GENETIC PARENTAGE ANALYSIS AS A TOOL FOR MEASURING LARVAL CONNECTIVITY IN A NETWORK OF MARINE RESERVES

Thesis submitted by

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in November 2012

for the degree of Doctor of Philosophy

within the School of Marine & Tropical Biology
James Cook University

and

in the École doctoral Systèmes Intégrés, Environment et Biodiversité
Specialty: Ecology & Population Genetics
École Pratique des Hautes Études

MÉHODES DE PARENTÉ GÉNÉTIQUE COMME OUTIL POUR MESURER LA CONNECTIVITÉE LARVAIRE DANS UN RÉSEAUX DE RESERVES MARINES

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en Novembre 2012

Thèse pour l'obtention du grade de docteur en Écologie

de la School of Marine & Tropical Biology
James Cook University

et

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STATEMENT OF CONTRIBUTION OF OTHERS

This project was funded by research grants from the Australian Research Council and the Marine and Tropical Sciences Research Facility to GP Jones, and a Graduate Research Scheme grant to HB Harrison. A James Cook University Postgraduate Research Scholarship supported me throughout the duration of my thesis. P Saenz-Agudelo provided assistance in designing the simulations in Chapter 2. KA Feldheim developed the microsatellite libraries for Chapter 3. DH Williamson and RD Evans oversaw the field component at the heart of Chapters 4 and 5, including the underwater visual census data. Field sampling was conducted under Marine Parks permit No. G06/17981.1 and Queensland General Fisheries permit No. 87381. The work was conducted under JCU Ethics approval A1001.

ACKNOWLEDGMENTS

As with any great journey, it is the people that we encounter that make it such a memorable experience.

First and foremost, I would like to thank my advisors, Geoffrey Jones, David Williamson, Garry Russ, Serge Planes and Lynne van Herwerden for having provided continuous support throughout my dissertation and for giving me the freedom to pursue my own interests. I was also very fortunate to receive support from Richard Evans, Glenn Almany and Michael Berumen, who took me under their wing, taught me the ropes, and with whom I shared incredible adventures. This work would not have been possible without generous financial support from Geoffrey Jones, the School of Marine and Tropical Biology and the Graduate Research School at JCU.

I owe a great deal to the fishermen and volunteers who generously offered their time, their boats, and their expertise to catch the many thousands of fish that are at the heart of this work. I also wish to thank Irene Fuertes-Jerez, Teresea Lambrick and Laura Stoltenberg for assistance in the lab, Thomas Mannering and Paul Costello for their work ageing juvenile fish, Alexia Pihier and James Moloney for their work with GIS.

A special thanks to Rene Abesamis, J Howard Choat, Severine Choukroun, Renata Ferrari-Legorreta, Whitney Goodell, Jonathan Kool, Ian McLeod, Mae Noble, Mark Priest, Pablo Saenz-Agudelo, Maya Srinivasan, Brett Taylor, Simon Thorrold, Colin Wen and “La Maison du Bonheur”: Jorge Alvarez-Romero, Alexander Anderson, Emmanuelle Botte, Clément Fay, Vanessa Messmer, Morena Mills, Véronique Mocellin, Jean-Baptiste Raina, and Rebecca Weeks for their support and stimulating discussions over the years.

Finally, I am grateful to Clive, Denise and Claire Harrison for encouraging me along this path, not knowing where it would lead. I am forever indebted to Stephen Simpson for opening my eyes to what lies beneath the ocean swell.

GENERAL ABSTRACT

Networks of no-take marine reserves are widely advocated as a means to conserve biodiversity and manage coastal fisheries. Reserves not only deliver rapid and long-term benefits within their boundaries, they also provide a broader framework that augments the resilience of coral reef ecosystems. Understanding the level of demographic connectivity between discreet populations is essential to determine a network's efficacy to supplement fisheries and protect biodiversity. In theory, the larger biomass of exploited fishes within reserves, and higher reproductive capacity, increase recruitment to nearby populations due to larval export, and connectivity between reserves support stable populations. However, for large exploited fishes, it has been seemingly impossible to determine where the larvae from populations within reserves go or assess the relative importance of the supply of juveniles from reserves. One of the major challenges is identifying methods that can be applied to large species at the scale at which reserve networks have been implemented. Recent developments in genetic parentage analysis show that this is possible for small reef species, but techniques have not been tested on and applied to important fishery species. *The overall aim of this thesis was to develop and apply parentage analysis to assess the conservation and fisheries objectives of a network of no-take marine reserves of the Great Barrier Reef Marine Park* (*GBRMP*)*, Australia.* It focuses on two of the most important inshore fishery species on the GBRMP, the coral trout (*Plectropomus maculatus*) and stripey snapper (*Lutjanus carponotatus*), and provides the first empirical description of the dispersal of larvae from marine reserves.

The use of parentage analysis has become an increasingly popular approach to investigate ecological processes in animal populations. While this is an extremely powerful technique, one aspect of parentage studies has received limited attention: How accurate are they, and what errors are they most likely to encounter? A number of different assignment methods have emerged in common use, and the accuracy of each may differ in relation to the number of loci examined, allelic diversity, incomplete sampling of all candidate parents, and the presence of genotyping errors. In **Chapter 2**, I examine how these factors affect the accuracy of three popular parentage inference methods to resolve true parent-offspring pairs.

Using simulated data, I was able to capture a wide diversity of conditions that are commonly encountered in parentage studies and identified key factors for the identification of true parent-offspring pairs in natural populations. The findings of this study clearly demonstrate that the number and diversity of loci were the most important factors in obtaining accurate assignments, while the proportion of candidate parents sampled had only a small impact on the susceptibility of each method to either false positive or false negative assignments.

Recent technical advances in the isolation of molecular markers and the high throughput screening of multi-locus genotypes have made it possible to screen large numbers of individuals with unprecedented resolution. Microsatellite markers, short tandem repeats in the nuclear genome, have become a marker of choice in parentage studies for their high level of allelic diversity (polymorphism). As identified in the previous chapter, this is an important factor in obtaining accurate parentage assignments. In **Chapter 3**, I develop novel sets of microsatellite loci specifically designed for parentage analyses in natural populations of coral trout (*P. maculatus*) and stripey snapper (*L. carponotatus*). This resulted in a panel of 11 and 13 highly polymorphic microsatellite markers for *P. maculatus* and *L. carponotatus*, respectively. These unique marker sets resulted in an exclusion power of over 99.98% for assignments to single parents, thus providing a high level of accuracy for parentage studies.

Our understanding of the spatial scale of dispersal in coral reef fishes has certainly altered our perception of how populations are regulated, however our knowledge-base largely stems from unique study systems involving small habitat-specialised species with high site fidelity. For large exploited species of commercial value and greatest need of effective management, it has been seemingly impossible to identify where or how far larvae go. In **Chapter 4,** I describe the first conclusive field evidence that larval supply from marine reserves benefits both fish and fisheries, which fills a major knowledge gap that has impeded wider acceptance of marine reserve networks as an effective fisheries management strategy. Over the course of an extensive field study, tissue samples were collected from adult coral trout and adult stripey snapper within three focal no-take marine reserves in the Keppel Island group, an inshore island archipelago of the GBRMP. During the following 15 months, juveniles of both species were collected throughout the

island group up to 30 km from focal reserves. Using DNA parentage analysis, I assigned juveniles collected in both fished and protected locations throughout the island group back to their parents sampled inside reserves. Based on the observed dispersal trajectories, I was able to show that populations within reserves were responsible for supplying approximately half of all juvenile recruitment within 30km of reserves. These findings settle a 20-year long debate as to whether marine reserves actually work as a fisheries management tool in reef systems.

In **Chapter 5**, I build on previous chapters to explore some of the majors assumptions in our understanding of population dynamics for the management of coral reef fishes: (1) whether the spatial patterns of recruitment are persistent over time; (2) whether patterns of larval supply are consistent across multiple cohorts; (3) whether larger adult fishes account for a greater proportion of local recruitment (settlement) than smaller adult fish; (4) whether fish below the legal length limit contribute to local recruitment; and (5) whether the more abundant and larger fish in reserves are important to local recruitment. By combining genetic and demographic data, this study offers a rare insight into the demographic processes of wild reef fish populations and provides critical information for the management of two commercially and recreationally important fish species. Over the course of three successive cohorts of juvenile coral trout and stripey snapper, recruitment was unevenly distributed throughout the Keppel Islands with three main 'recruitment hotspots'. However, spatial patterns were temporally consistent and successive cohorts were genetically homogeneous, suggesting that the adult source population supplying juvenile recruitment was consistent and largely local. Using genetic parentage analysis I identified which specific adults had contributed to local recruitment, providing a unique perspective on the reproductive success of individual size classes for these species and the influence of reserves on local recruitment.

In summary, this thesis provides a unique perspective on the accuracy of parentage studies in natural populations and identifies key recommendations for the development of microsatellite marker sets for parentage analysis. It provides the first conclusive evidence that larval supply from marine reserves benefits both fish and fisheries, and fills a major knowledge gap that has impeded wider

acceptance of marine reserve networks as a viable and effective fisheries management strategy.

RÉSUMÉ GÉNÉRAL

Les réseaux de réserves marines sont préconisés comme un moyen de conserver la biodiversité et administrer la gestion des ressources halieutique. La mise en place de réserves favorise non seulement des améliorations à cours et à long terme au sein de leurs frontières, mais fournissent également de manière plus générale un moyen d'augmenté la résilience des écosystèmes coralliens. Comprendre le niveau de connectivité démographique entre les populations est essentiel pour déterminer l'efficacité de ces réseaux afin d'accroître le rendement des pêcheries tout en protégeant la biodiversité marine. En théorie, l'augmentation de la biomasse et de la capacité reproductive des poissons à valeur commerciale au sein des réserves augmenterait le recrutement dans les populations avoisinantes en raison de l'exportation des larves. En outre, la connectivité entre réserves soutiendrait la stabilité de ces populations. Cependant, identifier où se dispersent les larves de poissons dans le milieu marin s'est avéré un défi de longue date. Le développement de méthode de parenté génétique démontre qu'il est possible de suivre la trajectoire de certain poisson de récifs. *L'objectif principal de cette thèse est de développer et d'appliquer les analyses de parenté génétique afin d'évaluer la valeur des réseaux de réserve marine pour la gestion des ressources halieutique de la Grande Barrière de Corail en Australie*. Cette thèse se concentre sur deux espèces de poissons à valeur commercial sur l'ensemble du parc : le mérou à point bleu (*Plectropomus maculatus*) et le vivaneau (*Lutjanus carponotatus*).

L'utilisation de l'analyse de parenté génétique est devenue une approche de plus en plus repandue permettant d'enquêter sur les processus écologiques dans les populations animales. Différente méthodes d'assignation sont fréquemment utilisées, et la précision de chacune peut différer en fonction du nombre de marqueurs génétiques examinés, de la diversité allélique, de l'échantillonnage de la population et de la présence d'erreurs de séquençage. Dans le **Chapitre 2**, j'examine comment ces facteurs affectent la précision de trois méthodes d'assignation pour identifier les relations de parenté. En utilisant des données simulées, j'ai été capable de capturer une grande diversité des conditions qui sont couramment rencontrés dans les études de parenté. Les résultats de cette étude démontrent clairement que le nombre et la diversité des marqueurs génétiques

sont les facteurs les plus importants pour l'obtention de résultats précis, tandis que la proportion de parents candidats échantillonnés n'a qu'un faible impact sur la sensitivité de chaque méthode.

Les microsatellites sont devenus le marqueur de choix dans les études de parenté a cause de leur grande diversité allélique. Comme il est indiqué dans le chapitre précédent, il s'agit d'un facteur important dans l'obtention de résultat précis. Dans le **Chapitre 3**, je développe de nouveaux marqueurs microsatellites spécialement conçus pour les analyses de parenté dans les populations naturelles de mérou à pointe bleu (*P. maculatus*) et de vivaneau (*L. carponotatus*). Cela s'est traduit par le développement de 11 et 13 marqueurs pour *P. maculatus* et *L. carponotatus*, respectivement. Cet ensemble de marqueurs abouti à une valeur d'exclusion de 99,99%, offrant ainsi un haut niveau de précision pour les études de parenté.

Notre compréhension de l'échelle spatiale de la dispersion des poissons de récifs a certainement changé notre perception de la règlementation des populations marines. Cependant notre base de connaissances provient en grande partie des systèmes d'études portant sur des espèces uniques utilisant des habitats spécialisées. Pour les espèces à valeur commerciale qui ont le plus besoin d'une gestion efficace, identifier où et dans quelle mesure les larves se dispersent reste un obstacle important. Le **Chapitre 4** démontre que les populations de poisson dense présentes dans les réserves marines contribuent au recrutement de juvéniles dans les populations avoisinantes. Au cours d'une étude de terrain approfondie, des échantillons de tissus ont été prélevés à partir de mérous et de vivaneaux adultes dans trois réserves marines des iles Keppel, un archipel côtier de la Grande Barrière de Corail. Au cours des 15 mois suivants, les juvéniles des deux espèces ont été recueillies tout autour de l'archipel jusqu'à 30 km des réserves. En utilisant une analyse de parenté, j'ai pu identifier les juvéniles qui provenaient de parents à l'intérieur des réserves. Basé sur leurs trajectoires de dispersion, je montre que les populations dans les réserves ont fourni environ la moitié de tout le recrutement dans l'archipel.

Dans le **Chapitre 5**, j'explore plusieurs hypothèses dans notre compréhension de la dynamique des populations de poissons récifaux: (1) si les structures spatiales de recrutement dérivée des adultes dans les réserves persistes

au fil du temps, (2) si les l'approvisionnement des larves sont uniformes à travers plusieur cohortes de recrutement; (3) si la taille de poissons adultes a un effet au niveau du recrutement local ; (4) si les limites actuelles de taille de captures sont efficaces pour protéger la biomasse reproductive, et (5) si l'abondance et la taille de poissons dans les réserves sont importantes pour le recrutement local.

En combinant les données génétiques et démographiques, cette étude offre un aperçu dans les processus démographiques des populations sauvages de poissons récifaux et fournit des informations essentielles pour la gestion de ces espèces de poisson. Au cours des trois cohortes successives, le recrutement a été inégalement répartie à travers les îles Keppel avec trois sites de recrutement principaux. Toutefois, les tendances spatiales étaient régulières avec une homogénéité génétique entre les trois cohortes. Cela suggère que la population locale était une source important du recrutement local. En utilisant les analyses de parenté génétique j'identifie les adultes qui ont contribué au recrutement localqui offrant une perspective unique sur le succès de reproduction des différentes taille de poisson pour ces espèces.

En résumé, cette thèse offre une perspective unique sur la précision des études de parenté dans les populations naturelles et identifie les points les plus importants à prendre en compte lors du design de nouveaux marqueurs microsatellites pour les analyses de parenté. Il fournit la première preuve concluante que l'approvisionnement en larves de réserves marines est un gain à la fois pour les stocks de poissons et pour les pêcheries, et comble une lacune majeure qui a empêché une plus large acceptation des réseaux de réserves marines comme stratégie viable et éfficace pour la gestion de la pêche.

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CHAPTER 1 - GENERAL INTRODUCTION

DISPERSAL AND CONNECTIVITY

Dispersal is a crucial ecological process, driving population dynamics and defining the structure and persistence of populations across ecological and evolutionary timescales (Krebs and Davies 1997; Hanski 1999; Clobert *et al.* 2001). At small spatial scales, the movement of individuals can greatly impact the spatial dynamics of local populations, connecting populations in fragmented landscapes and allowing for the replenishment of harvested populations (Clobert *et al.* 2001). Over larger spatial scales, dispersal drives the spread of invasive species (Mooney and Hobbs 2000; Gaither *et al.* 2010, 2011), enables species range shifts in response to environmental stressors (Simpson *et al*. 2011) and permits the re-colonisation of disturbed and depleted populations (Trakhenbrot *et al.* 2005; Lotze *et al.* 2011). Resolving patterns of connectivity and understanding the processes that influence dispersal are therefore fundamental to our understanding of population dynamics in all organisms.

The concept of population connectivity is broad and its definition remains discreetly linked to the scale at which it is considered and the method employed to measure it (Clobert *et al.* 2001; Lowe and Allendorf 2010). Genetic methods are commonly used to infer the magnitude of connectivity at large spatial scales. They rely on estimates of allelic frequency and diversity within subpopulations to estimate the degree of historical gene flow between subpopulations. Genetic connectivity is thus the degree to which gene flow affects evolutionary process within populations (Lowe and Allendorf 2010). In contrast, on ecologically relevant or contemporary timescales, connectivity refers to the degree to which population growth is affected by the dispersal of individuals among them (Sale *et al.* 2005; Lowe and Allendorf 2010). Unlike genetic connectivity, demographic

connectivity is a measure of migration between discreet populations and its relative contribution to the replenishment and growth of focal populations.

