, and w is the Akaike weight.

Model	AIC_c	ΔAIC_c	w
Sex ratio (L_F)			
$\boldsymbol{P_M = L_F * R * Z + \beta_{reef} + \varepsilon}$	**5788.37**	**0**	**0.99**
$P_M = L_F * R + \beta_{reef} + \varepsilon$	5800.06	11.7	<0.01
$P_M = L_F + R + Z + \beta_{reef} + \varepsilon$	5806.07	17.7	<0.01
$P_M = L_F * R + Z + \beta_{reef} + \varepsilon$	5810.00	21.6	<0.01
Sex ratio (A)			
$\boldsymbol{P_M = A * R * Z + \beta_{reef} + \varepsilon}$	**5912.72**	**0**	**0.82**
$P_M = A + R + \beta_{reef} + \varepsilon$	5917.36	4.59	0.08
$P_M = A + R + Z + \beta_{reef} + \varepsilon$	5921.23	4.64	0.08
$P_M = A * R + \beta_{reef} + \varepsilon$	5921.52	8.51	0.01
Vitellogenic females (L_F)			
$\boldsymbol{P_V = L_F + \beta_{reef} + \varepsilon}$	**1569.04**	**0**	**0.36**
$P_V = L_F + Z + \beta_{reef} + \varepsilon$	1569.13	0.09	0.34
$P_V = L_F * Z + \beta_{reef} + \varepsilon$	1571.11	2.08	0.13
$P_V = L_F + R + Z + \beta_{reef} + \varepsilon$	1572.99	3.96	0.05
Vitellogenic females (A)			
$P_V = A + Z + \beta_{reef} + \varepsilon$	1426.71	0	0.26
$\boldsymbol{P_V = A + \beta_{reef} + \varepsilon}$	**1426.82**	**0.11**	**0.24**
$P_V = A * Z + \beta_{reef} + \varepsilon$	1427.25	0.55	0.19
$P_V = A * R * Z + \beta_{reef} + \varepsilon$	1429.70	0.77	0.18
Spawning fraction (L_F)			
$\boldsymbol{P_S = R * Z + \beta_{reef} + \varepsilon}$	**1547.55**	**0**	**0.90**
$P_S = R + \beta_{reef} + \varepsilon$	1553.46	5.91	0.05
$P_S = R + Z + \beta_{reef} + \varepsilon$	1555.38	7.83	0.02
$P_S = L_F + R + \beta_{reef} + \varepsilon$	1555.42	7.87	0.02
Spawning fraction (A)			
$\boldsymbol{P_S = A * R * Z + \beta_{reef} + \varepsilon}$	**1477.75**	**0**	**0.70**
$P_S = A * R + \beta_{reef} + \varepsilon$	1480.24	2.49	0.20
$P_S = A * R + Z + \beta_{reef} + \varepsilon$	1482.23	4.48	0.08
$P_S = A + R + \beta_{reef} + \varepsilon$	1485.78	8.02	0.01

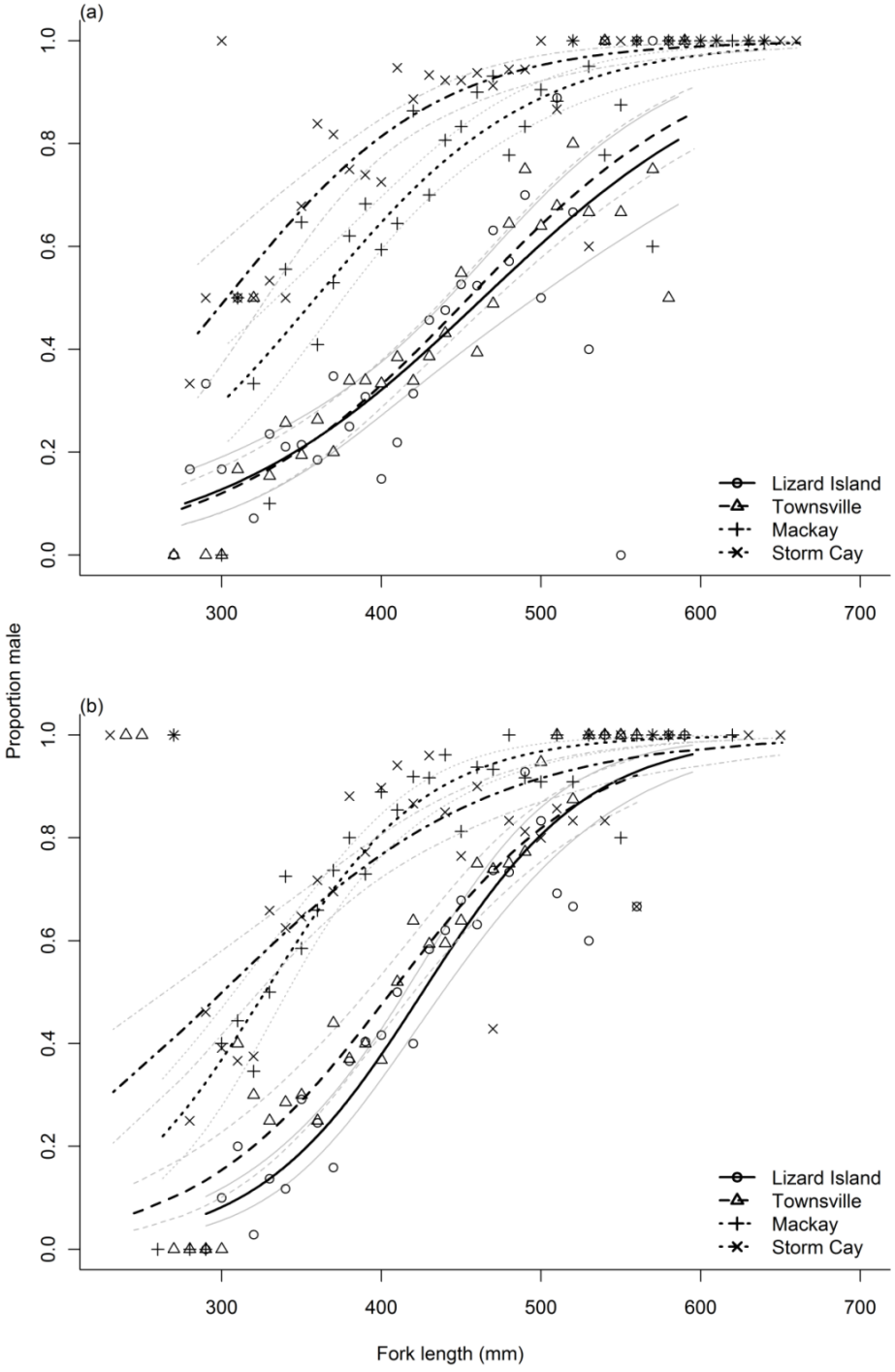


Figure 2.3 Observed and predicted trends in *Plectropomus leopardus* sex ratio (proportion male) (±95% confidence intervals) with fork length (mm) at Lizard Island, Townsville, Mackay and Storm Cay from (a) reserve reefs and (b) fished reefs. Predictions derived from best fit model described in Table 2.3.

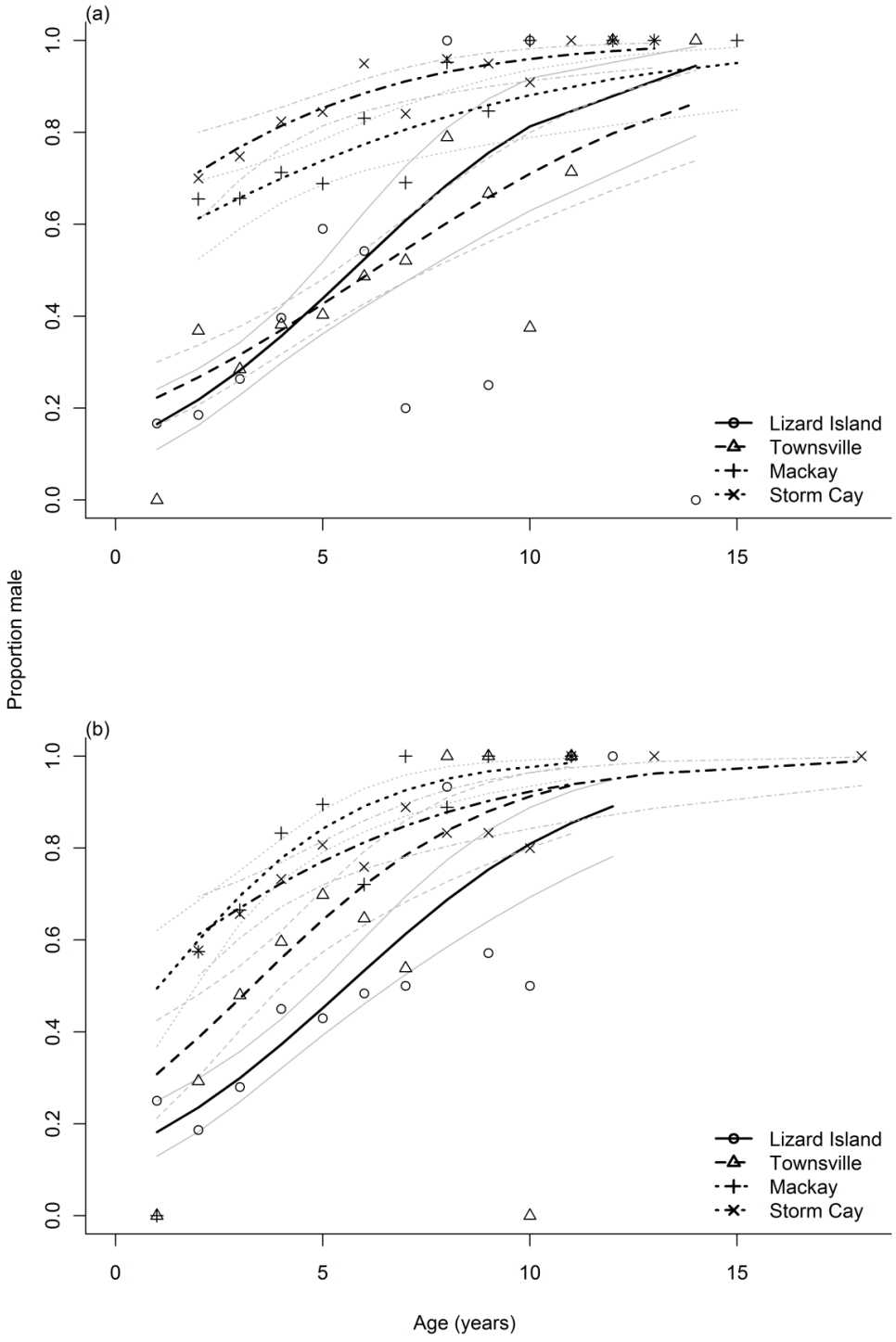


Figure 2.4 Observed and predicted trends in *Plectropomus leopardus* sex ratio (proportion male) (±95% confidence intervals) with age at Lizard Island, Townsville, Mackay and Storm Cay from (a) reserve reefs and (b) fished reefs. Predictions derived from best fit model described in Table 2.3.

2.3.2 Proportion of vitellogenic females

There was an expected increase in the proportion of vitellogenic females with length and age (Figure 2.5, Table 2.3). Fifty percent of females were vitellogenic at 263 mm L_F, and 80% of females were vitellogenic by 333 mm L_F (Figure 2.5). Fifty per cent of 1 year old females were vitellogenic, and 80% of females were vitellogenic by age 3 (Figure 2.5).

2.3.3 Spawning frequency

Ovaries from 23.5% of mature female *P. leopardus* contained 1-Day POFs, indicating spawning on the GBR occurred approximately every 4.3 days during the Austral spring spawning season (Table 2.2). Sixty five per cent of mature females with hydrated ovaries contained histological evidence of two potential spawning events. That is, the ovary contained both hydrated oocytes and 1-Day POFs, indicating that 18% of mature female spawners spawned on consecutive days. Only three ovaries contained "0-Day" POFs. These females were caught in the late afternoon (1530 – 1630), indicative of the narrow time period between ovulation and dusk spawning reported for *P. leopardus*. Ovaries with POFs were present during all lunar periods surveyed during the Austral spring spawning season (first quarter, full moon, and last quarter (Table 2.2).

A broad length range of mature *P. leopardus* was sampled (263 – 585 mm $L_{F)}$ but there was little support for any of the spawning fraction models that included L_F. Variation in *P. leopardus* spawning fraction was best described by a model that included an interaction between the terms region and zone (Table 2.3). Townsville's reserve reefs had the highest spawning fraction of any reefs at 0.43, equivalent to a spawning

frequency of 2.3 days, and greater than that for fished reefs in the same region of 0.19, reflecting a spawning frequency of 5.3 days. The pattern was opposite in the Lizard Island region, where 31% of mature females on Lizard Island fished reefs contained 1-Day POFs, indicating spawning occurred on average every 3.2 days, compared with 8% on neighbouring reserve reefs, indicating spawning every 12.0 days (Figure 2.6; Table 2.2). Spawning was less frequent at Mackay and Storm Cay reefs. Storm Cay's mature females spawned every 17.2 days on fished reefs and 28.8 days on reserve reefs (Table 2.2). There was no evidence of recent spawning on Mackay's fished reefs and on reserve reefs spawning occurred once every 83.2 days. The number of batches per season ranged from approximately 52 spawned by Townsville's females on reserve reefs, to no batches spawned on Mackay fished reefs.

The best model explaining spawning fraction involving age was an interaction between region, zone, and age (Table 2.3). One and 2 year old *P. leopardus* spawned most frequently on reserve reefs, after which spawning fraction on reserve reefs decreased with age in all regions (Figure 2.7). Spawning fraction decreased approximately linearly on Townsville and Lizard reserve reefs from 50% and 11% at age 1 respectively to 30% and 3% at age 10 respectively. Spawning fraction on Mackay and Storm Cay reserve reefs, however, declined sharply from 10 – 20% age 1 to zero at age 4 and did not change thereafter (Figure 2.7). Spawning fraction on fished reefs increased with age at Lizard Island from approximately 20% at age 1 to 60% at age 10 years (Figure 2.7) but spawning fraction at Townsville fished reefs remained approximately 20% irrespective of age. Females with POFs were recorded only in individuals aged 3 and 4 on Storm Cay fished reefs and no females with POFs were recorded on Mackay fished reefs (Figure 2.7).

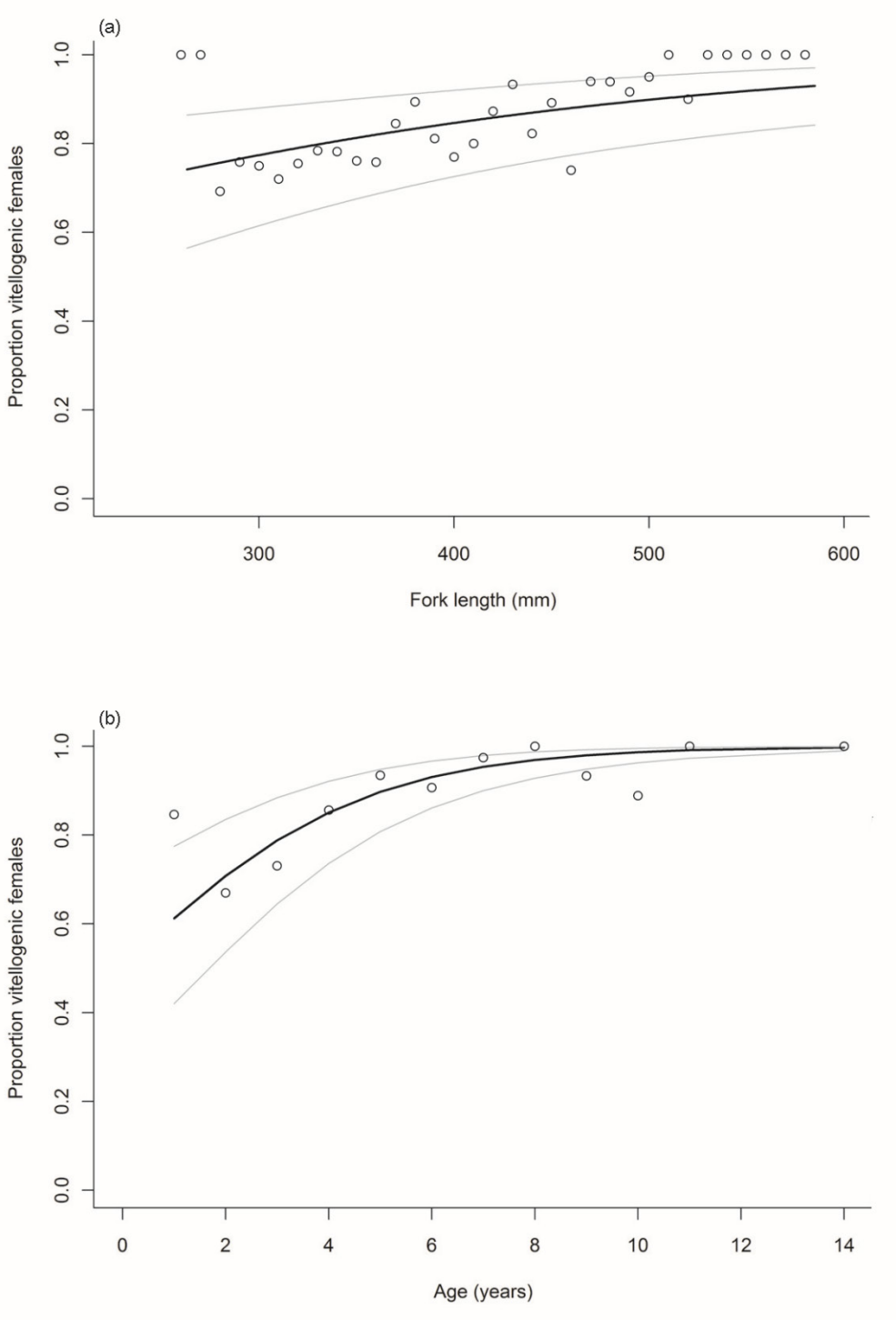


Figure 2.5 Predicted trends in the proportion of vitellogenic *Plectropomus leopardus* females (± 95% confidence intervals) with (a) fork length (mm) and (b) age. Predictions derived from best fit model described in Table 2.3.

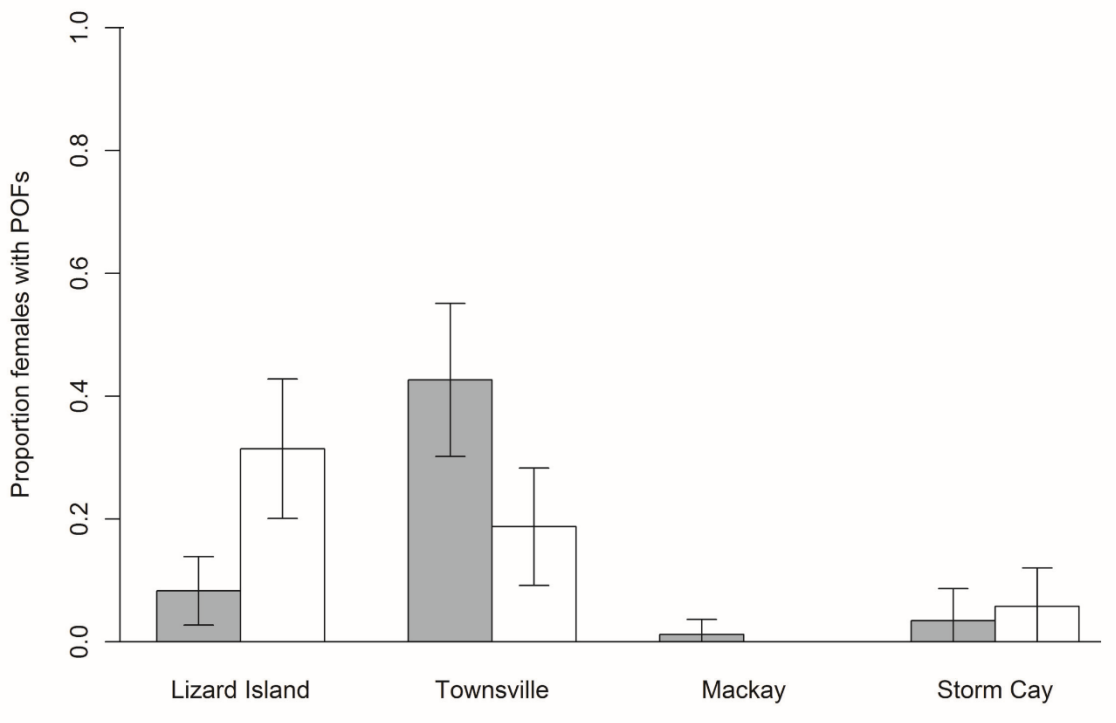


Figure 2.6 Predicted spawning fraction of female *Plectropomus leopardus* (proportion females with POFs) (± 95% confidence intervals) with region from fished reefs (white) and reserve reefs (grey). Predictions derived from best fit model described in Table 2.3.

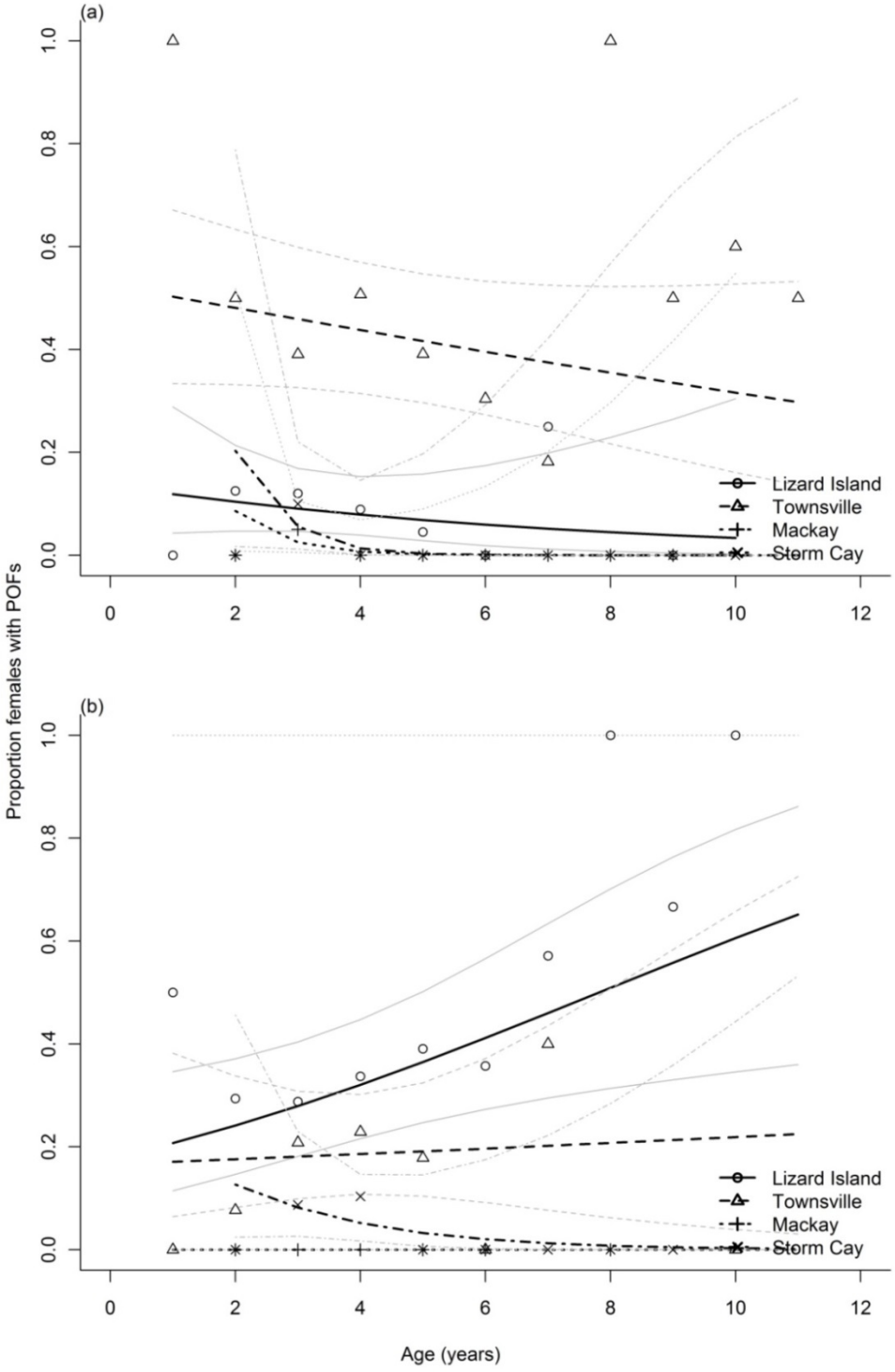


Figure 2.7 Observed and predicted trends in spawning fraction (proportion female *Plectropomus leopardus* with POFs) (±95% confidence intervals) with age at Lizard Island, Townsville, Mackay and Storm Cay from (a) reserve reefs and (b) fished reefs. Predictions derived from best fit model described in Table 2.3.

2.4 Discussion

This study of *P. leopardus*' reproductive dynamics provides one of the few comparisons of sex ratios, the proportion of vitellogenic females, and spawning frequency of fish collected within and outside of reserves across a broad geographic range. Spatial variation in reproductive dynamics of *P. leopardus* between reserves and fished reefs and among regions of the GBR was striking. Populations of *P. leopardus* at Mackay and Storm Cay in the southern GBR were characterised by male-bias and very infrequent spawning for a given size and age, relative to *P. leopardus* from Townsville and Lizard Island in the central and northern GBR. Spawning frequency was greater on reserves than on fished reefs off Townsville but the reverse in the Lizard Island region and effectively the same on both zones in Mackay and Storm Cay. There was no significant relationship between length and spawning frequency and the effect of age varied with GBR region. These results add to the knowledge of *P. leopardus'* reproduction required for management of this commercially important species, raise some striking questions, and highlight the need for understanding spatial variation in reproduction where spawning closures and reserves are used as fisheries or conservation management tools.

2.4.1 Effects of length and age on spawning frequency

The absence of a positive relationship between maternal length and spawning frequency for *P. leopardus* and the inconsistent relationship between maternal age and spawning frequency both are unusual. Larger and older females generally spawn more frequently than their smaller and younger counterparts (Claramunt et al. 2007, Lowerre-Barbieri et al. 2009). Assessments of the relationship between maternal length or age with

spawning frequency for commercially important tropical reef fish is lacking, although a positive relationship between maternal length or age, or both, with spawning frequency has been confirmed in a number of temperate (Bani et al. 2009, Ganias 2009, Mehault et al. 2010), tropical pelagic (Farley et al. 2013) and tropical estuarine (Lowerre-Barbieri et al. 2009) species. The lack of influence that female length has on spawning frequency is not unprecedented, however, and has been documented for northern anchovy *Engraulis mordax* (Hunter and Macewicz 1980). A previous assessment of *P. leopardus* from reefs adjacent to Cairns (central GBR) also reported no effect of female length on spawning frequency (Brown et al. 1994). Larger and older females commonly spawn more batches, however, if there is a positive relationship between female size and age with spawning season duration (Claramunt et al. 2007). For example, *E. mordax* displays no relationship between spawning frequency and female size (Hunter and Macewicz 1980), but older females spawn more batches annually compared with young females because spawning season duration increases with age (Parrish et al. 1986). Maternal effects on spawning season duration could not be determined for *P. leopardus* from this study due to the limited sampling intervals (one lunar period) in each year but warrant further research.

2.4.2 Spatial variation in reproductive dynamics

Significant spatial variation in *P. leopardus*' reproductive traits is not surprising given the large geographic scale of this study and the variation in oceanographic influences (Wolanski 1994, Hopley et al. 2007), population density of *P. leopardus*, fishing effort, and other species, including prey species of *P. leopardus* (Mapstone et al. 2004), along the GBR. Variation in reef fish population biology often occurs at much finer spatial scales, such as within individual reefs or among reefs within a single region (Gust

2004). Spatial variation in reproductive traits is influenced by genetics, the environment, or both (Wakefield et al. 2013). No significant genetic variation in *P. leopardus* exists along the GBR (van Herwerden et al. 2009), so spatial variation in *P. leopardus*' reproduction is more likely driven by environmental factors, which in turn affects demography and social dynamics.

Variation in water temperature is a common driver of spatial variation in fish reproduction (Danilowicz 1995, Pörtner et al. 2001), with reproductive activity commonly reduced at higher latitudes for marine fishes with broad geographic distributions (Chauvet 1991, Fennessy and Sadovy 2002, Williams et al. 2006, Wakefield et al. 2013). Relatively low water temperatures in the southern half of *P. leopardus*' range may cause reduced spawning frequency and influence sex ratios. Mean sea surface temperature (SST) in the Mackay and Storm Cay region of the GBR is generally 1 to 2° C below mean SST in the central and northern GBR (Lough 1999). Spawning omission at high latitudes, with reduced water temperature hypothesized to be the most likely cause, has been documented in fishes where a large proportion of female ovaries remain "resting" in a pre-vitellogenic stage during the spawning season (Fennessy and Sadovy 2002, Williams et al. 2006), the entire female population fails to mature (Wakefield et al. 2013), or females mature but fail to spawn (Chauvet 1991). Temperature can influence sex differentiation in hermaphrodite fishes. For example, eggs of the simultaneous hermaphrodite mangrove rivulus *Rivulus marmoratus* incubated at low temperatures resulted in the proportion of primary males increasing from 4% to 75% (Harrington Jr 1975). Male-bias was evident on fished and reserve reefs in the southern GBR, consistent with the hypothesis that male-bias is a natural phenomenon on the GBR rather than an effect of fishing (Adams et al. 2000).

Water temperature frequently operates as a "physiological switch" for the beginning and end of the spawning season (Conover 1992, Ganias 2009) and it may be argued that sampling was confounded by spatial variation in spawning season along the GBR. Delayed spawning has been correlated with low water temperatures in the temperate species *G. morhua* (Hutchings and Myers 1994, Kjesbu 1994), and capelin *Mallotus villosus* (Carscadden et al. 1997) and may confound spawning frequency estimates depending on when fish are sampled. *P. leopardus* also experience a delayed spawning season with increasing latitude (Table 2.4) but spawning frequency estimates in this study are unlikely to be confounded by latitudinal variation in the onset of spawning as spawning peaks in October – November regardless of latitude (Table 2.4). Mackay and Storm Cay reefs were sampled late October – early December, and therefore were sampled during the spawning peak for *P. leopardus* on the southern GBR (Table 2.4).

Social dynamics are known to play an important role in sex change and spawning behaviour of fishes (Munday et al. 2006, Godwin 2009), including *P. leopardus* (Goeden 1978, Samoilys and Squire 1994, Samoilys 2000). Environmental conditions and social dynamics may interact to affect sex ratios, the prevalence of diandry, and spawning frequency for *P. leopardus*. The relatively low water temperature in the southern GBR may inhibit spawning and therefore diminish the reproductive benefits of maintaining a female-biased sex ratio. Perhaps the male-biased sex ratio results because females that do not successfully spawn are genetically predisposed to change sex?

Table 2.4 Timing and duration of spawning season for *Plectropomus leopardus*. See Figure 2.1 for each location.

GBR Region	Location	Spawning peak	Spawning range (inclusive)	Vitellogenesis range (inclusive)	Indicator	Study duration	Author
North of GBR	Torres Strait	October-November	July – November	May - November	GSI, % frequency of gonadal stages	2004-2005	(Williams *et al.*, 2008)
Northern	Lizard Island	October	September – December*	August – December	GSI, % frequency of gonadal stages	1990 - 1992	(Brown *et al.*, 1994; Ferreira, 1995)
Central	Cairns	September-October	September – November/ December	August – December/ January	GSI, % mature females in vitellogenic state of development	1992 –1994	(Samoilys, 2000)
Central	Cairns	October	September -December	August - December	GSI, % mature	1989 - 1992	(Brown *et al.*, 1994)
Central	Townsville	October-November	September – December	July – January	GSI, % mature	1998-2000	(Davies *et al.*, 2006)
Central	Townsville	October	September – November*	July – November	GSI, % frequency of gonadal stages	1990 – 1992	(Brown *et al.*, 1994; Ferreira, 1995)
Central	Townsville	October	October – December	August – December	GSI, maximum oocyte diameter, % frequency of gonadal stages	2004 – 2005	(Frisch *et al.*, 2007)
Central	Townsville	ns.	September – November	March – November	Presence of ripe females	1990-1994	(Russ *et al.*, 1995)
Southern	Swains and Capricorn-Bunker Group	October-November	October – February	ns.	GSI	ns.	(Brown *et al.*, 1994)
Southern	Heron Island	November-December	October – January	Ovaries inactive by February	GSI	1971-1972	(Goeden, 1978)

* Defines “ripe” as female with oocytes ranging from tertiary yolk globule to hydrated; GSI, gonadosomatic index; ns., not specified.

The estimates of spawning frequency reported here are less than Samoilys' (2000) and Brown's (1994) estimates of *P. leopardus* spawning every 2 to 3 days during the spawning season on reefs off Cairns (Figure 2.1). My estimate of spawning frequency may be conservative because samples were not collected during the new moon period when *P. leopardus*' spawn most frequently (every 1.6 days) (Samoilys 2000). Lunar phase also may have a stronger influence on spawning frequency for *P. leopardus* at higher latitudes, but no sampling occurred during the new moon period, precluding further examination of lunar effects. Lunar periodicity in batch spawning is common for tropical marine fishes, with lunar phases often synchronizing reproduction (Taylor 1984, Takemura and Rahman 2004, Bushnell et al. 2010). *P. leopardus* on Cairns reefs favour group spawning during the new moon and pair spawning during remaining lunar phases (Samoilys 1997, Samoilys 2000). This study and previous work (Ferreira 1995, Samoilys 2000) demonstrates that *P. leopardus* are capable of frequent spawning outside of new moon periods (Table 2.2). Lizard Island and Mackay reefs were surveyed from first quarter to full moon phase, and Townsville and Storm Cay reefs were surveyed full moon to last quarter phase. New moon aggregations may play a more significant role in gathering spawning females in the male-biased southern GBR than in other regions.

It is unlikely that significant male bias and relatively infrequent spawning in the southern GBR reported here was due to a region-specific sampling bias where large females in spawning condition were unintentionally not accessed by the fishers. Post-settlement movement of *P. leopardus* is limited. Among-reef movement is rare (Davies 2000), and within-reef movement to and from aggregations typically ranges from hundreds of metres to several kilometres (Zeller 1998, Zeller and Russ 1998, Davies

2000). Sampling also avoided the new moon period when spawning aggregations were most likely to bias the catch-rate sampling prioritized by Mapstone et al. (2004). Even when new moon *P. leopardus* spawning aggregations do occur, they occur at multiple sites on a reef, possibly with only one or two major aggregation sites (Samoilys and Squire 1994). Sampling at each reef was highly structured around each reef and across the range of depths at which *P. leopardus* habitat occurred, making it unlikely that sex ratios and spawning frequency estimates were influenced by differences in reef-scale movements between sexes.

2.4.3 Effect of fishing on reproductive dynamics

There is no indication reproductive compensation occurs for *P. leopardus* at an individual level in the central and southern GBR where fishing pressure is greatest. Fishing-induced reproductive compensation is common in temperate fishes (Koslow et al. 1995), where per capita reproductive output of target species in fished areas increases due to reduced competition for food and space (Rose et al. 2001). For example, losses in total egg production due to increased exploitation were partially compensated for by approximately 25% due to changes in growth, maturation, and fecundity for North Sea plaice *Pleuronectes platessa*, sole *Solea solea*, and cod *Gadus morhua* (Rijnsdorp et al. 1991). This pattern was only evident at Lizard Island where females spawned three times more frequently on fished reefs than reserves. This region of the GBR receives less fishing pressure than more southern regions (Mapstone et al. 2004, Tobin et al. 2013) and reserve zoning has no measurable effect on mean size, age, and density of *P. leopardus* there (Mapstone et al. 2004). Reproductive compensation due to fishing does not seem a plausible explanation for increased spawning frequency

on fished reefs at Lizard Island, therefore, and it remains unclear why spawning frequency differed between zones in that region.

Female to male sex change occurred at smaller sizes and younger ages on fished than reserve reefs at Townsville, and on Townsville fished reefs spawning frequency was also reduced. The effect of reduced size and age at sex change with fishing pressure is well-documented for protogynous species (Hawkins and Roberts 2004, Hamilton et al. 2007, Götz et al. 2008). The effect on spawning frequency is consistent with the small amount of published research on tropical species regarding the effects of fishing on reproductive output. A complete lack of spawners of the protogynous hogfish *Lachnolaimus maximus* in fished areas was attributed to a total breakdown of social structure because of intense fishing pressure adjacent to the Florida Keys National Marine Sanctuary (Muñoz et al. 2010). Reduced spawning frequency for *P. leopardus* on Townsville fished reefs may indicate similar fishing-induced pressures on social structure. The lack of a positive relationship between maternal length and age with spawning frequency on Townsville, Mackay, and Storm Cay reefs indicates that the key benefit of reserves for *P. leopardus* spawning frequency comes from maintaining greater densities of *P. leopardus* inside reserves and perhaps reducing disturbance from fishing, rather than from protecting larger and older individuals from harvest. Protection also led to a stronger spawning response in Townsville's females. These results indicate that reserves are beneficial for maintaining "less disturbed" spawning populations of *P. leopardus* on the GBR.

Reserve design theory suggests that in the absence of information on larval connectivity and important larval sources reserves are best placed in areas considered pristine, or

where there is an abundance of targeted species (Botsford et al. 2003, Bode et al. 2012). Bode et al. (2012) recently modelled reserves using "connectivity surrogates" for *P. leopardus* and concluded that using the existing biomass characteristics of the species on a reef provided the best indication of transporting larvae in the GBR metapopulation. This logic would suggest that reserves would be best placed in the southern GBR where the *P. leopardus* fishery is concentrated, abundance is highest and, presumably, so too is reproductive output. Male-biased sex ratios and the lack of spawning activity on southern GBR fished and reserve reefs, however, indicates that applying biomass as a connectivity surrogate to measure the benefits of reserves for *P. leopardus* may be flawed. The importance of considering empirically measured larval dispersal and connectivity in the design and function of reserves is recognised increasingly (Almany et al. 2009, Jones et al. 2009, Gaines et al. 2010, Kininmonth et al. 2011, Bode et al. 2012, Harrison et al. 2012, Almany et al. 2013). This study highlights the importance of understanding and incorporating spatial variation in reproductive characteristics that affect the sources of larvae into the design of reserves, and when assessing any benefits of reserves for target species.

2.4.4 Management implications

Male-bias and lack of spawning activity in the southern GBR is surprising given the southern region supports the majority of commercial catch and effort for *P. leopardus*, with catch and catch per unit effort up to four times higher than in the northern GBR (Mapstone et al. 2004, Bergenius 2007, Tobin et al. 2013). Greater densities of *P. leopardus* in the southern GBR possibly indicate that southern recruits originate from more productive reefs in the central or northern GBR. The lack of genetic variation among regions indicates larval dispersal occurs along the GBR (van Herwerden et al.

2009). Pelagic larval duration for *P. leopardus* is approximately 25 days and recruitment of juveniles at larger spatial scales largely appears to be driven by current patterns and geomorphology (Doherty et al. 1994), with the predominant flow of water along the GBR (south of 14° S) southward due to the East Australia Current (EAC) (Wolanski and Pickard 1985). Larval dispersal modelling in the central GBR (Cairns region) supports the theory that there is net export of larvae from northern source to southern sink reefs, with self-recruitment accounting for less than 9% of the settling cohort in 80% of reefs (James et al. 2002, Bode et al. 2006). The hypothesis of long distance larval dispersal on a scale of hundreds of kilometres is contrary to recent genetic parentage analysis, however, which indicates the majority of larvae for congenerics *P. areolatus* (Almany et al. 2013) and *P. maculatus* (Harrison et al. 2012) and other reef fish (Jones et al. 2005, Planes et al. 2009, Saenz-Agudelo et al. 2009, Harrison et al. 2012) settle within tens of kilometres of their natal reef. These studies were conducted in different environments, however, where along-shore transport, such as the EAC, is not as influential and transport dynamics are presumably different to those present on mid-shelf reefs of the GBR. Parentage analysis should also be applied to determine the role that central GBR reefs play as a source of recruits for southern reefs.