New genetic tools, such as genetic parentage analysis, are now being developed and applied to directly measure ecologically important levels of migration between populations (Hellberg *et al.* 2002; Hedgecock *et al.* 2007). At large spatial scales, or where gene flow is restricted, genetic assignment tests can identify the origin of individuals provided that discreet populations are genetically distinct and that all populations have been sampled (Cornuet *et al.* 1999; Pritchard *et al.* 2000; Guillot 2005). At smaller spatial scales, parentage studies and family reconstructions have become increasingly popular to investigate a range of ecological processes in natural populations, including dispersal. Furthermore, recent technical advances in both the isolation of molecular markers and the high-throughput screening of multi-locus genotypes have made parentage studies more widely accessible to ecologists (Selkoe and Toonen 2006; Guichoux *et al.* 2011; Gardner *et al.* 2011). However, despite a proliferation of statistical approaches to infer pedigree structure or kinship in natural populations (Blouin 2003; Jones and Ardren 2003; Jones *et al*. 2010), parentage analysis remains a relatively new procedure and has successfully been applied to only a small range of species.

LARVAL CONNECTIVITY IN CORAL REEF FISH POPULATIONS

Coral reefs are inherently patchy and fragmented, consisting of spatially heterogeneous populations linked primarily by larval dispersal. In recent years, substantial effort has been made to identify patterns of connectivity in coral reef fishes to understand how fragmented reef populations are sustained (Sale 2004; Steneck *et al.* 2009), with the aim of informing conservation efforts (Kinlan *et al.* 2005; Trakhtenbrot *et al.* 2005; Jones *et al.* 2009, McCook *et al.* 2009) and better manage exploited fishes (Roberts *et al.* 2001; Sale *et al.* 2005; Steneck and Wilson 2010). Since the large majority of coral reef fish are site-attached as adults, connectivity between discreet populations depends on the successful migration of planktonic larvae (Shanks 2009). However, tracking their dispersal trajectories has

remained a long-standing challenge, leaving the dispersal potential of pelagic larvae largely unknown (Jones *et al.* 2009; Levin 2006).

While direct measures of demographic connectivity remain challenging, several approaches have been applied to track the dispersal of coral reef fish larvae. Capture-mark-recapture, perhaps one of the oldest methods to study the movement of animals, provides unequivocal individual dispersal patterns. The challenge is identifying ways to tag individual larvae, which are too small for physical tags and disperse in the thousands from their source of origin. One of the first attempts to obtain empirical measures of larval dispersal used mass marking of post-natal eggs using a tetracycline 'dye'. These could later be traced in the core of larval otoliths (Jones *et al.* 1999). Though ideal for small populations of benthic spawners, it has since been replaced by a new technique that allows trans-generational mass marking of pre-natal eggs (Jones *et al.* 2005; Thorrold *et al*. 2006) and has a wider range of applications. The approach is based on the *in vivo* transmission of natural elements to eggs from mothers that were injected with a saline solution of natural isotopes (Thorrold *et al.* 2006). Isotopic ratios in the core of the otolith can then trace the source of juvenile fish back to their natal population. Different source populations can be branded with different isotopic ratios allowing multiple populations to be targeted (Almany *et al.* 2007; Planes *et al.* 2009). While these techniques have altered our perceptions of how far larvae disperse, they are essentially mass-marking methods that provide no additional information on the dispersal trajectories of individual larvae.

Genetic parentage analysis offers a powerful alternative for quantifying dispersal and population connectivity in coral reef fishes (Jones *et al.* 2005; Planes *et al.* 2009). It was first used in conjunction with tetracycline tagging to establish local self-recruitment in clownfish populations (Jones *et al.* 2005; Saenz Agudelo *et al*. 2009), and later with trans-generational isotope labelling to measure the relative magnitude of self-recruitment and connectivity among populations (Planes *et al.* 2009). Applications of genetic parentage analysis have since been expanded to investigate the dispersal trajectories of larvae that encompass a range of other species (Christie *et al.* 2010a; Saenz-Agudelo *et al.* 2011; Berumen *et al.* 2012; Saenz-Agudelo *et al.* 2012). Parentage studies and sibship reconstructions have also given us unique insights into the mating behaviour and reproductive

success of coral reef fish (Araki *et al.* 2007; Beldade *et al*. 2012), and kin association of recruiting larvae (Buston *et al.* 2007; Piyapong *et al.* 2011; Bernardi *et al.* 2012). While it promises to be an extremely powerful approach to elucidating dispersal trajectories and community structure for coral reef fish, its performance in identifying long-distance dispersal has not been fully evaluated.

To date, evidence for long-distance dispersal (100-1000s km) comes from studies of evolutionary processes that measure historical gene flow (Purcell *et al*. 2006), while attempts to infer large-scale patterns of dispersal using oceanographic models (Cowen *et al.* 2006; Kool *et al.* 2011; Foster *et al.* 2012) are as yet, unvalidated estimates of demographic connectivity. However, the behavioural and ontogenetic characteristics of coral reef fish larvae (Shanks 2009; Leis *et al.* 2007, 2009), broad-scale genetic homogeneity (Purcell *et al.* 2006; Mora *et al.* 2011) and coupled-biophysical models (Roberts 1997; Cowen *et al.* 2006; Treml *et al.* 2008) all suggest larvae also have the potential to undertake long migrations during their pelagic phase. The application of parentage analysis over ever-greater spatial scales and novel approaches using a combination of genetic analyses and oceanographic data (Gerlach *et al.* 2007; Galarza *et al.* 2009; White *et al*. 2010; Schunter *et al.* 2011) now have the potential to validate other approaches to investigate broad-scale connectivity patterns.

Our understanding of the spatial scale of dispersal in coral reef fishes has certainly altered our perception of how populations are regulated (Cowen *et al.* 2000; Swearer *et al.* 2002; Mora and Sale 2002; Warner and Cowen 2002; Jones *et al*. 2009). While population were once considered largely 'open', the ubiquity of self-recruitment demonstrates that coral reef fish population are at least partially regulated by local demographic processes. Levels of self-recruitment, typically reported within the range of 30-60% (Jones *et al.* 2009), have important implications for fish conservation and fisheries management. However, our knowledge-base largely stems from unique study systems involving habitat-specialised species with high site fidelity. For large exploited species of commercial value and greatest need of effective management, it has been seemingly impossible to identify where or how far larvae go. Addressing this knowledge gap remains an important challenge in fisheries management that limits our understanding of the processes that regulate the replenishment of fished and protected populations.

MARINE RESERVES IN FISHERIES MANAGEMENT

Coral reefs are extremely fragile ecosystems, threatened by a rapidly changing environment, increasing coastal pollution, and overexploitation of natural resources (Pandolfi *et al.* 2003; Hughes *et al.* 2005; Hoegh-Guldberg *et al.* 2007; De'ath *et al.* 2012). Effective conservation and management of coral reef ecosystems is vital to mitigate the impacts of human activities and ensure the future of coral reefs. While there is no single solution to alleviate such impacts, no-take marine reserves represent one management action that can deliver tangible and often rapid benefits (Pauly *et al.* 2002; Gell and Roberts 2003; Lubchenco *et al.* 2003; Gaines *et al.* 2010). Thousands of marine reserves have been implemented globally in hope of restoring and sustaining fisheries and conserving biodiversity (Mora *et al.* 2006; Wood *et al.* 2008). While studies universally demonstrate that exploited fish are larger and more abundant within adequately protected reserves (Halpern 2003; Lester *et al.* 2009; Babcock *et al.* 2010; Russ *et al.* 2008), such benefits do little to sustain fisheries or protect populations at large, unless the benefits extend beyond individual reserve boundaries (Gell and Roberts 2003; Sale *et al.* 2005). Understanding the extent of larval dispersal is critical for the success of fisheries management and conservation policies (Palumbi 2003; Roberts 1997; Cowen *et al.* 2000; Sale *et al.* 2005).

Marine reserves certainly have the potential to simultaneously benefit both unfished and fished populations. In theory, the increased abundance, size and reproductive output of individuals inside reserves can provide substantial benefits to neighbouring fished areas through the 'spillover' of adults and the export of larval progeny. While there is evidence that both these processes occur (Russ *et al.* 2004; Abessamis and Russ 2005; Roberts *et al.* 2005; Pelc *et al.* 2010; Russ and Alcala 2011), adult spillover alone is unlikely to offset the displacement of fishing effort and the local recruitment contribution from larval export has yet to be quantified. The implementation of no-take marine reserves thus creates the possibility of a conflict between fishery and conservation objectives, where there is a compromise between maximising their conservation benefits (Almany *et al.* 2009) and minimising their impact on fisheries. Connectivity is increasingly recognised as a central tenet of this debate. In order to fulfil both conservation and

fisheries objectives, reserves must simultaneously enhance populations within reserves, through larval retention or dispersal amongst reserves, and provide substantial larval export to fished areas. To be considered as a viable fisheries management tool, no-take reserves must also provide demonstrable benefits to neighbouring areas open to fishing.

In 2004, the Great Barrier Reef Marine Park (GBRMP) was restructured in a comprehensive network of marine protected areas with the premise of (1) providing grounds for sustainable exploitations, which assumed 'spillover' from reserves, and (2) biodiversity conservation, which assumed some migration between reserves. In the re-zoning process, 33.4% of the Great Barrier Reef (GBR) was designated in no-take zones, creating the largest network of no-take marine reserves in the world. If the effectiveness of marine parks is fundamentally dependent on the dispersal of pelagic larvae then there is great incentive to understand processes of larval dispersal and introduce networks of marine reserves that encompass the full life cycles of target species and the diversity of habitats they require.

ASSESSING THE EFFECTIVENESS OF MARINE RESERVES IN THE GREAT BARRIER REEF MARINE PARK

This thesis is a first attempt at understanding the dispersal patterns and spatio-temporal recruitment dynamics of two important fisheries species within a network of no-take marine reserve on the Great Barrier Reef: the coral trout, *Plectropomus maculatus*, and the stripey snapper, *Lutjanus carponotatus*. This is an extremely challenging and demanding endeavour, which requires bridging the fundamentals of coral reef ecology and fisheries science, while developing new applications for individual level genetic profiling. Applying methods of parentage analysis to such species has never before been attempted. These are highly mobile species as adults and cryptic as juveniles and obtaining representative proportions of focal populations requires a large investment in sampling. Furthermore, as non-model organisms, it entails the development of novel genetic markers that are sufficiently informative to distinguish between individuals and identify true

parent-offspring pairs from erroneous assignments. Since a compromise must be made between the costs of developing and processing a large number of markers and sampling effort, it is also necessary to ensure that the methods we apply do not infringe on the accuracy of parentage assignments.

Parentage studies and family reconstructions have become increasingly popular for investigating a range of evolutionary, ecological and behavioural processes in natural populations. However, in natural populations where exhaustive sampling is prohibitive, variation in the proportion of sampled parents can have a significant impact on the accuracy of parentage reconstructions (Hadfield *et al.* 2006; Kock *et al.* 2008; Nielsen *et al.* 2001). Furthermore, a number of different assignment methods have emerged in common use, and the accuracy of each may differ in relation to the number of loci examined, allelic diversity, incomplete sampling of all candidate parents, and the presence of genotyping errors. I start in **Chapter 2**, by exploring how these different factors affect the accuracy of three distinct parentage inference methods to resolve true parent-offspring pairs. Using simulated data, I was able to capture a wide diversity of conditions that are commonly encountered in parentage studies and identified key factors for the identification of true parent-offspring pairs in natural populations.

Designing microsatellite markers for non-model organisms can be challenging. In **Chapter 3**, I describe the development of a novel suite of microsatellite markers for *P. maculatus* and *L. carponotatus* and their integration to multiplex PCRs for parentage analysis. Markers were selected from previously published marker sets developed for congeneric species or described here from species-specific cloning libraries. As identified in the previous chapter, the number and diversity of loci are the most important factors in obtaining accurate parentage assignments. I therefore preferentially selected highly polymorphic markers and maximised the number of loci given available technologies. I further describe their integration into multiplex PCRs for high-throughput genotyping, and their application to parentage analysis.

Identifying the extent to which larval offspring are exported and the relative contribution of reserves to recruitment in both fished and protected areas represent some of the major challenges of marine reserve research for the last 20 years. **Chapter 4** provides the first conclusive evidence that larval supply from

marine reserves benefits both fish and fisheries, and fills a major knowledge gap that has impeded wider acceptance of marine reserve networks as a viable and effective fisheries management strategy. This large field based study hinges on previous chapter to track the dispersal pathways of juvenile coral trout and stripey snapper throughout a network of no-take marine reserves in the Great Keppel islands, an island archipelago of the southern Great Barrier Reef. Using parentage analysis, I assigned juveniles collected in both fished and protected location throughout the island group back to their parents sampled inside reserves. Based on the observed dispersal trajectories, I was able to show that populations within reserves were responsible for supplying approximately half of all juvenile recruitment within 30km of reserves.

As a major source of local recruitment, no-take marine reserve networks are likely to play an important role in shaping local population dynamics. In **Chapter 5**, I explore some of the majors assumptions in our understanding of population dynamics for the management of coral reef fishes: (1) whether the spatial patterns of recruitment derived from adults in reserves are persistent over time; (2) whether patterns of larval supply are consistent across multiple cohorts; (3) whether larger adult fishes account for a greater proportion of local recruitment (settlement) than smaller adult fish; (4) whether fish below the legal length limit contribute to local recruitment; and (5) whether the more abundant and larger fish in reserves are important to local recruitment. This study represents the first explicit assessment of reproductive success and stock-recruitment relationships for these commercially targeted fish.

CHAPTER 2 - RELATIVE ACCURACY OF THREE COMMON METHODS OF PARENTAGE ANALYSIS IN NATURAL POPULATIONS

ABSTRACT

Parentage studies and family reconstructions have become increasingly popular for investigating a range of evolutionary, ecological and behavioural processes in natural populations. However, a number of different assignment methods have emerged in common use and the accuracy of each may differ in relation to the number of loci examined, allelic diversity, incomplete sampling of all candidate parents, and the presence of genotyping errors. Here I examine how these factors affect the accuracy of three popular parentage inference methods (COLONY, FAMOZ and an exclusion-Bayes' theorem approach by Christie (2010)) to resolve true parent-offspring pairs using simulated data. Our findings demonstrate that accuracy increases with the number and diversity of loci. These were clearly the most important factors in obtaining accurate assignments explaining 75-90% of variance in overall accuracy across 60 simulated scenarios. Furthermore, the proportion of candidate parents sampled had a small but significant impact on the susceptibility of each method to either false positive or false negative assignments. Within the range of values simulated, COLONY outperformed FAMOZ, which outperformed the exclusion-Bayes' theorem method. However, with 20 or more highly polymorphic loci, all methods could be applied with confidence. Our results show that for parentage inference in natural populations, careful consideration of the number and quality of markers will increase the accuracy of assignments and mitigate the effects of incomplete sampling of parental populations.

INTRODUCTION

Our ability to infer genealogical relationships among individuals has become an effective approach to investigate a wide variety of evolutionary, ecological and behavioural questions. Pedigrees, often based on a combination of observation and molecular data, have given us invaluable insights into mating systems, revealing the prevalence of extra-pair paternities and cooperative breeding in the wild (e.g. Richardson *et al.* 2001; Magrath *et al.* 2009), mating behaviour and reproductive success (Araki *et al.* 2007; Kanno *et al.* 2011; Rodrigues-Munoz *et al.* 2010; Ford *et al.* 2011; Beldade *et al.* 2012), and kin association (e.g. Reeve *et al.* 1990; Buston *et al.* 2007; Piyapong *et al.* 2010) in diverse animal groups. Parentage studies and sibship reconstructions have also become increasingly popular approaches to estimate population parameters such as self-recruitment (Jones *et al.* 2005; Saenz-Agudelo *et al.* 2009; Saenz-Agudelo *et al.* 2012), fine scale population structure (e.g. Nussey *et al.* 2005; Slavov *et al.* 2010) and population connectivity in the form of migration (Nathan *et al.* 2003; Harrison *et al.* 2010) or dispersal (e.g. Garcia *et al.* 2005, 2007; Jordano *et al.* 2007; Planes *et al.* 2009; Christie *et al.* 2010a; Saenz-Agudelo *et al.* 2011, 2012; Berumen *et al.* 2012). Parentage studies have also revealed new aspects of inbreeding and trait heritability (Ritland 2000; Garant and Kruuk 2005; Pemberton 2008; Nielsen *et al.* 2012), genetic adaptation of wild species to captivity (Christie *et al.* 2012) and assisted in the restoration of captive and endangered populations (Keller and Waller 2002; Herbinger *et al.* 2006). Individual level analyses can resolve family relationships in a wide range of taxa where this information has proven difficult to obtain from direct observations.

Recent technical advances in both the isolation of molecular markers, notably microsatellites or SNPs, and the high-throughput screening of multi-locus genotypes are likely to make parentage studies more widely accessible to ecologists studying wild populations (Selkoe and Toonen 2006; Guichoux *et al.* 2011; Gardner *et al.* 2011). However, despite a proliferation of statistical approaches to infer pedigree structure or kinship relationships among pairs of individuals in natural populations (reviewed in Blouin 2003; Jones and Ardren 2003; Jones *et al.* 2010), parentage analysis remains a relatively new procedure. A

number of different approaches are currently being used, but the factors affecting the relative accuracy of the different approaches have received little attention.