Alternatively, greater densities of adult *P. leopardus* may occur in the southern GBR because the relatively small numbers of eggs and larvae produced on southern reefs have a greater proportion survive to settlement compared with larvae produced at lower latitudes. Temperature has a well-known effect on larval development in marine fishes. As water temperature decreases larvae generally experience slower growth, longer larval stage duration, smaller energy budgets, decreased larval mortality rates, and

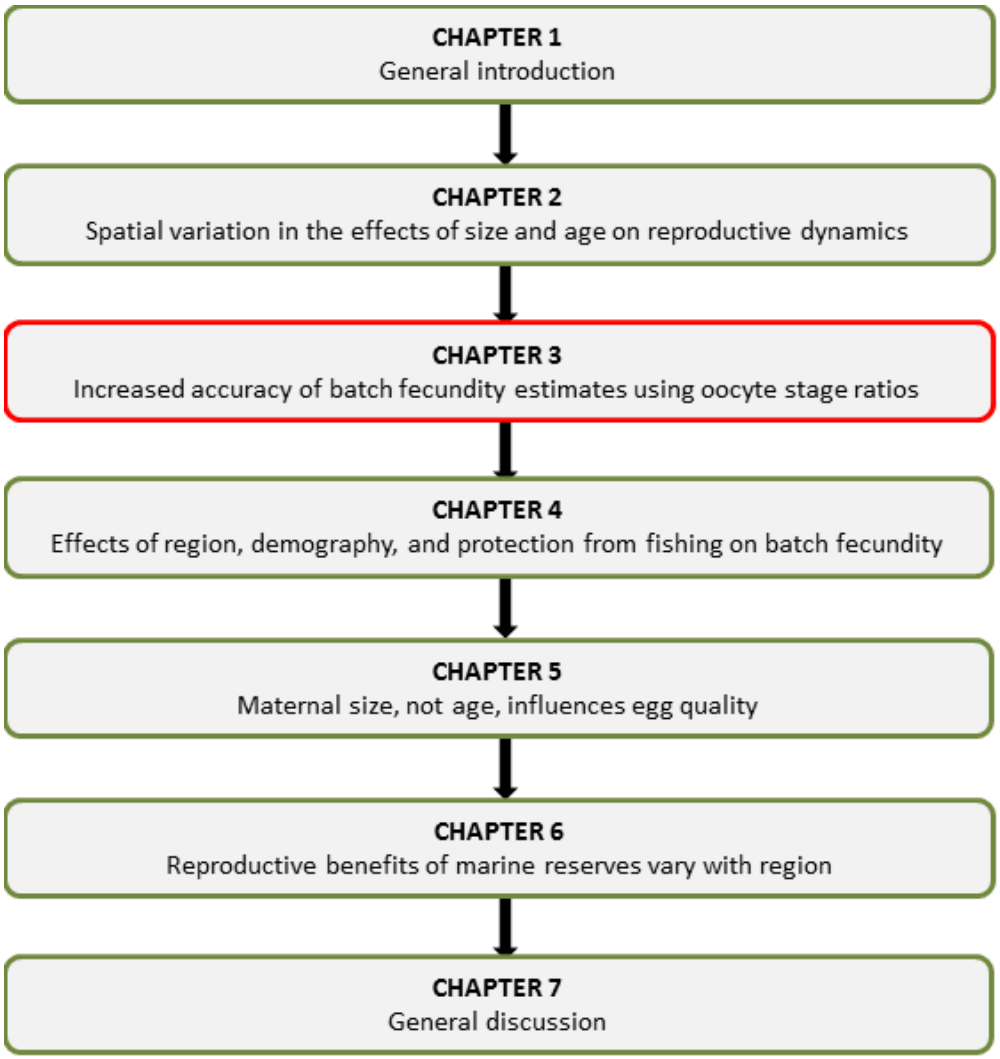
greater net survivorship (see review by Houde 1989). Higher survival rates for *P. leopardus* larvae at high latitudes possibly could compensate for reduced spawning frequency and male bias in the GBR region. Increased survival of larvae as they enter cooler waters also might mean that larvae from central and northern reefs that reach the southern GBR experience greater survival and so enhance recruitment to southern reef populations compared with larvae that remain in warmer, more northern waters.

2.4.5 Conclusion

Understanding spatial variation in reproductive traits is essential when assessing management policies (e.g. reserves, minimum sizes, and spawning closures) where the management objective is maintaining sufficient reproductive adults and reproductive output of exploited species. This study highlights the pitfalls of assuming reproductive traits are homogenous when a stock is distributed over a broad geographic area, even when the stock apparently is genetically homogeneous. The current management of *P. leopardus* as a homogenous population is likely to be ineffective for maximising reproductive output, given the striking regional differences demonstrated here. A regional approach to management of the *P. leopardus* fishery may be required if a specific and confined region is disproportionately responsible for maintaining populations at a much broader geographic scale through larval source and sink relationships. If this was the case, management of exploited species would benefit by understanding where these key reproduction areas are so that management measures can mitigate effects of fishing on key reproductive components of the population. Perhaps more importantly, this study clearly demonstrates that *P. leopardus* population(s) on the GBR do not fit the conventional model of reproductive biology and sex structure following protection from fishing and highlights the need for more

systematic long-term studies of the population biology of large protogynous hermaphroditic tropical reef fish.

CHAPTER 3 Increased accuracy of batch fecundity estimates using oocyte stage ratios



Chapter 3 has been published as: Carter AB, Williams AJ and Russ GR (2009). Increased accuracy of batch fecundity estimates using oocyte stage ratios in *Plectropomus leopardus*. Journal of Fish Biology **75**:716 – 722. doi:10.1111/j.1095-8649.2009.02313.x

3.1 Introduction

Fecundity data are important in fisheries science as a predictor of reproductive potential and vulnerability to recruitment overfishing (Plan Development Team 1990, Sadovy 1996). Fecundity data are also necessary when calculating egg production per unit area and estimating spawning stock biomass (Lasker 1985, Paddack and Estes 2000). In batch fecundity estimates for fish that spawn multiple batches, such as engraulids, hemiramphids and serranids, conventionally only hydrated oocytes are counted (Hunter et al. 1985, Samoilys 2000, McBride and Thurman 2003). Sampling time is therefore critical, as females generally hydrate oocytes on the day the batch is spawned (Hunter et al. 1986, Fitzhugh et al. 1993, Samoilys 2000).

Obtaining appropriate samples for batch fecundity estimates can be difficult, because samples are often acquired opportunistically during commercial fishing surveys operating at a sub-optimal time for collection of spawning fishes (Cooper et al. 2005). Published fecundity estimates for exploited fishes are therefore often based on limited sample sizes and restricted size and age ranges. The hydrated oocyte method also relies on the major assumption that only the hydrated oocytes present in the ovary at the time of capture will be spawned in the next batch (Hunter et al. 1985). Batch fecundity estimates may include oocytes at stages prior to hydration if there is relative certainty that these oocytes would have matured to be spawned in the next batch (Macewicz and Hunter 1993, Yoneda et al. 1998, Thorsen and Kjesbu 2001).

The only previous estimate of batch fecundity for *Plectropomus leopardus* used the hydrated oocyte method (Samoilys 2000). This method restricted the available samples

to ovaries collected predominantly by spear fishing in the Austral spring, during the new moon, after 1300 hours (Samoilys 2000). These criteria maximized the proportion of hydrated females sampled, as the greatest proportion of females with hydrated oocytes were collected between 1330 and 1545, just prior to dusk spawning (Samoilys 2000). The method, however, limited the sample size available to describe the size-fecundity relationship, and field sampling was often difficult, involving diver collections of eggs from spawning females in the afternoon, often in deep water (Samoilys 2000).

The objective of this chapter was to examine histologically the developmental stages of *P. leopardus* oocytes to determine whether oocytes at other developmental stages (i.e. non-hydrated) could be included in batch fecundity estimates.

3.2 Methods

3.2.1 Sample collection and histological processing

P. leopardus gonads were collected in 2001 on the Great Barrier Reef as part of the Effects of Line Fishing (ELF) Experiment, which conducted surveys during the Austral spring spawning season for this species (see Chapter 2.2 and Mapstone et al. 2004 for details). Sample collection and histological processing were conducted as described in Chapter 2.2.

3.2.2 Reproductive staging

Each gonad section was examined under a high-powered microscope to determine sex and ovarian developmental stage according to the most advanced oocyte present

(Samoilys 2000; Appendix A). Developmental stages were classified according to Samoilys and Roelofs (2000), Adams (2002) and Arocha (2002). A sub-sample of 30 histological sections (10 yolk globule stage, 8 migratory nucleus stage and 12 hydrated stage ovaries) were selected randomly for each developmental stage from 408 females classed as containing either ripe or running ripe (n=337 yolk globule and n=30 migratory nucleus) or hydrated (n=41) ovaries. Ovaries with post-ovulatory follicles were excluded as their presence indicates spawning has already commenced (Macewicz and Hunter 1993).

Ten replicate photographs were taken randomly of each of the 30 sectioned ovaries using a photomicroscope. The number of oocytes in each developmental stage (pre-vitellogenic, yolk vesicle, early yolk globule, late yolk globule, migratory nucleus and hydrated stages) were counted using ImageTool computer software (UTHSCSA ImageTool 3.0®; http://ddsdx.uthscsa.edu/dig/itdesc.html). The percentage of each oocyte stage relative to the total number of all oocytes present was then calculated.

3.2.3 Data analysis

The mean number of migratory nuclei and hydrated oocytes was calculated for each of the 12 fish with hydrated ovaries, and an additional 10 hydrated ovaries randomly selected to increase sample size. A Chi-square (χ^2) contingency test was used to determine whether the proportion of migratory nucleus to hydrated oocytes differed significantly according to the time of day the fish was caught: early morning (0800 – 1000), late morning (1000 – 1200) and late afternoon (1400 – 1700). The early afternoon period (1200 – 1400) was not sampled during the ELF surveys. Statistical analysis was conducted using SPSS.

3.3 Results

The most common oocytes present in mature *P. leopardus* ovaries were pre-vitellogenic, comprising 83.2, 82.1 and 79.1% of oocytes in yolk globule, migratory nuclei and hydrated ovaries, respectively. Vitellogenic oocytes not in the final stages of maturation (i.e. yolk vesicle and yolk globule) were observed with migratory nuclei and hydrated oocytes. Nine percent of oocytes present in the ovary were late yolk globule oocytes, a consistent percentage regardless of the maturity of the ovary. Oocytes in the most advanced stages of development comprised a relatively small portion of total oocytes present. In migratory nucleus and hydrated stage ovaries, 1.8 and 2.1% respectively, were at the migratory nucleus stage, while hydrated ovaries contained an average of only 4.6% hydrated oocytes. Spent ovaries were either pre-vitellogenic or at the yolk vesicle or yolk globule stages.

When individual yolk globule, migratory nucleus and hydrated stage ovaries were plotted against time of capture, a decrease in migratory nucleus oocytes over time was observed in hydrated ovaries (Figure 3.1). The proportion of migratory nucleus to hydrated oocytes was dependent on time of day ($\chi^2 = 22.74$, df =2, $p < 0.001$). Female *P. leopardus* with the greatest proportion of migratory nucleus oocytes were caught in the early morning (0800 – 1000; 34% of oocytes at migratory nucleus stage). The percentage of migratory nucleus oocytes decreased to 26% in samples caught in the late morning (1000 – 1200) and even further to 4% by the afternoon (1400 – 1700). Migratory nucleus oocytes were absent in 50% of the ovaries sampled in the afternoon, as these oocytes had progressed to the hydrated stage in these ovaries.

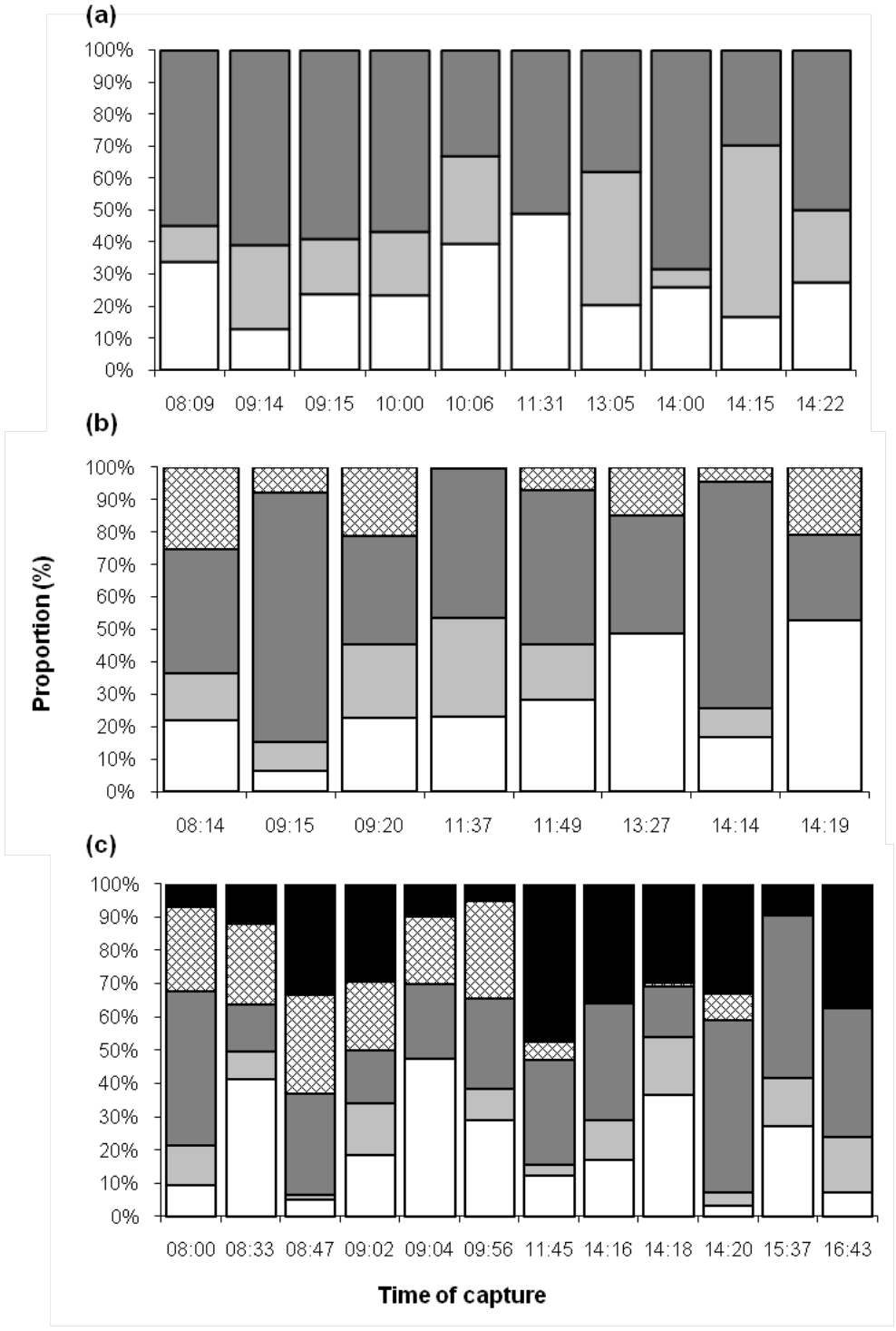


Figure 3.1 Percentage of vitellogenic oocytes at time of capture for individual female *Plectropomus leopardus* at three stages of ovarian maturity: (a) late yolk globule stage, (b) migratory nucleus stage, and (c) hydrated stage. Oocyte stages counted include yolk vesicle ( ), early yolk globule ( ), late yolk globule ( ), migratory nucleus ( ) and hydrated ( ).

3.4 Discussion

The examination of oocyte development in *P. leopardus* revealed that migratory nucleus oocytes present in the morning on the day of spawning progressed to the hydrated stage by late afternoon. Therefore, for *P. leopardus* samples with hydrated oocytes present, batch fecundity should be estimated by counting migratory nuclei and hydrated oocytes, rather than using the conventional method (Hunter et al. 1985) of counting hydrated oocytes only. Counting hydrated oocytes only would have underestimated batch fecundity by between 34% for samples caught in the early morning, and 4% for samples caught in the afternoon. The absence of migratory nucleus oocytes in spent ovaries further indicates these oocytes do hydrate on the day of spawning.

The duration of the migratory nucleus stage is currently unknown for *P. leopardus*, but hydration is known to begin 4 – 9 hours before spawning commences at dusk (Samoilys 2000). Individual *P. leopardus* with migratory nucleus oocytes but no hydrated oocytes were caught as late as 1630 hours. It is unlikely that these individuals would have spawned on the day of capture, and it is possible that an unknown portion of the yolk globule oocytes present at the time of capture would have progressed to the migratory nucleus stage by the day of hydration and spawning. A batch fecundity estimate based on counts of migratory nucleus oocytes from an ovary collected prior to the day of spawning would, therefore, be inappropriate and likely to underestimate batch fecundity.

Samoilys (2000) used the hydrated oocyte method in the only previous study of *P. leopardus* batch fecundity. Samoilys (2000) utilized samples collected after 1300 hours, so the effect of excluding migratory nucleus oocytes would have been minimal. Because fecundity studies are often limited to ovaries acquired opportunistically during commercial fishing surveys, rather than through structured sampling at the optimum (spawning) time (Cooper et al. 2005), the ability to broaden criteria to include more oocytes and thus more samples has important implications in a field that suffers from small sample sizes. For example, *c*. 68% of *P. leopardus* caught with hydrated ovaries in this study were caught before 1300 hours, suggesting that sample sizes could be increased more than two-fold for this species by including samples collected throughout the day of spawning.

Results from this study support more recent studies that use the more time-consuming oocyte size-frequency or oocyte stage-frequency methods, which indicate the most appropriate oocyte stage to include is species-specific (Macewicz and Hunter 1993, Yoneda et al. 1998). The size-frequency method has led to the inclusion of migratory nucleus oocytes in a growing number of fecundity studies (Macewicz and Hunter 1993, Cuellar et al. 1996, Karlou-Riga and Economidis 1997). Differentiating early-stage migratory nucleus from late-stage yolk globule oocytes, however, is difficult because whole oocytes are visually similar. For *P. leopardus* oocytes, the transition from late-stage yolk globule to early-stage migratory nucleus is characterised by an internal change in appearance rather than an increase in oocyte size or colour change (*personal observation*; Appendix A). This characteristic, also observed in yellowfin tuna *Thunnus albacares* (Schaefer 1996), jack mackerel *Trachurus symmetricus* (Macewicz and Hunter 1993) and vermilion snapper *Rhomboplites aurorubens* (Cuellar et al. 1996), has

led to the exclusion of early-stage migratory nucleus oocytes from batch fecundity estimates (Macewicz and Hunter 1993).

3.4.1 The ratio method for batch fecundity estimates

To avoid excluding early-stage migratory nucleus oocytes from batch fecundity estimates, an alternative method may be used for estimating the number of migratory nucleus and hydrated oocytes in a *P. leopardus* batch. First, the numbers of migratory nucleus stage oocytes and hydrated oocytes are counted using a histological section of the ovary of interest. Second, the ratio of migratory nucleus oocytes to hydrated oocytes is calculated for each fish. Third, all hydrated oocytes in an ovarian subsample are counted using the conventional hydrated oocyte method of Hunter et al. (1985). Finally, the number of migratory nucleus oocytes (M_S) in the ovarian sample is estimated using:

$$M_S = H_S\left(\frac{M_H}{H_H}\right) \qquad (1)$$

Where H_S is the number of hydrated oocytes in a sample taken from the whole gonad, and M_H and H_H are the number of migratory nucleus and hydrated oocytes in a histological section of the gonad. The total number of oocytes in a sample to be included in the batch fecundity estimate, O_S, is then calculated as:

$$O_S = M_S + H_S \qquad (2)$$

Batch fecundity (F) is then calculated using the conventional formula (adapted from Cooper et al. 2005)

$$F = \left(\frac{W_T}{W_S}\right) \times O_S \qquad (3)$$

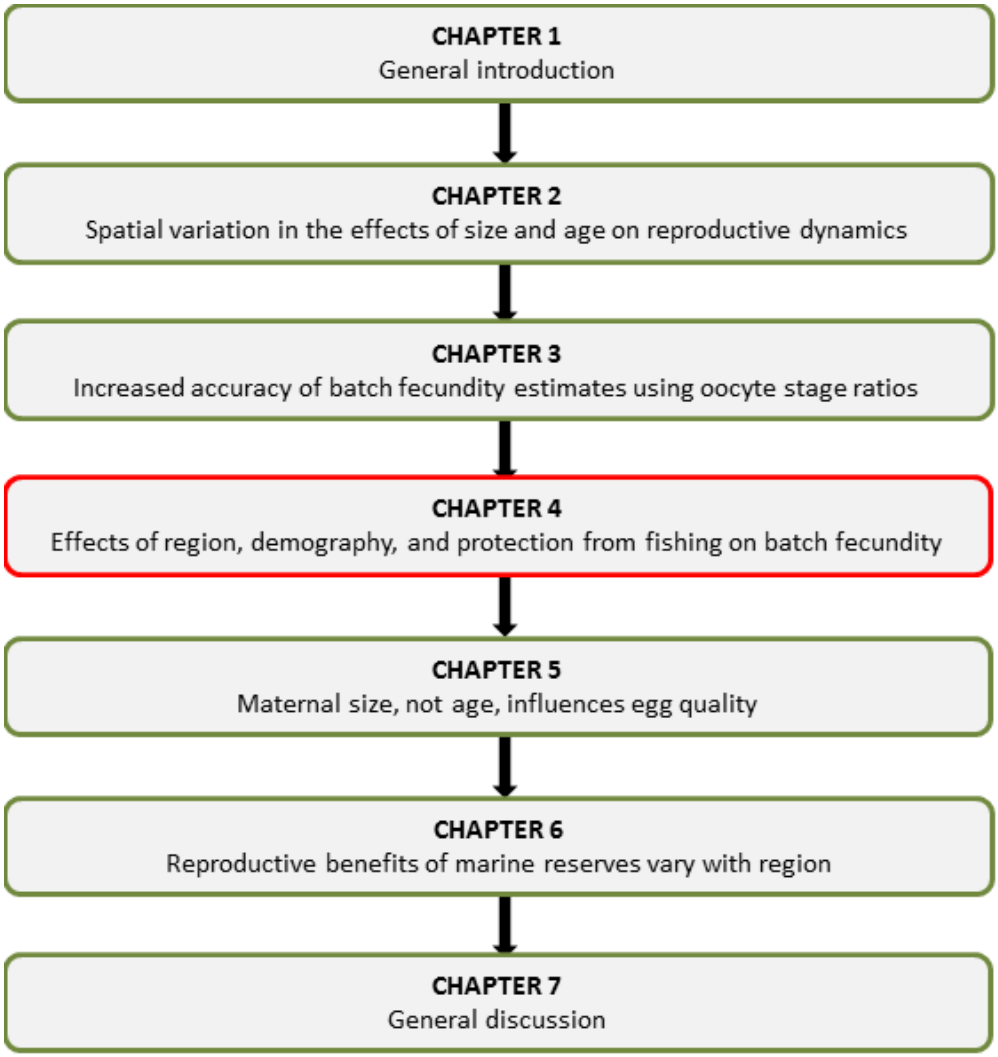
Where W_T is total ovary mass, and W_S is subsample mass.

The ratio method may be applied to future studies of any species where oocyte stages other than the visually distinct hydrated stage are appropriate to include in batch fecundity estimates. The method may be particularly useful when the oocyte size-frequency method is not applicable because of overlap in oocyte size between developmental stages. This is because the ratio method accounts for specific oocyte developmental stages, thus potentially increasing the accuracy in batch fecundity estimates that would have previously included or excluded inappropriate stage oocytes because of their size.

3.4.2 Conclusion

Understanding the reproductive biology of exploited fishes is essential for effective management, particularly when assessing replenishment of populations. Fecundity studies of exploited fishes provide the basis for estimates of the reproductive capacity of individual fishes (Murua et al. 2003). Accurate estimation of batch fecundity is dependent on determining the appropriate oocyte stages to count. Given the development of oocytes in *P. leopardus*, including counts of migratory nucleus oocytes is suitable for this species. The ratio method could potentially be applied to other oocyte stages suitable for inclusion in batch fecundity estimates for different species. The ratio method can increase sample size available for batch fecundity estimates, reduce potential biases in batch fecundity estimates, and avoid the potential danger to divers of collecting samples during dusk spawning aggregations.

CHAPTER 4 Effects of region, demography, and protection from fishing on batch fecundity



Chapter 4 has been published as: Carter AB, Davies CR, Mapstone BD, Russ GR, Tobin AJ and Williams AJ (2014) Effects of region, demography, and protection from fishing on batch fecundity of common coral trout (*Plectropomus leopardus*). Coral Reefs **33**:751 – 763. doi: 10.1007/s00338-014-1164-z

4.1 Introduction

Recruitment subsidy from reserves is expected because protection of an area from fishing generally leads to higher densities of larger and older target species relative to fished areas (Lester et al. 2009, Miller et al. 2012), and because fecundity often increases with female size and age (Sadovy 1996, Lambert 2008). The benefits of preserving the Big Old Fat Fecund Females (BOFFFs) for maximizing the reproductive potential of a population is well established (Birkeland and Dayton 2005, Hixon et al. 2014). It remains unresolved, however, whether recruitment subsidies compensate partially, fully, or more-than fully for the loss of fished area (and loss of catch) that occurs when a reserve is established (Russ 2002, Halpern and Warner 2003). The relative benefit of reserves for population conservation and yield are likely to be greater when areas outside reserves are heavily fished (Beverton and Holt 1957, Gerber et al. 2003).

Despite the popularity of the BOFFF Hypothesis, there are a lack of studies that quantify and compare fecundity for exploited fish populations using samples collected inside and outside of reserves, or studies assessing regional variation in the effect of reserves on individual fish fecundity. As discussed in Chapter 1, previous studies quantifying the benefits of reserves on egg production of targeted species generally combine density data from inside and outside a reserve with fecundity models based on females collected only from fished areas (Willis et al. 2003, Denny et al. 2004, Evans et al. 2008). This approach relies on the assumption that size-fecundity relationships from fished populations are representative of size-fecundity relationships within reserves, and that differences in egg production are driven by larger numbers of larger fish in reserves

compared with fished areas. This approach is often the only option due to the difficulty in obtaining permission for destructive sampling required for fecundity estimates within reserves. Several confounding factors limit the appropriateness of this approach. For example, decreased densities in fished areas may be reproductively compensated for by increased individual fecundity due to reduced intra-specific competition for food, i.e. "density dependent reproduction" (Rose et al. 2001). Conversely, reproductive output may be repressed in fished areas due to Allee-type depensation or fishing-related disruption of spawning behavior (Sadovy 2001).

The effect of female metrics (length, weight, and age) on fecundity also may vary spatially for reasons unrelated to fishing pressure, such as variations in water temperature and prey availability. Such variation in environmental factors that could affect fecundity has received little attention for tropical fish but is well-documented for temperate fishes such as Atlantic cod (*Gadus morhua*) (McIntyre and Hutchings 2003, Yoneda and Wright 2004). The majority of published length-fecundity, weight-fecundity and age-fecundity relationships also are biased towards temperate gonochores (e.g. Blanchard et al. 2003, McIntyre and Hutchings 2003, Cooper et al. 2005) and, to a lesser extent, tropical gonochores (Itano 2000, Kamukuru and Mgaya 2004, Evans et al. 2008). Predictors of fecundity, the extent of spatial variation in fecundity, and the effect of fishing on fecundity have received scant attention for hermaphrodites (but see Chan et al. 2012), despite the prevalence of protogynous hermaphroditism in tropical fish families (Jennings and Kaiser 1998).

As discussed in Chapter 1, regional variation in *Plectropomus leopardus* density and fishing pressure occurs where commercial effort is concentrated in the southern GBR

where densities are greatest, recreational effort is concentrated in the central GBR where reefs are most accessible, and relatively little fishing effort occurs in the isolated northern GBR region (Mapstone et al. 2004, Miller et al. 2012, Tobin et al. 2013). The combination of this variation in density and fishing pressure, plus regional variation in reproductive dynamics outlined in Chapter 2, suggests that female fecundity for *P. leopardus* is unlikely to be dependent on female length, weight, and age alone.

The objective of this chapter was to compare age-, length-, and weight-specific batch fecundity of *P. leopardus* caught within or outside reserves from the northern, central, or southern GBR. This is the first study to my knowledge to compare fecundity between reserves and fished areas for a commercially important coral reef fish using samples taken both within and outside reserves. This study also provides the first broad regional comparison of fecundity relationships for a tropical protogynous reef fish.

4.2 Methods

4.2.1 Sample collection and processing

P. leopardus were collected from 1998 to 2001 as part of the Effects of Line Fishing (ELF) Experiment on the GBR (see Chapter 2.2 and Mapstone et al. 2004). As in Chapter 2.2, fish used in this chapter were caught from four reef clusters spanning three regions of the GBR: Northern (Lizard Island reefs), central (Townsville reefs), and southern (Mackay and Storm Cay reefs). Mackay and Storm Cay reefs were pooled into one southern region for this analysis due to geographic proximity and the low number of spawning females on southern reefs as described in Chapter 2.3 (n=5288; Table 4.1). Within each cluster two reefs were zoned no-take marine reserve and the other two

reefs were historically open to fishing, although one of these reefs was closed to fishing for the four years of sampling and the other was closed to fishing from March 2000 onwards (Figure 2.1). As in Chapter 2.2, these historically fished reefs were considered to be fished reefs for the purposes of the analyses because the period of closure to fishing was short (< 2 – 4 years) relative to the longevity of *P. leopardus* (up to 16 years) and it was assumed that any fecundity responses to changes in the level of fishing would be relatively slow.

As explained in Chapter 2.2, *P. leopardus* spawn throughout the lunar cycle during the Austral spring but spawning activity peaks during new moon periods (Samoilys 1997, Samoilys 2000), a period avoided during the ELF Experiment to reduce potential bias in abundance estimates if spawning aggregations were encountered. The assumption was made that batch fecundity was not influenced significantly by lunar phase and that the relative differences in batch fecundity of *P. leopardus* among regions and zones would be consistent regardless of whether sampling occurred during or outside new moon periods.

Table 4.1 Numbers of male and female *Plectropomus leopardus* sampled from three Great Barrier Reef regions (northern, central, southern) and two management zones (reserve and fished reefs), by month and lunar quarter.

Region	Zone	Month sampled	Lunar quarter	Male	Female	Proportion male	Proportion spawners
North	Fished	Oct	First quarter - full moon	287	452	0.39	0.33
	Reserve	Oct	First quarter - full moon	172	323	0.35	0.08
Central	Fished	Oct – Nov	Full moon - last quarter	283	236	0.55	0.24
	Reserve	Oct– Nov	Full moon - last quarter	375	516	0.42	0.49
South (Mackay)	Fished	Nov	First quarter - last quarter	487	155	0.76	0.01
	Reserve	Oct – Nov	First quarter	475	183	0.72	0.04
South (Storm Cay)	Fished	Nov – Dec	Full moon - last quarter	503	189	0.73	0.06
	Reserve	Nov – Dec	Full moon - last quarter	550	113	0.83	0.12

Fork length (*FL*, nearest millimeter) and total weight (*W*, nearest gram) were measured for all fish at the time of capture. As described in Chapter 2.2, otoliths were removed and sectioned to determine age (*A*), and gonads were removed, preserved, and histological sections made to determine sex and female maturity. Sex ratios (proportion male) were calculated from the number of males divided by the sum of females and males (Table 4.1). Female maturity was classified based on the most advanced oocyte present (Appendix A). The proportion of spawners was calculated from the number of females with hydrated oocytes present in the ovary divided by the total number of females. Presence or absence of postovulatory follicles (POFs) in the ovary was recorded and, where present, POFs were classified as "0-Day POFs" (~several hours old) or "1-Day POFs" (~12 – 24 hours old) as described in Chapter 2.2.

4.2.2 Batch fecundity estimates

Batch fecundity (*BF*) was estimated for 329 spawners. Spawners were defined as females with hydrated oocytes present in the ovary. Batch fecundity was not estimated for females with evidence in histological sections of recent spawning, i.e. that afternoon (0-Day POFs present in the ovary). Females with 1-Day POFs were retained in the analysis for two reasons: (1) *P. leopardus* are capable of frequent, and in some cases daily spawning (particularly in the central GBR), so to exclude these females would be to exclude a potentially important group of females contributing to egg production; and (2) preliminary data analysis indicated *FL-BF*, *W-BF*, and *A-BF* relationships did not differ between females with and without 1-Day POFs (data not shown).

Batch fecundity was estimated using the gravimetric (Hunter et al. 1985) and ratio methods detailed in Chapter 3.4.1. The gravimetric method involves counting the

number of hydrated oocytes in a weighed ovarian sample and extrapolating this to estimate the number of hydrated oocytes in the whole ovary (Hunter et al. 1985). The ratio method was used in my calculations because the inclusion of only hydrated oocytes in batch fecundity estimates is not appropriate for *P. leopardus* as migratory nuclei oocytes progress to the hydrated stage by late afternoon on the day of spawning. As described in Chapter 3.4.1, the ratio method requires a count of the number of migratory nuclei and hydrated oocytes from histological sections to determine the ratio of migratory nuclei to hydrated oocytes for each fish. Hydrated oocytes in an ovarian subsample were then counted using the conventional hydrated oocyte method of Hunter et al. (1985). Batch fecundity was subsequently estimated using a series of equations outlined in Chapter 3.4.1. The ratio method allowed me to include a larger sample size of *P. leopardus* samples collected throughout the day of spawning in the analysis.

4.2.3 Data analysis

Generalized linear mixed-effects models (GLMM) were used to examine the fixed effects of *FL*, *W*, *A*, Region (*R*) and management Zone (*Z*; fished and reserve reefs) on batch fecundity of *P. leopardus*. Each factor was modelled as an additive term and as an interaction with other factors, and *FL*, *W*, and *A* were also modelled as cubic splines with between two and six degrees of freedom to model suspected non-linearity in the fecundity-somatic relationships (McKinley and Levine 1998). The factor reef was modelled as a random effect term in all models to eliminate potential bias resulting from the non-independence of samples collected at the same time within each reef. The response variable batch fecundity (*BF*) was modelled using a Gaussian distribution. Akaike's Information Criterion for small sample sizes (AICc) (Burnham and Anderson 2002) was used to determine the best set of explanatory factors for adequately

predicting *BF*, and to compare functional forms for the relationship between explanatory factors and *BF*. The best-fit model was considered to be the model with the lowest AICc (Burnham and Anderson 2002), with models with AIC values within 2 of each other considered equally good models. The best model(s) were used as a basis to predict the expected values of *BF* across a range of *FL*, *W* and *A*. Statistical analysis was performed in R (R Core Team 2014) using the *lme4* package (Bates et al. 2012). Annual data were pooled for analyses because sample sizes of spawners were small in many of the individual years surveyed for most regions and because preliminary analyses where data were sufficient (Townsville region) indicated non-significant effects of year on *BF*.

4.3 Results

None of the GLMM models was unambiguously the best model to describe the variation in *P. leopardus* batch fecundity but the models describing most information included terms for region and either *FL*, *W,* or *A* (Table 4.2). There also was strong support for the inclusion of zone for the effects of *W* in interaction with region (Table 4.2). The most informative models included linear variation in *FL* and *W*, but non-linear variation in *A* (Table 4.2).

Table 4.2 Form of generalized linear mixed effects models (GLMM) and their relative explanatory power examining the effects of fork length (*FL*), age (*A*), weight (*W*), region (*R*) and zone (*Z*) on *Plectropomus leopardus* batch fecundity (*BF*) from fish collected in three Great Barrier Reef regions (northern, central, southern) and two management zones (reserve and fished reefs) (see Figure 2.1).

Analysis	Model	AIC_c	ΔAIC_c	*w*
Batch fecundity (*FL*)	$\boldsymbol{BF = FL*R + \beta_{reef} + \varepsilon}$	8326.22	0	0.52
	$BF = FL*R + Z + \beta_{reef} + \varepsilon$	8327.96	1.75	0.22
	$BF = FL*R*Z + \beta_{reef} + \varepsilon$	8329.67	3.45	0.09
	$BF = FL + R + \beta_{reef} + \varepsilon$	8331.51	5.29	0.04
Batch fecundity (*A*)	$\boldsymbol{BF = s(A, df = 2) + R + \beta_{reef} + \varepsilon}$	8012.57	0	0.23
	$BF = s(A, df = 3) + R + \beta_{reef} + \varepsilon$	8013.60	1.03	0.14
	$BF = A + R + \beta_{reef} + \varepsilon$	8014.40	1.82	0.09
	$BF = s(A, df = 2) + R + Z + \beta_{reef} + \varepsilon$	8014.48	1.91	0.09
Batch fecundity (*W*)	$\boldsymbol{BF = W*R*Z + \beta_{reef} + \varepsilon}$	8042.85	0	0.31
	$BF = W*R + \beta_{reef} + \varepsilon$	8043.56	0.71	0.22
	$BF = W*Z + \beta_{reef} + \varepsilon$	8044.38	1.53	0.15
	$BF = W*R + Z + \beta_{reef} + \varepsilon$	8045.09	2.24	0.10

Note: The four best-fitting models are shown, and the final model selected (in bold). The effects of *FL*, *W* and *A* were modelled as cubic splines (*s*) with between two and six degrees of freedom (*df*). Models with interaction terms (*) also include main effects. Batch fecundity was modelled using a Gaussian distribution. β_{reef} is the random effect of reef, and ε is the error term. AIC_c is the small-sample bias-corrected form of Akaike's information criterion, Δ is the Akaike difference, and *w* is the Akaike weight.

Batch fecundity increased with length in the northern and, particularly, central regions but did not vary systematically with length in the southern region (Figure 4.1; Table 4.3; Table 4.4). Batch fecundity increased with age between 1 and 6 years before reaching an asymptote in northern and central regions although an asymptote was not observed for the southern region where there were no spawners captured older than five years (Figure 4.2). Females were most fecund for a given age on central GBR reefs, followed by females on northern and then southern reefs (Figure 4.2; Table 4.5).

Batch fecundity increased strongly with weight on reserve reefs in the central region and fished reefs in the northern region, increased moderately with weight on central fished reefs, and varied little with weight on northern reserve reefs and on reserve and fished reefs in the southern region (Figure 4.3; Table 4.3; Table 4.4).Small females at 0.5 kg were similarly fecund on reserve and fished reefs in the southern and northern GBR but more fecund on fished reefs than on reserve reefs in the central GBR (Table 4.3). Females at 2.0 kg were less fecund on northern reserve reefs than on the respective fished reefs, but batch fecundity per female on central reserve reefs was twice that on central fished reefs (Table 4.3). Central reefs generally contained females that were most fecund for a given length, weight, and age compared with northern and southern reefs (Figure 4.1; Figure 4.2; Figure 4.3; Table 4.3). Spawners from southern reefs were consistently least fecund for a given length, weight, and age, and mean batch fecundity was lowest, compared with spawners from central and northern reefs (Table 4.3; Table 4.5).

Mean batch fecundity was two to three times greater on central reefs compared with other regions, and spawners were on average several centimeters longer, several

hundred grams heavier, and up to 0.5 year older than on northern and southern reefs (Table 4.3). The proportion of spawners was also greatest on central reserve reefs where 49% of females sampled were hydrated compared with 8% on northern reefs, and 4% and 12% on southern reserve reefs (Mackay and Storm Cay, respectively) (Table 4.1). On fished reefs 33%, 24%, 1% and 6% of females sampled were hydrated from the northern, central, and southern (Mackay and Storm Cay, respectively) regions (Table 4.1). The spawners from the central reserve reefs represented 49% of all spawners sampled from all 16 reefs across all regions. Sex ratios became increasingly male-biased with latitude on the GBR, with the proportion male increasing from 35% – 39% in the northern, 42% – 55% in the central, and 72% – 83% in the southern GBR (Table 4.1).