The methods used to identify parent-offspring relationships can be broadly divided into four categories: strict exclusion, categorical assignment, fractional assignment and pedigree reconstruction (Jones *et al.* 2010). Among these, the most commonly used methods are strict exclusion and categorical assignment, whereby the genotype of each offspring is compared to the genotype of all candidate parents. For strict exclusion methods, any parent failing to share at least one allele at a given locus is excluded. If more than one parent cannot be excluded, categorical assignments measure the likelihood of each putative parent-offspring pair of being true given their respective multi-locus genotype and the observed allelic frequencies in the population (Marshall *et al.* 1998; Nielsen *et al.* 2001, Gerber *et al.* 2003; Kalinowski *et al.* 2007). Categorical assignment approaches offer several advantages over strict exclusion methods (Danzmann 1997; Goodnight and Queller 1999) as they can more easily accommodate scoring errors, missing data or null alleles that commonly occur in microsatellite datasets (Pemberton *et al.* 1995; Dakin and Avise 2004; Pompanon *et al.* 2005; Wang 2010). However, in the right circumstances, strict exclusion can be a powerful approach, and could prove useful to detect parent-offspring pairs in large open populations (Christie 2010). Recently, full-probability approaches for parental or sibship reconstructions have also become more accessible and widely applied. Rather than simply evaluating pairwise relationships, individuals are clustered into family groups and the likelihood of different clusters is evaluated to identify the most parsimonious configuration (Almudevar and Field 1999; Thomas and Hill 2002; Wang 2004; Hadfield *et al.* 2006; Wang and Santure 2009; Jones and Wang 2010a; Almudevar and Anderson 2012). In turn, accounting for the presence of family groups provides valuable information that significantly enhances the accuracy of assignments (Wang 2007; Walling *et al.* 2010).

All the above methods are subject to incorrect assignments that may be affected by the number and allelic diversity of loci examined (Bernatchez and Duchesne 2000; Nielsen *et al.* 2001), the proportion of the population sampled (Oddou-Muratorio *et al.* 2003; Koch *et al.* 2008), genotyping errors, mutations, allelic dropouts and miscalling (Bernatchez and Duchesne 2000; Hoffman and

Amos 2005). However, having only a limited number of genetic markers and incomplete sampling of all candidate parents are thought to have the largest effects on the accuracy of assignments (Marshall *et al.* 1998; Nielsen *et al.* 2001; Wilson and Ferguson 2002; Oddou-Muratorio *et al.* 2003; Jones *et al.* 2010). Some likelihood-based approaches such as CERVUS (Marshall *et al.* 1998; Kalinowski *et al.* 2007) and full likelihood methods such as COLONY (Wang 2004; Jones and Wang 2010a) account for incomplete sampling by defining *a priori* the probability that the true parent is present in the sample. This probability can be estimated from the proportion of putative parents sampled from the entire parental population, which requires prior knowledge, or approximation, of the size of the population. While COLONY is robust to uncertainty in this sampling rate (Wang and Santure 2009; Jones and Wang 2010b), mis-specification of this parameter in CERVUS can have significant impact on assignments made (Nielsen *et al.* 2001; Hadfield *et al.* 2006; Koch *et al.* 2008).

Other approaches have been developed to infer parentage without prior knowledge of population size or the proportion of candidate parents in the sample. Such methods have been favoured to assess population connectivity in large populations (mostly plants and marine fish) where accurate estimates of the breeding population size are often difficult to obtain. For instance, the pairwise-likelihood method implemented in FAMOZ (Gerber *et al.* 2003) estimates the likelihood ratios (LOD scores) of putative parent-offspring pairs being true and determines critical thresholds to accept or reject assignments by simulating true and false parent-offspring pairs. The calculation of LOD scores is based on the same approach as CERVUS (Meagher and Thompson 1986; Marshall *et al.* 1998; Gerber *et al*. 2000), however FAMOZ does not require *a priori* information of the proportion of candidate parents in the sample to determine critical LOD thresholds. The exclusion-Bayes' theorem approach by Christie (2010) is another method that follows in this category. It consists of calculating the probability of false parent-offspring pairs in a dataset to determine if all putative parent-offspring pairs can be accepted with strict exclusion. In situations where the dataset lacks sufficient power, Bayes' theorem is used to determine the probability of putative parent-offspring pairs being false given the frequencies of shared alleles. This approach was designed for situations where only a small fraction of all

candidate parents can be sampled, and does not require *a priori* information of the proportion of candidate parents in the sampled population or other demographic parameters (Christie 2010). While the effects of the mis-specification of the proportion of sampled candidate parents in CERVUS has been evaluated and discussed elsewhere (Hadfield *et al.* 2006; Koch *et al.* 2008; Nielsen *et al.* 2001), it is unclear how the absence of this parameter may affect the performance of exclusion and categorical assignment approaches, such as those implemented in FAMOZ and exclusion based approaches, especially under different sampling rates.

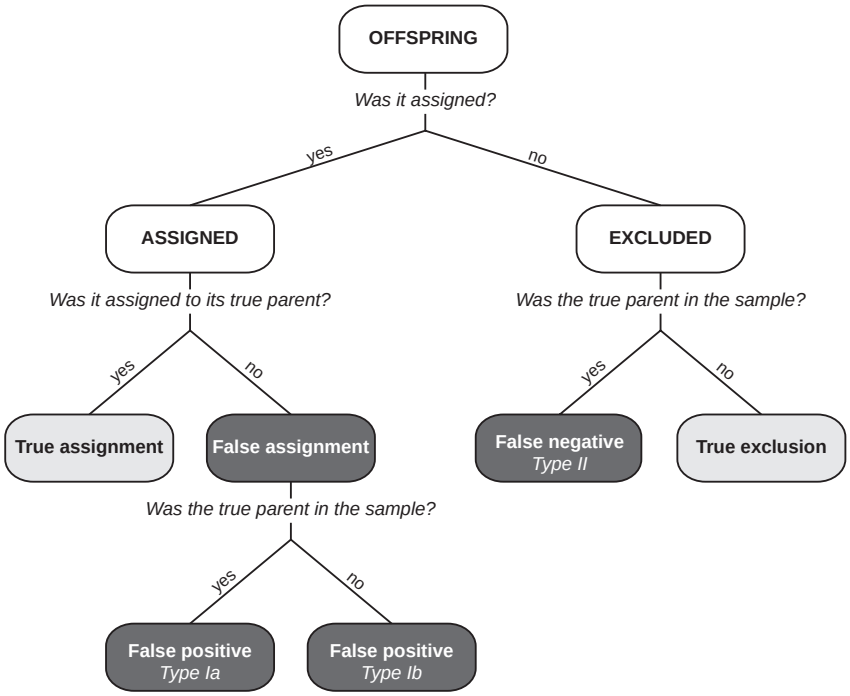

Figure 1 There are only two correct decisions with regards to single parent assignments, assigning the true parent when it is present in the sample (True assignment), and assigning no parent when the true parent is not in the sample (True exclusion). Assignment errors can be either false positive (falsely assigning an individual to a parent that is not its true parent) or false negative (falsely excluding a true parent). These are commonly referred to as Type I (false positive) and Type II (false negative) errors, respectively and can be estimated from simulations. False positives fall into two categories, falsely assigning to a parent when the true parent is in the sample, or when the true parent is not in the sample. To distinguish these from error estimates, I refer to these here as Type Ia and Type Ib errors, respectively. I refer to false negatives, falsely excluding a parent when it was in the sample as a Type II error. These errors cannot be calculated in real datasets unless the full pedigree is available.

The aim of the present study was to assess the accuracy of three popular methods of parentage analysis and investigate their susceptibility to error under 60 different scenarios that incrementally simulate the number of loci, allelic

diversity, adult sample size and genotyping error. Simulated offspring were assigned to single parents using the exclusion-Bayes' theorem approach developed by Christie (2010) (hereafter referred to as 'the Christie method'), the pairwise-likelihood method implemented in FAMOZ (Gerber *et al.* 2003), and the full probability approach implemented in version 2.0 of COLONY (Wang 2004). Putative parent-offspring pairs were validated against known true parents and assignment errors were classified as described in Figure 1. I then examined how the number of assignment errors was correlated with the number of loci, allelic diversity and proportion of adults sampled from the population.

MATERIALS AND METHODS

Simulated datasets

Two parental datasets, of different population sizes, were generated in EASYPOP (Balloux 2001) in order to achieve different levels of allelic diversity while maintaining all remaining simulation parameters constant. While the difference in population size between both datasets has little relevance to the accuracy of assignments, this procedure allowed us to explore the effects of allelic diversity on assignments. The two parental datasets were based on a finite island model with 5 subpopulations, each of constant size and equal sex ratio. The first dataset consisted of 500 reproductive individuals with 100 individuals per subpopulation. The second dataset consisted of 1000 reproductive individuals with 200 individuals per subpopulation. These will subsequently be referred to as the N500 (low diversity) and N1000 (high diversity) populations, respectively. For both datasets, random mating was simulated to produce diploid genotypes at 20 independent loci for 5000 generations to approximate mutation-drift equilibrium (Waples and Gaggiotti 2006). Migration between subpopulations occurred with a probability of 0.15 to simulate high gene flow and demographic connectivity among subpopulations. This is equivalent to 15 and 30 migrants per generation for the N500 and N1000 populations, respectively. All loci had the same mutation dynamics, which occurred according to the K-allele model (each mutation equally likely to occur at any of K possible sites). Mutation rate ($\mu=1x10^{-4}$) and number of

allelic states (20 possible allelic states) were considered to represent highly polymorphic markers, such as microsatellites, within the ranges published in eukaryotic genomes (Buschiazzo and Gemmel 2006). Our simulated datasets represented an assorted array of loci akin to most microsatellite datasets. Individual locus characteristics for each simulated dataset were calculated in GENALEX v6.4 (Peakall and Smouse 2006). The N1000 population represented a more diverse and, therefore, informative dataset with an average of 14.9 (11 to 18) alleles per locus and average observed heterozygosity of 0.769 ± 0.070 S.D. (0.650 to 0.877) per locus (Table 1). In comparison, the N500 populations had lower allelic diversity with an average of 10.7 alleles per loci (7 to 14) and an observed heterozygosity of 0.655 ± 0.144 S.D. (0.396 to 0.874; Table 2). The probability of exclusion of each locus and the cumulative probability of exclusion of each dataset were calculated according to Jamieson and Taylor (1997) as the probability of excluding a single parent (Table 1-2).

For each of the two parental datasets, 1000 offspring genotypes were generated using the software package P-LOCI (Matson *et al.* 2008). Adults were paired randomly within each subpopulation and four offspring were generated for each adult pair under a monogamous mating system. This resulted in 250 adult pairs, which was necessary to keep offspring sample size equal between datasets and reduce computation time of parentage analyses. Offspring were generated following Mendelian inheritance with 0.1% and 1% genotyping error, which are typical of microsatellite loci (Pompanon *et al.* 2005). Each parental population was randomly sampled into samples representing 20, 40, 60, 80, and 100 per cent of the parental population and the resulting datasets were further subset taking the first 10, 15 and 20 loci, totalling 60 independent datasets. All datasets were deposited in the Dryad digital repository. Each of these datasets was then analysed using the following three freely available software packages to identify parent-offspring pairs.

Table 1 Characteristics of 20 simulated loci for the N1000 high diversity population. Number of alleles (*Na*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), and the inbreeding coefficient (*F*) were calculated for each locus in GENALEX v6.4 (Peakall and Smouse, 2006). The probability of exclusion at each locus (*Pe*) and cumulative probability of exclusion (*Cum. Pe*) were measured for single parent assignments.

N1000	*Na*	*Ho*	*He*	*F*	*Pe*	*Cum. Pe*
Locus-1	16	0.679	0.675	-0.007	0.2897	0.2897
Locus-2	15	0.821	0.837	0.019	0.5133	0.6543
Locus-3	11	0.767	0.779	0.016	0.3930	0.7902
Locus-4	12	0.755	0.759	0.005	0.3988	0.8738
Locus-5	14	0.785	0.786	0.001	0.4288	0.9279
Locus-6	13	0.773	0.775	0.003	0.3953	0.9564
Locus-7	18	0.814	0.810	-0.005	0.4593	0.9764
Locus-8	18	0.819	0.827	0.010	0.4912	0.9880
Locus-9	18	0.844	0.837	-0.008	0.5249	0.9943
Locus-10	11	0.815	0.799	-0.020	0.4455	0.9968
Locus-11	15	0.667	0.679	0.017	0.2948	0.9978
Locus-12	17	0.827	0.840	0.015	0.5339	0.9990
Locus-13	17	0.797	0.821	0.030	0.4874	0.9995
Locus-14	15	0.650	0.658	0.012	0.2636	0.9996
Locus-15	17	0.877	0.871	-0.006	0.5952	0.9998
Locus-16	17	0.817	0.825	0.010	0.4936	0.9999
Locus-17	14	0.817	0.817	0.000	0.4586	1.0000
Locus-18	12	0.721	0.726	0.007	0.3385	1.0000
Locus-19	14	0.650	0.662	0.018	0.2719	1.0000
Locus-20	15	0.683	0.694	0.015	0.3004	1.0000
Average	14.95	0.769	0.774	0.007	0.4189	
SD	2.305	0.070	0.068	0.012	0.0984	

Table 2 Characteristics of 20 simulated loci for the N1000 high diversity population. Number of alleles (*Na*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), and the inbreeding coefficient (*F*) were calculated for each locus in GENALEX v6.4 (Peakall and Smouse, 2006). The probability of exclusion at each locus (*Pe*) and cumulative probability of exclusion (*Cum. Pe*) were measured for single parent assignments.

N500	***Na***	***Ho***	***He***	***F***	***Pe***	***Cum. Pe***
Locus-1	13	0.774	0.781	0.009	0.3980	0.3980
Locus-2	11	0.626	0.639	0.020	0.2381	0.5414
Locus-3	9	0.624	0.637	0.020	0.2509	0.6564
Locus-4	12	0.482	0.504	0.043	0.1395	0.7043
Locus-5	7	0.590	0.617	0.044	0.2251	0.7709
Locus-6	13	0.670	0.674	0.006	0.2807	0.8352
Locus-7	13	0.874	0.868	-0.006	0.5749	0.9299
Locus-8	10	0.810	0.821	0.013	0.4646	0.9625
Locus-9	9	0.396	0.389	-0.017	0.0817	0.9656
Locus-10	10	0.526	0.552	0.048	0.1590	0.9710
Locus-11	12	0.808	0.774	-0.044	0.4000	0.9826
Locus-12	12	0.434	0.430	-0.009	0.1075	0.9845
Locus-13	11	0.418	0.437	0.043	0.1088	0.9862
Locus-14	13	0.772	0.785	0.017	0.4284	0.9921
Locus-15	9	0.720	0.714	-0.009	0.3168	0.9946
Locus-16	7	0.634	0.643	0.014	0.2278	0.9958
Locus-17	14	0.832	0.829	-0.004	0.4994	0.9979
Locus-18	9	0.676	0.669	-0.011	0.2601	0.9985
Locus-19	10	0.696	0.716	0.028	0.3133	0.9989
Locus-20	10	0.728	0.699	-0.042	0.2829	0.9992
Average	10.7	0.655	0.659	0.008	0.288	
S.D.	2.029	0.144	0.138	0.026	0.138	

Exclusion-Bayes' Theorem – Christie method

The method described by Christie (2010) is an unbiased exclusion probability designed to identify true parent-offspring pairs in large populations where the proportion of sampled parents is low. For each dataset I calculated the probability of observing shared alleles between unrelated individuals using 1,000 simulations, then calculated the probability of each putative parent-offspring pair being false given the frequency of shared alleles. This method does not explicitly account for genotyping error or marker specific error rates, but allows for mismatches between parent-offspring pairs. Assignments are made to single parents only and parent pairs in the sample are not considered. When assigned, each parent-offspring pair is given a probability and several adults may be assigned to the same offspring. When two or more putative parents were assigned to the same offspring,

only the parent with the highest probability of assignment was kept for further analyses. This method was implemented in R v2.14.0 (R Development Core Team).

Pairwise-Likelihood - FAMOZ

The software program FAMOZ (Gerber *et al.* 2003) allows for the categorical allocation of parent-offspring pairs based on a maximum likelihood approach. The program computes log of the odds ratio (LOD) scores for assigning individuals to candidate parents based on the observed allelic frequencies at each locus. I allowed for genotyping errors by introducing an error rate of 0.01% in the LOD score calculation, which produces the lowest Type I and II error rates (Gerber *et al.* 2000; Morissey and Wilson 2005; Saenz-Agudelo *et al.* 2011; Harrison *et al.* 2012). For each dataset, 10,000 parent-offspring pairs were simulated based on the observed allelic frequencies at each locus and 10,000 parent-offspring pairs were generated from the putative parental genotypes. The frequency distributions of the two simulations were compared and the intersection was defined as the minimum threshold to accept a given parent-offspring pair or parent-pair trio. When two or more putative parents were assigned to the same offspring, only the parent with the highest LOD score was retained. When two parents were assigned as a parent pair, both were retained for further analyses.

Full-Likelihood - COLONY

The software program COLONY (Wang 2004; Jones and Wang 2010a) implements a full-likelihood approach to parentage analysis. In our analyses I considered both parent-offspring relationships and sibship amongst offspring samples. Adult samples were separated by sex and I assumed a polygamous mating system for diploid organisms. The prior probability that the true parent was present in the sample was considered in the assignment of parent-offspring pairs in accordance with the proportion of candidate parents included in the simulated datasets. Allelic frequencies were determined from the sample dataset, but did not take into account the relationship between individuals or inbreeding. All results were based on a single short run with high precision to maximise the accuracy of assignment whilst reducing the length of individual runs. This approach accounts for genotyping error at each locus of each sampled individual when estimating the

likelihood of a particular family cluster, and simulated error rates were taken into account in each analysis. Only the parent or parent pair with the highest likelihood is assigned, and all assigned parents were retained for further analysis.

Assignment errors

For each offspring, the assigned parent or parent-pairs were compared to the known true parents. When an offspring was assigned to a parent that was not its true parent or not assigned (excluded), I determined if the true parent was in the sample and identified it as either false positive (Type Ia or Type Ib) or false negative (Type II) errors (Figure 1). The overall accuracy is the sum of all errors over the total number of possible assignments. I then used a Generalized Linear Model (GLM) framework to quantify the effect of allelic diversity, number of loci, percentage of sampled parents, genotyping error and their possible interaction on the proportion of correct assignments of each method. Since the response variable was a proportion, GLMs were fitted using a logit link function (as fitted values are bounded between 0 and 1) and quasi-binomial errors (to account for non-normally distributed errors, non-constant variance and over-dispersion) (Crawley 2007). For each method I first fitted a maximal model (4 parameters and their interactions) and then removed non-significant terms until a minimal adequate model was reached (Crawley 2007). Processing of all software outputs and all model fitting were performed in R with scripts deposited in the Dryad digital repository (http://dx.doi.org/10.5061/dryad.2ht96).

RESULTS

Relative accuracy of the three methods

Given high diversity (N1000) and sufficient number of loci, all methods tested identified parent-offspring pairs with over 90% accuracy, regardless of the proportion of the population sampled or the presence of genotyping error (Figure 2 and Table 3). However, the performance of each method varied, with accuracy affected by the number and allelic diversity of loci, and the proportion of sampled parents. The full-likelihood method implemented in COLONY (Wang 2004; Jones and

Wang 2010a) outperformed the other two methods with a mean (± SD) accuracy across all scenarios of 98.4% ± 4.0 compared to 89.0% ± 11.3 for the pairwise-likelihood method (Gerber *et al.* 2003), and 65.3% ± 28.3 for the Christie method (Christie 2010). For each method, the number of loci and differences in allelic diversity between the low and high diversity populations had the largest effect on the overall accuracy of assignments. For most scenarios analysed, COLONY performed best, FAMOZ was intermediate and the Christie method was least accurate, with the disparity between methods increasing with increasing proportions of the population sampled (Figure 2).

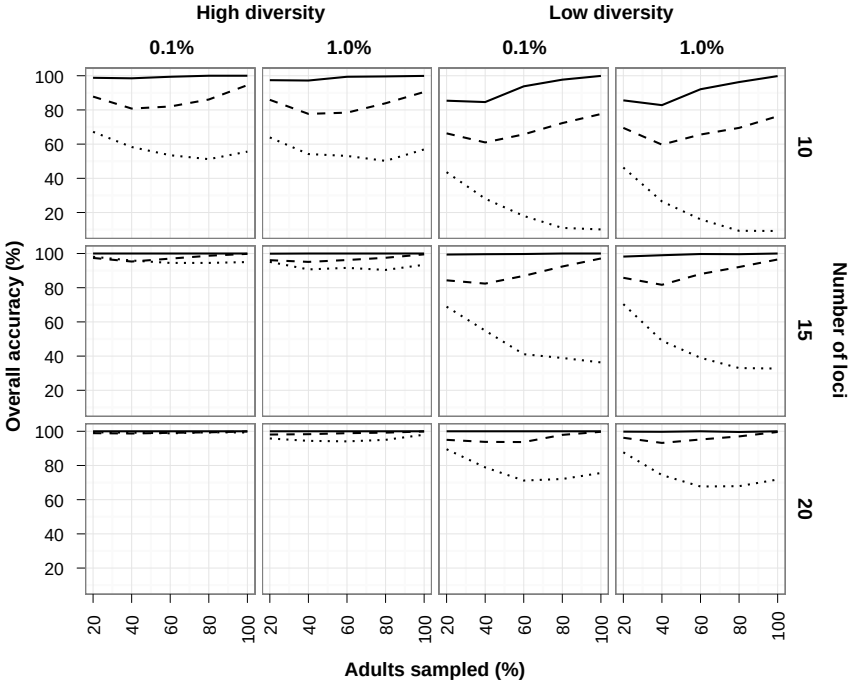

Figure 2 Proportion of accurate assignments of three approaches to parentage analyses. Each method was tested on high and low diversity simulated microsatellite datasets with high (1%) and low (0.1%) levels of genotyping error for varying levels of number loci and proportion of candidate parents sampled. Continuous lines correspond to the results from the full-likelihood method implemented in COLONY v2.0 (Wang 2004), dashed lines are the results from the pairwise-likelihood implemented in FAMOZ (Gerber *et al.* 2003), and dotted lines from the Christie method (Christie 2010).

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□□□t□□ □□□UO□□h1LGMNO □YLYUP□OZA MNL□FTLOZY□O RM□E □□R□PhFT□□R1LY R□YLYUP□ML RF□□R□□□FFTERA RM□OOLOFTLOMN □□j’’’ 1FTA UP□M□□ □□NMF M□

Population size	Percent error	No. loci	Percent adult sampled	Type Ia	Type Ib	Type II	Accurate Assignments	Accurate Exclusion	Overall Accuracy	Type Ia	Type Ib	Type II	Accurate Assignments	Accurate Exclusion	Overall Accuracy	Type Ia	Type Ib	Type II	Accurate Assignments	Accurate Exclusion	Overall Accuracy
High diversity (N1000)	0.1	10	20	80	246	2	278	394	0.672	12	61	49	295	583	0.878	12	61	49	295	583	0.878
			40	232	184	1	407	176	0.583	48	84	60	295	238	0.808	48	84	60	295	238	0.808
			60	356	107	2	462	73	0.535	54	58	68	690	130	0.820	54	58	68	690	130	0.820
			80	457	31	0	503	9	0.512	80	9	50	834	28	0.861	80	9	50	834	28	0.861
			100	444	0	0	556	0	0.556	44	0	11	945	0	0.945	44	0	11	945	0	0.945
		15	20	2	14	3	355	626	0.981	5	18	3	348	626	0.974	5	18	3	348	626	0.974
			40	19	21	1	620	339	0.959	8	28	11	637	316	0.953	8	28	11	637	316	0.953
			60	40	13	2	778	167	0.945	8	18	4	800	170	0.970	8	18	4	800	170	0.970
			80	55	0	0	905	40	0.945	6	0	7	951	36	0.987	6	0	7	951	36	1
			100	50	0	0	950	0	0.95	1	0	1	998	0	0.998	1	0	1	998	0	1
		20	20	1	2	3	356	638	0.994	0	10	1	355	634	0.989	0	10	1	355	634	1
			40	5	1	2	633	359	0.992	1	10	2	653	335	0.987	1	10	2	653	335	1
			60	5	4	3	812	176	0.988	4	4	1	807	184	0.991	4	4	1	807	184	1
			80	6	0	0	954	40	0.994	5	0	1	958	36	0.994	5	0	1	958	36	1
			100	7	0	0	993	0	0.993	0	0	1	999	0	0.999	0	0	1	999	0	1
	1.0	10	20	81	264	16	263	376	0.639	16	71	54	246	613	0.859	16	71	54	246	613	0.859
			40	230	204	24	386	156	0.542	57	84	82	485	292	0.777	57	84	82	485	292	0.777
			60	355	102	12	453	78	0.531	83	48	85	652	132	0.784	83	48	85	652	132	0.784
			80	458	25	15	487	15	0.502	84	18	59	809	30	0.839	84	18	59	809	30	0.839
			100	428	0	3	569	0	0.569	65	4	26	903	2	0.905	65	4	26	903	2	0.905
		15	20	5	14	30	325	626	0.951	1	25	13	302	659	0.961	1	25	13	302	659	0.961
			40	23	26	44	573	334	0.907	8	17	24	592	359	0.951	8	17	24	592	359	0.951
			60	31	10	43	746	170	0.916	5	13	20	795	167	0.962	5	13	20	795	167	0.962
			80	59	3	34	867	37	0.904	6	2	17	929	46	0.975	6	2	17	929	46	0.975
			100	53	0	13	934	0	0.934	1	0	4	995	0	0.995	1	0	4	995	0	1
		20	20	0	1	41	319	639	0.958	0	11	8	308	673	0.981	0	11	8	308	673	0.981
			40	0	1	55	585	359	0.944	0	11	6	618	365	0.983	0	11	6	618	365	0.983
			60	2	0	57	761	180	0.941	2	3	6	812	177	0.989	2	3	6	812	177	1
			80	4	0	46	910	40	0.95	0	1	7	945	47	0.992	0	1	7	945	47	0.992
			100	3	0	17	980	0	0.98	0	0	1	999	0	0.999	0	0	1	999	0	1

Population size	Percent error	No. loci	Percent adult sampled	Type Ia	Type Ib	Type II	Accurate Assignments	Accurate Exclusion	Overall Accuracy	Type Ia	Type Ib	Type II	Accurate Assignments	Accurate Exclusion	Overall Accuracy	Type Ia	Type Ib	Type II	Accurate Assignments	Accurate Exclusion	Overall Accuracy
Low diversity (N500)	0.1	10	20	122	293	149	81	355	0.436	53	257	27	256	407	0.663	15	80	51	286	568	0.854
			40	288	161	270	82	199	0.281	155	216	19	458	152	0.610	34	89	31	575	271	0.846
			60	396	52	372	108	72	0.180	186	89	68	590	67	0.657	15	29	18	843	95	0.938
			80	446	16	428	98	12	0.110	180	20	77	699	24	0.723	6	15	2	964	13	0.977
			100	460	0	439	101	0	0.101	177	0	47	776	0	0.776	1	0	0	999	-	0.999
		15	20	49	153	109	194	495	0.689	13	140	4	319	524	0.843	0	6	0	352	642	0.994
			40	133	101	217	290	259	0.549	34	134	8	590	234	0.824	0	4	0	640	356	0.996
			60	222	39	328	326	85	0.411	47	58	26	771	98	0.869	0	2	1	875	122	0.997
			80	255	12	344	373	16	0.389	53	12	11	892	32	0.924	0	0	0	972	28	1
			100	274	0	363	363	0	0.363	26	0	3	971	0	0.971	0	0	0	1000	-	1
		20	20	11	48	46	295	600	0.895	6	43	1	329	621	0.950	0	0	0	352	648	1
			40	39	32	140	461	328	0.789	5	50	7	620	318	0.938	0	0	0	640	360	1
			60	83	11	194	599	113	0.712	13	16	34	797	140	0.937	0	0	0	876	124	1
			80	82	5	192	698	23	0.721	12	7	2	942	37	0.979	0	0	0	972	28	1
			100	102	0	142	756	0	0.756	3	0	0	997	0	0.997	0	0	0	1000	-	1
	1.0	10	20	117	267	153	82	381	0.463	53	219	33	274	421	0.695	13	88	43	296	560	0.856
			40	267	175	293	80	185	0.265	127	212	64	439	158	0.597	45	94	33	562	266	0.828
			60	394	54	392	90	70	0.160	187	73	84	580	76	0.656	36	28	15	825	96	0.921
			80	439	18	450	83	10	0.093	198	29	78	672	23	0.695	14	20	3	955	8	0.963
			100	444	0	464	92	0	0.092	185	0	52	763	0	0.763	2	0	0	998	-	0.998
		15	20	46	144	106	200	504	0.704	16	115	11	333	525	0.858	0	13	5	347	653	0.982
			40	141	101	268	231	259	0.490	36	118	29	567	250	0.817	0	9	1	639	351	0.990
			60	225	28	357	294	96	0.390	48	45	27	777	103	0.880	0	3	0	876	121	0.997
			80	280	15	375	317	13	0.330	43	14	22	883	38	0.921	1	3	0	971	25	0.996
			100	286	0	387	327	0	0.327	26	0	9	965	0	0.965	0	0	0	1000	-	1
		20	20	13	36	74	265	612	0.877	3	26	9	348	614	0.962	0	2	0	352	646	0.998
			40	37	34	185	418	326	0.744	7	44	17	608	324	0.932	0	3	0	640	357	0.997
			60	68	14	241	567	110	0.677	12	23	13	827	125	0.952	0	0	0	876	124	1
			80	68	11	242	662	17	0.679	7	10	13	928	42	0.970	0	4	0	972	24	0.996
			100	85	0	197	718	0	0.718	3	0	1	996	0	0.996	0	0	0	1000	-	1

	Factors	Deviance explained by each term	d.f.	Residual deviance in model	F	Significance
Exclusion –Bayes Theorem	Null deviance		59	23777		
	Prop. Adult sample	835.3	58	22941.7	41.7	***
	Number of loci	10641.4	57	12300.3	531.7	***
	Allelic diversity	10309.2	56	1991.1	515.1	***
	Prop. Adult sample: Number of Loci	38.6	55	1952.4	1.9	NS
	Prop. Adult sample: Allelic diversity	613	54	1339.4	30.6	***
	Number of Loci: Allelic diversity	438.6	53	900.8	21.9	***
[illegible]	Null deviance		59	7532.9		
	Prop. Adult sample	370	58	7162.9	40.6	***
	Number of loci	5000.1	57	2162.9	548.5	***
	Allelic diversity	1500.1	56	662.8	164.6	***
	Prop. Adult sample: Number of Loci	89.5	55	573.3	9.8	**
	Number of Loci: Allelic diversity	57.5	54	515.8	6.3	*
[illegible]	Null deviance		59	3258.6		
	Prop. Adult sample	574.3	58	2684.2	97.2	***
	Number of loci	1690.9	57	993.4	286.1	***
	Allelic diversity	758.5	56	234.9	128.3	***

Significance codes: '***' 0.001; '**' 0.01; '*' 0.05; 'NS' not significant

The number of loci was always the most important single factor in determining the accuracy of assignments for all three methods investigated (Figure 2 and Tables 3-5). Across all scenarios, the Christie method was the most affected with an overall reduction of 47% in overall accuracy when reducing the number of loci from 20 to 10. However, this only determined ~45% of the variance in overall accuracy (GLM: $F_{1,57} = 531.7$, $p < 0.001$), suggesting other factors are influencing the discrimination of true parent-offspring pairs. In contrast, the accuracy of the pairwise-likelihood method was reduced by only 21% overall, and represented ~66% of the variance in overall accuracy ($F_{1,57} = 548.5$, $p < 0.001$). For the full-likelihood method, overall accuracy was only reduced by 4% between 20 and 10 loci, which represented ~52% of variance ($F_{1,57} = 286.1$, $p < 0.001$).

Differences in allelic diversity between the two simulated populations further accentuated the effect of the number of loci on the overall accuracy of assignment, with a significant interaction between these two factors (Table 5). The performance of both the Christie method and the pairwise-likelihood methods was most severely affected by their combined effect (as the sum of variances explained by each variable and their interaction), explaining a total of 90% and ~87% of variance in overall accuracy, respectively. For both methods, this included a low but significant interaction between the number of loci and allelic diversity (1.8%: $F_{1,53} = 21.9$, $p < 0.01$ and 0.8%: $F_{1,54} = 6.3$, $p < 0.05$, respectively). In contrast, these two factors explained ~75% of the overall variance in accuracy of the full-likelihood method, and there was no significant interaction between the two on the overall accuracy of assignment. Though the accuracy of the full-likelihood method was high overall, it is likely that the presence of full-sibs in our simulated data increased the accuracy of assignment for this method.

The proportion of sampled parents had a small but significant effect on the accuracy of all methods, which were only exacerbated by variation in the number and allelic diversity of loci. Variation in the proportion of sampled parents explained only ~6% of variance in overall accuracy of both the Christie method and the pairwise-likelihood methods (Table 5). This included a small but significant interaction with allelic diversity (2.6%: $F_{1,54} = 30.6$, $p < 0.001$) for the Christie method and a small but significant interaction with the number of loci (1.2%: $F_{1,55} = 9.8$, $p < 0.01$) for the pairwise-likelihood method. In contrast,

proportion of sampled parents explained ~17.6% of the overall variance in accuracy of the full-likelihood method, and showed no significant interaction with other variables.

Overall, the presence of genotyping error had negligible impact on the accuracy of assignments (Figure 2 and Tables 3-5). For each method, I compared the average accuracy in the high and low diversity datasets with either 0.1% or 1% genotyping error. Overall, a 10-fold increase in genotyping error, resulted in a 2-3% reduction in accuracy for the Christie methods and less than 1% reduction for the pairwise- and full-likelihood methods.

Trends in error types

The relative accuracy of each method was reflected in their susceptibility to Type Ia, Type Ib and Type II errors (Figure 1), and though incomplete sampling of candidate parents was not highly descriptive of the variance in overall accuracy (<10% for all methods), it was highly significant ($p < 0.001$) and defined clear trends in error rates, irrespective of the number of loci and allelic diversity.

For both the Christie method and pairwise-likelihood methods, the number of Type Ia errors (falsely assigning to a parent when the true parent is in the sample) increased as the proportion of candidate parents in the sample increased (Figure 3). In most scenarios investigated, the Christie method was the most susceptible to Type Ia errors, which represented 45% of all false assignments. The susceptibility of the pairwise-likelihood approach to Type Ia errors, though not as sensitive as the Christie method with fewer errors representing 38% of all errors overall, also increased with increasing proportion of the adult sample. While the number of Type Ia errors appears to asymptote when the proportion of adults reached 60% and 80% for the pairwise-likelihood approach and Christie method, respectively, it did not necessarily decrease beyond that point.