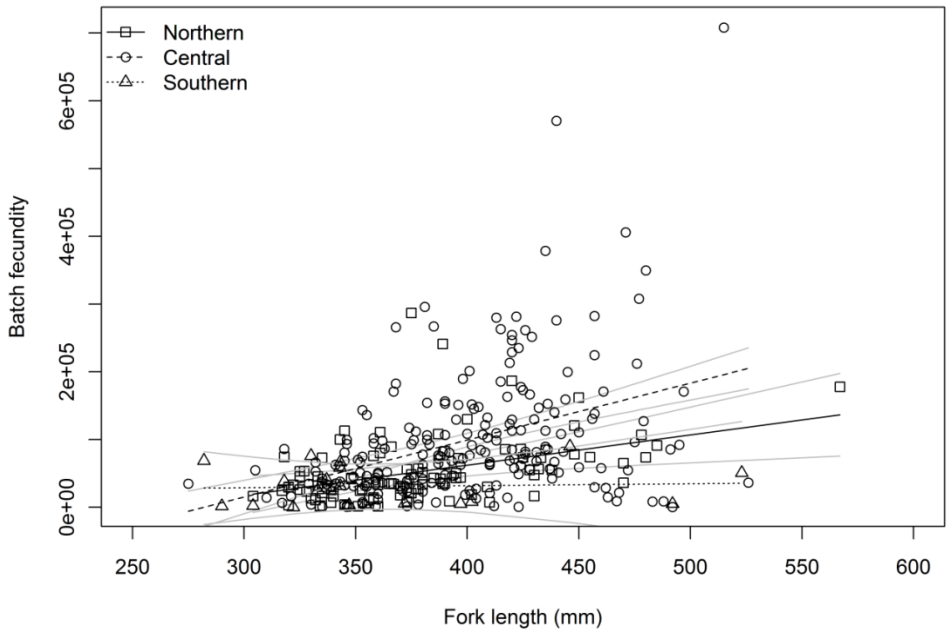


Figure 4.1 Batch fecundity (*BF*) of *Plectropomus leopardus* versus fork length (*FL*; ± 95% CIs) from the northern, central, and southern GBR. Predictions of *BF* from *FL* derived from best fit model described in Table 4.2**.**

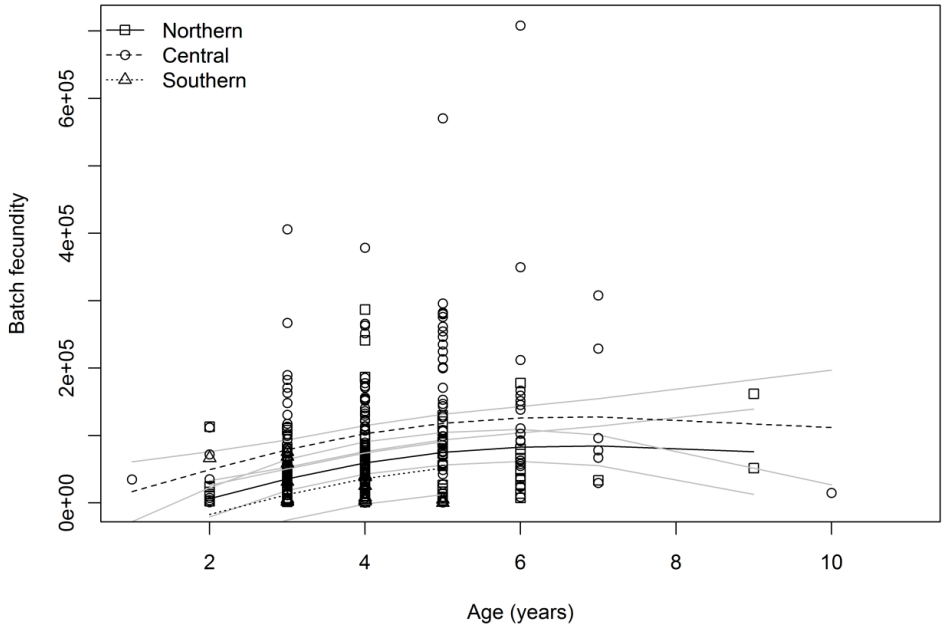


Figure 4.2 Batch fecundity (*BF*) of *Plectropomus leopardus* versus age (*A*; ± 95% CIs) from the northern, central, and southern GBR. Predictions of *BF* from *A* derived from best fit model described in Table 4.2.

Table 4.3 Batch fecundity (*BF*) estimates (±95% confidence intervals) for *Plectropomus leopardus* from three GBR regions (northern, central, southern) based on generalized linear mixed effects models (GLMM; see Table 4.2) for length (*FL*; mm), age (*A*; years), and weight (*W*; kg; fished and reserve reefs), and metrics of female spawners. *n* is sample size.

			Region		
Zone			North	Central	South
Combined	Length-specific *BF*	FL_{350}	39 925 ± 16 639	57 224 ± 15 008	30 238 ± 33 809
		FL_{500}	106 720 ± 41 029	183 251 ± 24 886	34 749 ± 80 084
		n	102	208	19
Combined	Age-specific *BF*	A_3	35 764 ± 17 201	78 969 ± 14 435	12 426 ± 38 010
		A_5	74 834 ± 18 553	118 039 ± 13 455	51 496 ± 39 121
		n	99	199	17
Fished	Weight-specific *BF*	$W_{0.5}$	36 908 ± 19 068	70 800 ± 28 661	30 313 ± 50 368
		$W_{2.0}$	136 235 ± 53 664	123 189 ± 57 179	48 825 ± 112 486
		n	83	43	8
Reserve	Weight-specific *BF*	$W_{0.5}$	38 900 ± 43 591	44 333 ± 17 213	35 334 ± 55 360
		$W_{2.0}$	60 917 ± 169 166	247 225 ± 35 087	33 192 ± 150 195
		n	14	161	9
Fished	Mean *BF*		53 626 ± 10 408	84 632 ± 23 146	31 686 ± 21 270
Reserve	Mean *BF*		41 500 ± 17 075	102 325 ± 15 155	29 809 ± 18 949
Fished	Mean *FL*		379 ± 9	394 ± 16	339 ± 56
Reserve	Mean *FL*		366 ± 23	401 ± 6	378 ± 16
Fished	Mean *A*		4.1 ± 0.2	3.8 ± 0.4	3.6 ± 0.8
Reserve	Mean *A*		3.2 ± 0.4	4.3 ± 0.2	3.7 ± 0.4
Fished	Mean *W*		0.77 ± 0.08	0.90 ± 0.14	0.61 ± 0.54
Reserve	Mean *W*		0.68 ± 0.17	0.93 ± 0.71	0.76 ± 0.29

Table 4.4 Change (%) in batch fecundity (*BF*) with length (FL_{350} - FL_{500}), age (A_3 - A_5), and weight ($W_{0.5}$ - $W_{2.0}$, fished and reserve reefs) for *Plectropomus leopardus* spawners in the northern, central and southern GBR.

			GBR Region		
Zone			North	Central	South
Combined	Length-specific *BF*	FL_{350} - FL_{500}	+167%	+220%	+15%
Combined	Age-specific *BF*	A_3 - A_5	+110%	+50%	+314%
Fished	Weight-specific *BF*	$W_{0.5}$ - $W_{2.0}$	+270%	+74%	+61%
Reserve	Weight-specific *BF*	$W_{0.5}$ - $W_{2.0}$	+57%	+458%	-6%

Table 4.5 Increase (%) in length-, age-, and weight-specific (fished and reserve reefs) batch fecundity for *Plectropomus leopardus* spawners in the central and northern GBR relative to the southern GBR.

			Region	
Zone			Northern	Central
Combined	Length-specific *BF*	FL_{350}	32%	89%
		FL_{500}	207%	427%
Combined	Age-specific *BF*	A_3	188%	534%
		A_5	45%	129%
Fished	Weight-specific *BF*	$W_{0.5}$	22%	134%
		$W_{2.0}$	179%	152%
Reserve	Weight-specific *BF*	$W_{0.5}$	10%	25%
		$W_{2.0}$	84%	645%

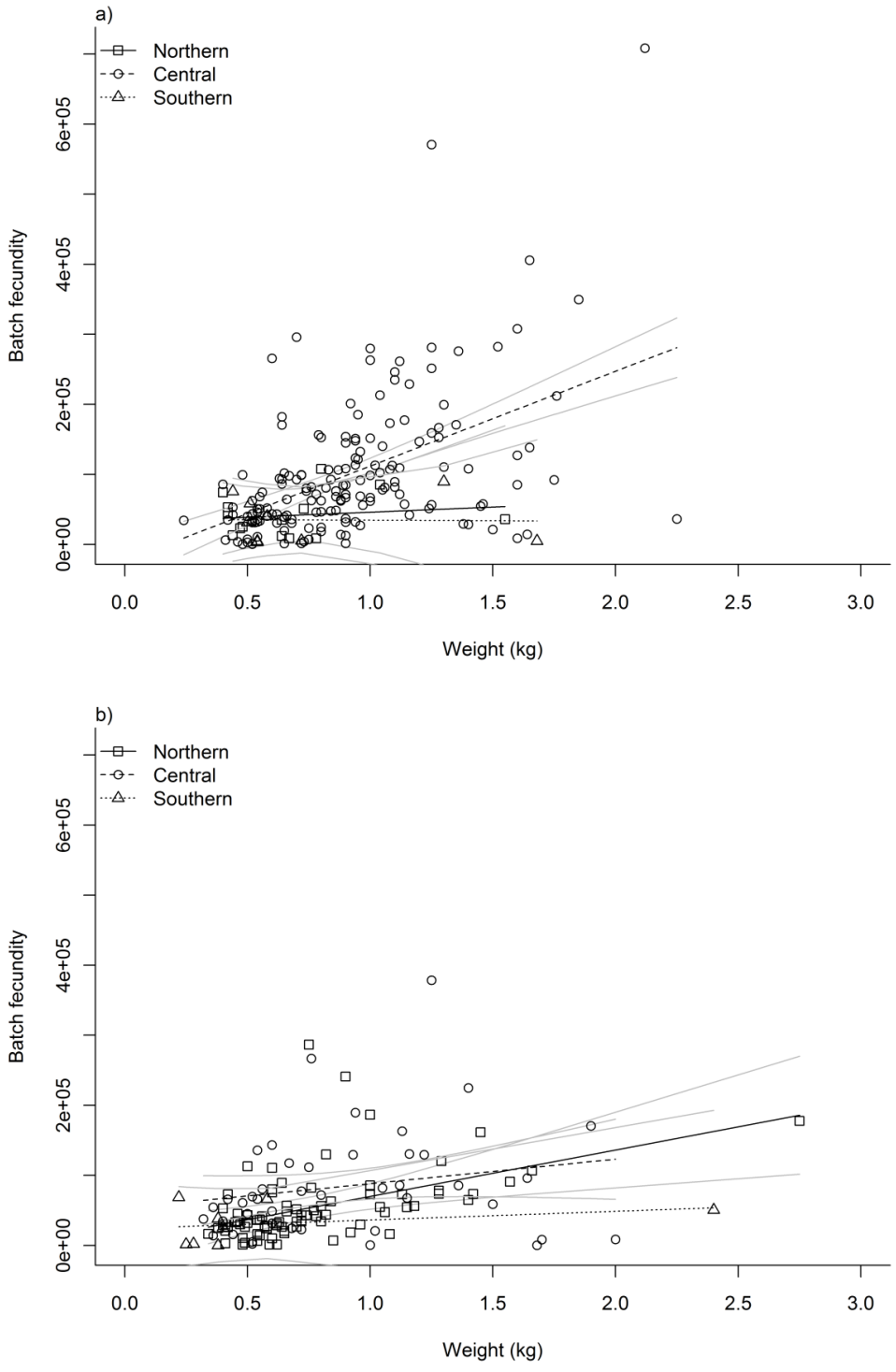


Figure 4.3 Batch fecundity (*BF*) of *Plectropomus leopardus* versus weight (*W*; ± 95% CIs) from the northern, central, and southern GBR on (a) reserve and (b) fished reefs. Predictions of *BF* from *W* derived from best fit model described in Table 4.2.

4.4 Discussion

The comparisons of batch fecundity relationships for *P. leopardus* presented in this study, using samples collected from fished and reserve reefs at a broad regional scale, to my knowledge are the first for a commercially exploited fish. Such comparisons are an important step in quantifying the effect that reserves may have on the reproductive potential of target species and for identifying any material spatial variation in reproductive potential of metapopulations that are managed and harvested as a single stock.

Reserves had a material effect on weight-related fecundity of *P. leopardus* in the central GBR, with heavier females (at 2.0 kg) more fecund and lighter females (0.5 kg) less fecund on reserve reefs than on fished reefs. Heavier females in the northern GBR, however, were more fecund on fished than on reserve reefs, whilst there was no evidence of an effect on southern reefs. Lighter females in the northern and southern GBR showed relatively little difference in batch fecundity between fished and reserve reefs. The magnitudes of differences in batch fecundity were much greater among regions, however, than between management zones. Females were least fecund on southern reefs where adult densities were highest and most fecund in the central region, at intermediate population densities (Mapstone et al. 2004). These results highlight the need for understanding spatial variation in reproduction in order to evaluate the potential effects of reserves beyond reserve boundaries, and to assess the appropriateness of single conservation or fishery management strategies for populations that span large, potentially diverse, spatial scales.

4.4.1 Regional variation in batch fecundity

Spawning females in the central GBR were generally more fecund for a given length, weight, and age, and had a greater mean batch size than spawners from northern and southern regions. Batch fecundity on central reefs was 84 632 $\pm$ 23 136 oocytes batch^{-1} (mean $\pm$ 95% confidence intervals) and 102 325 $\pm$ 15 155 oocytes batch^{-1} on fished and reserve reefs respectively (Table 4.3), similar to a previous estimate of 87 244 $\pm$ 49 898 oocytes batch^{-1} for *P. leopardus* spawners on fished reefs off Cairns in the central GBR that also used the gravimetric method (Samoilys 2000). Spatial variation in fish reproduction has been linked to genetic variation previously (McIntyre and Hutchings 2003). As discussed in Chapter 2, *P. leopardus* comprise a single genetic stock on the GBR (van Herwerden et al. 2009), so genetic variation therefore is unlikely to explain the regional variation in fecundity observed in this study. Variability in environmental conditions such as water temperature and prey availability are more likely drivers of regional differences in *P. leopardus* fecundity, perhaps along with regional variation in fishing effects.

Decreases in fecundity often are attributed to environmental factors, particularly decreases in water temperature (Kjesbu et al. 1998). Mean sea surface temperature (SST) in the southern region of the GBR is generally 1 to 2° C below mean SST in the central and northern GBR (Lough 1999). Average batch fecundity for spawners in the southern region was just 29% – 37% of the average batch fecundity of spawners in the central region. The proportion of females sampled from the southern region, and the proportion of those females that were spawners, was much smaller than from northern and central reefs. Reefs in the southern GBR, however, have two to four times greater densities of adult *P. leopardus* than other regions (Mapstone et al. 2004). Small batch

fecundity and low numbers of hydrated females on southern reefs may be indicative of spawners nearing their thermal limit in the southern half of the species' range. Similar "spawning omission" has been reported for other epinephelids such as *Epinephelus andersoni* on the east coast of South Africa (Fennessy and Sadovy 2002), *E. marginatus* in the Mediterranean (Chauvet 1991), and *Hyporthodus octofasciatus* in Western Australia (Wakefield et al. 2013) where an absence of spawning during the reproductive season toward the latitudinal limits of these species' range was attributed to decreased water temperature.

Female *P. leopardus* from the northern GBR produced batches approximately half the mean size of those produced by females in the central GBR. This difference was driven by spawners in the central region producing larger batches for a given body length, weight, and age, and having longer, heavier, and older spawners compared with the northern region (Table 4.3). Thermally-induced reproductive inhibition is an unlikely explanation for reduced fecundity on northern reefs as the distribution of *P. leopardus* extends throughout equatorial waters, north to southern Japan (Heemstra and Randall 1993). Prey availability may explain partly why females in the northern region produced smaller batches than those in the central region. Prey availability is often correlated with reproductive characteristics and fecundity, most commonly as a positive relationship between prey availability and batch fecundity (Lambert 2008). *P. leopardus* are carnivores (Goeden 1978) and underwater visual surveys conducted from 1995 to 2000 on the same reefs used for this study reported *P. leopardus*' prey densities were generally lowest in the northern region (for some prey species up to five-fold lower) compared with the southern region, and intermediate in the central region (Mapstone et al. 2004). Interestingly, greater prey density in the southern GBR did not

offset whatever other effects, perhaps such as water temperature, may have been driving lowered reproductive output in the south.

As discussed in Chapter 2, it might be argued that the regional variation in *P. leopardus* batch fecundity reported here is an artifact of sampling due to latitudinal variation in the spawning season for *P. leopardus* along the GBR, with females from the southern region sampled outside their peak spawning season. Water temperature is an important trigger for the commencement of spawning for fishes, and spawning often occurs later in the year at higher latitudes (Conover 1992). The spawning season for *P. leopardus* varies regionally, from approximately September to December in the central and northern GBR (Brown et al. 1994, Ferreira 1995, Russ et al. 1995, Samoilys 2000, Frisch et al. 2007), and October to February in the south (Goeden 1978, Brown et al. 1994), yet spawning consistently peaks in October – November regardless of region (Goeden 1978, Brown et al. 1994, Ferreira 1995, Samoilys 2000). Female *P. leopardus* sampled in late October to early December on southern reefs were, therefore, sampled during their peak spawning months.

The possibility needs to be considered that the regional patterns in fecundity of *P. leopardus* may be confounded in part with cross-shelf variation in reef fish communities that has been reported previously on the GBR (Williams 1982, Newman and Williams 1996). Reefs sampled in this study were inshore – mid-shelf reefs in the northern region, mid-shelf and outer-shelf reefs in the central region and all mid-shelf reefs in the southern region (Figure 2.1). Cross-shelf variation in maturation and sex change for two protogynous scarids was reported on a scale of tens of kilometres in the northern GBR (Gust 2004). This variation in reproductive traits primarily was driven by

different physical and biological environments that influenced local population density, mortality rates, and social systems (Gust 2004). It is unclear, however, how this variation may manifest in estimates of fecundity. The effect of reef shelf position on batch fecundity of *P. leopardus* remains unclear, and future studies would benefit from a sampling design that allowed the separation of shelf position and latitudinal effects.

4.4.2 Effect of reserves and fishing

A large component of reserve theory is based on the high predictability of fecundity relationships and the expectation that females within reserves will reach larger sizes and older ages, and therefore be more fecund, and occur in greater densities than in fished areas (Russ 2002). Fecundity of *P. leopardus* increased with length, weight, and age in the central and northern GBR, though at different rates and the relationships were not exponential as is frequently reported for fish (Sadovy 1996), and showed little variation with length or weight in the southern GBR. It is possible that if sampling were conducted during the peak new moon spawning period clearer length-fecundity, weight-fecundity and age-fecundity relationships may have been evident. However, the similarities in mean batch fecundity between this study and a previous estimate of batch fecundity for *P. leopardus* sampled during the new moon in the central GBR (Samoilys 2000) indicates batch fecundity does not change significantly between lunar phase. Further, all regions were sampled over a wide and overlapping range of lunar phases (Table 4.1), supporting my presumption that the relative differences in batch fecundity of *P. leopardus* among regions and zones would be consistent regardless of whether sampling occurred during or outside new moon periods. There was no general or consistent effect of zoning (reserves vs fished reefs) on fecundity, with only one instance of an expected benefit of reserves evident - fecundity increasing more steeply

with weight on reserve reefs than on fished reefs in the central region. The reverse effect was observed in the northern region, however, where there was a steeper rate of increase in fecundity with weight on fished reefs than on reserve reefs. The variable effect of reserves on batch fecundity presented here highlights potential shortcomings in egg production calculations where fecundity of target species is either assumed to always increase on reserve reefs or is assumed to be the same on reserve reefs as in fished areas.

Only the results from central GBR reefs supported the expectations of greater mean individual batch fecundity on reserve reefs relative to fished reefs (Russ 2002). This was driven by spawners on central reserve reefs having strong positive length-, weight- and age-fecundity relationships, and greater fecundity of larger (heavier) females on reserve reefs than their counterparts on fished reefs. The lightest females were less fecund on reserve than fished reefs in the central GBR, however, where greater numbers of heavy, fecund females were present. Social dynamics are an important influence on spawning behaviour of *P. leopardus* (Goeden 1978, Samoilys and Squire 1994, Samoilys 2000). Perhaps intraspecific social interactions between females also influence batch fecundity. Spawners were comparatively shorter, lighter, and younger on reserve reefs than on fished reefs in the northern GBR, contrary to reserve theory predictions. Similarities in abundance, length, and age of *P. leopardus*, across all sampled individuals not specifically for spawners, between fished and reserve reefs in the northern region have been reported previously (Mapstone et al. 2004). The lack of a positive zoning effect on mean fecundity in the northern region may be attributable to relatively low recreational and commercial fishing pressure, and therefore fishing impacts, in this isolated region of the GBR (Mapstone et al. 2004).

Mean batch fecundity of spawners from the southern GBR was similar on reserve and fished reefs despite spawners being longer and heavier, though not older, on reserve reefs. This similarity was likely due to very small increases in batch fecundity with female length (and weight) and similarities in the mean age of spawners between fished and reserve reefs (3.6 $\pm$ 0.8 and 3.7 $\pm$ 0.4 years, respectively). As described in Chapter 2.3, *P. leopardus* populations in the southern region are heavily male-biased (Table 4.1), meaning that increases in batch fecundity with length, weight, and age will be realized only in a very small proportion of the population compared with populations on central and northern reefs where sex ratios are more female biased and female-male sex change occurs at larger sizes and older ages.

4.4.3 Potential implications for recruitment subsidy

The very low densities of hydrated females and small batch fecundity on southern reefs were unexpected, especially given the relatively high population densities and comparable commercial harvests of *P. leopardus* from this region compared with the central region (Mapstone et al. 2004, Tobin et al. 2013). As discussed in Chapter 2, it is possible that greater densities of adult *P. leopardus* occur in the southern GBR due to higher survival rates for *P. leopardus* larvae at high latitudes. Larvae produced in colder waters generally experience decreased larval mortality rates and greater net survivorship, although this is often countered by slower growth and longer larval stage duration (Houde 1989).

Alternatively, greater densities of adult *P. leopardus* on southern reefs may indicate that recruits originate from reefs in the central GBR where females produce much larger egg batches and there is a greater abundance of spawners. This hypothesis is also discussed

in Chapter 2 and is consistent with the genetic similarity in *P. leopardus* along the GBR that indicates extensive larval dispersal and substantial larval connectivity among regions (van Herwerden et al. 2009). Pelagic larval duration of *P. leopardus* is almost four weeks (Doherty et al. 1994), which would allow for extensive dispersal. The predominant flow of water between the central and southern GBR is southward due to the East Australia Current (Wolanski and Pickard 1985). Current patterns and geomorphology appear to drive larval dispersal at large spatial scales for *P. leopardus* on the GBR (Doherty et al. 1994), and larval dispersal modelling in the central GBR (Cairns region) supports the theory that there is net export of larvae from northern source to more southerly sink reefs, with self-recruitment accounting for less than 9% of the settling cohort in 80% of reefs (James et al. 2002, Bode et al. 2006).

Recent genetic parentage analysis and estimation of larval dispersal of *Plectropomus areolatus* (Almany et al. 2013) in Papua New Guinea and *Plectropomus maculatus* (Harrison et al. 2012) on the southern-inshore GBR, however, is not completely consistent with this hypothesis of long distance larval dispersal of *Plectropomus* on a scale of hundreds of kilometres. Rather, these studies indicate that the majority of *Plectropomus* larvae settle within tens of kilometres of their natal reef. These studies were done in environments where strong long-shore currents, such as the East Australia Current, are not as influential, however, and might not reflect ubiquitous larval dispersal characteristics for *P. leopardus*. Parentage analysis should be applied to estimate larval dispersal of *P. leopardus* to determine whether central GBR reefs are an important source of recruits for southern reefs in the mid-shelf and off-shore areas characterised by strong long-shore transport.

A regional approach to management of the *P. leopardus* fishery may need to be considered to ensure adequate biomass of spawners in the central GBR if biomass and the sustainability of the southern GBR commercial fishery are reliant on replenishment of recruits from reefs to the north. A shift in fishing pressure from the southern GBR to the central and northern GBR could have cumulative detrimental consequences if the central GBR is a major source of recruits to the southern sink region.

Recruitment subsidy is one of the major prospective benefits proffered in support of declaring reserves (Russ 2002, Cudney-Bueno et al. 2009). Local reserves within each region are potentially important larval sources for fished reefs if the scale of larval dispersal is just tens of kilometres, as has been indicated for two *Plectropomus* spp. in two areas. Two-fold greater adult biomass, larger adult size, and greater per capita and per unit fecundity within reserves compared with fished reefs in the Keppel Island region of the GBR was attributed to reserves supplying 50% of total *P. maculatus* recruitment in that region despite covering just 28% of reef habitat (Harrison et al. 2012). Spawning females also were larger, older, more fecund, and more numerous in central GBR reserves in this study, characteristics that presumably also could lead to recruitment subsidy from reserves to fished reefs in that region, or potentially other regions further south. A future increase in fishing pressure in the northern GBR would potentially increase the importance of reserves for recruitment subsidy within this lightly fished northern region, though the importance of such potential subsidy for other regions is less clear as the direction and strength of long-shore transport in the northern GBR varies considerably with latitude north of about 18° S (Church 1987).

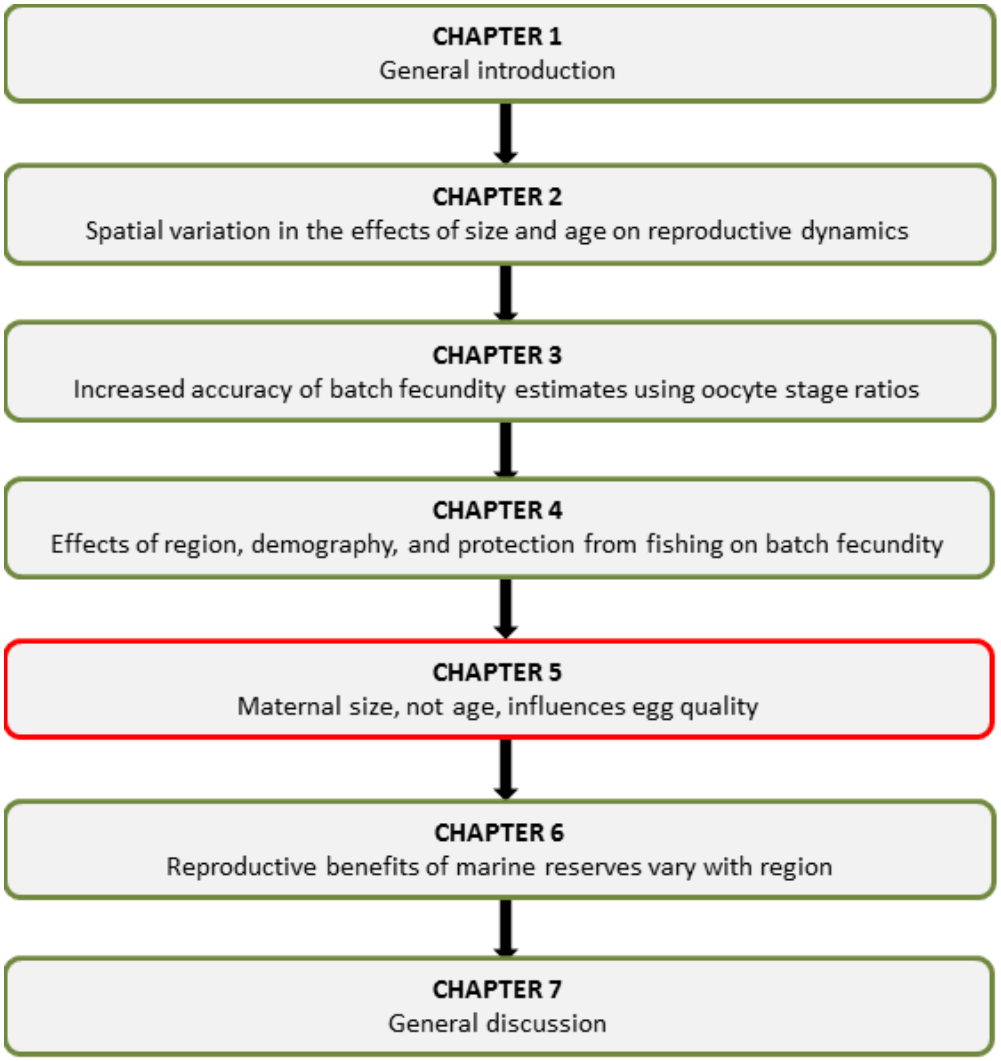
The potential for recruitment subsidy from reserves to fished reefs in the southern GBR may be limited, despite greater densities of *P. leopardus* on reserves than fished reefs, due to relatively smaller batch fecundity, limited positive effects of female length or weight on batch fecundity, the relatively younger age and smaller size in which sex change occurs, and strong male bias in southern reefs (Chapter 2.3). Recent modelling of the reproductive benefits of reserves suggests the recruitment subsidy benefits of reserves may be limited for *P. leopardus*, despite greater yields and population growth within reserves, because large females are "lost" through sex change due to protogyny (Chan et al. 2012). The results presented here support Chan et al.'s model and also indicate that regional variation in the rate of sex change in *P. leopardus* might reduce further the potential benefits of reserves for reproductive output and, potentially, recruitment subsidy in the southern GBR.

4.4.4 Conclusion

This is the only study to my knowledge worldwide to compare batch fecundity using fish collected within *and* outside of reserves across a broad geographic area. Spatial variation in batch fecundity, proportion of males, proportion of spawners, and length, weight, and age of *P. leopardus* demonstrates the varied outcomes that reserve reefs can have on reproductive output when the distribution of a target species spans a broad geographic range. In particular, this study demonstrates that the conditions that female *P. leopardus* experience on central GBR reefs serve to maximize their reproductive potential, particularly in the absence of fishing. Greater individual batch fecundity on central GBR reserves may have important implications for recruitment subsidy at a regional scale (north-to-south recruitment subsidy) as well as the local scale (reserve-to-fished reef recruitment subsidy), but there was no similar evidence of potential

subsidies from reserve reefs in other regions. This study highlights the need for further research on reproductive responses of target species to fishing, at appropriate spatial scales, and careful consideration of the suitability of single conservation or fishery management strategies for species distributed across large and diverse spatial scales.

CHAPTER 5 Maternal size, not age, influences egg quality



Chapter 5 has been published as: Carter AB, Carton AG, McCormick MI, Tobin AJ and Williams AJ (2015). Maternal size, not age, influences egg quality of a wild, protogynous coral reef fish, *Plectropomus leopardus*. Marine Ecology Progress Series 529:249 – 263. doi:10.3354/meps11277.*

5.1 Introduction

Maternal effects are an important source of non-genetic (phenotypic) variation within a population and are widely known to influence the reproductive potential of a fished stock (Green 2008). The quantity and quality of eggs is often strongly influenced by the female size and age structure of a population; larger, older females often have greater batch fecundity (Sadovy 1996), spawn more frequently (Lowerre-Barbieri et al. 2009), have a longer spawning season (Claramunt et al. 2007), and produce better quality eggs and larvae (Berkeley et al. 2004a) than smaller, younger conspecifics. Female body condition may also influence egg quality, with the hepatosomatic index commonly used as an indicator of body condition because of the liver's ability to store high levels of lipids used in egg production (Berkeley et al. 2004a, Macchi et al. 2013). Maternal effects on egg quality can have important implications for the reproductive potential of fished stocks because age or size truncation of a population through the removal of larger and older females by fishing can cause a greater reduction in the reproductive potential of that population than just a reduction in mature female biomass (Berkeley et al. 2004b, Venturelli et al. 2009, Hixon et al. 2014).

The size and biochemical composition of the egg is an important maternal contribution in fishes. Egg quality is commonly used to describe the condition of fish eggs, which can have a profound effect on fertilisation success, hatching success, larval growth and larval survival (Kjørsvik et al. 1990, Brooks et al. 1997). Fish eggs consist mostly of lipoprotein yolk that contain primarily polar lipids mainly in the form of phospholipids, which is a structural lipid used in catabolism for energy, and is rich in polyunsaturated fatty acids (*PUFA*), and some neutral lipids (Wiegand 1996). Many marine pelagic fish

eggs also contain an oil droplet that consists mostly of neutral lipids (mainly triacylglycerol [*TAG*], wax ester [*WE*] and/or steryl ester) that provide the egg with most of its energy (Wiegand 1996, Tocher 2003). These lipids provide an important endogenous energy source for the developing embryos and larvae during the period between fertilization and the onset of exogenous feeding (reviewed by Tocher 2003). Egg quality is, therefore, most frequently estimated using measurements of egg size, oil droplet size, and/or egg biochemical composition because larger eggs often indicate greater maternal investment into yolk and neutral lipids (Green 2008). Egg and/or oil droplet size has been positively correlated with fertilization rates, larval size at hatching, larval growth rates, and larval resistance to starvation (McCormick and Gagliano, Moodie et al. 1989, Hutchings 1991, Marteinsdottir and Begg 2002, Berkeley et al. 2004a). Egg lipid composition indicates the relative types of energy available to the developing embryo; eggs high in neutral lipids are often positively correlated with larval growth and survival (Berkeley et al. 2004a, Hilton et al. 2008).

Maternal effects on egg quality have been researched extensively, but the majority of studies have focused on temperate fishes, many of which are gonochores (see reviews by Wiegand 1996, Kamler 2005, Green 2008). Research questions are frequently framed in an aquaculture context where maximizing the number of viable larvae produced by female broodstock is a priority (Migaud et al. 2013). Aquaculture-related studies often use pooled egg samples rather than individual fish to examine the effects of diet or environment on broodstock egg quality (Brooks et al. 1997) or to compare egg quality between wild and captive spawners (Kjørsvik 1994). Less emphasis has been placed on egg quality of individual wild females (Bachan et al. 2012), and even fewer studies have investigated maternal effects on egg quality for tropical reef fishes

(Donelson et al. 2008, Maddams and McCormick 2012). Those studies that have examined maternal effects on egg quality for tropical reef fish have focused on populations of small species that have limited commercial food fisheries value, and are unlikely to be affected by fisheries or protection within reserves (McCormick and Gagliano, Green and McCormick 2005, Maddams and McCormick 2012). Maternal effects on egg quality for exploited protogynous fish are of particular interest for fisheries management because the implications of the direct removal of large females through fishing may be compounded because the loss of large males through fishing can induce female-male sex change at younger sizes and ages (Hawkins and Roberts 2004).

This chapter provides the first description of relationships between maternal traits and egg quality for *Plectropomus leopardus*. As discussed in Chapters 2 and 4, fishing for *P. leopardus* on the GBR has been implicated in reducing the size at sex change, reducing the mean size and age of spawning females, and reducing population density relative to reserve reefs (Emslie et al. 2015). If larger and older female *P. leopardus* that are in better condition can produce better quality eggs than smaller and younger females, the removal of the former, either directly by fishing or indirectly by fishing-induced early sex change, may reduce the quality of reproductive output on fished reefs relative to reserves.

The objective of this chapter was to examine the relationship between maternal traits (length, weight, age, HSI), egg morphometrics (egg diameter, oil droplet diameter) and egg biochemical composition (total lipid and lipid class composition) as indicators of egg quality in *P. leopardus*.

5.2 Methods

5.2.1 Sample collection

Gravid females in imminent spawning condition were collected from inner-shelf reefs located in the central section of the GBR, Queensland, Australia. Bramble Reef was sampled in 2008 (18.42° S, 146.72° E) and the neighbouring John Brewer Reef was sampled in 2009 (18.63° S, 146.05° E) (Figure 5.1). Mean monthly water temperature data for each sampling period was collected using the Australian Institute of Marine Science historic data tool for Davies Reef (DAVAWSL2 at 8.4m reef slope site, Site 2; http://data.aims.gov.au/aimsrtds/datatool.xhtml; Figure 5.1). Fishing surveys were conducted late October 2008 (mean monthly water temperature (MMWT) 27.0° C), mid-October 2009 (MMWT 26.7° C), and mid-November 2009 (MMWT 27.4° C) and coincided with the new moon lunar phase when spawning activity peaks for *P. leopardus* (Samoilys 2000). In 2008, live fish were transported to Orpheus Island Research Station at the end of each fishing trip, and in 2009, live fish were transported to Townsville prior to stripping for eggs in the lab.

Gravid females were identified by swollen abdomens and urogenital pores. In 2008, nine gravid females were successfully stripped of hydrated eggs with a further 28 successfully stripped in 2009. To collect eggs, a firm anterior to posterior stripping pressure was applied to the abdomen. Eggs released from the urogenital pore were collected in a sterile 600 mL sample jar whilst ensuring faeces, urine, blood and/or seawater did not contaminate egg samples, and that all eggs collected remained unfertilized. Egg samples from each female were divided in half for analysis of egg size characteristics (morphometry) and egg biochemistry.

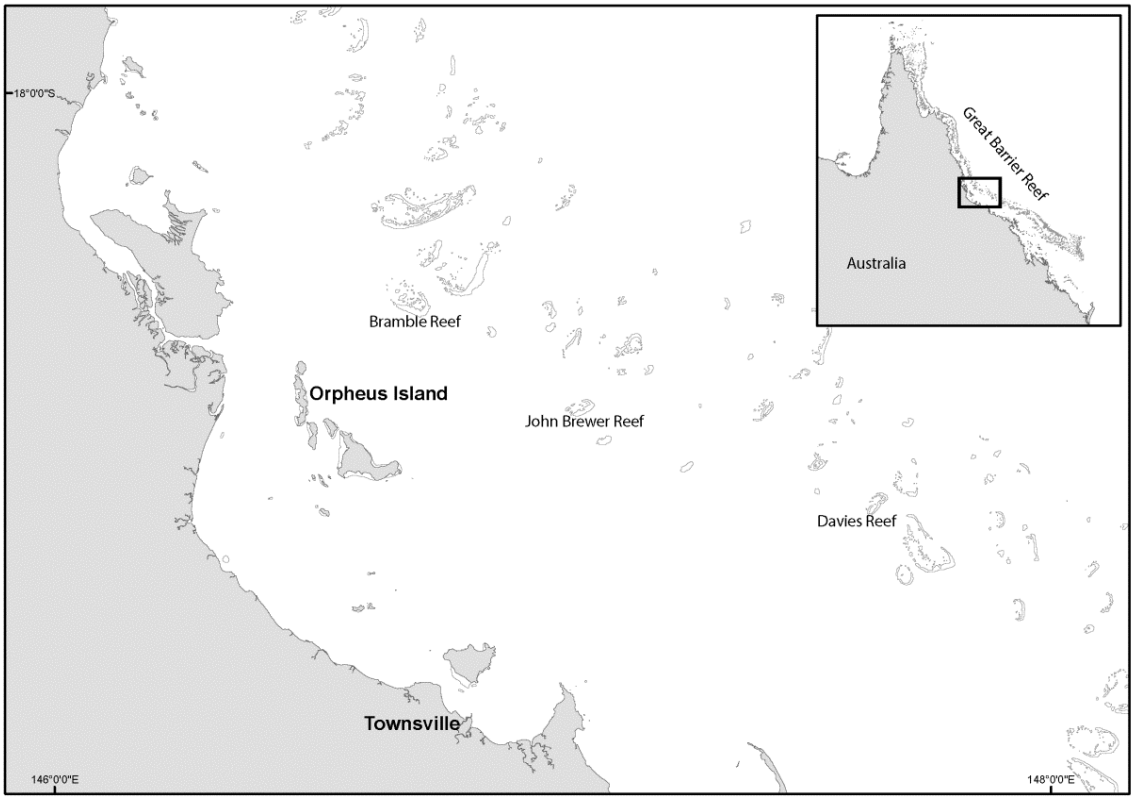


Figure 5.1. Egg quality estimates were obtained from *Plectropomus leopardus* sampled on reefs in the central Great Barrier Reef. Reef spatial layers courtesy of Great Barrier Reef Marine Park Authority © Commonwealth of Australia 2009.

Each stripped fish was anaesthetized, fork length (*FL*) measured to the nearest millimetre and weighed (total weight, *W*) to the nearest gram. I was unable to weigh all females due to a malfunction that developed with the scale during the November 2009 sampling period. The liver from each fish was also removed and weighed to determine hepatosomatic index (*HSI*) as an indicator of body condition and energy reserves using the formula:

HSI = (liver wet weight/total female wet weight)×100 (Wootton 1977).

Otoliths were removed, dried and stored in envelopes for later ageing as described in Chapter 2.2.

5.2.2 Egg morphometry and lipid composition

P. leopardus eggs contain a single oil droplet (Figure 5.2). For egg morphological measurements, fresh samples of whole, hydrated eggs were stored in a 1 mL cryovial with a small amount of sea water and refrigerated for < 24 h prior to being photographed using a high powered digital photo-microscope with 1 mm scale bar. Photographs were loaded into ImageTool computer software (UTHSCSA ImageTool 3.0®; http://ddsdx.uthscsa.edu/dig/itdesc.html), calibrated and measurements of egg diameter (*ED*) and oil droplet diameter (*ODD*) were taken to the nearest 0.01 mm of n = 30 randomly selected eggs per female.