Furthermore, both the Christie methods and the pairwise-likelihood method were also susceptible to Type Ib errors (falsely assigning to a parent when the true parent is not in the sample), with the number of errors decreasing as the proportion of sampled parents increased (Figure 4). These were the most common form of error for the pairwise-likelihood method (39%) and, while the overall trend and susceptibility was similar between the two approaches, these were the

least likely error for the Christie method (15% of errors overall). Given the accuracy of the full-likelihood method, clear trends were not easily identified. Low number and diversity of loci did appear to increase the susceptibility of this approach to both Type Ia and Type Ib errors, representing 20% and 57% of all errors, respectively. Both error types decreased with over 40% of the adult population sampled.

The occurrence of Type II errors (falsely excluding a parent when it was in the sample) remained low for both the pairwise-likelihood and full-likelihood methods, representing 22% and 23% of false assignments overall (Figure 5). However, these increased sharply with sample size for the Christie method under scenarios with low allelic diversity, representing 40% of false assignments overall. Furthermore, high genotyping error resulted in an increase in Type II errors for this method.

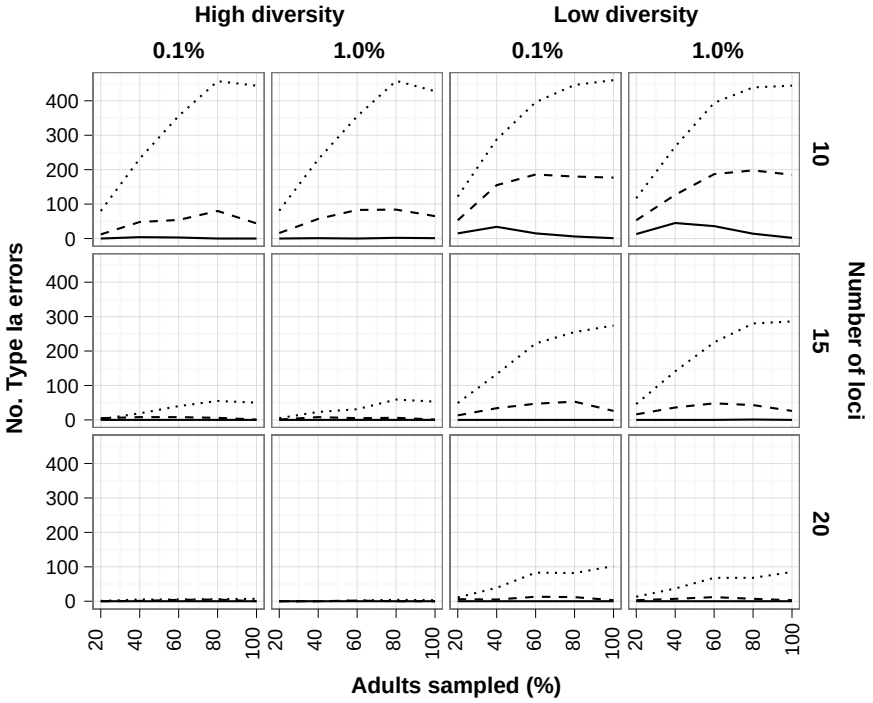

Figure 3 Susceptibility of three popular methods of parentage analysis to Type Ia errors under 60 independent scenarios. Number of false parent-offspring pairs where an offspring was assigned to a parent that was not its true parent when the true parent was in the sample varied with the number of loci (y-axis), allelic diversity in two simulated populations (N1000 & N500) level of genotyping error (0.1% & 1.0%). Continuous lines correspond to the results from the full-likelihood method implemented in COLONY v2.0, dashed lines are the results from the pairwise-likelihood implemented in FAMOZ, and dotted lines from the Christie method.

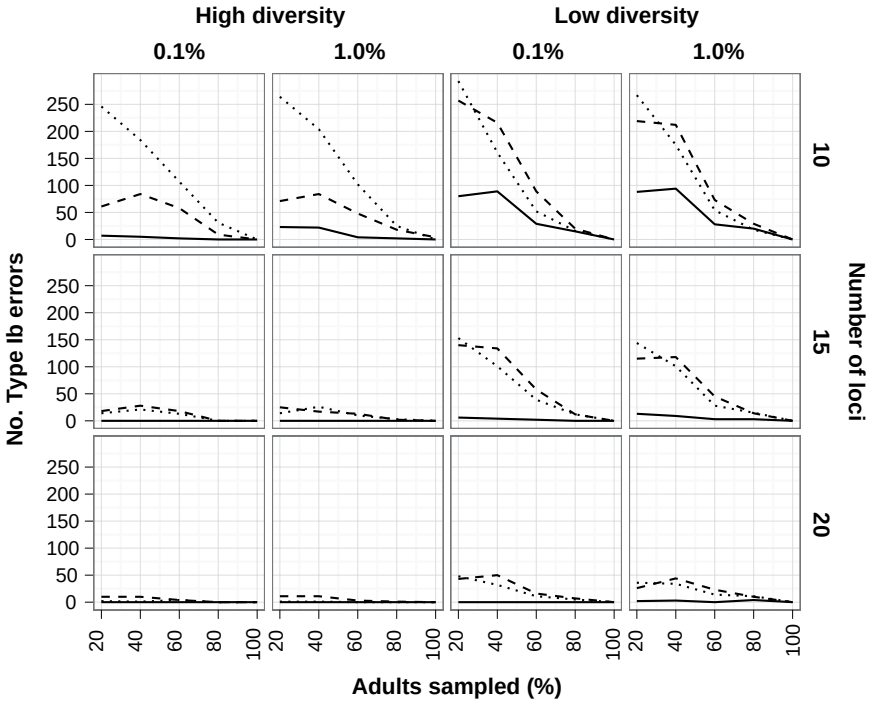

Figure 4 Susceptibility of three popular methods of parentage analysis to Type Ib errors under 60 independent scenarios. Number of false parent-offspring pairs where an offspring was assigned to a parent that was not its true parent when the true parent was not in the sample varied with the number of loci (y-axis), allelic diversity in two simulated populations (N1000 & N500) level of genotyping error (0.1% & 1.0%). Continuous lines correspond to the results from the full-likelihood method implemented in COLONY v2.0, dashed lines are the results from the pairwise-likelihood implemented in FAMOZ, and dotted lines from the Christie method.

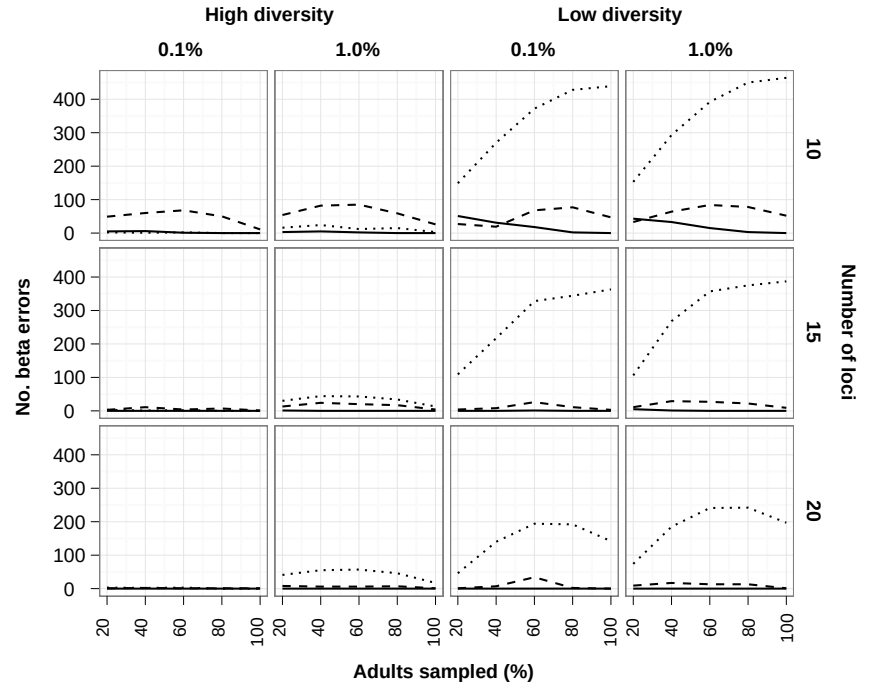

Figure 5 Susceptibility of three popular methods of parentage analysis to Type II errors under 60 independent scenarios. Number of false parent-offspring pairs where an offspring was not assigned when the true parent was in the sample varied with the number of loci (y-axis), allelic diversity in two simulated populations (high & low diversity) level of genotyping error (0.1% & 1.0%). Continuous lines correspond to the results from the full-likelihood method implemented in COLONY v2.0, dashed lines are the results from the pairwise-likelihood implemented in FAMOZ, and dotted lines from the Christie method.

DISCUSSION

This study evaluates the performance of three popular approaches to parentage analysis using microsatellite loci in open populations. In these simulated scenarios I was able to capture a wide diversity of conditions that are commonly encountered in parentage studies and identified key factors for the identification of true parent-offspring pairs in natural populations. I also identify the three main error types that lead to false assignments. Our results show that with many highly diverse loci, all three methods investigated identified true parent-offspring pairs with high levels of accuracy. However, as I reduced the number and allelic diversity of loci, and the proportion of parents sampled, the performance of each method responded differently. In general, accuracy declined with reduced number of loci and allelic diversity, while the response to the proportion of population sampled and effects of genotyping error varied with each method. In these simulated settings, the full-likelihood approach implemented in COLONY (Wang 2004; Jones and Wang 2010a), consistently outperformed both the pairwise-likelihood method implemented in FAMOZ (Gerber *et al.* 2003), and the Christie method (Christie 2010), which was subject to the most erroneous assignments.

Accounting simultaneously for parent-offspring pairs and full- and half-sibs clearly increases the accuracy of assignments for the full-likelihood approach implemented in COLONY (Wang 2007; Walling *et al.* 2010). While the inclusion of full-sibs in our simulated datasets was necessary to reduce the computational demands of this method, these may not be present at such frequencies in natural populations. Furthermore, low allelic diversity or the absence of many candidate parents makes the identification of family clusters much more difficult. While the most informative datasets (20 loci with 100% sampled parents and high allele

diversity) took several hours to complete, the least informative datasets (10 loci with 20% sampled parents and low allele diversity) took up to four months to complete (single run with high precision on a single CPU). For larger natural populations with mixed generations and complex genealogical relationships, it would take considerably longer. Consequently, the performance of COLONY may be reduced if the presence of large family groups is infrequent, a common characteristic of both terrestrial and marine systems (Selkoe *et al.* 2006; Buston *et al.* 2009). How the presence of full-sibs in the sample, as candidate parents or as offspring, affects performance remains unclear and requires further investigation. The computation time remains the major drawback of this method and thus its application may be restricted to studies with small sample sizes. However, during the development of this study, a new likelihood method was released (Wang 2012) that is less computationally demanding than the full-likelihood method and may overcome this limitation.

Though the pairwise-likelihood method implemented in FAMOZ (Gerber *et al.* 2003) was sensitive to the number and allelic diversity of loci used in the analysis, it is a good compromise to the full-likelihood approach. While it did not perform as well, it is well suited for parentage studies in large natural populations where knowledge of biological or demographic parameters is limited or unavailable, where sample sizes are large, or where the number and diversity of loci is limited. Furthermore, prior knowledge of the proportion of candidate parents sampled did not appear to be an important factor in determining true parent offspring pairs. The pairwise-likelihood method is also far less computationally intensive, with each run taking only minutes to complete on a standard laptop computer. The flexibility of FAMOZ allows for a broad range of applications and has made it an attractive approach to investigate mating patterns and dispersal in a variety of taxa where demographic parameters are often difficult to obtain.

The performance of the Christie method (Christie 2010) was clearly affected by the number and allelic diversity of molecular markers chosen in these simulated scenarios. However, provided that enough highly diverse markers are available, the accuracy of this method increases substantially. Low allelic diversity combined with large sample sizes increases the probability of false parent-offspring pairs in the sample, and would explain the susceptibility of this method

to Type II errors in low diversity datasets (Christie 2010). Setting a threshold whereby putative assignments are only accepted if the probability of false assignments is less than 0.10 or 0.05 was attempted to reduce the number of false positives, however the increase in false negatives outweighed the benefits and did not increase the overall accuracy of assignments. Overall, I found the approach computationally intensive, especially in scenarios where the number and diversity of loci was low, perhaps due to the standardised number of simulations I chose. One potential constraint of this approach is the inability to identify parent pairs, limiting its application for ecological studies if no demographic or mating information is available. Nevertheless, this method is well suited for situations where only small proportions of large populations can be sampled (e.g. Christie *et al*. 2010a) and has been successfully applied to infer reproductive success in a captive breeding program (Christie *et al.* 2011; Christie *et al.* 2012).

In natural populations where exhaustive sampling is prohibitive, variation in the proportion of sampled parents can have a significant impact on the accuracy of parentage reconstructions (Hadfield *et al.* 2006; Kock *et al.* 2008; Nielsen *et al.* 2001). Our results show that sampling higher proportions of the population decreases the likelihood of falsely assigning to a parent when the true parent was not in the sample (Type Ib). This is simply because more true parents are present in the sample. On the other hand, sampling higher proportions of the population increases the likelihood falsely assigning to a parent (Type Ia) or falsely excluding a parent (Type II) when the true parent was in fact in the sample. Sampling larger proportions of adults leads to exponential increases in the number of possible pairwise comparisons and limited genetic information leads to erroneous assignments. Nevertheless, our results showed that for all methods, increasing the number and allelic diversity of loci reduced the effects of incomplete sampling to the point where they became negligible.

Depending on the objectives of the study, different types of errors will have different consequences on the interpretation of the results. For example, if the objective is simply to assign offspring to a population or a group of individuals (e.g. to estimate self recruitment rates at the population level), Type Ia errors will have little bearing on the conclusions of a study because they will not affect the proportion of assignments. On the other hand, if one was to measure individual

reproductive success (e.g. Rodriguez-Muñoz *et al.* 2010; Beldade *et al*. 2012), any error type can have adverse consequences and assignments may not necessarily reflect true ecological processes. Regardless of the method used, performing simulations to estimate different error rates could help to identify the number of markers required to address specific questions. Striking a balance will be necessary to achieve the best performance or satisfy the objectives of a given parentage study.

CONCLUSION

This study highlights how the number and diversity of loci, the proportion of candidate parents sampled and the level of genotyping error can affect the accuracy of parentage assignments in three common methods of parentage analysis. Within the range of values simulated, COLONY outperformed FAMOZ, which outperformed the Christie method. However, with 20 or more highly polymorphic loci, all methods could be applied with confidence, though which method is most suitable is likely to depend on the size of the dataset and the size of the population investigated. When using fewer loci or less diverse loci, it is vital to be aware of the potential for assignments errors and the nature of these errors when choosing which method to apply. Parentage studies in natural populations are a challenging endeavor and obtaining accurate assignments is crucial to obtaining accurate representations of ecological processes. Whilst most studies will seek to minimise false assignments, a compromise between the cost of developing and processing a large number of loci and sampling effort is often necessary. Obtaining larger sample sizes of potential adults obviously increases the number of possible assignments. However, I found that increasing the number of loci, or selecting loci with greater allelic diversity can compensate for incomplete sampling of the parental population and still achieve high levels of accuracy.

CHAPTER 3 - DEVELOPMENT OF MULTIPLEX ASSAYS FOR PARENTAGE ANALYSIS FOR TWO CORAL REEF FISHES

ABSTRACT

Recently, microsatellite loci have been used to track individual patterns of dispersal and identify inter-generational relationships between individuals. However, to apply these methods accurately requires the development of numerous, highly polymorphic markers. Here, I describe the development of a novel set of 11 microsatellite loci for coral trout, *Plectropomus maculatus*, and 13 microsatellite loci for stripey snapper, *Lutjanus carponotatus*, two significant fishery species of the Great Barrier Reef (Australia). Markers were selected from a panel of microsatellite loci previously described for closely related species or species-specific, and described here following the development of novel libraries. Markers were integrated into multiplex PCRs for high-throughput genotyping of 466 and 1154 adult coral trout and stripey snapper, respectively. Each set of marker is composed of highly polymorphic loci delivering an overall exclusion power of over 99.98%. These provide a strong and robust basis with which to investigate parentage and other genealogical relationships in natural populations for these important fisheries species.

INTRODUCTION

Classical genetic analyses have been used for several decades to measure gene flow and estimate migration between discreet populations (Slatkin 1981; Hartl and Clark 1989). Each new genetic markers, first allozymes, then microsatellites, and more recently single nucleotide polymorphisms brought major advances in the efficiency, power and flexibility of genetic analyses, thus widening the use of genetics in the study of ecology and evolution (Cruzan 1998; Manel *et al.* 2005). After their discovery in the early 1990's, microsatellite markers were rapidly recognised as more versatile than allozyme markers and remain today the most popular marker type for ecological applications. Microsatellites commonly exhibit high levels of allelic diversity, therefore providing more information and power to describe demographic processes and the relationship between individuals. Developments in molecular techniques along with advances in computing technology continue to improve and broaden the scope of their application in ecology (Manel *et al.* 2005; Selkoe and Toonen 2006).