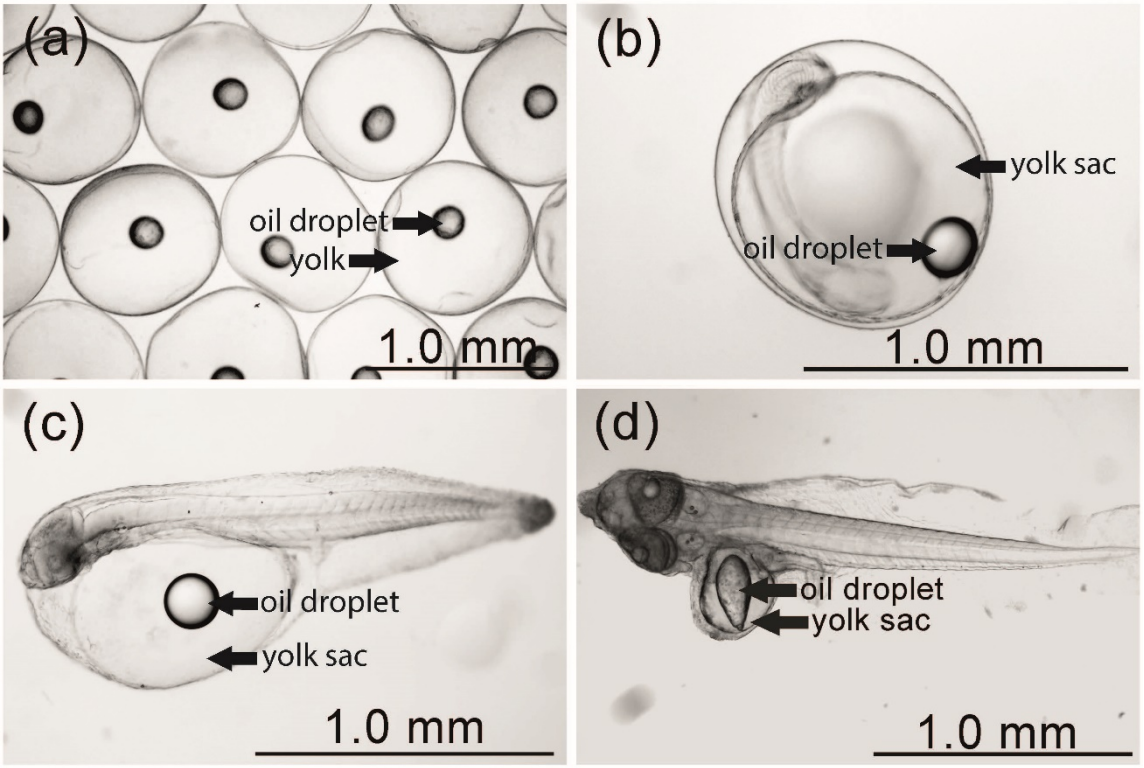


Figure 5.2 *Plectropomus leopardus* eggs with oil droplet at varying stages of development: (a) unfertilized eggs; (b) developing embryo, 3 hours post fertilization; (c) newly hatched larva still dependent on endogenous reserves, 3 hours post hatching; (d) larva at approximate onset of exogenous feeding with greatly depleted yolk sac, 43 hours post hatching. Photographs: A. Carter.

For lipid composition analysis, remaining whole, hydrated eggs were distributed into ~10 × 1 mL cryovials immediately after stripping, frozen in liquid nitrogen, and stored at -80° C for 14 – 15 months (2008 samples) and 2 – 3 months (2009 samples) prior to analyses. Eggs were emptied into a 750 mL vacuum flask and rinsed with MQ water for 2 – 3 minutes whilst under vacuum. Egg samples were filtered on Whatman 25 μm GF/F filters, removed from the filter paper using a clean stainless steel spatula, placed into pre-weighed 2.5 cm diameter glass vials, and the wet weights (± 0.01 g) of the egg samples were measured. Egg samples were homogenized for 5 – 10 minutes using a CAT hand-held homogenizer (X120). Accurate quantities of methanol, chloroform and MQ water (according to the Bligh and Dyer 1959 method) were used separately throughout the homogenization of samples with the intention of cleaning the homogenizer of any tissue residue. After each egg sample was processed, the homogenizer was dissembled and cleaned with MQ water and chloroform.

Total lipid was extracted from each egg sample by the modified Bligh and Dyer (Bligh and Dyer 1959) method using a one-phase methanol: chloroform: water solvent mixture (2:1:0.8 v/v/v). Approximately 0.5 g of each egg sample was weighed to the nearest 0.01 g before each sample was extracted. Egg samples and solvents were placed into 250 mL separatory funnels and shaken to form a miscible solution. Each sample was left overnight before breaking phase by the addition of chloroform and water so that the final ratio of solvents was 1:1:0.9. Samples were then left for approximately 4 hours to allow sufficient time for the separation of an upper aqueous phase and a lower chloroform phase. Total lipids were recovered from the lower chloroform phase into 250 mL round bottom flasks followed by the removal of chloroform *in vacuo* using a rotary evaporator at ~20 ºC. The total lipid extract (TLE) was transferred to chloroform

washed vials and concentrated by application of inert nitrogen gas, and diluted for further analyses. One mL of chloroform was added to samples so that the final concentration was ~10 mg of lipid in the vial. The TLE was weighed to determine total lipid content (%) where 1% lipid content (wet weight) is 10 mg g^{-1} (wet mass).

Lipid class composition of egg samples was determined using an Iatroscan Mark V TH10 thin layer chromatograph (TLC) coupled with a flame ionisation detector (FID) (Nichols et al. 1994). For each sample, the TLE was spotted and developed in a polar solvent system (60:17:0.1 v/v/v hexane: diethyl-ether: acetic acid); samples were also run in a non-polar solvent system (96:4 v/v hexane: ether) to resolve hydrocarbon (mainly Squalene, *SQ*) from wax esters (*WE*) and diacylglyceryl ethers (*DAGE*) from triacylglycerols (*TAG*). All samples were run in duplicate along with standard solutions, which contained known quantities of *WE*, *DAGE*, *SQ*, *TAG*, free fatty acids (*FFA*), sterols (*ST*), and polar lipids (*PL*). Chromarods were oven dried for 10 min at 100º C and analysed immediately. Peaks were quantified using DAPA Scientific Software (Kalamunda, Western Australia). Total lipid class content was obtained by summing the individual lipid classes determined using the Iatroscan TLC-FID. Total lipid content of egg samples was also obtained gravimetrically by TLC-FID. Total lipid (*TL*) content reported here was obtained by averaging total lipid content obtained gravimetrically and independently for all individual samples. Total lipid and lipid class values represent the average of two samples, except where one value was considered an outlier and removed when percentage accuracy was < 80%.

5.2.3 Data analysis

Egg samples from both years were pooled due to small sample size and Pearson's product-moment correlation coefficients were used to examine relationships between egg quality indicators *TL*, *WE*, *TAG*, *FFA*, *ST*, *PL*, *ED* and *ODD*. Egg diameter and *ODD* were log-transformed prior to correlation analysis but not for the generalized linear models (GLM). All statistical analyses were conducted in the statistical software environment R (v.3.0.2.) (R Core Team 2014).

The effects of maternal traits (factors: *FL*, *W*, *A* and *HSI*) on each indicator of egg quality (response variables: *TL*, *WE*, *TAG*, *FFA*, *ST*, and *PL*) were modelled using GLMs with a Gaussian distribution in the lme4 package (Bates et al. 2012). Explanatory variables were tested for collinearity prior to fitting the models using variance inflation factors (VIFs) (Zuur et al. 2009) in the car package (Fox and Weisberg 2009). The VIFs of *W* and *FL* were > 9, indicating that these factors were highly correlated. When either *W* or *FL* were removed and the VIFs recalculated, all VIFs were < 3, indicating that the collinearity among variables was within reasonable limits and would not substantially inflate the standard errors of each model's parameter estimates (Zuur et al. 2009). Separate *W* and *FL* models were then run that each included a three-way interaction with *A* and *HSI*.

To determine the optimal model, a global model was created for each egg quality indicator where all explanatory variables up to 3-way interactions were considered. Sub-model sets of the global model were then generated using the dredge function in the MuMIn package (Barton 2013). MuMIn requires no missing values in the dataset, so prior to analysis the *FL*-*W* relationship for *P. leopardus* was estimated and used to

generate missing *W* and *HSI* values. A second *W* model was also run without the 11 females for which *W* and *HSI* were estimated to assess the influence of using imputed weight data. The top set of models were defined as those that fell within two Akaike's Information Criterion corrected for small sample sizes (AICc) (Burnham and Anderson 2002) of the top ranked model in the set. The best-fit model was considered to be the simplest model with the lowest AICc (Burnham and Anderson 2002) and was used as a basis to predict the expected values of response variables. An overall test of the best-fit models were conducted by comparing that model to the intercept only (null) model with a Chi-squared test to determine whether the model selected was significantly better at predicting the measure of egg quality. A p-value of < 0.05 indicated there was a relationship between one or more of the maternal predictors and the egg quality indicator. Residual plots and q-q plots were examined for each best-fit model for violations of the assumptions of homogeneity of variance and normality.

5.3 Results

5.3.1 Egg characteristics

Egg diameter for *P. leopardus* ranged from 0.80 to 0.92 mm, and *ODD* ranged from 0.18 to 0.20 mm (Figure 5.2a, Table 5.1). Eggs consisted of approximately 3% *TL* content. The major portion of the egg lipid was composed of neutral lipids, with the short-term storage lipid *WE* comprising the largest proportion of *TL* (~40%) followed by the long-term storage lipid *TAG* (~34%). The next most dominant lipid class was *PL* (~24%), while *ST* accounted for < 2% of *TL*. Low levels of *FFA* were present in all egg samples (< 0.5%). No *SQ* or *DAGE* were detected in the eggs.

There was a significant positive relationship between *TL* and *TAG* ($r = 0.32$), and *PL* and *FFA* ($r = 0.33$) (Table 5.2). Sterols were significantly and negatively correlated with *TL* and *WE*, and *PL* was significantly and negatively correlated with *TL*, *WE* and *TAG* (Table 5.2). Sterols and *PL* were positively correlated ($r = 0.76$, $p < 0.001$) as were measurements of *ED* and *ODD* ($r = 0.42$, $p < 0.01$; Table 5.2).

Table 5.1 Minimum and maximum (all sampling periods combined), and mean ± standard error (SE) with sample size (*n*; for each sampling period and for all sampling periods combined) of mature females, and egg characteristics for *Plectropomus leopardus* collected from the central Great Barrier Reef, Australia. Percentage values refer to percent of total lipid within the egg unless otherwise stated.

		Minimum	Maximum	Mean ± SE (*n*)			
		All samples	All samples	Oct 2008	Oct 2009	Nov 2009	All samples
Mature females	Fork length (mm)	315	525	458 ± 12 (9)	409 ± 12 (19)	430 ± 12 (12)	426 ± 8 (40)
	Age (years)	3.0	9.0	4.4 ± 0.4 (9)	4.4 ± 0.4 (18)	4.1 ± 0.3 (11)	4.3 ± 0.2 (38)
	Total weight (g)	200	2400	1570 ± 133 (9)	862 ± 101 (18)	850 ± 50 (2)	1081 ± 96 (29)
	Hepatosomatic index	0.3	2.7	1.1 ± 0.1 (9)	1.2 ± 0.1 (18)	1.1 ± 0.6 (2)	1.2 ± 0.1 (29)
Eggs	Total lipid (% egg wet weight)	1.5	7.0	3.0 ± 0.6 (9)	3.2 ± 0.3 (19)	3.2 ± 0.4 (12)	3.1 ± 0.2 (40)
	Wax ester (%)	29.9	51.1	43.8 ± 1.6 (9)	38.3 ± 1.2 (19)	40.4 ± 1.6 (12)	40.2 ± 0.9 (40)
	Triacylglycerol (%)	21.1	41.2	31.9 ± 1.5 (9)	34.8 ± 1.1 (19)	35.6 ± 0.9 (12)	34.4 ± 0.7 (40)
	Free fatty acid (%)	0.00	0.51	0.06 ± 0.06 (9)	0.05 ± 0.03 (19)	0.06 ± 0.02 (12)	0.06 ± 0.02 (40)
	Sterols (%)	0.79	2.60	1.5 ± 0.1 (9)	1.8 ± 0.1 (19)	1.5 ± 0.1 (12)	1.6 ± 0.1 (40)
	Polar lipids (%)	10.6	34.6	22.7 ± 1.6 (9)	25.0 ± 1.6 (19)	22.4 ± 1.4 (12)	23.7 ± 0.9 (40)
	Egg diameter (mm)	0.80	0.92	0.88 ± 0.01 (9)	0.84 ± 0.004 (19)	0.83 ± 0.006 (12)	0.85 ± 0.004 (40)
	Oil droplet diameter (mm)	0.18	0.20	0.19 ± 0.002 (9)	0.18 ± 0.001 (19)	0.18 ± 0.001 (12)	0.19 ± 0.001 (40)

Table 5.2 Correlations for egg quality indicators total lipid (*TL*, proportion of egg wet weight), wax esters (*WE*, proportion of total lipid content [*TL*]), triacylglycerol (*TAG*, proportion of *TL*), free fatty acid (*FFA*, proportion of *TL*), sterols (*ST*, proportion of *TL*), polar lipids (*PL*, proportion of *TL*), egg diameter (*ED*, mm), and oil droplet diameter (*ODD*, mm). Pearson's product–moment correlation coefficients (*r*) are presented in the upper triangle and the 95% confidence intervals are presented in the lower triangle. Statistically significant correlations (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) and corresponding confidence intervals are in bold. Egg diameter and *ODD* were log-transformed prior to analysis.

	TL	*WE*	*TAG*	*FFA*	*ST*	*PL*	*ED*	*ODD*
TL	-	0.20	**0.32***	-0.15	**-0.36***	**-0.40***	-0.10	-0.0003
WE	-0.12 to 0.48	-	-0.21	-0.17	**-0.68*****	**-0.72*****	0.12	0.04
TAG	**0.01 to 0.57**	-0.49 to 0.11	-	-0.28	-0.28	**-0.52*****	-0.17	0.05
FFA	-0.44 to 0.17	-0.46 to 0.15	-0.54 to 0.04	-	0.20	**0.33***	0.21	-0.05
ST	**-0.60 to -0.05**	**-0.82 to -0.47**	-0.54 to 0.04	-0.12 to 0.48	-	**0.76*****	0.01	-0.13
PL	**-0.63 to -0.10**	**-0.85 to -0.53**	**-0.72 to -0.25**	**0.02 to 0.58**	**0.59 to 0.87**	-	0.01	-0.06
ED	-0.40 to 0.22	-0.20 to 0.41	-0.45 to 0.15	-0.11 to 0.49	-0.31 to 0.32	-0.30 to 0.32	-	**0.42****
ODD	-0.31 to 0.31	-0.28 to 0.34	-0.26 to 0.36	-0.36 to 0.27	-0.43 to 0.19	-0.37 to 0.25	**0.13 to 0.65**	-

5.3.2 Maternal influences on egg quality

A broad size and age range of female *P. leopardus* were examined for egg quality. These females ranged from 315 to 525 mm *FL*, 200 to 2400 g *W*, and 3 to 9 years *A* (Table 5.1). The relationships between maternal traits and indicators of egg quality were variable. The best-fit models that described most information on each indicator of egg quality generally included terms for either *FL* or *W* (Table 5.3, Table 5.4). None of the models selected included *A*, and *HSI* was a significant predictor of *ST* only in interaction with *W* (Table 5.3, Table 5.4).

Total lipid content within the eggs of *P. leopardus* did not vary with any of the maternal traits examined. The proportion of lipid classes within the *TL* of eggs, however, did vary. Wax esters as a proportion of *TL* was best described by a model that included *FL* or *W* (Table 5.3). Eggs spawned by large females (defined as 2000 g or 500 mm *FL*) contained approximately 15% and 16% more *WE*, respectively, than eggs spawned by small females (defined as 500 g or 350 mm FL) (Table 5.5). Sterols and *PL* as a proportion of *TL* decreased with female *FL* (Figure 5.3; Table 5.3, Table 5.4, Table 5.5). Triacylglycerol and *FFA* as a proportion of *TL* were not influenced by any of the maternal traits examined and remained constant despite variation in *ED* or *ODD* (Table 5.2, Table 5.3, Table 5.4).

Egg diameter increased with *W* and *FL*, and *ODD* also increased with *W* (Figure 5.3, Figure 5.4). Eggs spawned by large females were approximately 4% larger than eggs spawned by small females (Table 5.5). The models with *W* as a predictor of *ODD*, and *FL* as a predictor of *ED*, were selected as the best models despite the null model's

inclusion in the top model set (Table 5.3) because these models were significantly better predictors of *ODD* and *ED* than the respective null models (Chi-square tests, $p < 0.05$).

Table 5.3 Summary of generalized linear models (GLM) showing the set of top models ($AIC_c < 2$) predicting the relationship between maternal traits and egg quality indicators for *Plectropomus leopardus*. Two sets of global models were run to avoid collinearity between predictors: (a) weight (*W*), age (*A*), and hepatosomatic index (*HSI*), and (b) fork length (*FL*), *A*, and *HSI*. The best model selected for each egg quality indicator is in bold.

Global model	Egg quality indicator	Model	df	log Lik	AIC_c	ΔAIC_c	*w*
(a)	Total lipid	**Null**	**2**	**106.31**	**-208.3**	**0.00**	**0.39**
		HSI	3	106.61	-206.5	1.76	0.16
	Wax esters	***W***	**3**	**59.41**	**-112.1**	**0.00**	**0.29**
		*W + HSI + W*HSI*	5	61.31	-110.7	1.41	0.15
	Triacylglycerol	*HSI*	3	64.67	-122.6	0.00	0.27
		Null	**2**	**63.05**	**-121.7**	**0.88**	**0.17**
		W + HSI	4	65.22	-121.2	1.43	0.13
		A + HSI	4	64.98	-120.7	1.91	0.10
	Free fatty acids	**Null**	**2**	**196.74**	**-389.1**	**0.00**	**0.32**
		A	3	197.22	-387.7	1.41	0.16
		HSI	3	197.08	-387.4	1.69	0.14
	Sterols	***W + HSI + W*HSI***	**5**	**156.54**	**-301.1**	**0.00**	**0.53**
	Polar lipids	*W*	3	54.68	-102.6	0.00	0.18
		W + HSI	4	55.71	-102.2	0.46	0.14
		A	3	54.20	-101.7	0.97	0.11
		Null	**2**	**52.86**	**-101.4**	**1.26**	**0.10**
		A + W	4	55.17	-101.1	1.54	0.08
		*W + HSI + W*HSI*	5	56.43	-100.9	1.71	0.08
	Egg diameter	***W***	**3**	**83.58**	**-160.4**	**0.00**	**0.36**
		W + HSI	4	84.34	-159.4	1.00	0.22
	Oil droplet diameter [nb.]	***W***	**3**	**139.40**	**-272.1**	**0.00**	**0.26**
		*A + W + A*W*	5	141.39	-270.8	1.23	0.14
		W + HSI	4	139.87	-270.5	1.57	0.12
		Null	2	137.36	-270.4	1.71	0.11

Table 5.3 (Continued).

Global model	Egg quality indicator	Model	df	log Lik	AIC_c	ΔAIC_c	*w*
(b)	Total lipid	**Null**	**2**	**106.31**	**-208.3**	**0.00**	**0.37**
		HSI	3	106.61	-206.5	1.76	0.15
	Wax esters	***FL***	**3**	**59.32**	**-111.9**	**0.00**	**0.30**
	Triacylglycerol	*HSI*	3	64.67	-122.4	0.00	0.20
		FL + HSI	4	65.87	-122.3	0.13	0.19
		Null	**2**	**63.05**	**-121.5**	**0.88**	**0.13**
		*FL + HSI + FL*HSI*	5	66.54	-121.9	1.48	0.10
		A + HSI	4	64.98	-120.7	1.91	0.08
	Free fatty acids	**Null**	**2**	**196.74**	**-389.1**	**0.00**	**0.33**
		A	3	197.22	-387.7	1.41	0.16
		HSI	3	197.08	-387.4	1.69	0.14
	Sterols	*FL + HSI + FL*HSI*	5	155.99	-300.0	0.00	0.37
		FL	**3**	**152.74**	**-298.7**	**1.31**	**0.19**
	Polar lipids	***FL***	**3**	**55.43**	**-104.1**	**0.00**	**0.19**
		FL + HSI	4	56.55	-103.9	0.28	0.17
		*FL + A + FL*A*	5	57.47	-103.0	1.12	0.11
	Egg diameter[nb.]	***FL***	**3**	**82.52**	**-158.3**	**0.00**	**0.33**
		FL + HSI	4	82.91	-156.6	1.74	0.14
		Null	2	80.39	-156.4	1.90	0.13
	Oil droplet diameter	*FL + A + FL*A*	5	141.396	-270.9	0.00	0.23
		Null	**2**	**137.357**	**-270.4**	**0.50**	**0.18**
		FL	3	138.296	-269.9	0.99	0.14
		A	3	138.224	-269.7	1.13	0.13

Note: logLik: log likelihood; ΔAIC_c is the difference in AICc values between model *i* and the top ranked model of those considered; *w* is the probability that a model is the best model of the set. *W* was retained as a predictor in the *ODD* model, and *FL* was retained as a predictor in the *ED* model, because these models were significantly better predictors of *ODD* and *ED* than their respective null models (Chi-square test, $p < 0.05$).

Table 5.4 Parameter estimates and overall model fit of selected best model of maternal predictors for each measure of egg quality, including regression coefficient (*b*) and 95% confidence interval (CI) for *b*. The global model predicting each measure of egg quality was (a) weight (*W*)*age (*A*)*hepatosomatic index (*HSI*), and (b) fork length (*FL*)**A***HSI*. Statistically significant parameter estimates: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Global model	Egg quality indicator	Predictor	*b*	*t*-value	95% CI for *b*	*p*
(a)	Total lipid	(Intercept)	3.07×10^{-2}	13.47	2.62×10^{-2} to 3.52×10^{-2}	***
	Wax esters	(Intercept)	3.59×10^{-1}	16.86	3.18×10^{-1} to 4.01×10^{-1}	***
		W	3.73×10^{-5}	2.19	3.86×10^{-6} to 7.08×10^{-5}	*
	Triacylglycerol	(Intercept)	3.43×10^{-1}	46.70	3.28×10^{-1} to 3.57×10^{-1}	***
	Free fatty acids	(Intercept)	6.11×10^{-4}	3.09	2.23×10^{-4} to 10.00×10^{-4}	**
	Sterols	(Intercept)	7.22×10^{-3}	1.36	-3.19×10^{-3} to 1.76×10^{-2}	>0.05
		W	1.04×10^{-5}	2.29	1.50×10^{-6} to 1.93×10^{-5}	*
		HSI	8.38×10^{-3}	2.54	1.91×10^{-3} to 1.49×10^{-2}	*
		*W***HSI*	-9.91×10^{-6}	-3.17	-1.60×10^{-5} to -3.78×10^{-6}	**
	Polar lipids	(Intercept)	2.38×10^{-1}	24.61	2.19×10^{-1} to 2.57×10^{-1}	***
	Egg diameter	(Intercept)	8.24×10^{-1}	74.29	8.02×10^{-1} to 8.46×10^{-1}	***
		W	2.28×10^{-5}	2.57	5.42×10^{-6} to 4.02×10^{-5}	*
	Oil droplet diameter	(Intercept)	1.81×10^{-1}	73.91	1.77×10^{-1} to 1.86×10^{-1}	***
		W	3.97×10^{-6}	2.02	1.18×10^{-7} to 7.82×10^{-6}	0.05

Table 5.4 (Continued).

Global model	Egg quality indicator	Predictor	*b*	*t*-value	95% CI for *b*	*p*
(b)	Total lipid	(Intercept)	3.07×10^{-2}	13.47	2.62×10^{-2} to 3.52×10^{-2}	***
	Wax esters	(Intercept)	2.34×10^{-1}	2.97	7.98×10^{-2} to 3.89×10^{-1}	**
		FL	3.90×10^{-4}	2.14	3.33×10^{-5} to 7.46×10^{-3}	*
	Triacylglycerol	(Intercept)	3.43×10^{-1}	46.70	3.28×10^{-1} to 3.57×10^{-1}	***
	Free fatty acids	(Intercept)	6.11×10^{-4}	3.09	2.23×10^{-4} to 10.00×10^{-4}	**
	Sterols	(Intercept)	3.35×10^{-2}	5.31	2.11×10^{-2} to 4.59×10^{-2}	***
		FL	-3.94×10^{-5}	-2.70	-6.79×10^{-5} to -1.08×10^{-5}	*
	Polar lipids	(Intercept)	4.37×10^{-1}	4.99	2.65×10^{-1} to 6.08×10^{-1}	***
		FL	-4.61×10^{-4}	-2.28	-8.57×10^{-4} to -6.51×10^{-5}	*
	Egg diameter	(Intercept)	7.64×10^{-1}	18.13	6.81×10^{-1} to 8.46×10^{-1}	***
		FL	2.01×10^{-4}	2.07	1.06×10^{-5} to 3.91×10^{-4}	*
	Oil droplet diameter	(Intercept)	1.86×10^{-1}	188.8	1.80×10^{-1} to 1.90×10^{-1}	***

Table 5.5 Change (%) in *Plectropomus leopardus* egg quality indicators with body weight (grams; $W_{500} - W_{2000}$) and fork length (mm; $FL_{350} - FL_{500}$). Values are only included where the best model was a significantly better predictor than the null model for each egg quality indicator.

	Female metric	
	$W_{500} - W_{2000}$	$FL_{350} - FL_{500}$
Wax esters	+16%	+15%
Sterols	-25%	-30%
Polar lipids	-	-25%
Egg diameter	+4%	+4%
Oil droplet diameter	+3%	-

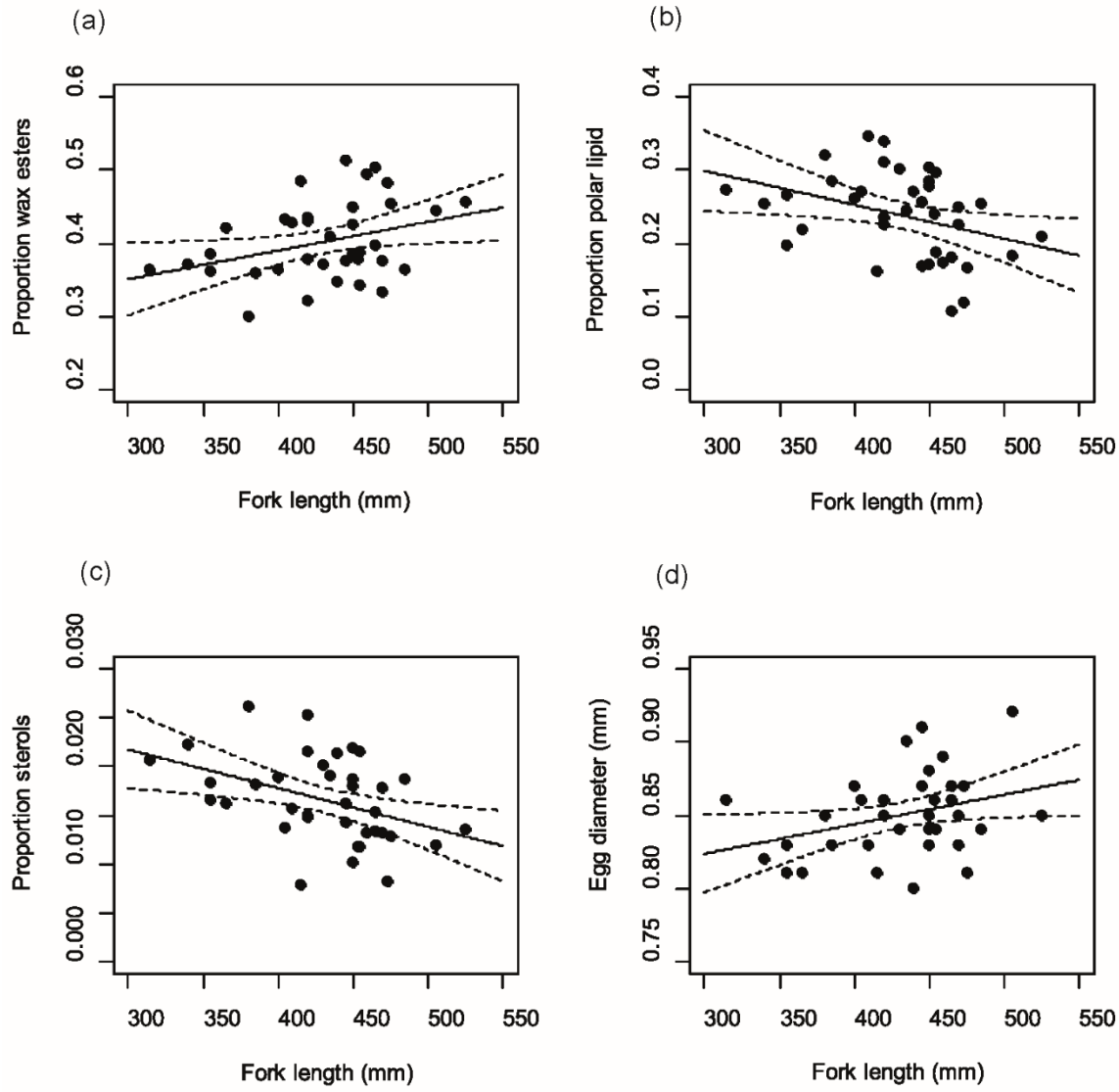


Figure 5.3 Relationship (± 95% CIs) between (a) fork length (*FL*) and wax esters as a proportion of total lipid (*TL*) content, (b) *FL* and polar lipid as a proportion of *TL*, (c) *FL* and sterols as a proportion of *TL*, and (d) *FL* and egg diameter (mm) in *Plectropomus leopardus* eggs. Predictions derived from best fit models described in Table 5.3.

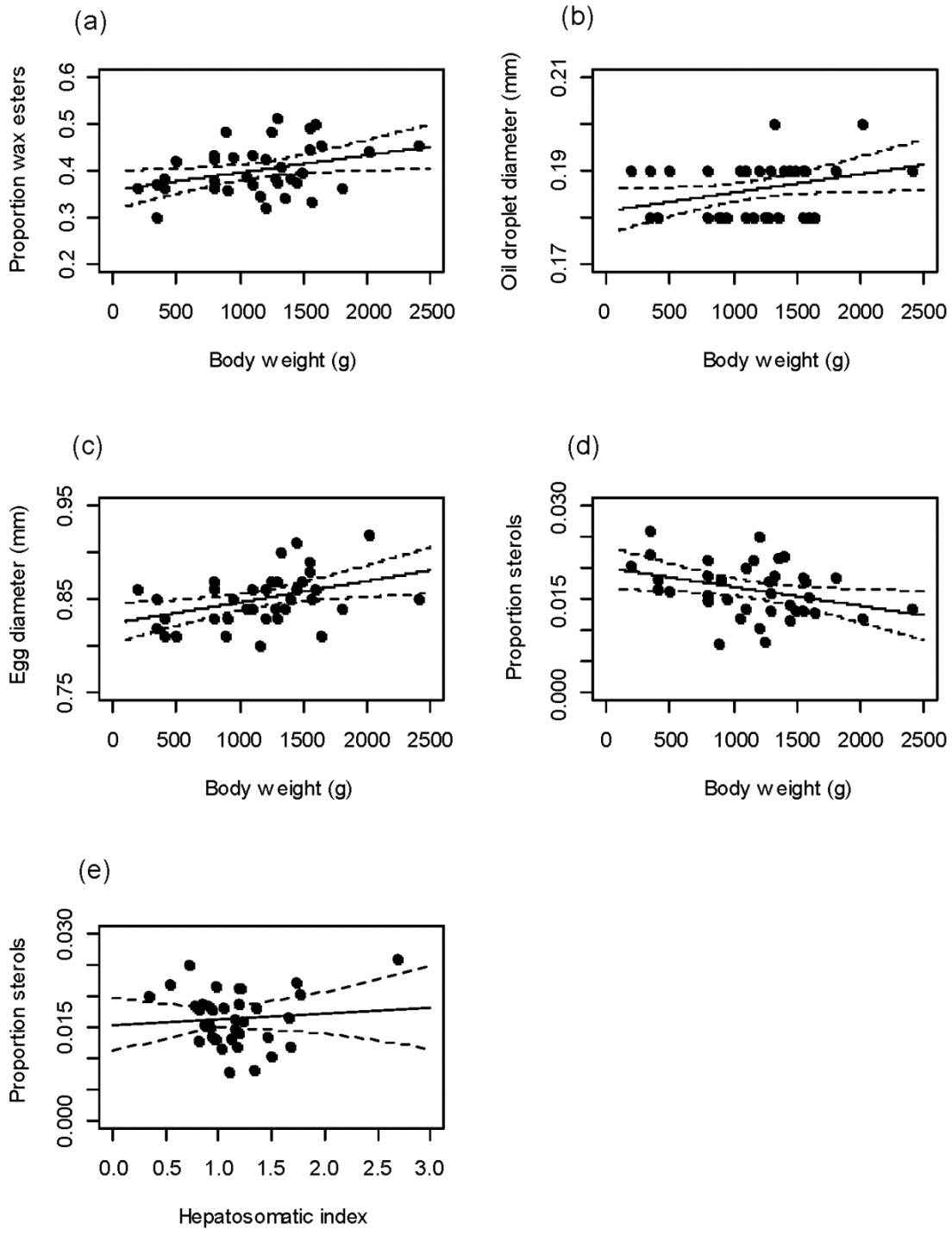


Figure 5.4 Relationships (± 95% CIs) between (a) body weight (*W*) and wax esters as a proportion of total lipid content (*TL*), (b) *W* and oil droplet diameter, (c) *W* and egg diameter, (d) *W* and sterols as a proportion of *TL*, and (e) hepatosomatic index and sterols as a proportion of *TL* in *Plectropomus leopardus* eggs. Predictions derived from best fit models described in Table 5.3.

5.4 Discussion

The relationships described here between maternal traits and egg quality for *P. leopardus* from wild populations is the first for a commercially exploited tropical protogynous reef fish. Longer and heavier females invested in larger eggs with greater concentrations of the neutral lipid *WE*, while maintaining concentrations of the neutral lipid *TAG* and *TL* content in the egg, relative to shorter and lighter females. Heavier females also were capable of increasing *WE* while maintaining *PL* concentrations in eggs that contained larger oil droplets, compared with eggs produced by lighter females. The positive relationship between female weight and oil droplet diameter was statistically weak, however, and future research would benefit from an increased sample size to confirm the results presented here. Maternal age had no effect on any indicators of egg quality. These results highlight the need for understanding maternal effects on reproduction in order to evaluate the potential effects of variation in population size-structure, and to assess the appropriateness of fishery management strategies for populations that assume all females are reproductively equal.

5.4.1 Maternal influences on egg quality

Maternal length and weight were the main factors that influenced indicators of egg quality for *P. leopardus*. The positive effect of female size on egg and/or oil droplet size is well quantified in fishes, including hake *Merluccius merluccius* (Mehault et al. 2010) and *M. hubbsi* (Macchi et al. 2013), haddock *Melanogrammus aeglefinnus* (Hislop 1988), Atlantic cod *Gadus morhua* (Marteinsdottir and Begg 2002), and stripey sea perch *Lutjanus carponotatus* (Evans et al. 2008). This phenotypic effect reflects differing investment in reproductive output by females (Bernardo 1996). The limited

influence of body condition (hepatosomatic index) on indicators of egg quality was, therefore, interesting because body condition is often used to indicate the energetic resources available for reproduction. Female body condition or liver indices have been positively correlated with egg and larval quality indicators, for example, such as egg and oil droplet size, fertilization success, and larval condition including size at hatching and survival (McCormick 2003, Berkeley et al. 2004a, Trippel and Neil 2004, Donelson et al. 2008, Bachan et al. 2012). The limited effect of body condition on egg quality observed may be due to co-variation with another factor not measured, such as coelomic fat.

In batch spawning fishes, egg quality can vary significantly depending on when in the season batches are spawned, with late season batches often displaying lower "quality" as the energetic resources available for reproduction diminish (Bachan et al. 2012). An examination of spawning season effects on egg quality indicators was beyond the scope of this study, but a combination of maternal size-related variability, and the probable changes in maternal condition during the spawning season for females of a given size, may explain the lack of relationship between condition and egg quality indicators for *P. leopardus*.

Maternal age was not included in any of the models that best described variation in egg or oil droplet size, or egg lipid composition for *P. leopardus*. Maternal age has a positive effect on egg and larval quality for many temperate fishes, including black rockfish *Sebastes melanops* (Berkeley et al. 2004a), Atlantic cod *G. morhua* (Marteinsdóttir and Steinarsson 1998), and haddock *M. aeglefinus* (Hislop 1988, Wright and Gibb 2005). Larval *S. melanops* produced by the oldest females, for example, grew

three times as fast (weight and length) when food was available, and survived twice as long when food was unavailable, compared to larvae from the youngest females (Berkeley et al. 2004a). Age is rarely the main determinant of egg or larval quality (although see Berkeley et al. 2004a), and usually covaries with maternal size which is often the better predictor of egg and larval condition (reviewed by Green 2008). The lack of an age effect on egg quality indicators may be indicative of the large amount of variation in female *P. leopardus* age-weight and age-length relationships (Williams et al. 2008), with female size a better indicator of reproductive investment potential than age for this species. As discussed in Chapter 4, batch fecundity increases with female age for *P. leopardus* indicating that older females invest energy into producing more eggs rather than better quality eggs. However, the positive relationship between age and batch fecundity asymptotes when females are approximately 5 years old and female length and weight remain better indicators of batch fecundity and egg quality indicators than age.

5.4.2 Potential implications for larval quality and recruitment

Polar lipids are the main component of lipoprotein yolk, with some neutral lipids present, while the oil droplet is comprised primarily of neutral lipids (Wiegand 1996). The yolk sac in *P. leopardus* is utilized during embryogenesis and the first 2 days post hatching. A large portion of the oil droplet remains at 2 days post-hatch when exogenous feeding commences (A. B. Carter, personal observation; Figure 5.2c,d), but the oil droplet is not present at 5 days post-hatching (Masuma et al. 1993). This pattern is consistent with many marine larvae where the lipoprotein yolk provides the principal source of energy during embryogenesis, and the oil droplet is consumed post-hatching (Anderson et al. 1990, Rønnestad et al. 1998). The compartmentalization of neutral

lipids between lipoprotein yolk and oil is most likely for nutritional reasons where the oil droplet is retained as an energy reserve in the event that food is limited in the early stages of exogenous feeding, and the oil is consumed to enhance growth when adequate food is available (Wiegand 1996). These endogenous energy reserves are important for larval survival because larval mortality during the transition from endogenous to exogenous feeding is frequently high (Moodie et al. 1989), and thought to be a significant source of recruitment fluctuations (Lasker 1981).

Egg and oil droplet size, and the polar and neutral lipid classes I measured, theoretically are good predictors of embryonic development and larval growth and survival. For many fishes, the oil droplet contains the majority of egg energy content, in particular the energy required to sustain larvae during development and the transition to exogenous feeding (Wiegand 1996, Rønnestad et al. 1998). Neutral lipids within the oil droplet provide endogenous energy for metabolism and growth, with *WE* and *TAG* the dominant neutral lipids in fish eggs with an oil droplet (Wiegand 1996). In *P. leopardus* eggs, *WE* and *TAG* accounted for approximately 74% of *TL*. The dominance of neutral lipids relative to polar lipids is typical of warmer water species (Mourente and Odriozola 1990, Navas et al. 1997), while polar lipids are generally the dominant lipid class in cold water fish eggs (Tocher and Sargent 1984). *TAG* lipids are a short-term energy storage lipid and constitute the major resource available to developing embryos (Hilton et al. 2008). *TAG* lipids are also the primary reserve lipid mobilized by larvae during starvation (Rainuzzo et al. 1997). The lack of maternal effects on *TAG* composition in *P. leopardus* eggs indicates that the provisioning of *TAG* is tightly regulated in this species irrespective of female size, age or condition. Maternal size did, however, have a positive effect on the provision of the long-term energy storage lipid

WE in *P. leopardus* eggs. The benefits of greater *WE* content in *P. leopardus* eggs produced by larger females is unclear, but higher *WE* levels are likely to be advantageous for larvae due to the potential role of *WE* in energy reserves and buoyancy. Previous studies on plankton have demonstrated that *TAG* lipids are the primary initial energy for larvae, but once these are depleted the starvation-related stress activates the wax-lipase and *WE* are consumed (Lee et al. 1974). An increased portion of *WE* in the egg may, therefore, provide larvae that are spawned from larger females with a potential survival advantage during the transition to exogenous feeding.

Polar lipids are important during embryogenesis as they provide energy reserves and are an important structural element in biomembrane formation (Wiegand 1996). For fishes that produce eggs with an oil droplet, the proportion of *PL* can range widely, e.g. 6% of *TL* in golden perch eggs *Macquaria ambigua* (Anderson et al. 1990) and 72% of *TL* in striped trumpeter eggs *Latris lineata* (Bransden et al. 2007). In *P. leopardus* eggs, approximately 24% of *TL* content was *PL*. The decrease in *PL* with female length does not necessarily indicate that larger *P. leopardus* produce eggs with lower quality yolk than eggs produced by smaller females. Decreasing *PL* concentrations in eggs spawned by larger females would be compensated to some extent by the increase in egg size and, therefore, an increase in the amount of total *PL* available for embryogenesis. Furthermore, the higher *PL* concentrations in eggs spawned by the smallest *P. leopardus* also generally had reduced *WE* and *TAG* concentrations and low *TL* content (Table 5.2), with potentially negative consequences for larval survival. For example, increases in *PL* and decreases in *TAG* have been reported over the spawning season in the striped trumpeter (Bransden et al. 2007), where the shift in lipid composition was

indicative of the typical decrease in egg quality experienced by batch spawning fishes as maternal resources diminish over time (Bachan et al. 2012).

Maternal reproductive success is a product of reproductive potential and offspring survival (Lambert 2008). The ability of large *P. leopardus* to produce relatively large eggs with a large oil droplet, with a constant *TAG* concentration and increased *WE* concentration likely has important implications for larval quality and survival. Larvae with high energy reserves are less susceptible to starvation during the period between endogenous and exogenous feeding (Moodie et al. 1989, Wiegand 1996, Brooks et al. 1997). The large number of studies that have examined the effect of egg and/or oil droplet size on larval quality and reproductive success have shown that larger eggs generally result in greater fertilization rates (e.g. common snook *Centropomus unidecimalis* (Neidig et al. 2000)), greater larval size at hatching that persists through time (e.g. Atlantic cod *G. morhua* (Marteinsdottir and Begg 2002) and brown trout *Salmo trutta* (Einum and Fleming 1999)), faster rates of larval growth (e.g. walleye *Stizostedion vitreum* (Moodie et al. 1989)), faster larval development and survival rates (e.g. black rockfish *S. melanops* (Berkeley et al. 2004a)), and greater larval feeding success (e.g. Atlantic cod *G. morhua* (Knutsen and Tilseth 1985)).

In terms of egg biochemistry, lipids are good indicators of egg quality due to the importance of lipids, particularly neutral lipids, in embryogenesis, hatching success, larval growth and larval survival (Wiegand 1996, Navas et al. 1997, Berkeley et al. 2004a, Hilton et al. 2008). Increased buoyancy of larger eggs and/or eggs with a greater proportion of *WE* may also have positive implications for the survival of larval *P. leopardus*, as buoyancy positions eggs and newly hatched larvae in the upper layers of

the water column where larval food sources are most abundant and oxygen conditions are best (Wright and Fyhn 2001, Mehault et al. 2010). Larger larvae with faster growth rates potentially have a survival advantage as they are able to outgrow smaller predators faster and therefore experience relatively less predation than smaller conspecifics (Vigliola and Meekan 2002), are able to access more of the water column in a retention area than smaller conspecifics due to increased swimming ability (Álvarez–Colombo et al. 2011), and can start feeding earlier and have higher feeding success as they are able to eat a wider variety of food items (Knutsen and Tilseth 1985).

Species-specific assessments of a wide range of indicators of egg and larval quality are required, as the relative importance of egg quality indicators is likely to be closely related to the early life history requirements of that species (Kaitaranta and Ackman 1981). Care should be taken in assuming the theory that egg size or biochemical composition will relate to embryo or larval performance in all species because there are many exceptions. For example, there is no relationship between egg morphometry and fertilization and hatching rates for Asian sea bass *Lates calcarifer* (Nocillado et al. 2000), no relationship between egg size and embryo and larval development for the winter flounder *Pseudopleuronectes americanus* (Butts and Litvak 2007), and no difference in egg *PL*, *TAG* and *TL* composition between viable and unviable eggs for Atlantic halibut *Hippoglossus hippoglossus* (Bruce et al. 1993). In addition, egg quality can vary within the spawning season for batch spawners (Bachan et al. 2012), and vary depending on maternal stress caused by social conditions such as crowding (McCormick 2006). Paternal effects may also influence the quality of fertilized eggs and subsequent larvae, including larval size, growth and performance (Brooks et al. 1997, Green and McCormick 2005, Butts and Litvak 2007). Further research is required

to determine the effect that variation in egg morphometry and lipid composition has on fertilization success, hatching rates, and larval survival for *P. leopardus*, and on other potentially important sources of variation in egg quality.

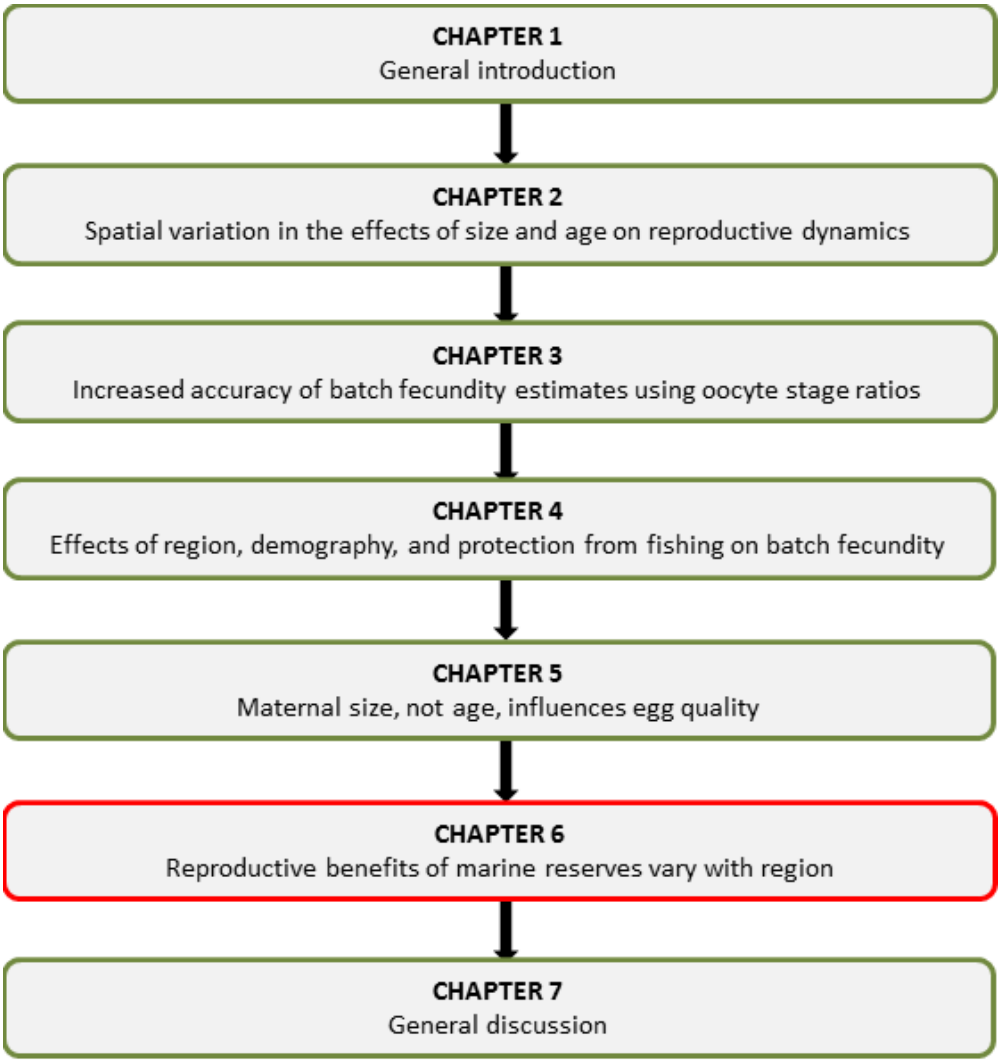
Recruitment success is positively influenced by larval growth and survival (Bergenius et al. 2002, Fontes et al. 2011). Variation in the egg quality traits that influence larval growth and survival for *P. leopardus*, particularly during the critical period between hatching and the establishment of exogenous feeding, may have significant implications for recruitment of this species. Fisheries models generally assume that eggs and larvae produced by females are of equal quality, regardless of the size and age structure of those populations (Birkeland and Dayton 2005). My study demonstrates that large female *P. leopardus* produce higher quality eggs than those spawned by smaller females. Batch fecundity also increases with female length and weight for *P. leopardus* (Chapter 4), as does the proportion of females that are reproductively mature (Chapter 2). Protection of large females is afforded in 33% of the GBR Marine Park (GBRMP) which is closed to fishing (reserves), where the mean length at female to male sex change, mean length and weight of female spawners, weight-specific batch fecundity (central GBR), and overall densities of coral trout, are greater relative to fished reefs (see Chapters 2 and 4; also Emslie et al. 2015). The combination of direct removal of large females through fishing, and indirect removal of large females via fishing-induced female-male sex change at smaller sizes relative to reserve reefs, is likely to have deleterious consequences for the quality of eggs produced by *P. leopardus* populations on reefs open to fishing. The "indirect" removal of females is of particular concern in tropical fisheries management where protogynous hermaphroditism is prevalent (Jennings and Kaiser 1998) and exploited species such as *P. leopardus* are likely to be

particularly susceptible to fishing-induced reductions in egg quality. Reserves in the GBRMP are, therefore, likely to have positive benefits on the quality and quantity of *P. leopardus* eggs produced, which are likely to supplement populations of *P. leopardus* on fished reefs (Harrison et al. 2012).

5.4.3 Conclusion

This is the first study to examine the effects of maternal length, weight, age and body condition on egg size and lipid composition for a commercially important tropical fish of wild origin. This study is an important addition to the growing body of literature on the importance of large female fish for population reproductive output in terms of the quality of eggs produced. My findings support the hypothesis that larger female fish produce better quality eggs, but not the hypothesis that older females produce better quality eggs. The reproductive importance of large females has important implications for the management of coral trout fisheries. Through the selective removal of the largest individuals, fishing removes, or in the very least diminishes, the reproductive benefits that larger females can provide for population viability. The effect of female size on egg quality and the potential implications for early survival and growth of larvae and recruitment is particularly relevant to exploited species like *P. leopardus* for which fishing removes larger and older females from the population.

CHAPTER 6 Reproductive benefits of marine reserves vary with region



Chapter 6 will be published as: Carter AB, Davies CR, Emslie M, Mapstone BD, Russ GR, Tobin AJ and Williams AJ (*in prep*) Reproductive benefits of marine reserves vary with region for an exploited coral reef fish.

6.1 Introduction

No-take marine reserves are expected to contribute to population recruitment disproportionately compared with fished areas because egg production per unit area (EPUA) should be greater inside reserves, with greater densities of larger and more fecund females (Roberts and Polunin 1991). Therefore, EPUA calculations are important in determining potential reserve recruitment subsidies to surrounding fished areas. The hypothesis that small reserves can produce the equivalent number of eggs as much larger exploited areas (Roberts and Polunin 1991) is supported by calculations of EPUA being consistently higher in reserves than fished areas for invertebrates (Babcock et al. 2007, Díaz et al. 2011), and temperate (Paddack and Estes 2000, Willis et al. 2003) and tropical fishes (Sluka et al., Evans et al. 2008). There was little empirical evidence for reserve recruitment subsidies (Sale et al. 2005) until recent work demonstrated that some reserves contribute more to recruitment in fished areas than do areas open to fishing (Harrison et al. 2012, Almany et al. 2013).

Reproductive output can be sensitive to the effects of fishing on the size of individuals, density of populations, batch fecundity, and spawning frequency, as demonstrated in Chapters 2 and 4, and previous studies (Russ 2002, Díaz et al. 2011). The reproductive potential of hermaphrodites can be particularly sensitive to fishing because size-selective mortality can result in sperm- or egg- limited populations and drive compensatory sex change at smaller sizes or ages (Sadovy 1996, Hawkins and Roberts 2004).

Direct empirical data for EPUA calculations for reserves are difficult to obtain where destructive sampling is restricted or not allowed. EPUA calculations frequently

combine size frequency distributions from reserve to fished areas with size-fecundity relationships estimated only from fished areas (Willis et al. 2003, Denny et al. 2004, Evans et al. 2008). This approach may confound estimates of EPUA from reserves because fishing (in open areas) can induce changes in reproductive traits such as Allee-type depensation (Sadovy 2001), reproductive compensation (Koslow et al. 1995), spawning behaviour (Muñoz et al. 2010), and sex ratios (Hawkins and Roberts 2004). Such effects would not be expected in reserves, from where samples often are not available.

Accurate assessments of reserve EPUA require data from multiple reserves at appropriate spatial and temporal scales. Reproductive characteristics of fishes have been reported to vary spatially (McIntyre and Hutchings 2003, Kokita 2004, Wakefield et al. 2013) and temporally (Bernal et al. 2011, Smith et al. 2014), often correlated with environmental variability or fishing pressure. Spatial variation in reproductive dynamics, such as sex ratios and reproductive output, is rarely considered in EPUA calculations, however, making spatial comparisons of EPUA inaccurate where reproductive traits are not spatially homogenous. Management of fish stocks should benefit from understanding spatial variation in reproductive biology through regulations that maximize egg production. Significant spatial variation in reproduction may undermine fisheries management if reserves created to enhance EPUA are placed where reproductive output is limited, or where high adult densities represent larval sinks rather than sources.

In this chapter, I examine spatial variation in effects of reserves on EPUA for *Plectropomus leopardus*, using reproductive traits collected from both fished areas and

reserves spread across 7° of latitude of the Great Barrier Reef (GBR). I incorporate known spatial variation in sex ratios, maturity schedules, batch fecundity and spawning frequency of *P. leopardus* as described in Chapters 2 and 4 of this thesis. Identification of EPUA hotspots could substantially benefit future spatial management and conservation of *P. leopardus*. The objective of this study was to integrate multiple reproductive datasets from Chapters 2 and 4 with available density and length-structure data from a long-term monitoring program (2004 – 2013) to determine spatial variation in EPUA on the GBR, including comparisons between fished and reserve reefs.

6.2 Methods

6.2.1 Population structure

Length and density data for *P. leopardus* were collected annually or biennially 2004–2013 using UVSs of 93 reefs (Table 6.1) in seven latitudinal sectors within the three GBR regions (Figure 6.1a) covering 150 000 km^2 of the GBR (for a detailed description see Halford and Thompson 1994). Replicate reserve and fished reefs were surveyed within three cross-shelf positions (inner-, mid-, and outer-shelf) in the Lizard Island, Cairns, Townsville and Whitsunday sectors, while only mid-shelf and outer-shelf reefs were surveyed in Pompey, Swain and Capricorn-Bunker sectors (Figure 6.1b). A standard habitat was surveyed at each reef: the reef slope at 6 – 9 m depth on the north-east flank. *P. leopardus* were counted and lengths (*TL*, cm) estimated along five permanently marked belt transects (50 x 5 m) parallel to the reef crest in each of three sites per reef (n = 15 transects per reef). Surveys were completed by trained observers on SCUBA moving 10 m per minute. Observers regularly calibrated length estimates

against model coral trout of known lengths. *TL*s were converted to fork lengths (*FL*) using the formula (A.B. Carter, unpublished data):

$$FL = 8.43 + 0.93 \times TL \qquad (1)$$

Table 6.1 Number of reefs sampled using underwater visual survey within each sector, 2004 – 2013. Regional distribution of sectors also is shown.

Region:	North	Central		South			
	Sector						
Year	Lizard Island	Cairns	Townsville	Whitsunday	Pompey	Swain	Capricorn-Bunker
2004	8	10	8	9	0	7	4
2005	8	10	8	9	0	7	4
2006	0	12	12	0	10	14	8
2007	8	10	9	9	0	6	4
2008	0	12	12	0	10	14	8
2009	8	10	9	9	0	7	4
2010	0	12	12	0	10	14	8
2011	8	10	9	9	0	7	4
2012	0	12	12	0	10	14	8
2013	8	10	9	9	0	7	4

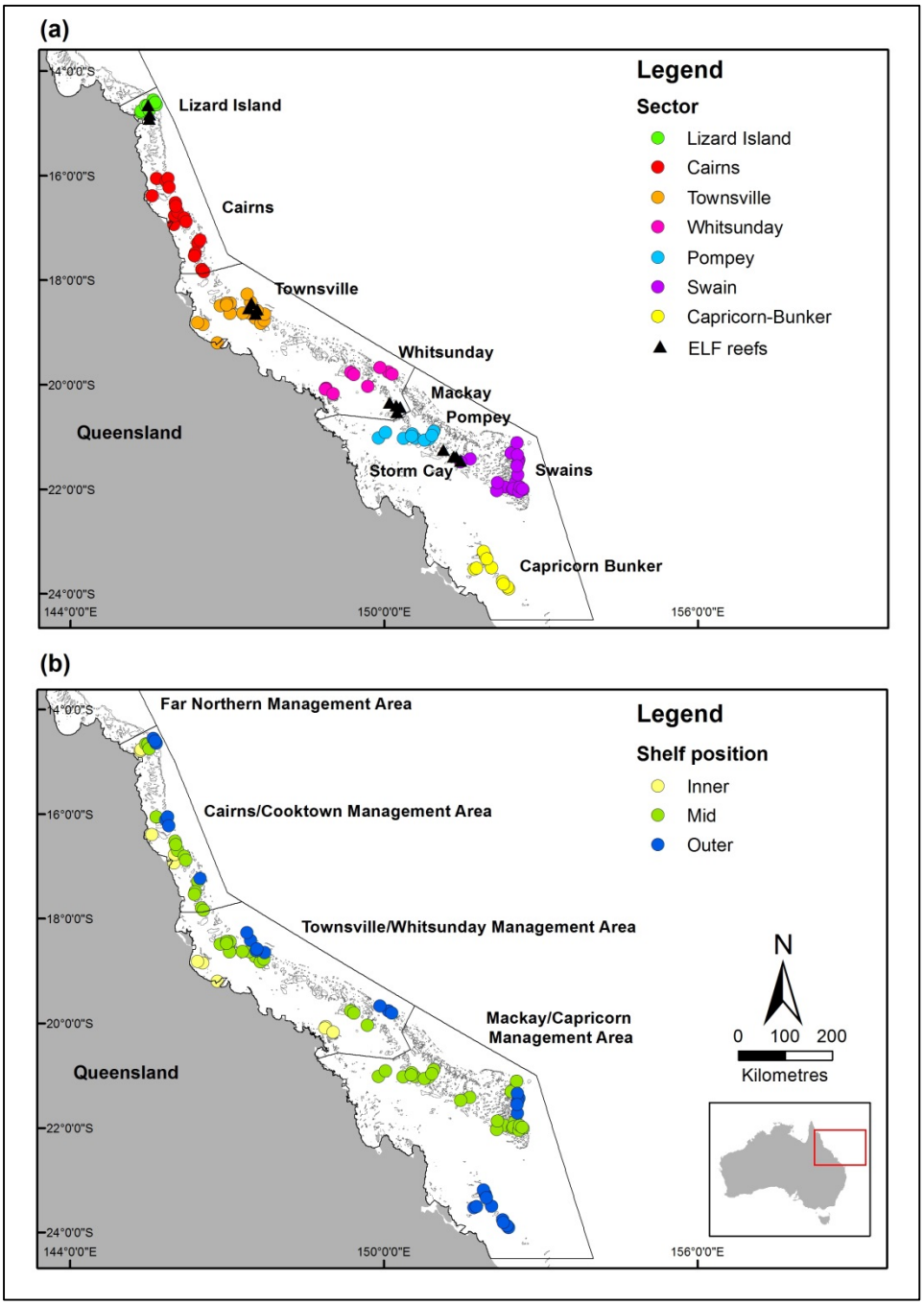


Figure 6.1 (a) *Plectropomus leopardus* densities and lengths were estimated using underwater visual survey (UVS) of 93 reefs (circles) in seven sectors along the Great Barrier Reef, while reproductive parameters were estimated from *P. leopardus* collected at four reef clusters (triangles; Lizard Island, Townsville, Mackay, Storm Cay). (b) UVS were conducted across three continental shelf positions (inner, mid, outer). Straight lines show boundaries of the Great Barrier Reef Marine Park and three Management Areas within the park.

6.2.2 Individual female fecundity

Annual individual female fecundity for *P. leopardus* was estimated from gonads collected from four mid-shelf clusters of reefs over 7° latitude (Lizard Island, Townsville, Mackay, Storm Cay, Figure 6.1a) as part of the Effects of Line Fishing (ELF) Experiment on the GBR (see Chapter 2.2 and Mapstone et al. 2004). Chapters 2 and 4 provide detailed descriptions of female reproductive parameters including sex ratio, female maturity, spawning frequency and batch fecundity from ELF Experiment samples. Reproductive data from the Lizard cluster of the ELF Experiment were applied to the UVS data from the Lizard Island survey sector. Townsville cluster reproductive data were applied to Townsville and Cairns sectors where *P. leopardus* have similar spawning frequency and batch fecundity (see Chapters 2 and 4 and Samoilys 2000). Reproductive parameters for the Pompey and Whitsunday UVS sectors were based on ELF Mackay reefs, and those for the Swain and Capricorn-Bunker sectors were based on ELF Storm Cay reefs (Figure 6.1, Figure 6.2).

Annual individual fecundity (I, no. oocytes female^{-1} year^{-1}) by FL, GBR reef sector (S), and management zone (Z), was calculated from:

$$I_{FL,Z,S} = R_{FL,Z,S} \times M_{FL} \times B_{Z,S} \times F_{FL,S} \quad (2)$$

where R is the sex ratio (proportion of females) by FL, zone, and sector (Figure 6.2a – b; Chapter 2.3);

M is the proportion of mature females (vitellogenic oocytes in ovary) by FL (Figure 6.2c; Chapter 2.3);

B is the annual number of batches spawned by mature females by zone and sector (=spawning season duration / spawning frequency), which does not vary with length (Table 6.2; Chapter 2.3); and

F is batch fecundity by *FL* and sector (Figure 6.2d; Chapter 4.3).

Individuals undergoing sex change and bisexuals accounted for <0.5% of populations and were excluded from sex ratio analyses. In Chapter 2.3, I reported that no spawning occurred on Mackay fished reefs over four years but in Chapter 4.3 several females (~1% of sample) were recorded on these reefs with hydrated ovaries, indicating imminent spawning. Spawning frequency was therefore set as the inverse of 0.01, indicating spawning every 100 days or 1.22 times per season. Spawning season duration was 122 days per year (September – December) based on previous data from the Cairns sector (Samoilys 2000) and assumed to be uniform along the GBR and over female length (reviewed in Chapter 2).

Negative values of *F* were calculated for 24 females (269–287 mm *FL*) from the central GBR because the intercept of the *FL*-*F* relationship in that region was <0. *F* for these females was estimated by scaling *F* to length using the first positive batch fecundity value (7431 oocytes batch^{-1}) for a 296 mm *FL* female.

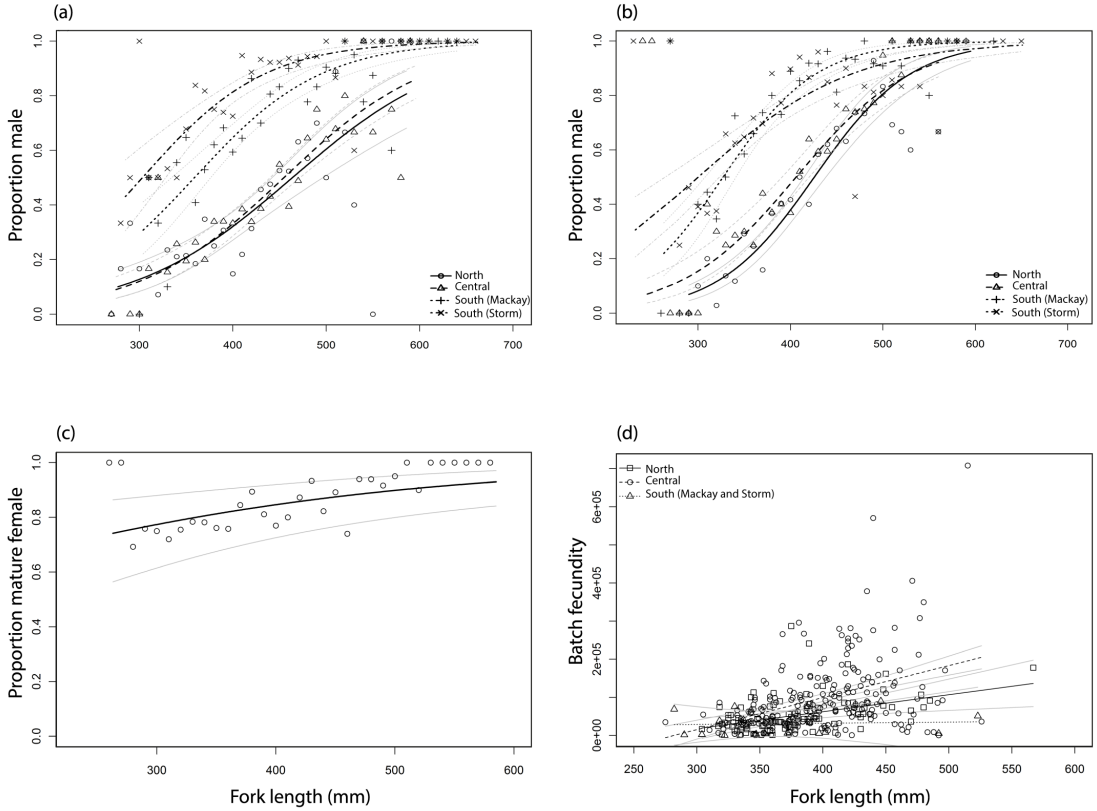


Figure 6.2 Relationships (±95% confidence intervals) between *Plectropomus leopardus* fork length (mm) and sex ratio (proportion male) on (a) reserve reefs and (b) fished reefs; (c) proportion of sexually mature females (vitellogenic ovaries); and (d) batch fecundity in the northern, central and southern (Mackay and Storm Cay) Great Barrier Reef. Source: Figure 2.3, Figure 2.5 and Figure 4.2.

Table 6.2 Spawning frequency (days) and number of batches spawned per four month spawning season for *P. leopardus* from fished and reserve reefs at Lizard Island (northern GBR), Townsville (central GBR), and Mackay and Storm Cay (southern GBR) (see Chapter 2.3 for more details).

	Fished		Reserve	
	Spawning frequency	Batches per season	Spawning frequency	Batches per season
Lizard Island	3.2	38.4	12.0	10.1
Townsville	5.3	22.9	2.3	52.0
Mackay	na.	na.	83.2	1.5
Storm Cay	17.2	7.1	28.8	4.2

6.2.3 Egg production 250 m^{-2}

EPUA (250 m^{-2}) was calculated for each transect (*T*) and year (*Y*) as:

$$EP_{T,Y} = \sum_{T,Y} I_{FL,Z,S} \times N_{FL,T,Y} \quad (3)$$

where *N* is the count of *P. leopardus* by *FL* per transect per year, and $I_{FL,Z,S}$ is from equation 2.

Females smaller than 263 mm *FL* are not reproductively mature (A. B. Carter, unpub. data) and were excluded from *EPUA* calculations.

6.2.4 Data analysis

The response (*EPUA*) and explanatory variables density (*D,* 250 m^{-2}), *I*, *FL* and *R* were averaged across 15 transects per reef in each year before analysis. Reef means were analysed because reefs are the 'experimental units' (and effective replicates) for the treatment effects of zoning (open or closed to fishing) and transects were stratified subsamples collected to account for the patchy distribution of *P. leopardus* within reefs. Using reef means also reduced zero-inflation inherent in the *P. leopardus* density data, with zero counts reduced from 60% of transects to 14% and 2% of reefs in the north-central and southern GBR datasets respectively.

Statistical analyses were done with R v.3.1.1 (R Core Team 2014). The north-central and southern GBR were analysed as separate models because one model fitted to the entire data set was unstable. The continuous covariates *FL, D*, *I*, *R*, and *Y* were tested for collinearity using variance inflation factors (VIFs) with the *car* package (Fox and

Weisberg 2011) prior to fitting models. *FL* and *R* always were collinear so *R* was removed and VIFs recalculated. The VIFs of *FL, D*, *I*, and *Y* were <2, indicating collinearity was within reasonable limits and unlikely to inflate standard errors of model parameter estimates (Zuur et al. 2009). Generalized additive mixed models (GAMM) were used to examine the effects of management zone (*Z*), sector (*S*), shelf position (*P*), *FL, D*, *I*, and *Y* on *EPUA* for the north-central and southern GBR regions using the *mgcv* package (Wood 2014). Separate global models for the north-central and southern regions were run initially to determine optimal models for final analysis. *FL, D*, *I*, and *Y* were modelled with smoothing splines in the global models after preliminary analysis indicated potential nonlinear effects on *EPUA:*

$$sqrt(EP) = s(FL) + s(D) + s(I) + s(Y) + Z * S + P + \beta\text{reef} + \varepsilon \qquad (4)$$

where β_{reef} is the random effect of reef, and ε is the random error term comprised of inter-annual variation within reefs. The error parts of the models were initially separated into a random component described by Gaussian temporally autocorrelated errors using cor AR1, and a normally distributed error term (Zuur et al. 2014). Including within-years autocorrelation did not improve the southern region model, however, and was excluded from the final model. Zone was included as a variance covariate in both models which improved model fit judged by Akaike's Information Criterion (AIC). Neither of the best-fit models included year effects.

Sub-sets of each global model were generated using the dredge function in the *MuMIn* package (Barton 2013) to find the most parsimonious model. The inferred best-fit model was the simplest model within two points of the lowest AIC corrected for small

sample sizes (AICc) (Burnham and Anderson 2002). Residual and q-q plots of normalised residuals of the best-fit models were inspected for heteroscedasticity and non-normality. *EPUA* was square-root transformed to correct for heterogeneity. The best-fit models were used to predict expected *EPUA* on reefs surveyed in the north-central and southern GBR.

6.2.5 Mapping

Reef means of *FL, D*, *I*, *R* and predicted values of *EPUA* from the best-fit GAMMs were used to estimate these variables for all reefs within the GBRMP south of the Far Northern Management Area (since Far Northern reefs were not surveyed; see Figure 6.1 for Management Area boundaries). Estimates were made using an inverse distance weighting (IDW) interpolation in ArcGIS 10.1, which assumes that each point (reef) is more influenced by nearby points than by those farther away (Lo and Yeung 2002, Chang 2006). Separate interpolations were made for fished and reserve reefs to avoid neighboring reefs with different zoning influencing each interpolation. These IDW interpolations applied only to the *P. leopardus* standard habitat surveyed during the UVSs (aka reefs) within the GBRMP and not to the inter-reef areas. The likelihood that UVS provide under-estimates of absolute abundance (because some individuals will not be available to observers during counts) means that these analyses provide estimates of patterns in relative egg production rather than absolute estimates of production. *R* interpolations are presented because *R* was excluded from GAMM analyses due to collinearity with *FL*, making *R* potentially interchangeable with *FL* in each model.

6.3 Results

EPUA varied considerably among sectors of the GBR, with greatest production on Cairns and Townsville reserve reefs (781 600 ± 122 600 and 804 000 ± 157 100 oocytes 250 m^{-2} $year^{-1}$ respectively; Figure 6.3a). *EPUA* was lower on southern reefs than on northern and central reefs (Whitsunday sector: 7 000 ± 1 800 oocytes 250 m^{-2} $year^{-1}$, Swain sector: 49 000 ± 6 000 oocytes 250 m^{-2} $year^{-1}$; Figure 6.4a). Management zoning significantly affected *EPUA* in the southern GBR, and interacted with sector in the northern and central GBR (Table 6.3, Table 6.4). *EPUA* was 178% and 182% greater on reserve reefs than fished reefs in the Cairns and Townsville sectors respectively, and 16% greater on reserve reefs in the southern GBR (Figure 6.3a). *EPUA* was 88% greater on fished reefs than on reserve reefs, however, in the northern GBR (Figure 6.3a).

P. leopardus density was greatest on southern GBR reefs, lowest on central region reefs, and intermediate on northern reefs (Figure 6.3c). Densities were greater on reserve reefs than on fished reefs in every sector, with densities 120% greater on reserve reefs than on fished reefs across the southern region and 33%, 16% and 5% greater on reserve reefs than on fished reefs in the Lizard Island, Cairns, and Townsville sectors respectively (Figure 6.3c).

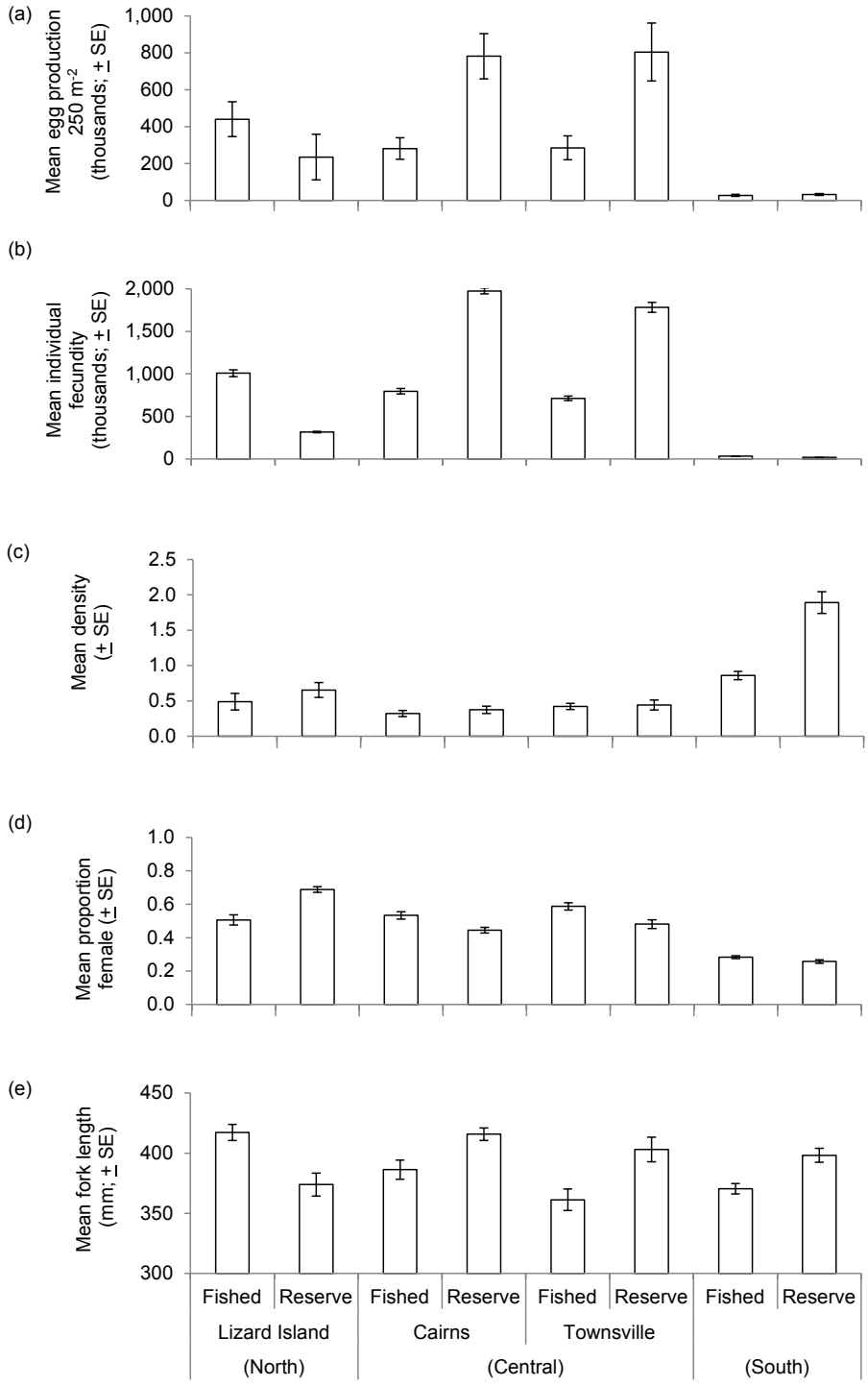


Figure 6.3 Mean ± standard error (SE) of *Plectropomus leopardus* (a) estimated egg production 250 m^{-2}; (b) individual fecundity (oocytes $female^{-1}$ $year^{-1}$); (c) density (individuals 250 m^{-2}); (d) proportion female and (e) fork length (mm) on fished and reserve reefs in the northern, central and southern Great Barrier Reef.

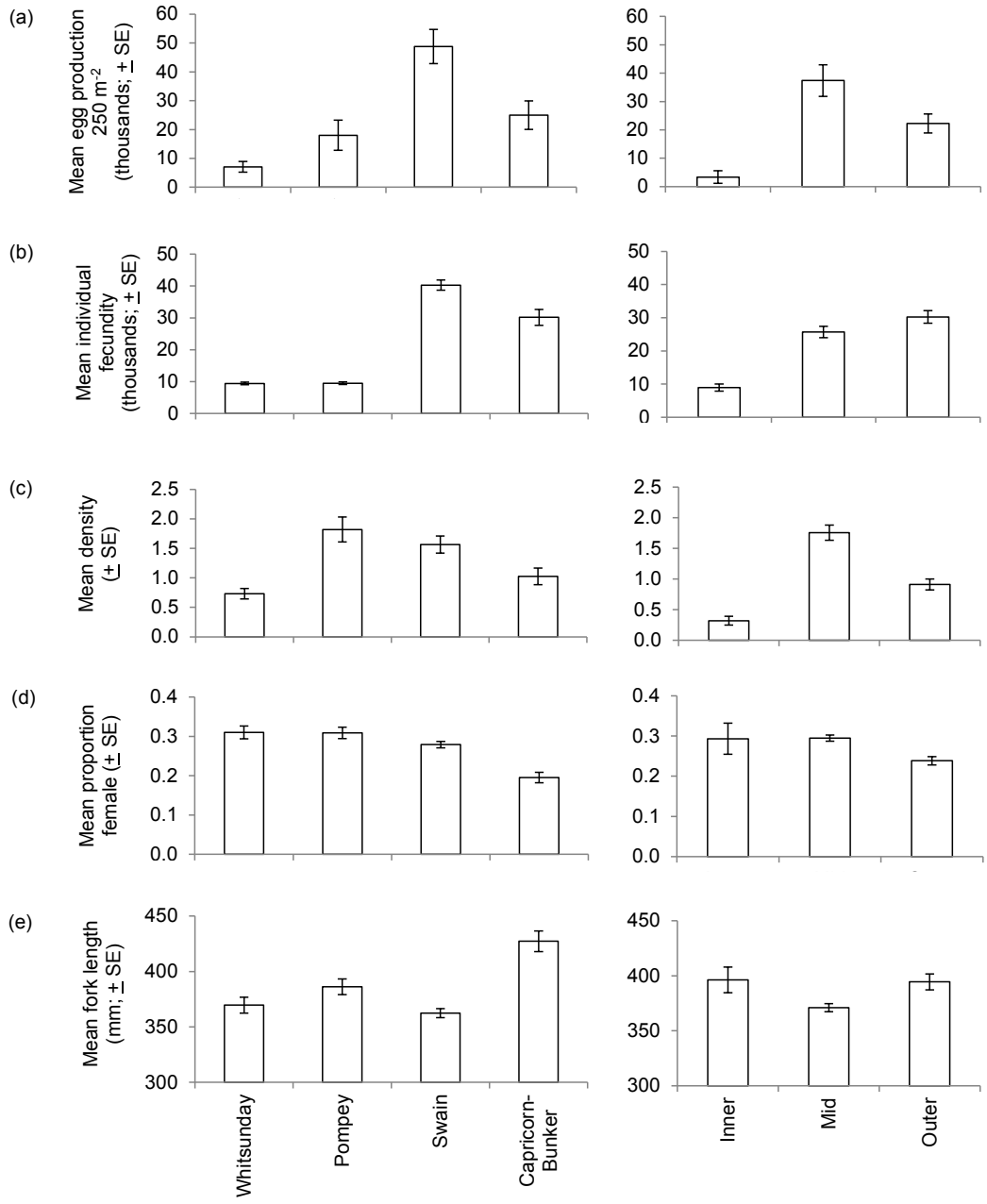


Figure 6.4 Mean ± standard error (SE) of *Plectropomus leopardus* estimated egg production 250 m^{-2}; (b) individual fecundity (oocytes $female^{-1}$ $year^{-1}$); (c) density (individuals 250 m^{-2}); (d) proportion female and (e) fork length (mm) on reefs from the Whitsunday, Pompey, Swain and Capricorn-Bunker sectors (first column) and inner, mid and outer shelf reefs (second column) in the southern Great Barrier Reef.