Microsatellite markers provide numerous advantages in ecological studies. Their high information content and mutation rate can provide insight into ecological processes over short spatial and temporal scales. Furthermore, unique genetic profiles can be generated for each individual in a population with relatively few markers, thus providing an effective approach to infer genealogical relationships among individuals. For example, microsatellite markers have been used to track the movement of individuals (e.g. Nathan 2003; Harrison *et al.* 2010), their reproductive behaviour and mating patterns (e.g. Araki *et al.* 2007; Kanno *et al.* 2011; Rodrigues-Munoz *et al.* 2010; Ford *et al.* 2011; Beldade *et al.* 2012) and, the dispersal and survivorship of their offspring (e.g. Garcia *et al.* 2005, 2007; Jordano *et al.* 2007; Planes *et al.* 2009; Christie *et al.* 2010a; Saenz-Agudelo *et al.* 2011; Berumen *et al.* 2012; Saenz-Agudelo *et al.* 2012). Individual level genetic analyses are an important tool in ecological studies as they can often be the only means with which to identify and track the movement of individuals. While several analytical approaches exist, obtaining unique genetic profiles for each individual in a population requires numerous markers. Identifying the relationship between

these individuals requires even more, with parent-offspring and full-sibs being the easiest relationships to identify.

Despite the many advantages and versatility of microsatellite markers, their application has, until recently, been limited by the time and expense of developing novel markers for non-model organisms and screening large numbers of individuals (Selkoe and Toonen 2006). In order to develop a novel set of markers for a given target species, markers can either be obtained from pre-existing microsatellite libraries for closely related species, or isolated *de novo*. The success rate of each approach will depend on whether species are closely related or the abundance of microsatellite in the target genome (Zane *et al.* 2002). Once candidate markers are identified, they must also satisfy certain characteristics that will ensure reliable PCR amplification across multiple samples. Markers that suffer from non-amplification, mispriming, artifacts, excessive stutter or low yield can result in poor data quality. When processing a large number of samples it is also necessary to minimise the number PCR reactions by 'multiplexing' several markers into a single PCR reaction. It can often take several rounds of optimisation before a set of markers amplifies successfully in multiplex reactions.

Depending on whether markers are intended for classical population genetics or individual level genetic analyses, the ideal marker will have very distinct characteristics. In general, the allelic diversity of markers should reflect the size of target populations and the number of individuals sampled in each population. For classical population genetics, the objective is often to obtain an accurate representation of allelic frequencies with which to distinguish target populations. The sample of each population should capture the full diversity of alleles, therefore small sample sizes require marker with small allelic diversity. For individual level genetic analyses, such as parentage analyses, markers with high allelic diversity are preferred as these increase the accuracy of parentage assignments (see **Chapter 2**). However, parentage analyses measure the likelihood of each assignment against the frequency of alleles in the target population. Therefore, it is also necessary that the sample of individuals is sufficiently large to provide an accurate representation of allelic frequencies in the population.

The aim of this chapter was to develop multiplex PCR assays for parentage analysis in two species of coral reef fish, the bar-cheeked coral trout *Plectropomus*

maculatus, and stripey snapper *Lutjanus carponotatus*. Microsatellite markers were selected from pre-existing microsatellite libraries for closely related species and isolated from a novel genomic DNA library enriched for microsatellites. Markers were selected based on the quality of amplification and the level of allelic polymorphism in order to maximise the accuracy of subsequent parentage analyses. For each marker, I measured the number of alleles and allelic richness, the level of heterozygosity, and tested for departure from Hardy-Weinberg equilibrium as evidence of null alleles or inbreeding. I used the polymorphic information content and probability of exclusion of each marker as an indication of their suitability for parentage analyses.

MATERIAL AND METHODS

Marker development - *Plectropomus maculatus*

A total of 34 microsatellite loci were tested in *Plectropomus maculatus* (Table 1). This included 29 primer pairs previously described for *P. maculatus* (Zhu *et al.* 2005) or for 4 congeneric or closely related fish species: *P. leopardus* (Ding *et al.* 2009) and *P. laevis* (van Herwerden *et al.* 2000), and *Lethrinus miniatus* (van Herwerden *et al.* 2000). A further 14 microsatellite loci were tested from a cloning library developed using an enrichment protocol described below. Loci obtained from congenerics were sequenced to confirm the presence of uninterrupted simple sequence repeats.

Novel primer pairs were selected in PrimerSelect (DNASTAR) on the basis of fragment size, hetero-dimer duplexing and complementarity of melting temperatures. Each locus was first tested in simplex with each PCR reaction containing 4µl 5x buffer (Kapataq, 10mM Tris-HCl, 50mM KCl, pH 8.3), 0.2 mM each dNTPs, 1.5 mM $MgCl_2$ and 1 U of Hotstart *Taq* (Kapataq). PCR cycling conditions began with an initial denaturation step at 94°C for 4 min followed by 20 cycles at 94°C for 30 s, 20 s at 58°C reducing the annealing temperature by 0.1°C every cycle, 72°C for 20 s, a further 5 cycles were then performed at an annealing temperature 56°C to maximize product amplification followed by a final extension step at 72°C for 15 min.

Microsatellite development - *Lutjanus carponotatus*

A total of 52 microsatellite loci were tested in *Lutjanus carponotatus* (Table 2). This included 39 microsatellite primer pairs previously described for 5 congeneric or closely related fish species: *Lutjanus russelli* (Guo *et al.* 2007), *L. argentimaculatus* (Zhang *et al.* 2006), *L. erythropterus* (Lo *et al.* 2006), and two Western Atlantic species *L. campechanus* (Gold *et al.* 2001), and *Rhomboplites aurorubens* (Bagley and Geller 1998). A further 13 microsatellite loci were tested from a cloning library developed using an enrichment protocol described by Glenn and Schable (2005). Loci obtained from congenerics and closely related species were sequenced to confirm the presence of uninterrupted simple sequence repeats.

Novel primer pairs were selected in PrimerSelect (DNASTAR) on the basis of fragment size, hetero-dimer duplexing and complementarity of melting temperatures. Each locus was first tested in simplex with each PCR reaction containing 4µl 5x buffer (Kapataq, 10mM Tris-HCl, 50mM KCl, pH 8.3), 0.2 mM each dNTPs, 1.5 mM $MgCl_2$ and 1 U of Hotstart *Taq* (Kapataq). PCR cycling conditions began with an initial denaturation step at 94°C for 4 min followed by 20 cycles at 94°C for 30 s, 20 s at 58°C reducing the annealing temperature by 0.1°C every cycle, 72°C for 20 s, a further 5 cycles were then performed at an annealing temperature 56°C to maximise product amplification followed by a final extension step at 72°C for 15 min.

Microsatellite enrichment protocol

Microsatellite markers were developed using an enrichment protocol developed by Glenn and Schable (2005). Approximately 4mg of genomic DNA (gDNA) from one individual was digested with RsaI and XmnI, and SuperSNX24 linkers were ligated onto the ends of gDNA fragments. Linkers act as priming sites for polymerase chain reactions (PCR) in subsequent steps. Five biotinylated tetranucleotide probes were hybridised to gDNA: $(AAAT)_8$, $(AACT)_8$, $(AAGT)_8$, $(ACAT)_8$ and $(AGAT)_8$ in the case of *P. maculatus,* and $(ACAT)_8$, $(AGAT)_8$, $(ACCT)_6$, and $(ACAG)_6$ for *L. carponotatus*. The biotinylated probe-gDNA complex was added to magnetic beads coated with steptavidin (Dynabeads® M-280 Invitrogen,

Carlsbad, California). This mixture was washed twice with 2xSSC, 0.1% SDS and four times with 1xSSC, 0.1% SDS at 52 °C. For the final two washes, the mixture was incubated for 1 minute in a 52°C water bath. Between washes, a magnetic particle-collecting unit was used to capture the magnetic beads, which are bound to the biotin-gDNA complex. Enriched fragments were removed from the biotinylated probe by denaturing at 95°C and precipitated with 95% ethanol and 3M sodium acetate.

To increase the amount of enriched fragments, a "recovery" PCR was performed in 25ml reactions containing 1x PCR buffer (10mM Tris-HCl, 50mM KCl, pH 8.3), 1.5mM $MgCl_2$, 0.16mM of each dNTP, 10X BSA, 0.52mM of the SuperSNX24 forward primer, 1U *Taq* DNA polymerase, and approximately 25ng enriched gDNA fragments. Thermal cycling was performed in an MJ Research DYAD as follows: 95°C for 2min followed by 25 cycles of 95°C for 20s, 60°C for 20s, and 72°C for 90s, and a final elongation step of 72°C for 30min.

Subsequent PCR fragments were cloned using the TOPO-TA Cloning® kit (Invitrogen) following the manufacturer's protocol. Bacterial colonies containing a vector with gDNA (i.e. white colonies) were used as a template for subsequent PCR in a 25ml reaction containing 1x PCR buffer (10mM Tris-HCl, 50mM KCl, pH 8.3), 1.5mM MgCl2, 0.12 mM of each dNTP, 10x BSA, 0.25mM of the M13 primers, and 1U *Taq* DNA polymerase. Thermal cycling was as follows: an initial denaturing step of 95 C for 7 min was followed by 35 cycles of 95°C for 20s, 50 °C for 20s, and 72°C for 90s. These PCR products were cleaned using MultiScreen-PCR Filter Plates following the manufacturer's protocol (Millipore, Billerica, Massachusetts).

DNA sequencing was performed using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California). Sequencing reactions were precipitated with ethanol and 125mM EDTA and run on an ABI 3730 DNA Analyzer.

Multiplex reactions

DNA extractions were optimised for fish fin tissue (Gentra Puregen, QIAGEN) and eluted to 200 µl TE buffer (pH 8.0). Primers were fluorescently labelled (TET, HEX, FAM) and pooled in multiple multiplex PCR reactions taking into account fragment overlaps, primer complementarity and optimal annealing temperatures. In the

case of *P. maculatus*, three multiplex PCRs were designed with similar conditions, all contained 10-40ng of genomic DNA, primer concentrations ranged from 0.08 – 0.6 μM. For *L. carponotatus*, loci were initially pooled in three multiplex reactions, which were later reduced to two. I report here only the final PCR protocol. All multiplex PCRs successfully amplified using the Type-it Microsatellite PCR kit (QIAGEN) following the manufacturer's protocol with annealing temperatures ranging from 59°C to 53°C and 28 to 30 cycles. All PCR products were purified using Sephadex G-50 chromatography and screened on a MegaBACE 1000 sequencer (GE Healthcare) at the Genetics Analysis Facility, Advanced Analytical Centre, James Cook University. Allele sizes were verified visually in the fragment analysis software FRAGMENT PROFILER 1.2 (GE Healthcare) and binning of alleles was done manually, taking into account allelic drift for larger fragments.

Population genetics statistics

A total of 466 adult *P. maculatus* and 1154 adult *L. carponotatus* were genotyped with a panel of 11 and 13 microsatellite markers respectively, resulting in unique genotype profiles for each individual (see **Chapter 4** for sample collection details). Observed genotypes were tested for departures from Hardy-Weinberg equilibrium due to heterozygote deficiency at each locus as implemented in FSTAT v. 2.9.3.2 (Goudet 1995). Significance of multiple *P*-values (α = 0.05) was assessed with strict Bonferroni correction applied for multiple comparisons ($P < 0.005$) (Rice 1989). Nei's unbiased expected and observed heterozygosity (Nei 1987) was calculated over all samples in FSTAT. The number of alleles, allelic richness and Weir & Cockerham's (1984) estimator of the inbreeding coefficient F_{IS} were also computed in FSTAT across all samples. Allelic richness was assessed relative to the Egg Rock sample, for which we had the smallest number of adult samples. The occurrence of null alleles and large allele drop-outs were assessed at each locus using MICROCHECKER v.2.2.3 (van Oosterhout *et al.* 2004). Genotyping error was estimated at each locus by pairwise comparisons of 20 *P. maculatus* and 92 *L. carponotatus* recaptured individuals. Polymorphic information content for each locus was estimated in CERVUS v. 3.0.3 (Marshall *et al.* 1998; Kalinowski *et al.* 2007) based on the frequency of alleles per locus ($PIC = 1 - \sum x_i^2$, where x_i is the frequency of the i^{th} allele). The probability of exclusion of each locus and the

cumulative probability of exclusion of each multiplex panel were calculated according to Jamieson and Taylor (1997) as the probability of excluding a single parent and parent pair.

Table 1 Microsatellite loci tested in *P. maculatus*

Species	Locus	Repeat motif	Forward primer (5'-3')	Reverse primer (5'-3')
P. maculatus (published libraries)	Pm01	TG	CTCGCTGCTTTGGAGGCAGTATA	TGGCTCAGTAGGTGGTTAAATTTTAGG
	Pm02	CA	GATCAGCCTGTTAGCCCTGGATAA	CCCCCTGGCCAAGTCACAG
	Pm03	CA	AGCTGCATAAGCACTTACCGTCACTG	CACGACCCCCAGAGCAAACAC
	Pm05	CA	TCAAGGGACACAGAAATGGTTCA	CCTGCGCTGACCCTGTTTTA
	Pm06	CA	AGCTACTGTCCGCCTCTGTTAATGCTA	AACAGGATGCTGAAATAGAATTTGG
	Pm08	TG	CTTAGGGAGCAGAAATGGGAACA	AGCAGATGCACAGGACTTGACA
	Pm10	TG	GCGGACAGGCTGGAAAACTG	CCGCTGACTGTGATCTCCCAA
	Pm11	CT-CA	CGAGCTTGGCACGAATGTATA	ACAGGCGATGTGAGATGTTGTC
	Pm12	TG	AGAAAAAGCTCCACAACACAACAA	GAGCCCCAGTCCCAAATATTG
P. maculatus (developed here)	Pma036	TAGA	GGGTCTGCAGGCAACACAAAGACAT	TGGAGAAAATTGTTGAGTGAAGAGTGG
	Pma063	TATC	AGCTACCCATGTGTGTCGTATGTCCA	TGTGTGCTGGCCCTTAACCCTATCA
	Pma067	TATC	ACCCAAGTCTAACTCTCAATGTGGA	GCTGGTTGCGAGGTAGCTAGATAAG
	Pma082	TATC	TCCCATACAGGCCGTTATATTGCAC	GCGACACTCACAGCAAGTTAGAAAGA
	Pma093	ATAG	GATGAGGGCGCTCTTGCCTTTGTAG	GCAGTTCCTGACAGTGCCCAGTAGA
	Pma097	ATCT	AGTGGGGCCATGTTTAACAACAGCA	ACGAGTTTTGTGAGATGGATGGGTGGA
	Pma101	TATC	TGCCCGACTCGATTTGTAACAGTGC	GGGGATAGACAAGAGGAAAGAGAGGGGA
	Pma104	TATC	CCATAACGGGGACTTTGGCCAATCA	CTGCACTTGTAGAACAGCCATGGGA
	Pma106	GATA	CAGGAGCCATTGAGACAGGGAGAGG	AGTGTTGGTGGTTTCGCTGATGCTT
	Pma112	TATC	GGGGGTCTGTTTGCACTTTTTATTACG	AACCAGCCACCTTTTAAGCCTCTGC
	Pma114	TATC	CTTGAACAGGCAGTGTAAAGGGGGC	ACCTGGAGCCAGTCATGTTCATGGT
	Pma121	ATAG	GCCTTGGGCACACACATATGCACTT	TTGCCATTATAGTTGCCAGGGACGC
	Pma128	TATC	TTGTGTCCTCTAGTCGGGTTGCACA	TTGTGGTTGCAGCTCTCTGTTAGCC
	Pma133	TATC	TGTTCTTACTGCATGGCGCTGCTAC	CCCCCTAAACCCTACACACTCAACC
P. leopardus	PL03	CA	TTTGTAGTTCAGTTCAGAAGAGC	AACTAAGGTTCAATCCAAGTCCAAT
	PLL04	GT	ACCCATCCACCTCCCATCCCTAA	TGTCTGCGTGCTCCAATCTATCT
	PLL05	CA	GCTGCCATTTATTTCGGCTTGA	TTCAATTTAGCTCCACTTGCTT
	PLL12	AC	CTTGATGACTCGGGCTCCTTTC	GTGTTTGGCAGGACCTTGAGTG
P. leavis	2.22	CA	GGTCCTCGTGGATGTGTAACC	CACATGGGATGAACTTCAGC
	3.62	CA	TTTGTGCAGCCAGACTTAAGC	ACATGCTGATTGGATCCTAGG
	7.90	TG	ATCCTAATCCACACAGAATGC	GGTTTAATCGTGTTTATGACCG
L. miniatus	19RTE	AC	CAGCCAGGTTCCTCTTCATCC	TGTACTCTATCAATGACAGACG
	58RTE	TG	TGCCAGTGTGTGGGCAGTAGG	TTCGACAAATTGTTTACTAATTTGC
	67RTE	GT	TGGCTTTGAACAGATCCAGC	TTTGGGCTATTAATGCCTTGG
	95TGRTE	TG	TCTGTATTTGGTATTCCAACG	TCAGTGTCAGAGAGACAGC