Table 6.3 Summary of final generalized additive mixed models (GAMMs) examining the categorical covariates management zone (*Z*), sector (*S*) and shelf position (*P*) and the continuous covariates density (*D*; individuals 250 m^{-2}), individual fecundity (*I*; oocytes $female^{-1}$ $year^{-1}$), fork length (*FL*, mm), and year (*Y*) on reef means of *Plectropomus leopardus* egg production 250 m^{-2} (*EPUA*). GAMMs were fitted separately to data from the north-central and southern regions.

Model	**AIC_c**	**ΔAIC_c**	***w***	**Adj. R^2**
North-central region				
$Sqrt(EP) = s(FL) + s(D) + s(I) + Z * S + \beta_{reef} + \varepsilon$	2570.5	0.00	0.59	0.94
Southern region				
$Sqrt(EP) = s(FL) + s(D) + s(I) + Z + S + P + \beta_{reef} + \varepsilon$	2153.2	0.00	0.50	0.96

Note: β_{reef} is the random effect of reef, and ε is the error term; AIC_c is the small-sample bias-corrected form of Akaike's information criterion; Δ is the Akaike difference; *w* is the Akaike weight; Adj. R^2 is adjusted coefficient of determination.

Table 6.4 Overall fit of selected best models of *Plectropomus leopardus* egg production per unit area (250 m^{-2}) from the north-central and southern GBR, including degrees of freedom (*df*), *F*-statistic and *p*-values. Categorical covariates are management zone (*Z*), sector (*S*) and shelf position (*P*). Smooth terms are fork length (*FL*, mm), density (*D*; individuals 250 m^{-2}), and individual female fecundity (*I*; oocytes $female^{-1}$ $year^{-1}$).

North-central region		***df***	***F***	***p*-value**
Parametric terms	*Z*	1	1.65	2.00×10^{-1}
	S	2	0.12	8.80×10^{-1}
	*Z*S*	2	7.26	$< 1.00 \times 10^{-3}$
Smooth terms	s(*FL*)	3.53	21.27	2.18×10^{-13}
	s(*D*)	5.71	473.33	$< 2.00 \times 10^{-16}$
	s(*I*)	1.00	93.54	$< 2.00 \times 10^{-16}$
Southern region		***df***	***F***	***p*-value**
Parametric terms	*Z*	1	7.86	$< 1.00 \times 10^{-2}$
	S	3	16.28	$< 1.37 \times 10^{-9}$
	P	2	6.26	$< 1.00 \times 10^{-2}$
Smooth terms	s(*FL*)	7.27	293.80	$< 2.00 \times 10^{-16}$
	s(*D*)	5.11	5.33	$< 1.00 \times 10^{-2}$
	s(*I*)	1.98	69.01	$< 2.00 \times 10^{-16}$

Note: Degrees of freedom for smooth terms are estimated *df*.

Individual fecundity was greatest on reserve reefs in the central GBR (>1.7 million oocytes female^{-1} year^{-1}, Figure 6.3b), intermediate on fished reefs in the central GBR and fished and reserve reefs in the northern GBR (300 000 – 1 million oocytes female^{-1} year^{-1}, Figure 6.3b), and lowest on Whitsunday and Pompey sector reefs (~9 000 oocytes female^{-1} year^{-1}) and Capricorn-Bunker and Swain reefs (30 000 – 40 000 oocytes female^{-1} year^{-1}, Figure 6.4b). Differences in individual fecundity among regions or sectors were driven primarily by regional variation in batch fecundity and spawning frequency. Batch fecundity increased at significantly steeper rates with *FL* in the central and northern regions than in the southern GBR, where *FL* had a negligible effect (Figure 6.3d). Females spawned significantly more frequently in the central GBR compared with the north (Table 6.2). Mean individual fecundity was ~150% greater on reserve reefs than fished reefs in the central GBR, but 218% and 70% greater on fished reefs than NTMR reefs in the northern and southern GBR respectively (Figure 6.3b). These patterns were driven by greater spawning frequency and larger mean fork length of *P. leopardus* on northern fished reefs compared with reserve reefs (Figure 6.3e; Table 6.2) and a weak length-batch fecundity relationship and greater spawning frequency on fished reefs compared with reserves in the southern region (Figure 6.2d; Table 6.2).

There was a general inverse relationship between mean fork length and mean proportion females on fished and reserve reefs within a region (Figure 6.3d, e). The proportion of females decreased as latitude increased along the GBR (Figure 6.3d), with southern GBR reefs male-biased, particularly in the southernmost Capricorn-Bunker sector where the proportion of females was just 0.20 $\pm$ 0.01 (Figure 6.4d).

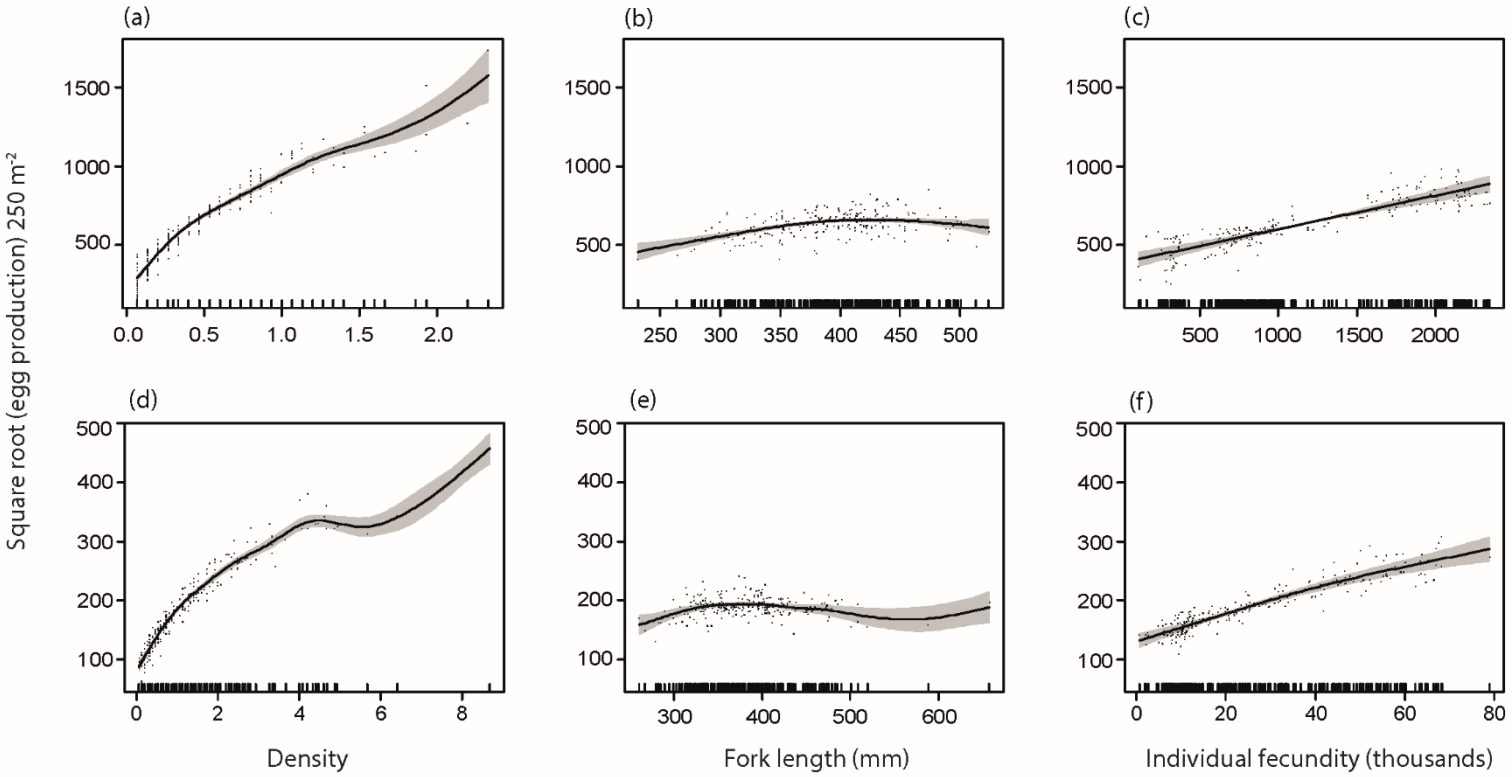
Shelf position was a significant predictor of *EPUA* in the southern GBR (Table 6.3, Table 6.4), with *EPUA* on mid shelf reefs (39 500 $\pm$ 3 500 oocytes year^{-1}) 68% and 1015% greater than on outer shelf and inner shelf reefs respectively (Figure 6.4a). Greater *EPUA* on mid shelf reefs was driven by two and five times higher densities of *P. leopardus* relative to inner shelf and outer shelf reefs (Figure 6.4c), and three times greater individual fecundity relative to inner shelf reefs (Figure 6.4b). *EPUA* did not vary with shelf position in the northern or central regions.

Density, individual fecundity and fork length all were significant terms in the north-central and southern GAMMs (Table 6.3, Table 6.4). Density of *P. leopardus* per reef had a significant, positive effect on reef *EPUA* within each GBR region, particularly in the northern and central regions (Figure 6.5), despite the qualitatively inverse relationship across regions between *EPUA* and regionally averaged density (Figure 6.3a, c). Greater densities of *P. leopardus* on northern reserve reefs did not equate to greater mean *EPUA* because individual fecundity and mean fork length were lower on reserve reefs than fished reefs in that region (Figure 6.3). Individual fecundity also had a significant and positive effect on *EPUA* in each GBR region (Figure 6.5). Fork length had a positive effect on *EPUA* until fish reached ~400 mm *FL* after which *EPUA* peaked and became asymptotic (northern and central GBR) or decreased slightly (southern GBR) (Figure 6.5), most likely due to female-male sex change beyond this size.

Interpolation of *EPUA* and its predictors across the southern two-thirds of the GBRMP showed conspicuous spatial patterns in *EPUA* and reproductive and population characteristics of *P. leopardus* (Figure 6.6). Distinction between high *EPUA* on reserve

reefs and lower *EPUA* on fished reefs in the Cairns and Townsville sectors likely reflected fishing-related reductions in population density, size of individuals, and individual fecundity in these regions with greater fishing pressure (Figure 6.6). The absence of similar patterns in *EPUA* in the southern GBR, despite similar patterns between fished and reserve reefs in population density and *FL*, are likely caused by the very low individual fecundity and low proportion of females in the southern region, irrespective of exposure to or protection from fishing (Figure 6.6).

Figure 6.5 Predicted egg production 250 m^{-2} (*EPUA*) with changes in density (individuals 250 m^{-2}), fork length (mm), and individual fecundity (oocytes $female^{-1}$ $year^{-1}$) of *Plectropomus leopardus* from (a-c) north-central and (d-f) southern Great Barrier Reef (GBR) regions. Non-linear trends are the fit of a Gaussian generalized additive mixed model (GAMM) with *EPUA* as response. Grey areas are 95% confidence intervals and black dots are residuals. x-axis and y-axis scales vary between GBR regions.



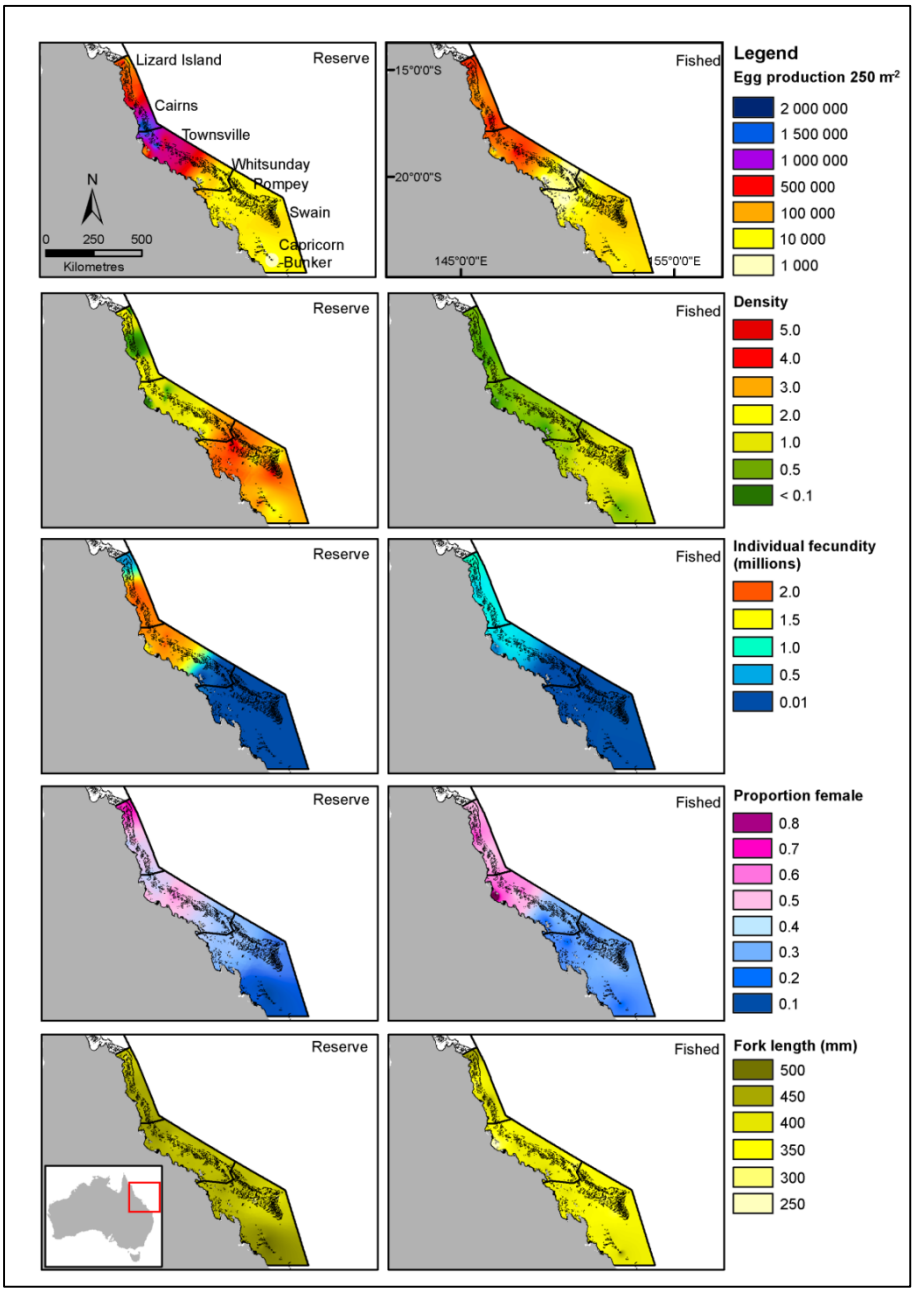


Figure 6.6 Estimated mean egg production 250 m^{-2} (*EPUA*), density (individuals 250m^{-2}), individual fecundity (oocytes $female^{-1}$ $year^{-1}$), proportion female, and fork length (mm) of *Plectropomus leopardus* on fished and reserve reefs within the Great Barrier Reef Marine Park (GBRMP). Estimated *EPUA* values are based on the fit of two Gaussian generalized additive mixed models (north-central and southern GBR), with *EPUA* as response. Black lines show boundaries of three Management Areas within the GBRMP.

6.4 Discussion

Reserves introduced to bolster fisheries productivity via recruitment subsidy are best placed where they will provide greatest net-recruitment to fished areas. EPUA is a key measure of potential reproductive output. This study highlights the extent to which EPUA can vary at both small and large spatial scales. This study demonstrates that EPUA should not be assumed to follow theoretical predictions based on proxies such as population density or adult size. For example, EPUA in the southern GBR was at least one order of magnitude lower than in the central and northern GBR, despite the southern GBR having 2 – 4 times the densities of *P. leopardus* as other regions. Importantly, these results are unlikely to be an artefact of temporal variation in reproductive output due to the extended period of the study (10 years). These results raise important questions regarding a key assumption of the role of reserves – that greater densities of larger individuals will lead to greater EPUA. Rather than using theoretical estimations, egg production needs to be quantified before site selection of reserves occurs if larval subsidy is to be an expected benefit.

6.4.1 Regional and cross-shelf variation in EPUA

Regional variation in life history of *P. leopardus* is unlikely due to genetic variability (van Herwerden et al. 2009). Environmental factors are, therefore, likely driving spatial variation in reproductive characteristics of *P. leopardus*. As discussed in Chapters 2 and 4, spatial variation in fish reproduction often is correlated with variation in environmental conditions (e.g. water temperature, food availability) or fishing pressure (Kokita 2004, Dimmlich et al. 2009, Collins and McBride 2014). Lower water temperatures on the southern GBR compared to the central and northern GBR may be approaching reproductive limits for *P.*

leopardus that cause reduced spawning frequency and batch fecundity, and increased male bias (see more detailed discussions in Chapters 2 and 4).

Spatial variation in EPUA among shelf positions also was evident in the southern GBR, with lower EPUA on inner shelf reefs driven by lower density and reduced individual fecundity relative to mid and outer shelf reefs. This cross-shelf variation in EPUA should be treated with some caution, however, as (a) inner-shelf density data in the southern GBR were collected only from the Whitsunday sector, (b) reproductive data were gathered from mid-shelf reefs, and (c) I assumed the relationship between size, density, and reproductive parameters were constant across shelf locations. Future studies would benefit from sampling that provided reproductive data from all shelf positions.

6.4.2 Reserves and EPUA

The largest effect of reserves on the reproductive output of *P. leopardus* occurred in the central GBR. A central paradigm of reserve theory is that preservation of "Big Old Fat Fecund Females" (BOFFFs) will lead to greater egg production from reserves and recruitment subsidy to surrounding fished areas (Hixon et al. 2014). Near-doubling of EPUA on central GBR reserve reefs was driven largely by greater individual fecundity of females in reserves than in fished reefs (Figure 6.3b). Reserve reefs had larger individuals (Figure 6.3e) that spawned more frequently (Table 6.2) and changed sex to male at a larger size on reserve reefs than on fished reefs (Figure 6.2a, b). This result is consistent with theory about the existence of BOFFFs in reserves. Similar effects of reduced fishing on individual fecundity were reported for the tropical protogynous hogfish *Lachnolaimus maximus* in the Gulf of Mexico, where females from lightly

fished offshore areas were larger, more fecund, and had a longer spawning season than females from inshore areas with greater fishing pressure (Collins and McBride 2014).

The results from the southern GBR illustrate that variation in protogynous dynamics, particularly early sex change, can change the prevalence or effects of BOFFFs, EPUA, and potential recruitment subsidy. Reserve EPUA was just 16% greater than on fished reefs despite densities of *P. leopardus* being 120% greater on reserves. Conditions in the southern GBR apparently are not conducive to the existence of BOFFFs, irrespective of zoning status, as the overall proportion of females is low, they spawn infrequently, and batch fecundity increases negligibly with length. Male-bias and presence of females only in smaller size classes in southern reefs, meant a relatively large proportion of females did not contribute at all to egg production. These patterns likely were driven by a combination of environmental conditions and fishing pressure that caused sex change at smaller sizes on fished reefs (Figure 6.2a, b). Sex change mediated by environment and fishing pressure also was reported for the tropical protogynous hogfish, where sex change occurred earlier and at smaller sizes when either males were removed through fishing or the conspecific density was high (Collins and McBride 2011).

Reserves consistently demonstrate increases in density, biomass and body size of target organisms relative to fished areas (Lester et al. 2009, Molloy et al. 2009, Edgar et al. 2014) and predictions of recruitment subsidy from reserves is reiterated often (Graham et al. 2011). Implications of protogyny on BOFFF theory have received limited attention, with reviews often focussing predominately on temperate gonochore species (Hixon et al. 2014). Recent modeling, however, suggests recruitment subsidy from

reserves may be limited for protogynous species relative to gonochores as large females are "lost" through sex change (Chan et al. 2012). Sex change should be given particular consideration in managing protogynous species because older, larger and potentially more fecund females are "removed" by both direct mortality of large females through fishing and fishing-induced earlier sex change following removals of large males (Hawkins and Roberts 2004, Hamilton et al. 2007, Götz et al. 2008). These "removals" may depress female fecundity to very low levels (Sadovy 1996, Jennings and Kaiser 1998). The characteristics of southern GBR *P. leopardus* indicate the BOFFF paradigm should not be assumed automatically for targeted protogynous species. The reserves in the southern GBR would be erroneously considered significant sources of reproductive output if size and density alone were considered.

It is unlikely that reproductive characteristics resulting in diminished EPUA in the southern GBR were artefacts of some unintentional region-specific sampling bias for several reasons: (1) Post-settlement movement between reefs for *P. leopardus* is rare (Davies 2000, Matley et al. 2015), and within-reef movement to and from spawning aggregations also is limited (Zeller 1998, Zeller and Russ 1998, Davies 2000); (2) sampling avoided the new moon period when spawning aggregations were most likely to bias sampling (Mapstone et al. 2004); (3) new moon spawning aggregations do occur but at multiple sites on a reef (Samoilys and Squire 1994); and (4) sampling was highly structured around each reef and across the depth range of *P. leopardus* habitat. Further, although the spawning season for *P. leopardus* varies regionally, from approximately September to December in the central and northern GBR (Brown et al. 1994, Ferreira 1995, Russ et al. 1995, Samoilys 2000, Frisch et al. 2007), and October to February in the southern GBR (Goeden 1978, Brown et al. 1994), spawning consistently peaks in

October – November regardless of region (Goeden 1978, Brown et al. 1994, Ferreira 1995, Samoilys 2000) when southern reefs were sampled.

Larger and more fecund females with frequent spawning were found on northern fished reefs compared to reserves. Increased individual fecundity in fished areas often is attributed to fishing-induced reproductive compensation, where individual fecundity increases due to reduced competition for space and food (Koslow et al. 1995, Fudge and Rose 2008). This pattern was evident only in the northern GBR, where fishing pressure is lower than in other regions (Mapstone et al. 2004, Tobin et al. 2013) and where reserve zoning had no measurable effect on overall mean size, age, and density of *P. leopardus* when reproductive samples were collected (Mapstone et al. 2004). Reproductive compensation due to fishing does not, therefore, seem a plausible explanation for increased individual fecundity on fished reefs and it remains unclear why individual fecundity differed between zones in that region.

6.4.3 Management and monitoring implications

Effective fisheries management benefits from knowledge of the reproductive potential of targeted populations at relevant spatial and temporal scales. The location and relative importance of areas with high reproductive potential is critical information when reproductive potential is non-uniform across the range of a population. Protecting regional hotspots of reproductive potential of sedentary species with disproportionate EPUA is analogous to protecting spawning aggregation sites of transient species.

Significant spatial variation in EPUA has important management implications for *P. leopardus* on the GBR. The first GBR stock assessment of *P. leopardus* was completed recently without incorporating region-specific biological relationships despite the authors acknowledging known regional variation in life history traits (Leigh et al. 2014). Incorporating such spatial differences at regional, cross-shelf and management (reserves and fished reefs) scales will add complexity to the "single biological stock" approach used by Leigh et al. (2014). However, future development of assessment models for *P. leopardus* is likely to benefit from explicit consideration of spatial patterns in EPUA within the stock. Such structural improvements in assessment models are likely to provide more reliable estimates of potential yields, and ultimately provide the foundation for more robust management decisions.

This study is unique in comparing EPUA using reproductive data from outside *and* within reserves across a broad geographic area. The region-specific zoning effects on spawning frequency and size at sex change highlight the importance of incorporating such data into EPUA calculations, rather than assuming homogeneity between fished and protected populations. Individual fecundity and EPUA would have been underestimated significantly in reserves were calculations based only on reproductive parameters estimated for fished reefs.

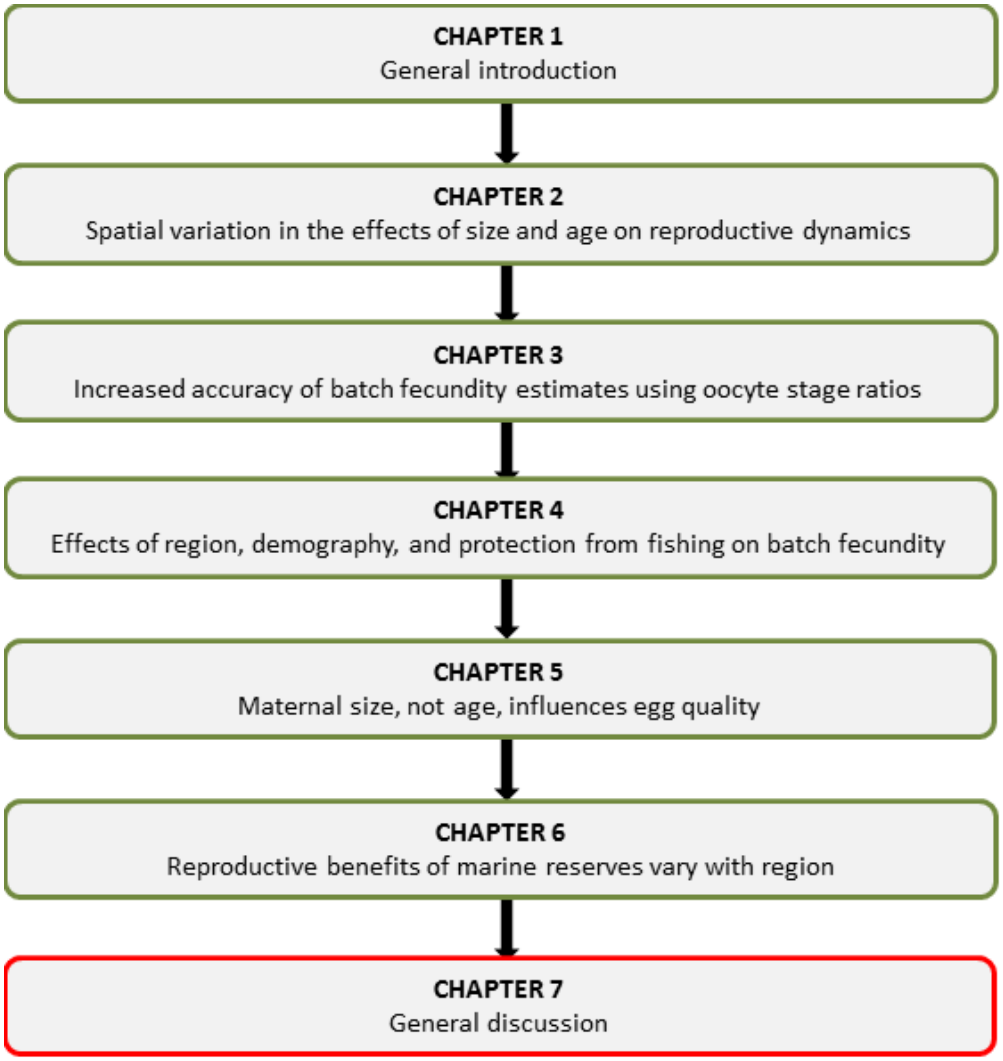
The combination of high densities and diminished EPUA on southern GBR reefs suggests that this region may be a larval sink benefiting from recruitment subsidies from reefs further to the north. As discussed in Chapters 2 and 4, larval dispersal modelling in the central GBR indicates larval export from a northern source to southern sink reefs driven

largely by the East Australia Current (EAC) on a scale of tens to hundreds of kilometres (James et al. 2002, Bode et al. 2006). The four week duration of pelagic larvae of *P. leopardus* (Doherty et al. 1994) would allow for extensive dispersal. Future parentage analysis (see Harrison et al. 2012, Almany et al. 2013) might provide estimates of the extent of *P. leopardus* larval dispersal and determine whether central GBR reefs are an important source of recruitment subsidy for southern reefs. Recruitment subsidy from southern GBR reserves is unlikely to be realized in that region if southern reefs are indeed larval sinks with recruits supplied predominantly from more northern reefs.

6.4.4 Conclusion

Spatial variation in *P. leopardus* individual fecundity, density, sex ratios and length among regions, and management zones within a region, demonstrates the importance of spatial variation in population dynamics and for siting of reserves based on reproductive output. Conditions on central GBR reefs support high reproductive potential of *P. leopardus* females, particularly in the absence of fishing. Greater individual fecundity on central GBR reserves may have important implications for recruitment subsidy both within and among regions. There was little evidence of potential subsidies from reserves to fished reefs within other regions of the GBR. Further research is required to determine the importance of the central GBR in replenishment of southern reefs. This study highlights the need to understand the reproductive responses of target species to fishing at appropriate spatial scales if reserves are to be a useful recruitment subsidy tool.

CHAPTER 7 General Discussion



7.1 General discussion

No-take marine reserves are popular worldwide as a means to conserve biodiversity and manage fisheries (Russ 2002, Sale et al. 2005, Edgar et al. 2014). Reserves are expected to be net exporters of recruits because egg production is particularly sensitive to the removal through fishing of Big Old Fat Fecund Females (BOFFFs) (Birkeland and Dayton 2005, Hixon et al. 2014). BOFFFs are expected to contribute disproportionately to population reproductive output by producing more eggs (Hixon et al. 2014) and better quality eggs (Berkeley et al. 2004a, Green 2008) relative to smaller and younger conspecifics. However, the majority of studies in this field have focused on temperate, gonochoristic (non-sex changing) fishes (Wiegand 1996, Green 2008, Venturelli et al. 2009, Hixon et al. 2014), and used reproductive data collected only from fished areas (Willis et al. 2003, Denny et al. 2004, Evans et al. 2008). The applicability of BOFFF theory to tropical fisheries management may be limited in tropical seas where protogyny is common (Chan et al. 2012). Reserves should also be assessed at appropriate spatial scales (Graham et al. 2011, Smith et al. 2014) as spatial variation in fish reproduction is often correlated with environmental conditions or fishing pressure (McIntyre and Hutchings 2003, Kokita 2004, Wakefield et al. 2013).

The goal of this thesis was to contribute key information on the reproductive biology of *Plectropomus leopardus* required to effectively manage the fishery at the Great Barrier Reef Marine Park (GBRMP) scale. To achieve this goal I (1) examined relationships between maternal traits of *P. leopardus* and egg quantity and quality; (2) compared relationships between maternal traits with egg quantity at a broad spatial scale that incorporated fished and reserve reefs among the northern, central and southern GBR;

and (3) quantified the extent of spatial variation in *P. leopardus*' egg production per unit area (EPUA) along the GBR.

7.2 Thesis outcomes

Objective 1: Quantify the effects of size and age on sex ratios and female sexual maturity of *P. leopardus* among regions, and between management zones on the GBR.

In Chapter 2, I demonstrated that sex ratios were more male-biased on southern GBR reefs than central and northern reefs across all lengths and ages. Fishing had a measurable effect on the length at sex change, with female-male transition occurring at consistently smaller lengths on fished reefs than reserve reefs. The difference varied among regions, however, and was most pronounced on central GBR reefs where 50% sex change occurred at ~50 mm shorter, and 3 years younger, on fished than on reserve reefs. The proportion of vitellogenic females increased with length and age but this relationship did not differ between management zones or among regions.

Objective 2: Quantify the effects of maternal size and age on the quantity of eggs produced by *P. leopardus* (spawning frequency, batch fecundity) between management zones and among regions on the GBR.

Results presented in Chapters 2 and 4 demonstrated variable maternal effects on egg production. Length had no effect on spawning frequency. Length, weight, and age had positive effects on batch fecundity of spawners from northern and central reefs but negligible effects on spawners from southern reefs. Mature females in the southern GBR spawned as infrequently as once every 2 – 3 months during the spawning season, compared with every 2 – 3 days on central GBR reserve reefs and northern GBR fished

reefs. Zoning influenced spawning frequency depending on which region the reserve was placed. Southern GBR females also were least fecund for a given length, weight and age. The effects of length and age on batch fecundity did not differ significantly between management zones in any region, but weight-specific fecundity was 100% greater for large (2 kg or greater) females on reserve reefs compared with fished reefs in the central GBR.

Objective 3: Quantify the maternal effects of size, age and body condition on indicators of egg quality for *P. leopardus*.

Chapter 5 detailed relationships between maternal traits (length, weight, age, and hepatosomatic index) and indicators of egg quality (egg size, oil droplet size, total lipid content and lipid classes) for *P. leopardus* in the central GBR. Egg size and oil droplet size increased positively with maternal weight, and egg size increased positively with maternal length. The proportion of long-term storage lipid wax esters also increased with maternal length and weight. Maternal age had no effect, and body condition had a very limited effect, on the egg quality indicators examined.

Objective 4: Estimate and compare EPUA of *P. leopardus* among regions and between management zones on the GBR

Egg production was greatest on reserve reefs in the central GBR (780 000 – 800 000 oocytes 250 m^{-2} $year^{-1}$) and lowest on southern GBR reefs (28 000 – 32 000 oocytes 250 m^{-2} $year^{-1}$). EPUA was ~180% and 16% times higher on reserve reefs than on fished reefs in the central GBR and southern GBR respectively. Individual fecundity positively affected EPUA. Reef-specific population density positively affected EPUA within each region, particularly in the northern and central regions. Fork length

positively affected EPUA until ~400 mm *FL*, after which EPUA became asymptotic or decreased slightly. Male-biased sex ratios and low individual fecundity limited the prospective reproductive benefits expected from higher population densities or larger fish in southern GBR reserves.

7.3 Implications of main findings

Reserve theory suggests that the relative benefit of reserves for population conservation and fisheries yield is likely to be greatest when areas outside reserves are heavily fished (Beverton and Holt 1957, Gerber et al. 2003, Buxton et al. 2014). Greater EPUA inside reserves relative to fished areas requires the presence of BOFFFs, greater densities of target species, or both. In the central GBR, the near-doubling of EPUA on reserve than on fished reefs was driven largely by BOFFFs in reserves in the central GBR and other GBR regions. The northern GBR receives relatively less fishing pressure than central and southern reefs (Mapstone et al. 2004, Tobin et al. 2013), and reserve zoning had no measurable effect on mean size, age, and density of *P. leopardus* in the north during the period when samples were collected (Mapstone et al. 2004). It was not surprising that northern reserve reefs did not demonstrate any potential for recruitment subsidy to fished reefs, although this is likely to change if commercial fishing pressure shifts northward.

The most surprising finding in this thesis was the greatly diminished egg production on southern GBR reefs, despite southern reefs, and particularly reserve reefs, containing two to four times greater densities of *P. leopardus* than in the central and northern GBR. Diminished egg production in the southern GBR was influenced by almost every reproductive parameter quantified – sex ratios were heavily male-biased, spawning was

infrequent, batch fecundity was relatively small, and maternal size and age had a negligible effect on batch fecundity. In short, the southern GBR lacked BOFFFs. These findings provide a dramatic example of the effects protogynous hermaphroditism, particularly early female-male sex change, can have on the prevalence of BOFFFs and on potential recruitment subsidy.

As the first study to examine spatial variation in egg production for a protogynous hermaphrodite across a broad geographic area, using fish collected within *and* outside of reserves, this thesis provides a model for future research on the effectiveness of reserves for target species whose distribution spans broad geographic scales. As discussed in Chapter 2, the theory of reserve design suggests that when information on larval connectivity and important larval sources is unavailable, reserves are best placed where there is an abundance of target species (Botsford et al. 2003, Bode et al. 2012). Without the reproductive data gathered during this thesis the logical endpoint of this theory is that reserves would be best placed in the southern GBR where the *P. leopardus* fishery is concentrated, abundance is greatest and, presumably, so too is reproductive output. Male-bias and diminished individual fecundity on southern GBR reefs suggests that applying biomass as a recruitment subsidy surrogate to measure the benefits of reserves for *P. leopardus* is flawed.

This thesis highlights the importance of examining the interplay between protogyny and BOFFFs at appropriate spatial scales, when a management goal is to maximize reproductive output. The stated purpose of GBRMP zoning is conservation and multiple use (Fernandes et al. 2005) and not explicitly for fisheries management. This is despite zones being defined primarily on what extractive activities are allowed and prohibited

(Figure 1.2). A shift in management focus from conservation of bioregions to fisheries management for the GBRMP is unlikely for both ideological and jurisdictional reasons. If, however, the management focus did shift to fisheries in the future, the spatial positioning of the current reserve network should be reviewed. For example, to manage against the risk of recruitment overfishing of coral trout, reserves placed in the central GBR would maximize the reproductive output of *P. leopardus*.

In Chapters 2, 4 and 6, I hypothesized that central GBR reserves may play an important role in recruitment subsidy at a regional scale (north-to-south recruitment subsidy) as well as at the local scale (reserve-to-fished reef recruitment subsidy). This hypothesis offers a potential explanation for high densities of adult *P. leopardus* on southern GBR reefs, where populations are characteristically male-biased and have reduced individual fecundity. The importance of considering empirically measured larval dispersal and connectivity in the design and function of reserves is increasingly recognized (Almany et al. 2009, Jones et al. 2009, McCook et al. 2009, Gaines et al. 2010, Kininmonth et al. 2011, Bode et al. 2012, Harrison et al. 2012, Almany et al. 2013, Green et al. 2014). The potential role of the central GBR in recruitment subsidy at a regional scale in particular requires further investigation.

A major objective of fisheries management is the prevention of recruitment overfishing by maintaining spawning stock biomass (Myers et al. 1994). The range of management tools applied to the coral trout fishery across zones of the GBRMP open to fishing contribute to achieving this objective and include protection of potential spawners and limits on catch and effort. A combination of tools to release fishing pressure is likely to remain the most appropriate approach to manage this species given the spatial

heterogeneity in fishing pressure and biology of the stock. These tools include allowing females to spawn at least once before entering the fishery through a minimum retention size limit of 380 mm *TL* (~360 mm *FL*), protecting spawning females during two 5-day closures to all fishing in peak *P. leopardus* spawning times around new moons in the Austral spring, limiting the number of coral trout that can be taken via bag limits for recreational and charter fishers, limited commercial entry to the fishery, and Individual Transferrable Quotas (ITQs) operating under a Total Allowable Commercial Catch (TACC) (Leigh et al. 2014). Significant regional variation in reproductive traits identified in this thesis raises important questions, however, regarding the appropriateness of managing this species as a single stock on the GBR.

Any future re-evaluation of fisheries management within the GBRMP zones open to fishing will need to occur within the framework of a multi-species Management Strategy Evaluation (MSE) that identifies the trade-offs in different management options for target and non-target species. The principles of ecosystem-based fishery management (EBFM) should also be applied that consider, for example, biological responses of *P. leopardus* to spatial variation in environmental and ecological conditions such as habitat, predators, and prey of the target species (Pikitch et al 2004). Region-specific management strategies for the *P. leopardus* fishery would require periodic re-evaluation as part of an adaptive management cycle. This is particularly important because habitat quality can change over time and commercial fishers are potentially highly mobile. This mobility was demonstrated recently when fishing effort shifted to the north due to decreased catch rates of *P. leopardus* in the southern GBR following Tropical Cyclone Hamish in 2009 (Tobin et al. 2010). If the hypothesis is correct that the central GBR is a source of recruits for southern reefs, any increase in

fishing effort in the central GBR that removes BOFFFs, either directly through fishing or indirectly through fishing-induced early sex change, may have deleterious consequences on recruitment to, and sustainability of, the southern fishery. If commercial fishing pressure shifts from the southern GBR, future MSEs should consider region-specific TACC and protection of BOFFFs. For example, in the central GBR an upper limit on TACC, a reduction in recreational and charter bag limits, an extension of the duration of the spawning season closure, and the introduction of an upper retention size limit (e.g. >450 mm *FL*) would protect *P. leopardus* BOFFFs in the region where they are most productive. This could be extended to the northern GBR if fishing pressure increases to the extent that reserves begin to provide measurable reproductive benefit in that region. Alternately, for the southern GBR where reproductive output is limited, the need for temporal spawning closures and stringent size limits could be reviewed.