Table 2 Microsatellite loci tested in L. carponotatus

Species	Locus	Repeat motif	Forward primer (5'-3')	Reverse primer (5'-3')
L. carponotatus (developed here)	Lca053	ATCC	TGGCCCCTAAGTTTATTGATGAA	TGCCTCTCCCCCAGTGTC
	Lca059	TGGA	CTGCCACTGCGACCTGAC	AAGCTTGTTTTGGGGATTGT
	Lca075	AGAT	TAGGGGGCTGGGCATAAGT	GAGCCAAAGAGCAAGCAAAAC
	Lca080	ATGG	CTTCTGCAGGAGTCTTTAGG	CATTTGTATTTTGTTGCTTATC
	Lca103	TAGA	TCCACAGTGCTCCTATATTCCTTA	TTCACCGCAACCTAAAGACC
	Lca109	ATAG	GCATGTTGCACAGCAATAGA	TATCAGCCAGTTAGCCAGAAA
	Lca130	TATC	TCTCTTTCTCCTGCTCCTCTAACTC	CACTCTTCTCCCCTCCTCTGAC
	Lca141	ATGG	TAAAAGGGCAGATACCTCATAGTAGTAA	GTCTTTATGATCGAACAGGGATTTCTAT
	Lca145	ATCT	CCTTAGCACCACAAGCCCAGTG	ATATCCAGTTTGTAGTGGTGTGAGTTGC
	Lca146	ATAG	AAGCCATTTTGTTTCTGTGGTTTTTAT	GATTAGATGCCATCATGACATTGTGA
	Lca156	ATGG	TTAAGTGGAATAAGTGGAAGAGATAAGT	CAATAGAGTCTTGATCACCTTTACACT
	Lca167	TATC	GTGGTCAACCGGGTGGGAGTG	TCTCTGTGAGGTGAGCGTCAGTGTTACT
	Lca216	ATGG	ACCCATTTCAGATAAGCGGTTGA	GTCTCTGCTGACTTTGAATCACTCTTCC
L. russelli	Lru001	CA	TCCCTCTGTTGTTGAAAG	CCTGATCTCGATAGTGCC
	Lru002	AC	AGGTCTCCCCTGCAACAG	CACAACCCCACTTCAAAA
	Lru003	AC	GCATCTGCCTGGGAACTT	GCAAGAGGCTGTCGGTGT
	Lru004	CA	GATGGCAATGGAAGGCACA	CTGGGATCTATGAAAGCAAGAG
	Lru005	AC	AACAGGCACATTTTCACA	GAAGGAGCAGTACCAAGA
	Lru008	CA	CAGTCTTCCACTTTCATT	TTGCTACAGTTTCAACCC
	Lru010	CA	GCAAACGGAGGAAACAAA	CTGAAGCTCGGATGAGGA
	Lru011	AC	TGTGCTGCTGAGGACTGA	CACCCTGCGTGCGTAAGT
	Lru012	AC	ATGTTGGCTGAATCGTAG	GACCAGGTCTCCTTGAGGTT
	Lru013	CA	CATCGGGTATTTAGACAA	AGTGCCAACTACTGCTTT
	Lru014	AC	TGGAGGAAAATCTGTCTA	AGAGTAGCAGGTTTGATG
	Lru019	AC	AAATGCGACATCACCAAC	TTAGCGTAACCTCAAACTCC
	Lru020	CA	CACCCAAGTACACTCATG	GTGCAGCTTTCTCCGTAT
	Lru021	CTC	TGAGGGCATACCGATTTT	GACCAGGTCTCCCACAGC
	Lru022	CA	TTGGGGACGGCAGATACA	GAGGTGGAGTGAAAGAAGATAA
	Lru024	AC	CACCCGTTGCGTCAGATT	TCGTGCAGCGTGGTTTGG
	Lru027	AC	GGCTACAGCAGGAAGACT	TTGGAGGTTGTTGAGGAC
	Lru029	AC	CCGTTACGAAATCATCAG	TGCCTCCAGACTCAAATA
	Lru034	AACA	ACCACCAAAAGTCACAGA	ATACACCCCTCACGCATC
	Lru035	CA	ACCAGGTCTCCTTCATCC	CTCCAGTCTTCCCTACAT
	Lru036	AC	AGGTGCCAGATGAGGTAG	GGTTGGTAGGTGGAAGAG
	Lru039	AC	AGTTCTTGTTAGCACCTTT	TTGTAGTTTCACGAGCAG
	Lru041	ATTTT	CACTTTGCTCTTCTCCCTG	CTCCTCCATCATTCATTCTC
	Lru042	CA	TTGGGGACGGCAGATACA	GAGGTGGAGTGAAAGAAGATAA
	Lru043	CACT	CACAAATGGGCACAATAA	GGCAACATGGACGTGTAA

Table 2 (continued)

Species	Locus	Repeat motif	Forward primer (5'-3')	Reverse primer (5'-3')
L. argentimaculatus	Lar03	complex	CGCAGCAAGGACACTTACCA	GCCTGGATGTTTCAGAGGGA
	Lar04	AC	GAGGCTGTAGTGCTCTGCCC	GTTCACCTTCATGGCGACAG
L. erythropterus	Ler01	GACA	TCCCCGGATGTGTCTGTTAGCA	CCTCGCGTCAAAGCATGTGTCAAT
	Ler02	GACA	GTTTCAGGTCTACGATGGCAAGTT	GCGATAAAAGCGGTAATGGAAATG
	Ler03	GATA	CGTTCATTTTTAGCCTCTTCCTG	ACTCATGTAGTGTATTAGATATGGGATAAA
	Ler04	GATA	ATCATGGTGGCATCATTGGCTAAT	AAGGCTGCCGTCGTGCTTGTA
	Ler10	CA	CAGCATTACACCCGCCAAGTTA	CTCCCATGTGTTGATTTATTTAGG
	Ler11	GACA	AGTTGGCGATGGCTTTTCTGC	CCACATATTGCCCCACATTTTGAT
	Ler13	GACA	GGCCGTCCTGCTCACATC	TTTCAGTCATTTTAAGGCATTTTG
	Ler14	GATA	TTATCAAAGCCACAAAAACAATCA	CACAATAAAAGCCACAAATCCTG
	Ler16	GACA	GTGGGGCGCTGTTGCTCA	AGTTTTTGTTGCCCTTCTGCCTGTG
L. campechanus	Lca091	CA	GCATCCACCCTAAACATTTT	GTTCATCAGAGCAGCATCCT
	Prs055	TG	AGTTAGGGTTAGTCAGAGGAG	TAATGTCGTCAAAAATAGTGG
	Prs257	AAG	AAAGTTCTTGTGATGTGT	GAGAAAATGTTGGAATGA

RESULTS

Microsatellites development

Overall, 23 (68%) microsatellite markers for *P. maculatus* and 39 (75%) markers for *L. carponotatus* that were tested failed to meet the desired requirements multiplex PCR or parentage analysis. While the large majority of these markers successfully amplified microsatellite loci for each target species, the development of these markers was discontinued due to either excessive stutter, amplification artifact, low yield or insufficient polymorphism. Several markers showed promising results but could not be included in the multiplex reactions due to fragment size overlap (e.g. *Lca075*, *Pma093*, and *Pma097*) or excessive hetero-dimer duplexing (*Lru039*, *Lru001*, *Lru020*, *Ler13*, *3.62*, and *Pm01*). Others were included in multiplexes but were later found to have an excess of homozygotes, suggesting null alleles were present (*Pma082, Lru004, Lru041, Lru013*, and *Ler10*). Eleven and thirteen microsatellites were retained and successfully integrated to multiplex PCR reactions for the analysis of parentage in *P. maculatus* (Table 3) and *L. carponotatus* (Table 4), respectively.

Multiplex PCR assays for parentage analyses

Each microsatellite panel was characterised for a total of 466 adult *P. maculatus* and 1154 adult *L. carponotatus*. Each panel was composed of highly polymorphic microsatellite loci that showed no signs of null alleles, or significant departure from Hardy-Weinberg expectations. Missing data accounted for 0.2% in the *P. maculatus* dataset and 0.8% in the *L. carponotatus* dataset. Genotyping error rates across all loci were estimated as less than 2% in the *P. maculatus* dataset and less than 1% in the *L. carponotatus* dataset. All novel sequences were submitted to GenBank (Accession no. JN222550-JN222555).

Among 466 *P. maculatus*, the mean number of alleles and allelic richness per locus was 24.8 and 17.4, respectively, with an average observed heterozygosity of 0.753 ± 0.067 SE across all samples (Table 3). Most loci showed considerable polymorphic information content (*PIC*) with only two loci with *PIC* < 0.5 (*Pm10* and *Pm12*). This also resulted in a low probability of exclusion for these loci. However, across the panel of 11 loci, the average *PIC* per locus was 0.734 ± 0.070

SE with an exclusion probability of single and parent pair assignments of 0.4772 ± 0.0811 SE and 0.7662 ± 0.0755 SE, respectively. These exceptionally high levels polymorphism in the marker panel resulted in a cumulative exclusion probability above 99.98%. In other words, the probability that an individual is falsely identified as the parent of an offspring based strict Mendelian incompatibilities is less than 1 in 5,000.

Among 1154 *L. carponotatus*, the mean number of alleles and allelic richness per locus was 24.0 and 15.5, respectively, with average observed heterozygosity of 0.760 ± 0.048 SE across all samples (Table 4). Among 1154 *L. carponotatus*, the mean number of alleles and allelic richness per locus was 24.0 and 15.5, respectively, with average observed heterozygosity of 0.760 ± 0.048 SE across all samples (Table 4). Most loci showed considerable polymorphic information content (PIC) with only two loci with *PIC* < 0.5 (*Lru019* and *Lru024*). This also resulted in a low probability of exclusion for these loci. However, across the panel of 13 loci, the average *PIC* per locus was 0.738 ± 0.061 SE with an exclusion probability of single and parent pair assignments of 0.4585 ± 0.0784 SE and 0.7400 ± 0.0728 SE, respectively. These exceptionally high levels polymorphism in the marker panel resulted in a cumulative exclusion probability above 99.99%. In other words, the probability that an individual is falsely identified as the parent of an offspring based strict Mendelian incompatibilities is less than 1 in 10,000.

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DISCUSSION

In this chapter, I described the characterization of a novel set of microsatellite loci for the resolution of parentage in populations of coral trout, *P. maculatus*, and stripey snapper, *L. carponotatus*. Markers were selected based on the quality of amplification and the level of allelic polymorphism in order to maximise the accuracy of subsequent parentage analyses. Suitable markers were pooled into multiplex PCR reaction for high-throughput screening and characterised for several hundred individuals in each species. Overall, each set of markers showed high levels of polymorphism, which resulted in a cumulative probability of exclusion of at least 99.98%. While the power of exclusion is not directly relevant to all parentage methods (e.g. categorical assignments), it indicates that each set of markers is suitable to distinguish parent-offspring pairs with a high level of confidence.

The ideal marker set for parentage studies will depend entirely on the size of the target population, the proportion of individuals sampled and the level of polymorphism in the population. For small population, with less than 100 individuals, markers with low allelic diversity should be favoured to ensure accurate representation of allelic frequencies. Increasing the number of markers can compensate for the reduction in the accuracy of subsequent parentage analyses (see **Chapter 2**). In large populations, where large numbers of individuals can be sampled, it is possible to obtain accurate allelic frequencies for markers with a much greater allelic diversity. These are ideal for parentage studies as they greatly increase the accuracy of parentage assignments (see **Chapter 2**). Given the high polymorphism of markers developed here, fewer markers were necessary to achieve a *Pe* > 99.9 than those simulated in **Chapter 2** (Tables 1 & 2), and thus providing a high level of accuracy and confidence in parentage assignments.

While these highly diverse loci greatly increase the power of exclusion, the presence of rare alleles and uneven distribution of alleles may also increase the likelihood of false positive assignments (see **Chapter 2**). Where two individuals share the same rare alleles, these may be identical in state but not by descent and mis-representation of allelic frequencies can artificially increase the likelihood that the two individuals are related. Though this is not likely to be the case in our

populations, where a large proportion of adults have been sampled, this marker set may be of limited value to studies that do not sample large proportions of focal populations. What constitutes a representative proportion of a given population, and how this may affect the allelic frequencies at a particular locus, has not yet been adequately addressed (but see Neff *et al.* 2000; Bernatchez and Duschesne 2000; Kalinowski 2005, Hale *et al.* 2012). Assuming our sample of the adult population for *P. maculatus* (466 individuals) and *L. carponotatus* (1154 individuals) in the Keppels captured the full allelic distribution at each locus, we can measure the proportion of alleles that are likely to be represented in a subset of the population. If only 50 individuals were sampled, which is common for most studies investigating population structure and gene flow, the mean number of alleles sampled across all loci (± exact pooled variance) is only 67% ± 8% and ~52% ± 8% of all known alleles in the *P. maculatus* and *L. carponotatus* datasets, respectively. Small sample sizes are therefore likely to have significant effect on the accuracy of allelic frequencies for these marker set. What constitutes a large enough sample remains to be determined, however, it clear that there is an optimum sample size for any given number of alleles per locus.

CONCLUSION

In conclusion, the multiplex assays developed for coral trout, *P. maculatus*, and stripey snapper, *L. carponotatus*, provide a strong and robust basis with which to investigate parentage and other genealogical relationships in natural populations. While their application to classical population genetics analysis may be limited to studies with large sample size, they serve as an important baseline for future microsatellite development for these species. With the advent of next generation sequencing, the development of large sets of microsatellite loci has become easier, faster, and creates additional flexibility for the development of multiplex assays. The technical developments in both isolating molecular markers and high throughput screening of multi-locus genotypes have laid the foundation for a new era linking genetic approaches to ecological research.

CHAPTER 4 - LARVAL EXPORT FROM MARINE RESERVES AND THE RECRUITMENT BENEFIT FOR FISH AND FISHERIES

ABSTRACT

Marine reserves, areas closed to all forms of fishing, continue to be advocated and implemented to supplement fisheries and conserve populations. However, while the reproductive potential of important fishery species can dramatically increase inside reserves, the extent to which larval offspring are exported and the relative contribution of reserves to recruitment in fished and protected populations are unknown. Using genetic parentage analyses, we resolve patterns of larval dispersal for two species of exploited coral reef fish within a network of marine reserves on the Great Barrier Reef. In a 1,000km^2 study area, populations resident in three reserves exported 83% (coral trout – *Plectropomus maculatus*) and 55% (stripey snapper - *Lutjanus carponotatus*) of assigned offspring to fished reefs, with the remainder having recruited to natal reserves or other reserves in the region. We estimate that reserves, which account for just 28% of the local reef area, produced approximately half of all juvenile recruitment to both reserve and fished reefs within 30km. This is commensurate with an observed two-fold greater adult biomass within reserves. Our results provide compelling evidence that adequately protected reserve networks can make a significant contribution to the replenishment of populations on both reserve and fished reefs at a scale that benefits local stakeholders.

INTRODUCTION

Coastal marine ecosystems have been subjected to a long history of human impacts that are predicted to escalate over the next century (Jackson *et al.* 2001; Halpern *et al.* 2008). While there is no single solution to alleviate such impacts, no-take marine reserves represent one management action that can deliver tangible and rapid benefits (Pauly *et al.* 2002; Gell and Roberts 2003; Lubchenco *et al.* 2003; Gaines *et al.* 2010). Thousands of marine reserves have been implemented globally in the hope of restoring and sustaining fisheries, and conserving biodiversity (Mora *et al.* 2006; Wood *et al.* 2008). Hundreds of studies have demonstrated that exploited species have higher abundance, biomass and reproductive potential within adequately protected reserves (Halpern 2003; Lester *et al.* 2009; Babcock *et al.* 2010; Russ *et al.* 2008). In theory, the elevated populations in reserves can simultaneously provide both fisheries and conservation benefits, as long as there is both substantial larval export from reserves to fished areas, and either self-recruitment within or dispersal among reserves (Gaines *et al.* 2010; Sale *et al.* 2005; Botsford *et al.* 2003; Hastings and Botsford 2006; Pelc *et al.* 2010). In practice, there is little concrete evidence that reserves provide recruitment benefits beyond their immediate boundaries (Gell and Roberts 2003; Sale *et al.* 2005; Steneck *et al.* 2009). Recent breakthroughs in larval tagging and genetics have shown that it is possible to track the dispersal trajectories of larvae for small marine fishes over increasingly large distances (Swearer *et al.* 2002; Jones *et al.* 2005; Planes *et al.* 2009; Jones *et al.* 2009; Almany *et al.* 2007; Saenz-Agudelo *et al.* 2010; Christie *et al.* 2010a; Berumen *et al.* 2012; Hogan *et al.* 2012). However, for large exploited fishes, it has been seemingly impossible to determine where the larvae from populations within reserves go, or to assess the relative importance of the supply of juveniles from reserves. These are critical knowledge gaps that limit our understanding of the wider benefits of marine reserve networks for fish conservation and fisheries management.