7.4 Evaluation of the ELF Experiment reproductive data and future research directions

Assessing the effect of reserves and fishing on the reproductive dynamics of target species presents challenges due to the destructive sampling of large numbers of fish required within reserves. This makes the large samples of fish collected during the Effects of Line Fishing (ELF) Experiment that were used in Chapters 2, 4 and 6 of this thesis so valuable. The analysis for Chapter 2 utilized nearly 6 000 fish collected over four years, more than half of which were caught within no-take reserves. For such an ambitious research program to become a reality required a serendipitous mix of political will, long-term (10 years) financial commitment, scientific expertise, and support from the fishing industry, conservation groups and the public. It is unlikely that such a broad

scale experiment involving destructive sampling within reserves will be repeated on the GBR. The ELF Experiment sampling design was not without its limitations, however, when used to gather the reproductive data that was used in this thesis. Below I identify some of these limitations and, where applicable, suggest future research directions to address these.

7.4.1 Spawning season duration

The short sampling period (approximately 1 week per year in each of the four sampling regions) that reproductive data were collected meant that I was unable to assess whether maternal size or age influenced spawning season duration for *P. leopardus*. Larger/older females often spawn more batches annually than their smaller conspecifics by either spawning more frequently (Claramunt et al. 2007, Lowerre-Barbieri et al. 2009), which I established in Chapter 2 was not the case for *P. leopardus*, or by spawning relatively more batches annually through their ability to maintain spawning activity over a longer spawning season (Parrish et al. 1986, Claramunt et al. 2007). Regional variation in spawning season duration also could not be determined from ELF Experiment samples. I used the only previous estimate of spawning season duration of four months (Samoilys 2000) for my egg production estimates, with the assumption that this would be consistent along the GBR. The estimate by Samoilys (2000) was calculated from *P. leopardus* samples collected in the central GBR, but considering the regional variation in most reproductive parameter traits, the existence of regional variation in spawning season duration would not be surprising. Potential future research directions include determining whether larger female *P. leopardus* spawn for a longer period each year, thereby contributing a greater number of batches annually, and whether females in different GBR regions spawn for differing lengths of time.

7.4.2 Lunar phase

Lunar periodicity in batch spawning is common for tropical marine fishes (Taylor 1984, Takemura and Rahman 2004, Bushnell et al. 2010) and *P. leopardus* spawn most frequently during the new moon (Samoilys 1997, Samoilys 2000). For this reason, ELF Experiment sampling avoided new moons when spawning aggregations were most likely to bias catch-rate sampling pivotal to the ELF Experiment (Mapstone et al. 2004). My estimates of spawning frequency (Chapter 2) may therefore be conservative, although my comparison of spawning frequency among regions remains valid because reefs were sampled at similar lunar periods. Lunar phase does not appear to influence batch size in the central GBR (Chapter 4; Samoilys 2000). It is possible lunar phase may have a stronger influence on spawning frequency and female investment in batch fecundity for *P. leopardus* at higher latitudes, whereby the new moon plays a more significant role in gathering females and eliciting a spawning response in the southern GBR where reefs have a strong male bias. Future research on lunar phase and spawning activity of *P. leopardus* in the southern GBR would provide a much needed missing piece of the *P. leopardus* reproductive puzzle; that is, how are such high densities of adult *P. leopardus* possible on the southern GBR when there are so few females, and reproductive output from those females is low?

7.4.3 Cross shelf variation

Fine-scale spatial variation in reef fish population biology is common (Williams 1982, Newman and Williams 1996, Gust 2004). As discussed in Chapters 4 and 6, the possibility needs to be considered that the regional patterns in *P. leopardus* reproduction may be driven partly by cross-shelf variation in density and measures of

reproductive output for *P. leopardus*. Reefs sampled during the ELF Experiment were inshore-mid shelf reefs in the northern region, mid-shelf and outer-shelf reefs in the central region and all mid-shelf reefs in the southern region (Figure 2.1). It remains unclear how cross-shelf variation may manifest in estimates of individual fecundity. Future studies on the GBR would benefit from a sampling design that examines the influence of shelf position on reproductive output.

7.4.4 Batch fecundity and time of day

One potential pitfall of using the ELF Experiment samples was determining batch fecundity from samples collected throughout the day. The only previous estimate of batch fecundity for *P. leopardus* was restricted to ovarian samples collected after 1300 hours, predominantly by spear fishing, from two reefs in the central GBR (Samoilys 2000). The approach was appropriate for that study as only hydrated oocytes were counted, so sampling near dusk when *P. leopardus* are known to spawn maximized the number of hydrated oocytes available for counting (Samoilys 2000). Sample size can be significantly reduced, however, in studies dependent on samples acquired as part of a larger research project with multiple objectives (such as the ELF Experiment), or acquired opportunistically from commercial fishing surveys operating at a sub-optimal time for collection of spawning fishes. The ratio method I developed in Chapter 3, where migratory nucleus oocytes that would reach hydration by dusk were incorporated into batch fecundity estimates, allowed me to include ELF Experiment samples collected throughout the day. The alternatives were to reduce my sample size by approximately two thirds by including only ovaries collected after 1300 hours, or to underestimate batch fecundity by up to one third by using fish caught in the morning.

7.4.5 Region-specific sampling bias

In Chapters 2 and 6, I postulated that erroneous conclusions of male bias, reduced individual fecundity and EPUA could be made if a region-specific sampling bias caused large females in spawning condition not to be sampled as effectively on southern reefs. This is unlikely for reasons outlined in Chapters 2 and 6, including (1) limited post-settlement movement between reefs (Davies 2000, Matley et al. 2015) and limited within-reef movement to and from aggregations (Zeller 1998, Zeller and Russ 1998, Davies 2000); (2) sampling was avoided during new moon periods when spawning aggregations were most likely to bias sampling (Mapstone et al. 2004); (3) sampling at each reef was highly structured and meant the range of depths at which *P. leopardus* habitat occurred were sampled; and (4) southern reefs were sampled during the known peak spawning months for *P. leopardus* (see Chapter 2, Table 2.4). Further, male bias in the youngest of age classes (1 and 2 years) on southern reefs as described in Chapter 2 indicates sex change occurs at a much younger age than central and northern reefs. It is therefore unlikely the regional differences in reproduction reported in this thesis were caused by a region-specific sampling bias.

7.4.6 Environmental variation

The GBR is a huge reef system with documented regional variation in oceanographic influences (Wolanski 1994, Hopley et al. 2007). Significant regional variation in reproductive dynamics of *P. leopardus* was therefore not surprising. Environmental influences reported to affect reproduction are not mutually exclusive, and include primary productivity (Ganias 2009), food availability and quality (El-Sayed and Kawanna 2008), body condition (Kjesbu 1994, Somarakis et al. 2012), fishing pressure

(Brown-Peterson et al.), lunar phase (Samoilys 2000) and water temperature (Conover 1992). I hypothesize in Chapters 2, 4 and 6 that spatial variation in sex ratios and egg production is likely driven by environmental factors, most likely water temperature or prey availability. Relatively greater rates of female spawning omission and proportions of primary (pre-maturational) males in the southern GBR, and significantly greater individual female fecundity on reserves compared with fished reefs in the central GBR, indicates significant phenotypic plasticity of *P. leopardus*' life history strategies whereby population maintenance is achieved through differential allocation of energy in response to varying environmental conditions along the GBR. Such plasticity has the potential to interact with mortality rates and stock-recruitment relationships (Jørgensen et al., 2006). The relationship between egg production, pre-maturational sex change, growth rates and associated mortality rate should to be further explored.

Colder water temperatures are commonly attributed to delayed or reduced reproduction for spawners near their thermal limit (Collins and McBride 2014). Water temperature is a strong candidate for further investigation on the effects of reproductive dynamics. This would require rearing *P. leopardus* under different water temperature treatments to determine the effect on sex change, spawning frequency and batch fecundity. A successful experiment of this kind would likely involve permissions to manipulate the conditions experienced by *P. leopardus* brood stock, for example in an aquaculture setting.

7.4.7 Larval quality and fertilization success

This thesis provides an extensive analysis of the reproductive potential of *P. leopardus*; however another important component of reproductive success is the quality of the

offspring, measured as their survival to maturity (Lambert 2008). As discussed in Chapter 5, longer and heavier *P. leopardus* invested in larger eggs with greater concentrations of the neutral lipid wax ester, while maintaining concentrations of triacylglycerol and total lipid in the egg, relative to shorter and lighter females. This has potentially important implications for larval survival during the transition from endogenous to exogenous feeding. Variation in egg quality traits that influence larval growth and survival during the critical period between hatching and successful exogenous feeding may have significant implications for *P. leopardus* recruitment. The 2014 stock assessment for *P. leopardus* assumed that eggs and larvae produced by females were of equal quality (Leigh et al. 2014). Direct removal of large females through fishing, and indirect removal of large females through fishing-induced female-male sex change at smaller sizes as demonstrated in Chapter 2, is likely to have deleterious consequences for the quality of *P. leopardus* eggs produced by females on reefs open to fishing. Reserves on the GBR therefore have positive benefits on the quantity of eggs produced, as demonstrated in Chapter 6, and the quality of eggs produced, which are likely to supplement populations of *P. leopardus* on fished reefs (Harrison et al. 2012). Further research is required to unequivocally make this link.

Future research also should examine maternal effects and egg characteristics on measures of reproductive success, including fertilization and hatching success, and measures of larval quality including larval development, size, growth rates, survival, time to first feeding, feeding success, and pelagic larval duration. Future research also should investigate the effect of removing older and larger males in terms of sperm limitation and subsequent fertilization success, which exploited protogynous species can be particularly susceptible to (Alonzo and Mangel 2004). The egg quality study

presented in Chapter 5 used samples collected from reefs in the central GBR. Considering the extent of regional variation in other reproductive traits presented in this thesis, future assessments of egg and larval quality would benefit from samples collected from different GBR regions. Similarly, fertilization rates should be examined in the context of significant regional variation in sex ratios and male bias in the southern GBR.

7.5 Conclusion

Managing the exploitation of any fish stock is challenging, particularly a stock of sex-changing fish with a broad distribution. This thesis fills many of the gaps in knowledge regarding spatial variation in the reproductive biology of *P. leopardus* on the GBR and highlights the importance of examining the relationship between protogyny and BOFFFs when assessments of reproductive output are made. Results presented here should be incorporated into future stock assessments of *P. leopardus*. This thesis also supports the call for assessing the effects of reserves at large and appropriate spatial scales, incorporating multiple reserves within the same ecosystem (Graham et al. 2011, Smith et al. 2014). This is particularly important when a goal of management is protecting BOFFFs in reserves for recruitment subsidy purposes, but the species has an extensive distribution. From a fisheries management and conservation perspective, extending the techniques described in this thesis to identify reproductive "hotspots" in Indo-Pacific countries where *P. leopardus* are heavily exploited, such as the Philippines (Cornish and Kiwi 2004), and assessing spatial variation in reproduction for other protogynous species, would be beneficial. This thesis highlights the need for further research on reproductive responses of target species to fishing at appropriate spatial

scales, and careful consideration of the suitability of single conservation or fishery management strategies for species distributed across large and diverse spatial scales.

REFERENCES

Abesamis R. A. & Russ G. R. (2005). Density-dependent spillover from a marine reserve: Long-term evidence. *Ecological Applications,* **15**: 1798-1812.

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

Adams S., Mapstone B. D., Russ G. R. & Davies C. R. (2000). Geographic variation in the sex ratio, sex specific size, and age structure of *Plectropomus leopardus* (Serranidae) between reefs open and closed to fishing on the Great Barrier Reef. *Canadian Journal of Fisheries and Aquatic Sciences,* **57**: 1448-1458.

Almany G. R., Connolly S. R., Heath D. D., Hogan J. D., Jones G. P., McCook L. J., Mills M., Pressey R. L. & Williamson D. H. (2009). Connectivity, biodiversity conservation and the design of marine reserve networks for coral reefs. *Coral Reefs,* **28**: 339-351.

Almany G. R., Hamilton R. J., Bode M., Matawai M., Potuku T., Saenz-Agudelo P., Planes S., Berumen M. L., Rhodes K. L. & Thorrold S. R. (2013). Dispersal of grouper larvae drives local resource sharing in a coral reef fishery. *Current Biology,* **23**: 626-630.

Alonzo S. H. & Mangel M. (2004). The effects of size selective fisheries on the stock dynamics of and sperm limitation in sex-changing fish. *Fishery Bulletin,* **102**: 1-13.

Álvarez–Colombo G., Dato C., Macchi G., Palma E., Machinandiarena L., Christiansen H. E., Betti P., Derisio C., Martos P., Castro–Machado F., Brown D., Ehrlich M., Mianzan H. & Acha M. (2011). Distribution and behavior of Argentine hake larvae: Evidence of a biophysical mechanism for self–recruitment in northern Patagonian shelf waters. *Ciencias Marinas,* **37**: 633–657.

Anderson A., Arthington A. & Anderson S. (1990). Lipid classes and fatty acid composition of the eggs of some Australian fish. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry,* **96**: 267-270.

Arocha F. (2002). Oocyte development and maturity classification of swordfish from the north western Atlantic. *Journal of Fish Biology,* **60**: 13-27.

Babcock R. C., Phillips J. C., Lourey M. & Clapin G. (2007). Increased density, biomass and egg production in an unfished population of Western Rock Lobster (*Panulirus cygnus*) at Rottnest Island, Western Australia. *Marine and Freshwater Research,* **58**: 286-292.

Bachan M., Fleming I. & Trippel E. (2012). Maternal allocation of lipid classes and fatty acids with seasonal egg production in Atlantic cod (*Gadus morhua*) of wild origin. *Marine Biology,* **159**: 2281-2297.

Bani A., Moltschaniwskyj N. & Pankhurst N. (2009). Reproductive strategy and spawning activity of sand flathead, *Platycephalus bassensis* (Platycephalidae). *Cybium,* **33**: 151-162.

Barton K. (2013). MuMIn: Multi-model inference. R package version 1.9.13. Available: http://CRAN.R-project.org/package=MuMIn [Accessed 04/06/13].

Bates D., Maechler M. & Bolker B. (2012). lme4: Linear mixed-effects models using S4 classes. R package version 0.999999-0. Available: http://CRAN.R-project.org/package=lme4 [Accessed 04/06/13].

Bean K., Mapstone B. D., Davies C. R., Murchie C. D. & Williams A. J. (2003). Gonad development and evidence of protogyny in the red-throat emperor on the Great Barrier Reef. *Journal of Fish Biology,* **62**: 299-310.

Begg G. A., Mapstone B. D., Williams A. J., Adams S., Davies C. R. & Lou D. C. (2005). Multivariate life-history indices of exploited coral reef fish populations used to measure the performance of no-take zones in a marine protected area. *Canadian Journal of Fisheries and Aquatic Sciences,* **62**: 679-692.

Bergenius M. A. (2007). *Stock structure of a coral reef fish, Plectropomus leopardus: identification and implications for harvest strategy evaluation.* PhD, James Cook University.

Bergenius M. A., Mapstone B. D., Begg G. A. & Murchie C. D. (2005). The use of otolith chemistry to determine stock structure of three epinepheline serranid coral reef fishes on the Great Barrier Reef, Australia. *Fisheries Research,* **72**: 253-270.

Bergenius M. A., Meekan M. G., Robertson R. D. & McCormick M. I. (2002). Larval growth predicts the recruitment success of a coral reef fish. *Oecologia,* **131**: 521-525.

Berkeley S. A., Chapman C. & Sogard S. M. (2004a). Maternal age as a determinant of larval growth and survival in a marine fish, *Sebastes melanops*. *Ecology,* **85**: 1258-1264.

Berkeley S. A., Hixon M. A., Larson R. J. & Love M. S. (2004b). Fisheries sustainability via protection of age structure and spatial distribution of fish populations. *Fisheries,* **29**: 23-32.

Bernal M., Somarakis S., Witthames P. R., van Damme C. J. G., Uriarte A., Lo N. C. H. & Dickey-Collas M. (2012). Egg production methods in marine fisheries: An introduction. *Fisheries Research,* **117**: 1-5.

Bernal M., Stratoudakis Y., Wood S., Ibaibarriaga L., Valdés L. & Borchers D. (2011). A revision of daily egg production estimation methods, with application to Atlanto-Iberian sardine. 2. Spatially and environmentally explicit estimates of egg production. *ICES Journal of Marine Science: Journal du Conseil,* **68**: 528-536.

Bernardo J. (1996). The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. *American Zoologist,* **36**: 216-236.

Beverton R. J. (1983). Science and decision-making in fisheries regulation. *FAO Fisheries Report,* **291**: 919-936.

Beverton R. J. H. & Holt S. J. (1957). *On the Dynamics of Exploited Fish Populations,* London, Chapman and Hall.

Birkeland C. & Dayton P. K. (2005). The importance in fishery management of leaving the big ones. *Trends in Ecology & Evolution,* **20**: 356-358.

Blanchard J. L., Frank K. T. & Simon J. E. (2003). Effects of condition on fecundity and total egg production of eastern Scotian Shelf haddock (*Melanogrammus aeglefinus*). *Canadian Journal of Fisheries and Aquatic Sciences,* **60**: 321-332.

Bligh E. G. & Dyer W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology,* **37**: 911-917.

Bode M., Armsworth P. R., Fox H. E. & Bode L. (2012). Surrogates for reef fish connectivity when designing marine protected area networks. *Marine Ecology Progress Series,* **466**: 155.

Bode M., Bode L. & Armsworth P. R. (2006). Larval dispersal reveals regional sources and sinks in the Great Barrier Reef. *Marine Ecology Progress Series,* **308**: 17-25.

Botsford L. W., Micheli F. & Hastings A. (2003). Principles for the design of marine reserves. *Ecological Applications,* **13**: 25-31.

Branch G. & Odendaal F. (2003). The effects of marine protected areas on the population dynamics of a South African limpet, *Cymbula oculus*, relative to the influence of wave action. *Biological Conservation,* **114**: 255-269.

Bransden M., Battaglene S., Goldsmid R., Dunstan G. & Nichols P. (2007). Broodstock condition, egg morphology and lipid content and composition during the spawning season of captive striped trumpeter, *Latris lineata*. *Aquaculture,* **268**: 2-12.

Brooks S., Tyler C. R. & Sumpter J. P. (1997). Egg quality in fish: What makes a good egg? *Reviews in Fish Biology and Fisheries,* **7**: 387-416.

Brown-Peterson N. J., Burns K. M. & Overstreet R. M. (2009). Regional differences in Florida red snapper reproduction. Proceedings of the 61st Gulf and Caribbean Fisheries Institute, November 10 - 14, 2008, Gosier, Guadeloupe, French West Indies. 149-155.

Brown-Peterson N. J., Wyanski D. M., Saborido-Rey F., Macewicz B. J. & Lowerre-Barbieri S. K. (2011). A standardized terminology for describing reproductive development in fishes. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science,* **3**: 52-70.

Brown I. W., Doherty P. J., Ferreira B., Keenan K. C., McPherson G., Russ G., Samoilys M. & Sumpton W. (1994). Growth, reproduction and recruitment of Great Barrier Reef food fish stocks. *Fisheries Research and Development Corporation Project No. 90/18.* Brisbane: Southern Fisheries Centre, Queensland Department of Primary Industries.

Bruce M., Shields R., Bell M. & Bromage N. (1993). Lipid class and fatty acid composition of eggs of Atlantic halibut, *Hippoglossus hippoglossus* (L.), in relation to egg quality in captive broodstock. *Aquaculture Research,* **24**: 417-422.

Burnham K. P. & Anderson D. R. (2002). *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach,* New York, Springer-Verlag.

Bushnell M. E., Claisse J. T. & Laidley C. W. (2010). Lunar and seasonal patterns in fecundity of an indeterminate, multiple-spawning surgeonfish, the yellow tang *Zebrasoma flavescens*. *Journal of Fish Biology,* **76**: 1343-1361.

Butts I. A. & Litvak M. K. (2007). Parental and stock effects on larval growth and survival to metamorphosis in winter flounder (*Pseudopleuronectes americanus*). *Aquaculture,* **269**: 339-348.

Buxton C. D., Hartmann K., Kearney R. & Gardner C. (2014). When is spillover from marine reserves likely to benefit fisheries? *PLoS ONE,* **9**: e107032.

Carscadden J., Nakashima B. S. & Frank K. T. (1997). Effects of fish length and temperature on the timing of peak spawning in capelin (*Mallotus villosus*). *Canadian Journal of Fisheries and Aquatic Sciences,* **54**: 781-787.

Chan N. C. S., Connolly S. R. & Mapstone B. D. (2012). Effects of sex change on the implications of marine reserves for fisheries. *Ecological Applications,* **22**: 778-791.

Chang K. (2006). *Introduction to Geographic Information Systems,* New York, McGraw Hill.

Chauvet C. (1991). Statut d'*Epinephelus guaza* (Linnaeus, 1758) et éléments de dynamique des populations méditerranéen et atlantique. *In:* Boudouresque C. F., Avon M. & Gravez V. (eds.) *Les Espèces Marines à Protéger en Méditerranée.* France: GIS Posidonie.

Church J. (1987). East Australian Current adjacent to the Great Barrier Reef. *Marine and Freshwater Research,* **38**: 671-683.

Claramunt G., Serra R., Castro L. R. & Cubillos L. (2007). Is the spawning frequency dependent on female size? Empirical evidence in *Sardinops sagax* and *Engraulis ringens* off northern Chile. *Fisheries Research,* **85**: 248-257.

Collins A. & McBride R. (2011). Demographics by depth: Spatially explicit life-history dynamics of a protogynous reef fish. *Fishery Bulletin,* **109**: 232-242.

Collins A. & McBride R. (2014). Variations in reproductive potential between nearshore and offshore spawning contingents of hogfish in the eastern Gulf of Mexico. *Fisheries Management and Ecology*.

Conover D. O. (1992). Seasonality and the scheduling of life history at different latitudes. *Journal of Fish Biology,* **41**: 161-178.

Cooper D. W., Pearson K. E. & Gunderson D. R. (2005). Fecundity of shortspine thornyhead (*Sebastolobus alascanus*) and longspine thornyhead (*S. altivelis*) (Scorpaenidae) from the northeastern Pacific Ocean, determined by stereological and gravimetric techniques. *Fishery Bulletin,* **103**: 15-22.

Cornish A. & Kiwi L. (2004). *Plectropomus leopardus*. *IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2* [Online]. Available: www.iucnredlist.org [Accessed 24/02/14].

Cudney-Bueno R., Lavin M., Marinone S., Raimondi P. & Shaw W. (2009). Rapid effects of marine reserves via larval dispersal. *PLoS ONE,* **4**: e4140.

Cuellar N., Sedberry G. R. & Wyanski D. M. (1996). Reproductive seasonality, maturation, fecundity, and spawning frequency of the vermilion snapper, *Rhomboplites aurorubens*, off the southeastern United States. *Fishery Bulletin,* **94**: 635-653.

Dadzie S. & Abou-Seedo F. (2008). Reproductive guilds (maturation, spawning frequency and fecundity) in the black pomfret, *Parastromateus niger* (Carangidae) in the Kuwaiti waters of the Arabian Gulf. *Journal of Applied Ichthyology,* **24**: 562-568.

Danilowicz B. S. (1995). The role of temperature in spawning of the damselfish *Dascyllus albisella*. *Bulletin of Marine Science,* **57**: 624-636.

Davies C. R. (1995). *Patterns of movement of three species of coral reef fish on the Great Barrier Reef.* PhD, James Cook University.

Davies C. R. (2000). Inter-reef movement of the common coral trout, *Plectropomus leopardus*. *GBRMPA Research Publication No. 61.* Townsville: Great Barrier Reef Marine Park Authority.

Davies C. R., Williams A. J., Mapstone B. D., Benzie J., van Herwerden L., Choat J. H., Adams S., Murchie C. D., Bean K., Carlos G., Tobin A. & Ackerman J. (2006). Stock structure and regional variation in population dynamics of the Red Throat Emperor and other target species of the Queensland tropical reef line fishery. *CRC Reef Research Centre Technical Report No. 61.* Townsville: CRC Reef Research Centre.

Denny C. M., Willis T. J. & Babcock R. C. (2004). Rapid recolonisation of snapper *Pagrus auratus*: Sparidae within an offshore island marine reserve after implementation of no-take status. *Marine Ecology Progress Series,* **272**: 183-190.

Díaz D., Mallol S., Parma A. M. & Goñi R. (2011). Decadal trend in lobster reproductive output from a temperate marine protected area. *Marine Ecology Progress Series,* **433**: 149-157.

Dimmlich W. F., Ward T. M. & Breed W. G. (2009). Spawning dynamics and biomass estimates of an anchovy *Engraulis australis* population in contrasting gulf and shelf environments. *Journal of Fish Biology,* **75**: 1560-1576.

Doherty P. J., Fowler A. J., Samoilys M. A. & Harris D. A. (1994). Monitoring the replenishment of coral trout (Pisces: Serranidae) populations. *Bulletin of Marine Science,* **54**: 343-355.

Donelson J. M., McCormick M. I. & Munday P. L. (2008). Parental condition affects early life-history of a coral reef fish. *Journal of Experimental Marine Biology and Ecology,* **360**: 109-116.

Edgar G. J., Stuart-Smith R. D., Willis T. J., Kininmonth S., Baker S. C., Banks S., Barrett N. S., Becerro M. A., Bernard A. T. & Berkhout J. (2014). Global conservation outcomes depend on marine protected areas with five key features. *Nature,* **506**: 216-220.

Einum S. & Fleming I. A. (1999). Maternal effects of egg size in brown trout (*Salmo trutta*): Norms of reaction to environmental quality. *Proceedings of the Royal Society of London. Series B: Biological Sciences,* **266**: 2095-2100.

El-Sayed A. M. & Kawanna M. (2008). Effects of dietary protein and energy levels on spawning performance of Nile tilapia (*Oreochromis niloticus*) broodstock in a recycling system. *Aquaculture,* **280**: 179/184.

Emslie M. J., Logan M., Williamson D. H., Ayling A. M., MacNeil M. A., Ceccarelli D., Cheal A. J., Evans R. D., Johns K. A., Jonker M. J., Miller I. R., Osborne K., Russ G. R. & Sweatman H. P. (2015). Expectations and outcomes of reserve network performance following re-zoning of the Great Barrier Reef Marine Park. *Current Biology,* **25**: 983-992.

Evans R. D., Russ G. R. & Kritzer J. P. (2008). Batch fecundity of *Lutjanus carponotatus* (Lutjanidae) and implications of no-take marine reserves on the Great Barrier Reef, Australia. *Coral Reefs,* **27**: 179-189.

Farley J. H., Williams A. J., Hoyle S. D., Davies C. R. & Nicol S. J. (2013). Reproductive dynamics and potential annual fecundity of South Pacific Albacore Tuna (*Thunnus alalunga*). *PLoS ONE,* **8**: e60577.

Fennessy S. T. & Sadovy Y. (2002). Reproductive biology of a diandric protogynous hermaphrodite, the serranid *Epinephelus andersoni*. *Marine and Freshwater Research,* **53**: 147-158.

Fernandes L., Day J., Lewis A., Slegers S., Kerrigan B., Breen D., Cameron D., Jago B., Hall J. & Lowe D. (2005). Establishing representative no-take areas in the Great Barrier Reef: Large-scale implementation of theory on marine protected areas. *Conservation Biology,* **19**: 1733-1744.

Ferreira B. P. (1995). Reproduction of the common coral trout *Plectropomus leopardus* (Serranidae: Epinephelinae) from the central and northern Great Barrier Reef, Australia. *Bulletin of Marine Science,* **56**: 653-669.

Ferreira B. P. & Russ G. R. (1994). Age validation and estimation of growth rate of the coral trout, *Plectropomus leopardus*, (Lacepede 1802) from Lizard Island, Northern Great Barrier Reef. *Fishery Bulletin,* **92**: 46-57.

Ferreira B. P. & Russ G. R. (1995). Population structure of the leopard coralgrouper, *Plectropomus leopardus*, on fished and unfished reefs off Townsville, Central Great Barrier Reef, Australia. *Fishery Bulletin,* **93**: 629-642.

Fitzhugh G. R., Thompson B. A. & Snider T. G. (1993). Ovarian development, fecundity, and spawning frequency of black drum *Pogonias cromis* in Louisiana. *Fishery Bulletin,* **91**: 244-244.

Fletcher W. J., Kearney R. E., Wise B. S. & Nash W. J. (2015). Large-scale expansion of no-take closures within the Great Barrier Reef has not enhanced fishery production. *Ecological Applications,* **25**: 1187-1196.

Fontes J., Santos R. S., Afonso P. & Caselle J. E. (2011). Larval growth, size, stage duration and recruitment success of a temperate reef fish. *Journal of Sea Research,* **65**: 1-7.

Fox J. & Weisberg S. (2009). Car: Companion to applied regression. R package version 02-14. Available: http://cran.r-project.org/web/packages/car [Accessed 24/02/14].

Fox J. & Weisberg S. (2011). *An R Companion to Applied Regression, Second Edition,* Thousand Oaks CA, Sage.

Frisch A. J., McCormick M. I. & Pankhurst N. W. (2007). Reproductive periodicity and steroid hormone profiles in the sex-changing coral-reef fish, *Plectropomus leopardus*. *Coral Reefs,* **26**: 189-197.

Froese R., Zeller D., Kleisner K. & Pauly D. (2012). What catch data can tell us about the status of global fisheries. *Marine Biology,* **159**: 1283-1292.

Fudge S. B. & Rose G. A. (2008). Life history co-variation in a fishery depleted Atlantic cod stock. *Fisheries Research,* **92**: 107-113.

Gaines S. D., White C., Carr M. H. & Palumbi S. R. (2010). Designing marine reserve networks for both conservation and fisheries management. *Proceedings of the National Academy of Sciences,* **107**: 18286-18293.

Ganias K. (2009). Linking sardine spawning dynamics to environmental variability. *Estuarine, Coastal and Shelf Science,* **84**: 402-408.

Ganias K. (2012). Thirty years of using the postovulatory follicles method: Overview, problems and alternatives. *Fisheries Research,* **117-118**: 63-74.

Geoscience Australia. (2011). *Moon phase data table* [Online]. Available: http://www.ga.gov.au/geodesy/astro/moonphases/moonphases.jsp [Accessed 30/01/11].

Gerber L. R., Botsford L. W., Hastings A., Possingham H. P., Gaines S. D., Palumbi S. R. & Andelman S. (2003). Population models for marine reserve design: A retrospective and prospective synthesis. *Ecological Applications,* **13**: 47-64.

Godwin J. (2009). Social determination of sex in reef fishes. *Seminars in Cell & Developmental Biology,* **20**: 264-270.

Goeden G. B. (1978). A Monograph of the Coral Trout, *Plectropomus leopardus* (Lacepede). *Research Bulletin No 1.* Brisbane: Queensland Fisheries Service.

Götz A., Kerwath S., Attwood C. & Sauer W. (2008). Effects of fishing on population structure and life history of roman *Chrysoblephus laticeps* (Sparidae). *Marine Ecology Progress Series,* **362**: 245-259.

Graham N. A. J., Evans R. D. & Russ G. R. (2003). The effects of marine reserve protection on the trophic relationships of reef fishes on the Great Barrier Reef. *Environmental Conservation,* **30**: 200-208.

Graham N. A. J., Ainsworth T. D., Baird A. H., Ban N. C., Bay L. K., Cinner J. C., De Freitas D. M., Diaz-Pullido G., Dornelas M., Dunn S. R., Fidelman P. I. J., Foret S., Good T. C., Kool J., Mallela J., Penin L., Pratchett M. J. & Williamson D. H. (2011). From microbes to people: tractable benefits of no-take areas for coral reefs. *Oceanography and Marine Biology: An Annual Review,* **49**: 105-136.

Green A. L., Maypa A. P., Almany G. R., Rhodes K. L., Weeks R., Abesamis R. A., Gleason M. G., Mumby P. J. & White A. T. (2014). Larval dispersal and movement patterns of coral reef fishes, and implications for marine reserve network design. *Biological Reviews*.

Green B. S. (2008). Maternal effects in fish populations. *Advances in Marine Biology,* **54**: 1-105.

Green B. S. & McCormick M. I. (2005). Maternal and paternal effects determine size, growth and performance in larvae of a tropical reef fish. *Marine Ecology Progress Series,* **289**: 263-272.

Gust N. (2004). Variation in the population biology of protogynous coral reef fishes over tens of kilometres. *Canadian Journal of Fisheries and Aquatic Sciences,* **61**: 205-218.

Halford A. R. & Thompson A. A. (1994). Visual Census Surveys of Reef Fish. *Long-term monitoring of the Great Barrier Reef Standard Operating Procedure Number 3.* Townsville: Australian Institute of Marine Science.

Halpern B. S., Lester S. E. & Kellner J. B. (2010). Spillover from marine reserves and the replenishment of fished stocks. *Environmental Conservation,* **36**: 268-276.

Halpern B. S. & Warner R. R. (2003). Review Paper. Matching marine reserve design to reserve objectives. *Proceedings of the Royal Society of London. Series B: Biological Sciences,* **270**: 1871-1878.

Hamilton S. L., Caselle J. E., Standish J. D., Schroeder D. M., Love M. S., Rosales-Casian J. A. & Sosa-Nishizaki O. (2007). Size-selective harvesting alters life histories of a temperate sex-changing fish. *Ecological Applications,* **17**: 2268-2280.

Harrington Jr R. W. (1975). Sex determination and differentiation among uniparental homozygotes of the hermaphroditic fish *Rivulus marmoratus* (Cyprinodontidae: Atheriniformes). *Intersexuality in the Animal Kingdom.* Berlin, Heidelberg, New York: Springer Verlag.

Harrison H. B., Williamson D. H., Evans R. D., Almany G. R., Thorrold S. R., Russ G. R., Feldheim K. A., van Herwerden L., Planes S. & Srinivasan M. (2012). Larval export from marine reserves and the recruitment benefit for fish and fisheries. *Current Biology,* **22**: 1023-1028.

Hawkins J. P. & Roberts C. M. (2004). Effects of fishing on sex-changing Caribbean parrotfishes. *Biological Conservation,* **115**: 213-226.

Heemstra P. C. & Randall J. E. (1993). Groupers of the World (Family Serranidae, Subfamily Epinephelinae). An annotated and illustrated catalogue of the grouper, rockcod, hind, coral grouper and lyretail species known to date. FAO Species Catalogue. Rome: Food and Agriculture Organization of the United Nations.

Heppell S. S., Heppell S. A., Coleman F. C. & Koenig C. C. (2006). Models to compare management options for a protogynous fish. *Ecological Applications,* **16**: 238-249.

Hilborn R., Stokes K., Maguire J.-J., Smith T., Botsford L. W., Mangel M., Orensanz J., Parma A., Rice J. & Bell J. (2004). When can marine reserves improve fisheries management? *Ocean & Coastal Management,* **47**: 197-205.

Hilton Z., Poortenaar C. W. & Sewell M. A. (2008). Lipid and protein utilisation during early development of yellowtail kingfish (*Seriola lalandi*). *Marine Biology,* **154**: 855-865.

Hislop J. (1988). The influence of maternal length and age on the size and weight of the eggs and the relative fecundity of the haddock, *Melanogrammus aeglefinus*, in British waters. *Journal of Fish Biology,* **32**: 923-930.

Hixon M. A., Johnson D. W. & Sogard S. M. (2014). BOFFFFs: On the importance of conserving old-growth age structure in fishery populations. *ICES Journal of Marine Science: Journal du Conseil,* **71**: 2171-2185.

Hopley D., Smithers S. G. & Parnell K. E. (2007). *The Geomorphology of the Great Barrier Reef: Development, Diversity, and Change,* Cambridge, Cambridge University Press.

Houde E. D. (1989). Comparative growth, mortality, and energetics of marine fish larvae: Temperature and implied latitudinal effects. *Fishery Bulletin,* **87**: 471-495.

Hughes T. P. (1994). Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. *Science,* **265**: 1547-1551.

Hunter J. R. & Goldberg S. R. (1980). Spawning incidence and batch fecundity in northern anchovy, *Engraulis mordax*. *Fishery Bulletin,* **77**: 641-652.

Hunter J. R., Lo N. C. H. & Leong R. J. H. (1985). Batch fecundity in multiple spawning fishes. *In:* Lasker R. (ed.) *An Egg Production Method for Estimating Spawning Biomass in Pelagic Fish: Application to the Northern Anchovy, Engraulis mordax.* NOAA Technical Report NMFS.

Hunter J. R. & Macewicz B. J. (1980). Sexual maturity, batch fecundity, spawning frequency, and temporal pattern of spawning for the northern anchovy, *Engraulis mordax*, during the 1979 spawning season. *California Cooperative Oceanic Fisheries Investigations,* **21**: 139-149.

Hunter J. R. & Macewicz B. J. (1985). Measurement of spawning frequency in multiple spawning fishes. *In:* Lasker R. (ed.) *An Egg Production Method for Estimating Spawning Biomass in Pelagic Fish: Application to the Northern Anchovy, Engraulis mordax.* NOAA Technical Report NMFS.

Hunter J. R., Macewicz B. J. & Sibert J. R. (1986). The spawning frequency of skipjack tuna, *Katsuwonus pelamis*, from the South Pacific. *Fishery Bulletin,* **84**: 895-903.

Hutchings J. A. (1991). Fitness consequences of variation in egg size and food abundance in brook trout *Salvelinus fontinalis*. *Evolution,* **45**: 1162-1168.

Hutchings J. A. & Myers R. A. (1994). Timing of cod reproduction: Interannual variability and the influence of temperature. *Marine Ecology Progress Series,* **108**: 21-32.

Itano D. G. (2000). The reproductive biology of yellowfin tuna (*Thunnus albacares*) in Hawaiian waters and the western tropical Pacific Ocean: project summary. SOEST 00-01, JIMAR Contribution 00-328. University of Hawaii, Joint Institute for Marine and Atmospheric Research, Pelagic Fisheries Research Program.

Jackson J. B. C., Kirby M. X., Berger W. H., Bjorndal K. A., Botsford L. W., Bourque B. J., Bradbury R. H., Cooke R., Erlandson J., Estes J. A., Hughes T. P., Kidwell S., Lange C. B., Lenihan H. S., Pandolfi J. M., Peterson C. H., Steneck R. S., Tegner M. J. & Warner R. R. (2001). Historical overfishing and the recent collapse of coastal ecosystems. *Science,* **293**: 629.

Jakobsen T., Fogarty M. J., Megrey B. A. & Moksness E. (eds.) (2009). *Fish reproductive biology: implications for assessment and management.,* Chichester, UK: Wiley-Blackwell Scientific Publications.

James M. K., Armsworth P. R., Mason L. B. & Bode L. (2002). The structure of reef fish metapopulations: Modelling larval dispersal and retention patterns.

Proceedings of the Royal Society of London. Series B: Biological Sciences, **269**: 2079-2086.

Jennings S. & Kaiser M. J. (1998). The effects of fishing on marine ecosystems. *Advances in Marine Biology,* **34**: 201-352.

Jones G. P., Almany G. R., Russ G. R., Sale P. F., Steneck R. S., van Oppen M. J. H. & Willis B. L. (2009). Larval retention and connectivity among populations of corals and reef fishes: History, advances and challenges. *Coral Reefs,* **28**: 307-325.

Jones G. P., Planes S. & Thorrold S. R. (2005). Coral reef fish larvae settle close to home. *Current Biology,* **15**: 1314-1318.

Jørgensen C., Ernande B., Fiksen Ø. & Dieckman U. (2006). The logic of skipped spawning in fish. *Canadian Journal of Fisheries and Aquatic Sciences,* **63**: 200-211.

Kaitaranta J. K. & Ackman R. G. (1981). Total lipids and lipid classes of fish roe. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry,* **69**: 725-729.

Kamler E. (2005). Parent–egg–progeny relationships in teleost fishes: an energetics perspective. *Reviews in Fish Biology and Fisheries,* **15**: 399-421.

Kamukuru A. T. & Mgaya Y. D. (2004). Effects of exploitation on reproductive capacity of blackspot snapper, *Lutjanus fulviflamma* (Pisces: Lutjanidae) in Mafia Island, Tanzania. *African Journal of Ecology,* **42**: 270-280.

Karlou-Riga C. & Economidis P. S. (1997). Spawning frequency and batch fecundity of horse mackerel, *Trachurus trachurus* (L.), in the Saronikos Gulf (Greece). *Journal of Applied Ichthyology,* **13**: 97-104.

Kininmonth S., Beger M., Bode M., Peterson E., Adams V. M., Dorfman D., Brumbaugh D. R. & Possingham H. P. (2011). Dispersal connectivity and reserve selection for marine conservation. *Ecological Modelling,* **222**: 1272-1282.

Kjesbu O. S. (1994). Time of start of spawning in Atlantic cod (*Gadus morhua*) females in relation to vitellogenic oocyte diameter, temperature, fish length and condition. *Journal of Fish Biology,* **45**: 719-735.

Kjesbu O. S., Witthames P. R., Solemdal P. & Greer Walker M. (1998). Temporal variations in the fecundity of Arcto-Norwegian cod (*Gadus morhua*) in response to natural changes in food and temperature. *Journal of Sea Research,* **40**: 303-321.

Kjørsvik E. (1994). Egg quality in wild and broodstock cod *Gadus morhua* L. *Journal of the World Aquaculture Society,* **25**: 22-29.

Kjørsvik E., Mangor-Jensen A. & Holmeford I. (1990). Egg quality in fishes. *Advances in Marine Biology,* **26**: 71-113.

Knutsen G. & Tilseth S. (1985). Growth, development, and feeding success of Atlantic cod larvae *Gadus morhua* related to egg size. *Transactions of the American Fisheries Society,* **114**: 507-511.

Kokita T. (2004). Latitudinal compensation in female reproductive rate of a geographically widespread reef fish. *Environmental Biology of Fishes,* **71**: 213-224.

Korta M., Domínguez-Petit R., Murua H. & Saborido-Rey F. (2010). Regional variability in reproductive traits of European hake *Merluccius merluccius* L. populations. *Fisheries Research,* **104**: 64-72.

Koslow J. A., Bell J. D., Virtue P. & Smith D. C. (1995). Fecundity and its variability in orange roughy: Effects of population density, condition, egg size, and senescence. *Journal of Fish Biology,* **47**: 1063-1080.

Lambert Y. (2008). Why should we closely monitor fecundity in marine fish populations. *Journal of Northwest Atlantic Fishery Science,* **41**: 93-106.

Lasker R. (1981). *The Role of a Stable Ocean in Larval Fish Survival and Subsequent Recruitment,* Seattle, WA, University of Washington Press.

Lasker R. (ed.) (1985). *An Egg Production Method for Estimating Spawning Biomass in Pelagic Fish: Application to the Northern Anchovy, Engraulis mordax*.

Le Quesne W. J. & Codling E. A. (2009). Managing mobile species with MPAs: The effects of mobility, larval dispersal, and fishing mortality on closure size. *ICES Journal of Marine Science: Journal du Conseil,* **66**: 122-131.

Lee R. F., Nevenzel J. C. & Lewis A. (1974). Lipid changes during life cycle of marine copepod, *Euchaeta japonica* Marukawa. *Lipids,* **9**: 891-898.

Leigh G., Campbell A., Lunow C. & O'Neill M. (2014). Stock assessment of the Queensland east coast common coral trout (*Plectropomus leopardus*) fishery. Brisbane: State of Queensland.

Lester S. E., Halpern B. S., Grorud-Colvert K., Lubchenco J., Ruttenberg B. I., Gaines S. D., Airamé S. & Warner R. R. (2009). Biological effects within no-take marine reserves: A global synthesis. *Marine Ecology Progress Series,* **384**: 33-46.

Little L. R., Punt A. E., Mapstone B. D., Begg G. A., Goldman B. & Ellis N. (2009). Different responses to area closures and effort controls for sedentary and migratory harvested species in a multispecies coral reef linefishery. *ICES Journal of Marine Science: Journal du Conseil,* **66**: 1931.

Lo C. & Yeung A. (2002). *Concepts and Techniques of Geographic Information Systems,* Upper Saddle River, NJ, Prentice Hall.

Lough J. M. (1999). Sea surface temperatures on the Great Barrier Reef: A contribution to the study of coral bleaching. *GBRMPA Research Publication No. 57.* Townsville, Australia: Great Barrier Reef Marine Park Authority.

Lowerre-Barbieri S. K., Brown-Peterson N. J., Murua H., Tomkiewicz J., Wyanski D. M. & Saborido-Rey F. (2011). Emerging issues and methodological advances in fisheries reproductive biology. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science,* **3**: 32-51.

Lowerre-Barbieri S. K., Henderson N., Llopiz J., Walters S., Bickford J. & Muller R. (2009). Defining a spawning population (spotted seatrout *Cynoscion nebulosus*) over temporal, spatial, and demographic scales. *Marine Ecology Progress Series,* **394**: 231-245.

Macchi G. J., Leonarduzzi E., Diaz M. V., Renzi M. & Rodrigues K. (2013). Maternal effects on fecundity and egg quality of the Patagonian stock of Argentine hake (*Merluccius hubbsi*). *Fishery Bulletin,* **111**: 325-336.

Macewicz B. J. & Hunter J. R. (1993). Spawning frequency and batch fecundity of jack mackerel, *Trachurus symmetricus*, off California during 1991. *California Cooperative Oceanic Fisheries Investigations,* **34**: 112-121.

Maddams J. & McCormick M. (2012). Not all offspring are created equal: Variation in larval characteristics in a serially spawning damselfish. *PLoS ONE,* **7**: e48525.

Maki Jenkins K. L. & McBride R. S. (2009). Reproductive biology of wahoo, *Acanthocybium solandri*, from the Atlantic coast of Florida and the Bahamas. *Marine and Freshwater Research,* **60**: 893-897.

Mapstone B., Davies C., Little L., Punt A., Smith A., Pantus F., Lou D., Williams A., Jones A., Ayling A., Russ G. & McDonald A. (2004). The effects of line fishing on the Great Barrier Reef and evaluations of alternative potential management strategies. *CRC Reef Research Centre Technical Report No. 52.* Townsville: CRC Reef Research Centre.

Mapstone B., Little L., Punt A., Davies C., Smith A., Pantus F., McDonald A., Williams A. & Jones A. (2008). Management strategy evaluation for line fishing in the Great Barrier Reef: Balancing conservation and multi-sector fishery objectives. *Fisheries Research,* **94**: 315-329.

Marteinsdottir G. & Begg G. A. (2002). Essential relationships incorporating the influence of age, size and condition on variables required for estimation of reproductive potential in Atlantic cod *Gadus morhua*. *Marine Ecology Progress Series,* **235**: 235-256.

Marteinsdóttir G. & Steinarsson A. (1998). Maternal influence on the size and viability of Iceland cod *Gadus morhua* eggs and larvae. *Journal of Fish Biology,* **52**: 1241-1258.

Masuma S., Tezuka N. & Teruya K. (1993). Embryonic and morphological development of larval and juvenile coral trout, *Plectropomus leopardus*. *Japanese Journal of Ichthyology,* **40**: 333-342.

Matley J., Heupel M. & Simpfendorfer C. (2015). Depth and space use of leopard coralgrouper *Plectropomus leopardus* using passive acoustic tracking. *Marine Ecology Progress Series,* **521**: 201-216.

McBride R. S. & Thurman P. E. (2003). Reproductive biology of *Hemiramphus brasiliensis* and *H. balao* (Hemiramphidae): Maturation, spawning frequency, and fecundity. *Biological Bulletin,* **204**: 57-67.

McCook L., Almany G., Berumen M., Day J., Green A., Jones G., Leis J., Planes S., Russ G. & Sale P. (2009). Management under uncertainty: Guidelines for incorporating connectivity into the protection of coral reefs. *Coral Reefs,* **28**: 353-366.

McCormick M. & Gagliano M. (2008). Carry-over effects: The importance of a good start. 11th International Coral Reef Symposium. National Coral Reef Institute, NOVA Southeastern University, Fort Lauderdale, 7-11 July, 2008. 305-310.

McCormick M. I. (2003). Consumption of coral propagules after mass spawning enhances larval quality of damselfish through maternal effects. *Oecologia,* **136**: 37-45.

McCormick M. I. (2006). Mothers matter: Crowding leads to stressed mothers and smaller offspring in marine fish. *Ecology,* **87**: 1104-1109.

McIntyre T. M. & Hutchings J. A. (2003). Small-scale temporal and spatial variation in Atlantic cod (*Gadus morhua*) life history. *Canadian Journal of Fisheries and Aquatic Sciences,* **60**: 1111-1121.

McKinley S. & Levine M. (1998). Cubic Spline Interpolation. *College of the Redwoods* [Online]. Available: http://online.redwoods.edu/instruct/darnold/laproj/Fall98/SkyMeg/Proj.PDF [Accessed 15/08/13].

Mehault S., Domínguez-Petit R., Cerviño S. & Saborido-Rey F. (2010). Variability in total egg production and implications for management of the southern stock of European hake. *Fisheries Research,* **104**: 111-122.

Migaud H., Bell G., Cabrita E., McAndrew B., Davie A., Bobe J., Herraez M. P. & Carrillo M. (2013). Gamete quality and broodstock management in temperate fish. *Reviews in Aquaculture,* **5**: S194-S223.

Miller I., Cheal A. J., Emslie M., Logan M. & Sweatman H. (2012). Ongoing effects of no-take marine reserves on commercially exploited coral trout populations on the Great Barrier Reef. *Marine Environmental Research,* **79**: 167-170.

Molloy P. P., McLean I. B. & Cote I. M. (2009). Effects of marine reserve age on fish populations: A global meta-analysis. *Journal of Applied Ecology,* **46**: 743-751.

Moodie G., Loadman N., Wiegand M. & Mathias J. (1989). Influence of egg characteristics on survival, growth and feeding in larval walleye (*Stizostedion vitreum*). *Canadian Journal of Fisheries and Aquatic Sciences,* **46**: 516-521.

Mourente G. & Odriozola J. (1990). Effect of broodstock diets on lipid classes and their fatty acid composition in eggs of gilthead sea bream (*Sparus aurata* L.). *Fish Physiology and Biochemistry,* **8**: 93-101.

Munday P. L., Buston P. M. & Warner R. R. (2006). Diversity and flexibility of sex-change strategies in animals. *Trends in Ecology & Evolution,* **21**: 89-95.

Muñoz R. C., Burton M. L., Brennan K. J. & Parker R. O. (2010). Reproduction, habitat utilization, and movements of hogfish (*Lachnolaimus maximus*) in the Florida Keys, U.S.A.: Comparisons from fished versus unfished habitats. *Bulletin of Marine Science,* **86**: 93-116.

Murua H., Kraus G., Saborido-Rey F., Witthames P. R., Thorsen A. & Junquera S. (2003). Procedures to estimate fecundity of marine fish species in relation to their reproductive strategy. *Journal of Northwest Atlantic Fishery Science,* **33**: 33-54.

Murua H., Quincoces I., Garcia D. & Korta M. (2010). Is the Northern European hake, *Merluccius merluccius*, management procedure robust to the exclusion of reproductive dynamics? *Fisheries Research,* **104**: 123-135.

Myers R., Rosenberg A., Mace P., Barrowman N. & Restrepo V. (1994). In search of thresholds for recruitment overfishing. *ICES Journal of Marine Science: Journal du Conseil,* **51**: 191-205.

Myers R. A. & Mertz G. (1998). The limits of exploitation: A precautionary approach. *Ecological Applications,* **8**: S165-S169.

Navas J., Bruce M., Thrush M., Farndale B., Bromage N., Zanuy S., Carrillo M., Bell J. & Ramos J. (1997). The impact of seasonal alteration in the lipid composition of broodstock diets on egg quality in the European sea bass. *Journal of Fish Biology,* **51**: 760-773.

Neidig C. L., Skapura D. P., Grier H. J. & Dennis C. W. (2000). Techniques for spawning common snook: Broodstock handling, oocyte staging, and egg quality. *North American Journal of Aquaculture,* **62**: 103-113.

Newman S. J. & Williams D. M. (1996). Variation in reef associated assemblages of the Lutjanidae and Lethrinidae at different distances offshore in the central Great Barrier Reef. *Environmental Biology of Fishes,* **46**: 123-138.

Nichols P. D., Nichols D. S. & Bakes M. J. (1994). Developments with marine oil products in Australia. *International News on Fats, Oils and Related Materials,* **5**: 254-261.

Nocillado J., Penaflorida V. & Borlongan I. (2000). Measures of egg quality in induced spawns of the Asian sea bass, *Lates calcarifer* Bloch. *Fish Physiology and Biochemistry,* **22**: 1-9.

Paddack M. J. & Estes J. A. (2000). Kelp forest fish populations in marine reserves and adjacent exploited areas of central California. *Ecological Applications,* **10**: 855-870.

Parrish R. H., Mallicoate D. L. & Klingbeil R. A. (1986). Age dependent fecundity, number of spawnings per year, sex ratio, and maturation stages in northern anchovy, *Engraulis mordax*. *Fishery Bulletin,* **84**: 503-518.

Pauly D. (2009). Beyond duplicity and ignorance in global fisheries. *Scientia Marina,* **73**: 215-224.

Pauly D., Christensen V., Guénette S., Pitcher T. J., Sumaila U. R., Walters C. J., Watson R. & Zeller D. (2002). Towards sustainability in world fisheries. *Nature,* **418**: 689-695.

Picquelle S. & Stauffer G. (1985). Parameter estimation for an egg production method of northern anchovy biomass assessment. *In:* Lasker R. (ed.) *An Egg Production Method for Estimating Spawning Biomass in Pelagic Fish: Application to the Northern Anchovy, Engraulis mordax.* NOAA Technical Report NMFS.

Pikitch E. K., Santora C., Babcock E. A., Bakun A., Bonfil R., Conover D. O., Dayton P., Doukakis P., Fluharty D., Heneman B., Houde E. D., Link J., Livingston P. A., Mangel M., McAllister M. K., Pope J. & Sainsbury K. J. (2004). Ecosystem-based fishery management. *Science*, **305**:346-347.

Plan Development Team (1990). The potential of marine fishery reserves for reef fish management in the US South Atlantic. *NOAA Technical Memorandum. NMFS-SEFC-261.*

Planes S., Jones G. P. & Thorrold S. R. (2009). Larval dispersal connects fish populations in a network of marine protected areas. *Proceedings of the National Academy of Sciences,* **106**: 5693-5697.

Pörtner H. O., Berdal B., Blust R., Brix O., Colosimo A., De Wachter B., Giuliani A., Johansen T., Fischer T., Knust R., Lannig G., Naevdal G., Nedenes A., Nyhammer G., Sartoris F. J., Serendero I., Sirabella P., Thorkildsen S. & Zakhartsev M. (2001). Climate induced temperature effects on growth performance, fecundity and recruitment in marine fish: Developing a hypothesis for cause and effect relationships in Atlantic cod (*Gadus morhua*) and common eelpout (*Zoarces viviparus*). *Continental Shelf Research,* **21**: 1975-1997.

Punt A., Smith A., Davidson A., Mapstone B. & Davies C. (2001). Evaluating the scientific benefits of spatially explicit experimental manipulations of common coral trout, *Plectropomus leopardus*, populations on the Great Barrier Reef, Australia. *In:* Kruse G. H., Bez N., Booth A., Dorn M. W., Hills S., Lipcius R. N., Pelletier D., Roy C., Smith S. J. & Witherell D. (eds). Lowell Wakefield Fisheries Symposium. Alaska Sea Grant College Program, University of Alaska Fairbanks, 67-103.

R Core Team. (2014). *R: A language and environment for statistical computing* [Online]. R Foundation for Statistical Computing, Vienna, Austria. Available: http://www.R-project.org/ [Accessed 02/01/14].

Rainuzzo J. R., Reitan K. I. & Olsen Y. (1997). The significance of lipids at early stages of marine fish: a review. *Aquaculture,* **155**: 103-115.

Rijnsdorp A. D., Daan N., Van Beek F. A. & Heessen H. J. L. (1991). Reproductive variability in North Sea plaice, sole, and cod. *ICES Journal of Marine Science: Journal du Conseil,* **47**: 352-375.

Roberts C. M. & Polunin N. V. C. (1991). Are marine reserves effective in management of reef fisheries? *Reviews in Fish Biology and Fisheries,* **1**: 65-91.

Rønnestad I., Koven W., Tandler A., Harel M. & Fyhn H. J. (1998). Utilisation of yolk fuels in developing eggs and larvae of European sea bass (*Dicentrarchus labrax*). *Aquaculture,* **162**: 157-170.

Rose K. A., Cowan J. H., Winemiller K. O., Myers R. A. & Hilborn R. (2001). Compensatory density dependence in fish populations: Importance, controversy, understanding and prognosis. *Fish and Fisheries,* **2**: 293-327.

Russ G. (2002). Yet another review of marine reserves as reef fishery management tools. *In:* Sale P. F. (ed.) *Coral Reef Fishes: Dynamics and Diversity in a Complex Ecosystem.* San Diego: Academic Press.

Russ G. R., Lou D. C. & Ferreira B. P. (1995). A long-term study on population structure of the coral trout *Plectropomus leopardus* on reefs open and closed to fishing in the central Great Barrier Reef. *CRC Reef Research Centre Technical Report No. 3.* Townsville: CRC Reef Research Centre.

Sadovy de Mitcheson Y., Craig M. T., Bertoncini A. A., Carpenter K. E., Cheung W. W., Choat J. H., Cornish A. S., Fennessy S. T., Ferreira B. P. & Heemstra P. C. (2013). Fishing groupers towards extinction: A global assessment of threats and extinction risks in a billion dollar fishery. *Fish and Fisheries,* **14**: 119-136.

Sadovy Y. (2001). The threat of fishing to highly fecund fishes. *Journal of Fish Biology,* **59 (Supplement A)**: 90-108.

Sadovy Y. & Shapiro D. Y. (1987). Criteria for the diagnosis of hermaphroditism in fishes. *Copeia,* **1987**: 136-156.

Sadovy Y. J. (1996). Reproduction of reef fishery species. *In:* Polunin N. V. C. & Roberts C. M. (eds.) *Reef Fisheries.* London: Chapman and Hall.

Saenz-Agudelo P., Jones G. P., Thorrold S. R. & Planes S. (2009). Estimating connectivity in marine populations: an empirical evaluation of assignment tests and parentage analysis under different gene flow scenarios. *Molecular Ecology,* **18**: 1765-1776.

Sale P. F., Cowen R. K., Danilowicz B. S., Jones G. P., Kritzer J. P., Lindeman K. C., Planes S., Polunin N. V. C., Russ G. R. & Sadovy Y. J. (2005). Critical science gaps impede use of no-take fishery reserves. *Trends in Ecology & Evolution,* **20**: 74-80.

Samoilys M. (2000). *Reproductive dynamics of an exploited serranid on the Great Barrier Reef.* PhD, James Cook University.

Samoilys M. A. (1997). Periodicity of spawning aggregations of coral trout *Plectropomus leopardus* (Pisces: Serranidae) on the northern Great Barrier Reef. *Marine Ecology Progress Series,* **160**: 149-159.

Samoilys M. A. & Roelofs A. (2000). Defining the reproductive strategy of a large serranid, *Plectropomus leopardus*. *CRC Reef Research Centre Technical Report No. 31.* Townsville: CRC Reef Research Centre.

Samoilys M. A. & Squire L. C. (1994). Preliminary observations on the spawning behaviour of coral trout, *Plectropomus leopardus* (Pisces: Serranidae), on the Great Barrier Reef. *Bulletin of Marine Science,* **54**: 332-342.

Schaefer K. M. (1996). Spawning time, frequency, and batch fecundity of yellowfin tuna, *Thunnus albacares*, near Clipperton Atoll in the eastern Pacific Ocean. *Fishery Bulletin,* **94**: 98-112.

Shuter B. J., Lester N. P., LaRose J., Purchase C. F., Vascotto K., Morgan G., Collins N. C. & Abrams P. A. (2005). Optimal life histories and food web position: Linkages among somatic growth, reproductive investment, and mortality. *Canadian Journal of Fisheries and Aquatic Sciences,* **62**: 738-746.

Sluka R., Chiappone M., Sullivan K. & Wright R. (1997). The benefits of a marine fishery reserve for Nassau grouper *Epinephelus striatus* in the central Bahamas. Proceedings of the 8th International Coral Reef Symposium, Panama. 1961-1964.

Smith A. N., Anderson M. J., Millar R. B. & Willis T. J. (2014). Effects of marine reserves in the context of spatial and temporal variation: an analysis using Bayesian zero-inflated mixed models. *Marine Ecology Progress Series,* **499**: 203-216.

Somarakis S., Schismenou E., Siapatis A., Giannoulaki M., Kallianiotis A. & Machias A. (2012). High variability in the Daily Egg Production Method parameters of an eastern Mediterranean anchovy stock: Influence of environmental factors, fish condition and population density. *Fisheries Research,* **117-118**: 12-21.

State of Queensland (2013). Coral Reef Fin Fish Fishery: 2011-12 Fishing Year Report. Brisbane: Department of Agriculture, Fisheries and Forestry.

Stobart B., Warwick R., Gonzalez C., Mallol S., Diaz D., Renones O. & Goni R. (2009). Long-term and spillover effects of a marine protected area on an exploited fish community. *Marine Ecology Progress Series,* **384**: 47-60.

Takemura A. & Rahman M. D. (2004). Lunar cycles and reproductive activity in reef fishes with particular attention to rabbitfishes. *Fish and Fisheries,* **5**: 317-328.

Taylor M. H. (1984). Lunar synchronization of fish reproduction. *Transactions of the American Fisheries Society,* **113**: 484-493.

Thorsen A. & Kjesbu O. S. (2001). A rapid method for estimation of oocyte size and potential fecundity in Atlantic cod using a computer-aided particle analysis system. *Journal of Sea Research,* **46**: 295-308.

Tobin A., Currey L. M. & Simpfendorfer C. (2013). Informing the vulnerability of species to spawning aggregation fishing using commercial catch data. *Fisheries Research,* **143**: 47-56.

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

Tocher D. R. (2003). Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews in Fisheries Science,* **11**: 107-184.

Tocher D. R. & Sargent J. R. (1984). Analyses of lipids and fatty acids in ripe roes of some northwest European marine fish. *Lipids,* **19**: 492-499.

Trippel E. A. & Neil S. R. E. (2004). Maternal and seasonal differences in egg sizes and spawning activity of northwest Atlantic haddock (*Melanogrammus aeglefinus*) in relation to body size and condition. *Canadian Journal of Fisheries and Aquatic Sciences,* **61**: 2097-2110.

van der Velde T. D., Griffiths S. P. & Fry G. C. (2010). Reproductive biology of the commercially and recreationally important cobia *Rachycentron canadum* in northeastern Australia. *Fisheries Science,* **76**: 33-43.

van Herwerden L., Choat J. H., Newman S. J., Leray M. & Hillersøy G. (2009). Complex patterns of population structure and recruitment of *Plectropomus leopardus* (Pisces: Epinephelidae) in the Indo-West Pacifc: Implications for fisheries management. *Marine Biology,* **156**: 1595-1607.

Venturelli P. A., Shuter B. J. & Murphy C. A. (2009). Evidence for harvest-induced maternal influences on the reproductive rates of fish populations. *Proceedings of the Royal Society of London. Series B: Biological Sciences,* **276**: 919-924.

Vigliola L. & Meekan M. G. (2002). Size at hatching and planktonic growth determine post-settlement survivorship of a coral reef fish. *Oecologia,* **131**: 89-93.

Wakefield C. B., Newman S. J., Marriott R. J., Boddington D. K. & Fairclough D. V. (2013). Contrasting life history characteristics of the eightbar grouper *Hyporthodus octofasciatus* (Pisces: Epinephelidae) over a large latitudinal range reveals spawning omission at higher latitudes. *ICES Journal of Marine Science: Journal du Conseil,* **70**: 485-497.

Watson R. A., Cheung W. W., Anticamara J. A., Sumaila R. U., Zeller D. & Pauly D. (2013). Global marine yield halved as fishing intensity redoubles. *Fish and Fisheries,* **14**: 493-503.

West G. (1990). Methods of assessing ovarian development in fishes: A review. *Australian Journal of Marine and Freshwater Research,* **41**: 199-222.

Wiegand M. D. (1996). Composition, accumulation and utilization of yolk lipids in teleost fish. *Reviews in Fish Biology and Fisheries,* **6**: 259-286.

Williams A. J., Currey L. M., Begg G. A., Murchie C. D. & Ballagh A. C. (2008). Population biology of coral trout species in eastern Torres Strait: Implications for fishery management. *Continental Shelf Research,* **28**: 2129-2142.

Williams A. J., Davies C. R. & Mapstone B. D. (2006). Regional patterns in reproductive biology of *Lethrinus miniatus* on the Great Barrier Reef. *Marine and Freshwater Research,* **57**: 403-414.

Williams D. M. (1982). Patterns in the distribution of fish communities across the central Great Barrier Reef. *Coral Reefs,* **1**: 35-43.

Willis T. J., Millar R. B. & Babcock R. C. (2003). Protection of exploited fish in temperate regions: High density and biomass of snapper *Pagrus auratus* (Sparidae) in northern New Zealand marine reserves. *Journal of Applied Ecology,* **40**: 214-227.

Wolanski E. (1994). *Physical Oceanographic Processes of the Great Barrier Reef,* Boca Raton, Florida, CRC Press.

Wolanski E. & Pickard G. L. (1985). Long-term observations of currents on the central Great Barrier Reef continental shelf. *Coral Reefs,* **4**: 47-57.

Wood S. (2014). Package 'mgcv', version 1.8-3. Available: http://cran.stat.auckland.ac.nz/web/packages/mgcv/mgcv.pdf [Accessed 08/08/14].

Wootton R. (1977). Effect of food limitation during the breeding season on the size, body components and egg production of female sticklebacks (*Gasterosteus aculeatus*). *Journal of Animal Ecology,* **46**: 823-834.

Wright P. & Fyhn H. (2001). Ontogeny of nitrogen metabolism and excretion. *Fish Physiology,* **20**: 149-200.

Wright P. J. & Gibb F. M. (2005). Selection for birth date in North Sea haddock and its relation to maternal age. *Journal of Animal Ecology,* **74**: 303-312.

Yoneda M., Tokimura M., Fujita H., Takeshita N., Takeshita K., Matsuyama M. & Matsuura S. (1998). Ovarian structure and batch fecundity in *Lophiomus setigerus*. *Journal of Fish Biology,* **52**: 94-106.

Yoneda M. & Wright P. J. (2004). Temporal and spatial variation in reproductive investment of Atlantic cod *Gadus morhua* in the northern North Sea and Scottish west coast. *Marine Ecology Progress Series,* **276**: 237-248.

Zeller D. C. (1998). Spawning aggregations: patterns of movement of the coral trout *Plectropomus leopardus* (Serranidae) as determined by ultrasonic telemetry. *Marine Ecology Progress Series,* **162**: 253-263.

Zeller D. C. & Russ G. R. (1998). Marine reserves: Patterns of adult movement. *Canadian Journal of Fisheries and Aquatic Sciences,* **55**: 917-924.

Zeller D. C., Stoute S. L. & Russ G. R. (2003). Movements of reef fishes across marine reserve boundaries: Effects of manipulating a density gradient. *Marine Ecology Progress Series,* **254**: 269-280.

Zuur A. F., Ieno E. N., Walker N. J., Saveliev A. A. & Smith G. M. (2009). *Mixed Effects Models and Extensions in Ecology with R,* New York, Springer.

Zuur A. F., Saveliev A. A. & Ieno E. N. (2014). *A beginner's guide to generalized additive mixed models with R,* Newburgh, UK, Highland Statistics.

APPENDICES

Appendix A

Table A.1 Oocyte developmental stages in female *Plectropomus leopardus* viewed as histological sections and as whole oocytes. Descriptions adapted from Samoilys and Roelofs (2000), Adams (2002) and Arocha (2002), plus personal observations. Letters in column 2 refer to corresponding histological photographs in Figure A.1. See Figure A.2 for photographs of corresponding whole oocytes.

Oocyte developmental stage	Stage in Figure A.1	Description
Pre-vitellogenic stages		
Chromatin nucleolus (CN)	A	Chromosomes are not well separated and form a network in the nucleus. The smallest oocyte stage (~20 μm diameter) stains very dark purple. Oocytes often in clusters. Whole oocytes appear opaque and white.
Early perinucleolus (EP)	B	Nucleoli in periphery of nucleus. Oocytes small (~50 μm). EP stage stains lighter than CN stage. Whole oocytes appear opaque and white.
Late perinucleolus (LP)	C	Similar to EP but oocytes larger (~80 μm) and stains lighter purple. Whole oocytes appear opaque and white.
Vitellogenic stages		
Yolk vesicle (YV)	D	Lipid droplets appear in cytoplasm and zona radiata visible in periphery of oocyte. Granulosa cells' nuclei start to differentiate. Much larger than pre-vitellogenic oocytes (~150 μm). Stains predominantly purple. Whole oocytes are cream with translucent periphery.

Early yolk globule (EYG)	E	Small, well differentiated yolk granules in inner part of cytoplasm that stain orange. Zona radiata well defined. Similar in size to YV stage (~150 – 200 μm). Whole oocytes are light yellow.
Late yolk globule (LYG)	F	Distinct, large yolk globules throughout cytoplasm. Lipid droplets can appear empty in the inner portion of oocyte. Thick zona radiata. Larger than EYG stage (250 – 400 μm). Whole oocytes are yellow.
Migratory nucleus (MN)	G	Yolk globules begin to coalesce. Usually 1 – 2 large empty spaces present. Nucleus migrates to the cell membrane. Similar size to LYG stage (250 – 400 μm), particularly in early MN stage. Whole oocytes are yellow in early stage, late stage MN will have a partially translucent yolk mass.
Hydrated (HY)	H	Yolk globules have all coalesced and separated from cell membrane. Yolk plates may be visible. The zona radiata appears to have thinned and can appear "crinkly". The centre of the cell is uniform colour with a single oil droplet that appears empty. Diameter is at least double the width of LYG and MNS stages. Whole oocytes are large and clear or slightly cloudy with a yellow oil droplet visible.

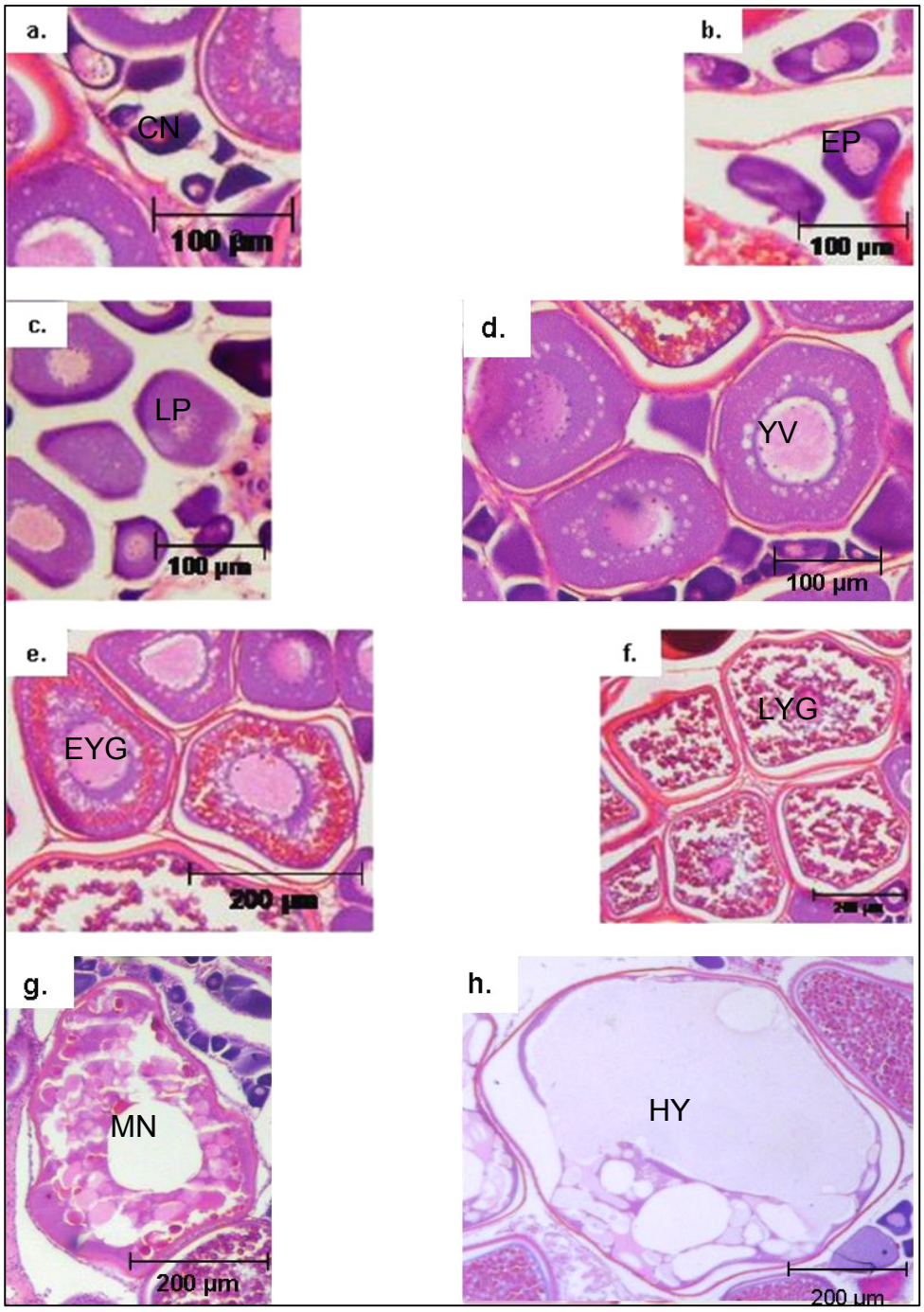


Figure A.1 Developmental stages of *Plectropomus leopardus* oocytes: (a) chromatin nucleolus (CN); (b) early perinucleolus (EP); (c) late perinucleolus (LP); (d) yolk vesicle (YV); (e) early yolk globule (EYG); (f) late yolk globule (LYG); (g) migratory nucleus (MN), (h) hydrated (HY). See Table A.1 for descriptions of oocyte stages. Photographs: A. Carter.

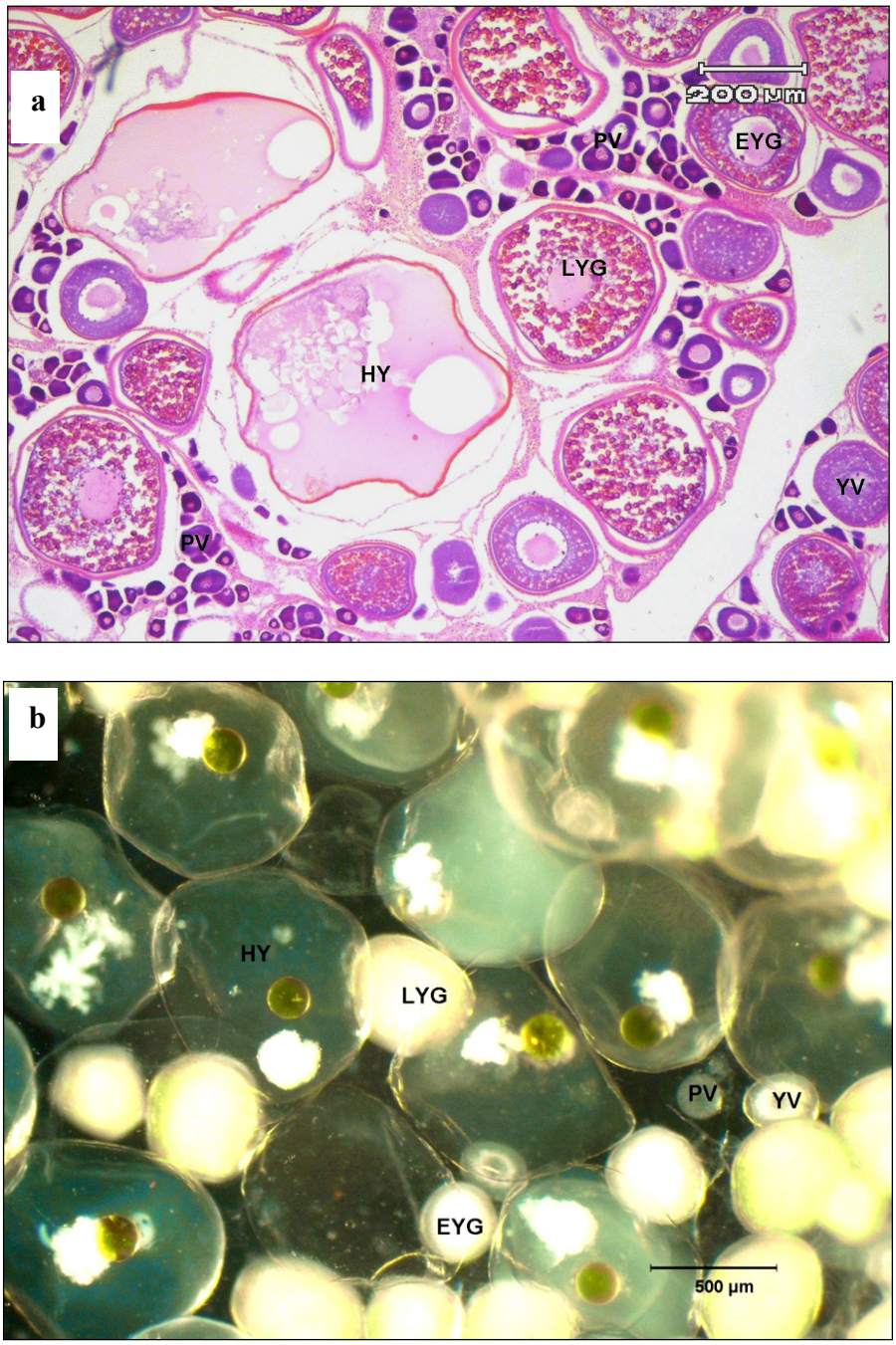


Figure A.2 Asynchronous development of *Plectropomus leopardus* oocytes. (a) Histological section of a hydrated ovary viewed under high powered microscope (40X). (b) Whole oocytes viewed under dissecting microscope (15X). Oocyte stage abbreviations: PV, pre-vitellogenic; YV, yolk vesicle; EYG, early yolk globule; LYG, late yolk globule; HY, hydrated. See Table A.1 for descriptions of oocyte stages. Photographs: A. Carter.

Appendix B

Prior to making batch fecundity estimates I examined whether samples taken from different positions in the ovary could lead to significantly different batch fecundity estimates for *P. leopardus*. Batch fecundity was calculated using the gravimetric method (see Chapters 3 and 4) for five randomly selected hydrated ovaries from three ovarian positions (proximal, medial and distal; Figure B.1). The proximal and distal samples were taken about one-quarter the distance from either end of the ovary. Batch fecundity among ovarian position was compared using a one-way analysis of variance (ANOVA) on square-root transformed data. Sample position did not have a significant effect on batch fecundity estimates ($F_{2,14} = 0.005$, $p > 0.05$).

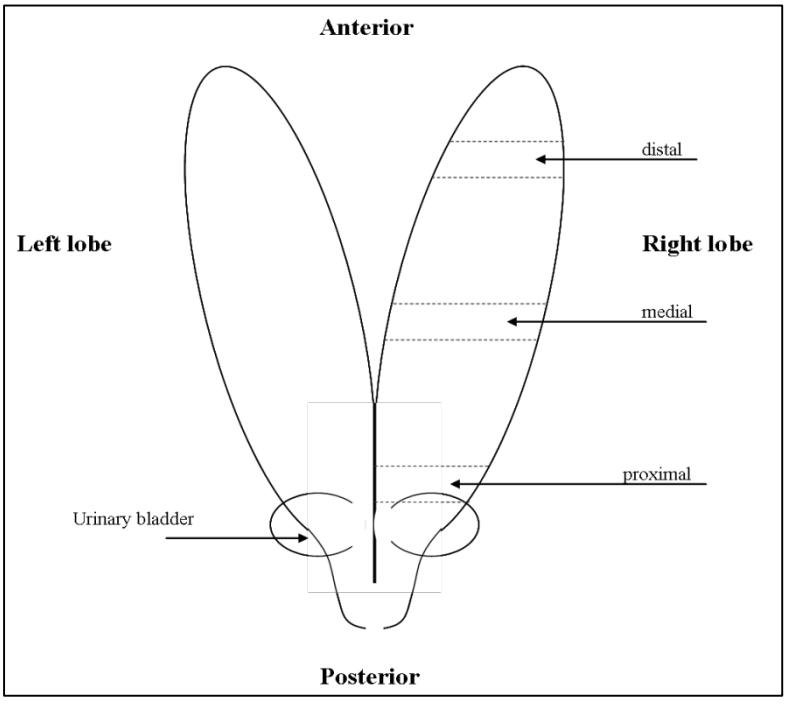


Figure B.1 Diagram of a *Plectropomus leopardus* ovary (dorsal view). Ovarian samples were taken from transverse sections at three positions (distal, medial and proximal).

Appendix C

Permission statement from Copyright holder for Chapter 5.

This administrative form has been removed