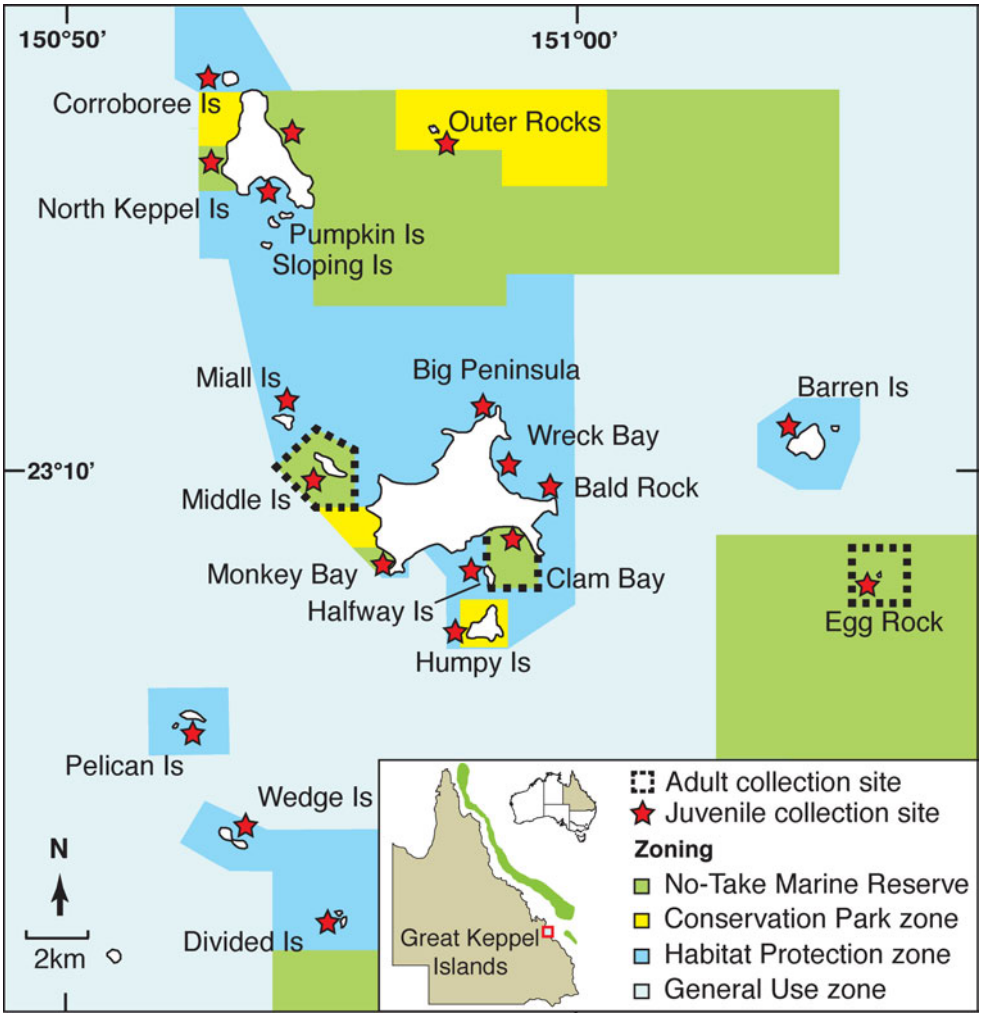

Figure 1 Sampling locations of adult and juvenile fish. The Keppel Islands includes six no-take marine reserves (Marine National Park zones – shaded green) protecting 28% of coral reefs. Adult *P. maculatus* and *L. carponotatus* were sampled within three no-take reserves (dashed line borders) and juveniles were sampled from 19 locations (red stars) within both reserves and areas open to fishing (See also Table 1). Conservation Park zones (yellow) permit limited recreational hook-and-line and spear fishing. Habitat Protection zones (dark blue) exclude demersal trawling but permit hook-and-line and spear fishing. General Use zones (light blue) allow all types of fishing.

We applied DNA parentage analysis to provide a unique assessment of the importance of reserves as a source of juveniles to both fished and protected populations in a coastal archipelago of the Great Barrier Reef (GBR). Here, 28% of coral reefs are protected in a network of six no-take marine reserves (Keppel Island group; Figure 1). Our field study focused on two commercially and recreationally targeted fish species: the coral trout, *Plectropomus maculatus*

(Serranidae), and stripey snapper, *Lutjanus carponotatus* (Lutjanidae), for which the mean biomass of reproductive adults was approximately twice as high on no-take reserve reefs than on surrounding fished reefs (Figure 2). Over 4 weeks during the peak reproductive season (Austral summer), we collected tissue samples from 466 adult *P. maculatus* and 1154 adult *L. carponotatus* within three focal reserves (Figure 1) representing 26.9% ± 8.3% SE and 35.7% ± 7.1% SE of focal populations, respectively. During the following 15 months, juveniles of both species were collected from 19 protected and fished locations up to 30 km from the focal reserves (Figure 1; Table 1). By recording the sampling locations of all adult and juvenile fishes, and assigning offspring to one or both parents, we were able to establish the dispersal distance and direction of juveniles spawned in the focal reserves.

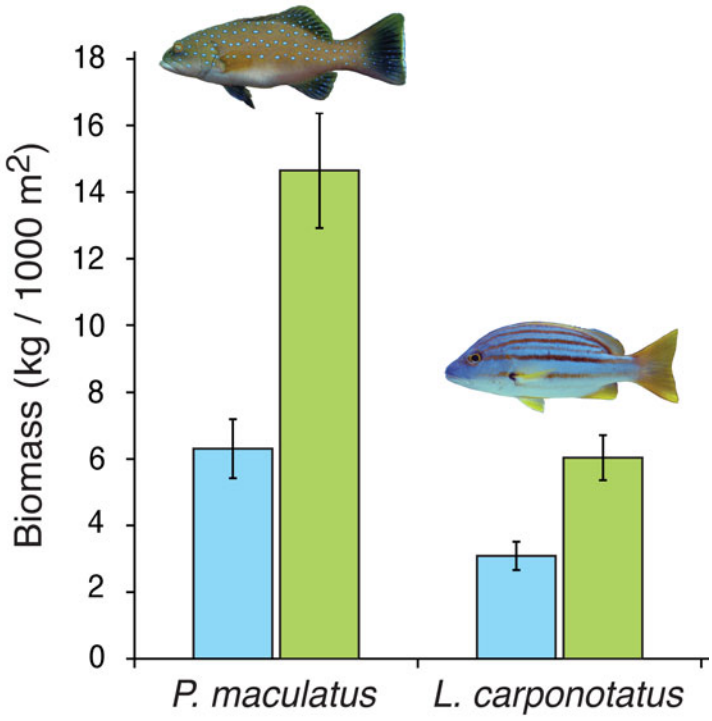

Figure 1 Relative fish biomass in protected and fished areas. Mean biomass (kg per 1000m^2) of *P. maculatus* and *L. carponotatus* within no-take marine reserves (green bars) and fished areas (blue bars) in the Keppel Islands study area as estimated from December 2007 UVC surveys. The mean biomass of both species was significantly greater inside no-take reserves than in adjacent areas which are open to fishing (ANOVA: $F_{1,20} = 12.87$, $P = 0.002$ for *P. maculatus*, and $F_{1,20} = 6.97$, $P = 0.016$ for *L. carponotatus*). Error bars indicate ± 1 SE.

MATERIAL AND METHODS

Natural history and fishery information

Coral trout, *Plectropomus maculatus* (Serranidae, Bloch 1790), and stripey snapper *Lutjanus carponotatus* (Lutjanidae, Richardson 1842), occur throughout the Great Barrier Reef Marine Park (GBRMP), the north and west coast of Australia, and most of the western tropical Pacific. Within the GBRMP, *P. maculatus* are predominantly found on inner-continental-shelf reefs, whereas *L. carponotatus* are widely distributed across both inshore and offshore reefs. Coral trout are protogynous hermaphrodites (change sex from female to male) that form small haremic social groups consisting of a single larger male and several smaller females (Heemstra and Randall 1993), whereas stripey snapper are gonochoristic (determinate sex throughout life history), typically occurring in 50:50 sex ratios (Kritzer 2004) and forming schools of several to tens of individuals (Allen 1985). Our underwater observations of the two species in the study area indicate that adult coral trout were most abundant on reef slopes, particularly in areas of high habitat complexity, whereas adult stripey snapper were commonly found on both reef flats and slopes. Extensive searches in all reef habitats revealed that the juveniles of both species were predominantly confined to reef flats and shallow lagoon areas (1-3m depth) with patch reefs dispersed within expanses of coral rubble and sand. Juvenile coral trout were also encountered in much lower densities in rubble-dominated habitats at the base of reef slopes, particularly in the vicinity of small patch reefs.

Both coral trout and stripey snapper are highly exploited in Australia and throughout the Indo-Pacific region. The commercial coral reef hook and line fishery in Queensland waters currently generates $39.5 million annually (DEEDI). During 2008/09, the total commercial catch of coral trout (*Plectropomus* spp.) from Queensland waters was 1230 tonnes (61% of the total catch of all species), while 44 tonnes of stripey snapper were harvested during the same period (DEEDI). Both species are also targeted by the recreational fishing sector, particularly on the inner-shelf reefs of the GBRMP. The total recreational catch of coral trout or stripey snapper has not been determined, but it can be stated with confidence that levels of recreational fishing effort are high enough to significantly

reduce the mean density, size and biomass of these species in areas that are open to fishing on inshore reefs of the GBRMP (Russ *et al.* 2008; McCook *et al.* 2010; Williamson *et al.* 2004).

Study location and sample collection

This study was carried out in the Keppel Island group (23°10´ S, 150°57´ E) within the Great Barrier Reef Marine Park, Australia (Figure 1). There are six no-take marine reserves in the island group, which had been protected for between 3 and 19 years at the time of the present study (Figure 1; Table 1). Adults were sampled between November 2007 and February 2008 from four coral reefs in three focal marine reserves (Figure 1; Table 1). A total of 466 adult coral trout and 1154 adult stripey snapper were sampled from the three focal marine reserves (Table 2; Table 3) and all individuals were released alive at the capture site. Fish were captured using baited, barbless hooks and line. Each fish was measured to the nearest centimetre and a uniquely-numbered, brightly-coloured t-bar tag (Hallprint, Australia) was inserted into the dorsal musculature. A small section of the pectoral fin was removed from each fish and stored in 85% ethanol for genotyping and parentage analysis. Juveniles were sampled in May 2008 and February 2009 from 19 locations scattered throughout the study area (Figure 1; Table 1). A total of 493 juvenile coral trout and 474 juvenile stripey snapper were collected, with slightly fewer samples collected in fished areas compared to reserves relative to the available reef area (*P. maculatus*: 1:1.04; *L. carponotatus*: 1:1.52). Juveniles of both species were predominantly found in specific reef habitats, however all reef habitats within the study area were searched. Juveniles were collected on SCUBA using a variety of methods, including spears, barrier nets, hand nets and clove oil (as an anesthetic). Each juvenile was measured to the nearest millimetre using dial callipers, otoliths (ear bones) were removed for age-determination, and a tissue sample was preserved in 85% ethanol for parentage analysis. During adult sampling, there were 109 recaptures of tagged adult *P. maculatus* and 471 recaptures of tagged adult *L. carponotatus* within the focal reserves. Over 80% of the *P. maculatus* recaptures and 90% of the *L. carponotatus* recaptures were within 500 m of their original tagging location, suggesting that adults were largely resident within reserves during the sampling period.

Population and density estimates

Underwater visual census (UVC) surveys were conducted at 22 sites within the Keppel Island group in December 2007 to estimate population density, size structure and biomass of *P. maculatus* and *L. carponotatus* within no-take reserves and fished zones (Figure 2). Eleven of the UVC sites were located within reserves and eleven sites were located in areas that were open to fishing. At each site, five replicate 50 m x 6 m transects were surveyed along the reef slope, oriented parallel to the reef crest. The total length (TL) of all sighted *P. maculatus* and *L. carponotatus* was estimated to the nearest 5 cm. In December 2007, the mean biomass of adult *P. maculatus* within Keppel Island group reserves was 2.3 times higher than it was in adjacent areas that were open to fishing. Similarly, the mean biomass of *L. carponotatus* was 1.9 times higher in reserves than in fished areas (Figure 2). For both species, the approximately two-fold differential in mean biomass between reserve and fished zones of the Keppel Islands in 2007, was consistent with the findings of previous studies that have examined the effects of reserves on hectares (~ 28%) was within six no-take reserves (Table 1). The three focal reserves from which adults were sampled contained 100.65 hectares of coral reef and accounted for 51.3% of these species on inshore reefs of the GBRMP (Russ *et al.* 2008; McCook *et al.* 2010; Williamson *et al.* 2004). In December 2007, the mean TL (± SE) of *P. maculatus* in Keppel Island group reserves was 355.4 mm ± 6.1 mm, while in fished areas the mean TL was 334.5 mm ± 6.1 mm. For *L. carponotatus,* the mean TL (± SE) was 261.1 mm ± 3.4 mm in reserves and 236.1 mm ± 4.5 mm in fished areas.

Table 1 Juvenile sampling locations and the distribution of coral reef area in reserves and fished locations in the Keppel Islands.

Juvenile sampling site	Reef status & year established	Total reef area (ha)	Number of juveniles collected	
			P. maculatus	*L. carponotatus*
Clam Bay & Halfway Island (East)	Reserve (1988 & '04)	60.21	98	118
Egg Rock	Reserve (1988)	4.07	0	0
Middle Island	Reserve (1988)	36.36	42	53
Monkey Bay	Reserve (2004)	17.67	2	5
North Keppel Island (East)	Reserve (2004)	59.67	0	0
North Keppel Island (West)	Reserve (2004)	18.06	0	0
Bald Rock	Fished	3.34	1	5
Barren Island	Fished	41.06	0	2
Big Penninsula	Fished	17.60	0	2
Corroboree Island	Fished	32.58	9	5
Divided Island	Fished	13.20	1	4
Halfway Island	Fished	29.96	159	109
Humpy Island	Fished	66.10	31	29
Miall Island	Fished	17.12	7	20
North Keppel Island	Fished	160.85	132	100
Outer Rocks	Fished	5.69	2	2
Pelican Island	Fished	16.81	0	3
Wedge Island	Fished	19.16	2	8
Wreck Bay	Fished	23.27	7	9
Other (not sampled)	Fished	58.04	0	0
Total	Reserves	196.04	142	176
Total	Fished	504.78	351	298
Grand Total		700.83	493	474

At the conclusion of adult fish sampling in February 2008, repeated timed swim UVC surveys were conducted within the three focal reserves to quantify the relative proportion of tagged to untagged individuals. A minimum of three 45-minute timed UVC swims were conducted by a single observer within each of the three focal reserves (10 replicate UVC swims in total). Replicate UVC counts of tagged and untagged fish were averaged to produce an overall estimate of the proportion of tagged (sampled) individuals within each of the focal reserves. The total adult population size for *P. maculatus* and *L. carponotatus* within each focal reserve was estimated using a modified Lincoln–Petersen tag-release-resight

method (Seber 2002). The proportion of each reserve population sampled was calculated by dividing the total number of fish sampled by the estimated population size within each of the focal reserves.

Analysis of juvenile otoliths

Sagittal otoliths (ear bones) were removed from each juvenile, cleaned and dried for age determination. Preparation of otoliths and determination of juvenile ages using daily growth increments was conducted in accordance with previously established protocols (Stevenson and Campana 1992). One of each pair of sagittal otoliths was individually mounted on glass slides using thermoplastic cement and then ground and polished to the point where daily growth increments were clearly visible. Analysis of daily otolith increments and identification of the 'settlement mark' from collected juvenile fish revealed that planktonic larval durations (PLDs) were between 24-29 days for *P. maculatus* and 21-27 days for *L. carponotatus*. The age-length relationship derived from the ageing analysis indicated that all collected juveniles had settled and recruited to the reefs between August 2007 and February 2009. Average fork length (± 1 SE) of the 58 assigned juvenile *P. maculatus* was 152.0 mm ± 9.2 SE, which corresponds to an age range of 179-206 days. Average fork length (± 1 SE) of the 74 assigned juvenile *L. carponotatus* was 123.8 mm ± 6.1 mm, corresponding to an age range of 256-296 days.

Measuring available coral reef area

The available coral reef area at each location was calculated from satellite imagery in ArcGIS (ESRI, Redlands). The total reef area within the study area was 700.83 hectares, of which 196.04 no-take reserve reefs within the study area. Concentric rings were drawn from the perimeter of each of the six reserves in ArcGIS (ESRI, Redlands) to calculate the available reef area with increasing distance (up to 30km) from reserves (Figure 4).

Parentage analysis

Samples were genotyped with a panel of 11 to 13 microsatellite loci previously described in Chapter 3. Categorical allocation of parent-offspring relationships was assessed based on a maximum likelihood approach implemented in the software

program FAMOZ (Gerber *et al.* 2003), the only method for which assignments of marine species have been independently verified using chemical markers (Jones *et al.* 2005). The program computes log of the odds ratio (LOD) scores for assigning individuals to candidate parents based on the observed allelic frequencies at each locus. We simulated 10,000 parent-offspring pairs based on the observed allelic frequencies to determine a minimum threshold beyond which the probability of incorrectly assigning juveniles to candidate parents was less than 5%. Genotyping error was introduced to simulate inconsistencies in the data (0.01%) and though this may underestimate true error in the data, it reduces both Type I and Type II errors in parentage assignments (Gerber *et al.* 2000; Morrisey and Wilson 2005). Minimum LOD score thresholds for single-parent assignments were identified as 5.55 for *P. maculatus* and 6.00 for *L. carponotatus* and 15.00 for the assignment to parent-pairs in both species. The resulting probability of assigning a juvenile to a parent that was not its true parent knowing that the true parent was not sampled was less than 5% (Type II error). Conversely, the probability of a true parent-offspring pair not being identified knowing that the true parent was sampled was less than 1% (Type I error). All collected juveniles were screened against the total pool of adult samples to identify parent-offspring relationships. No more than 2 mismatches were ever allowed to occur to accommodate for genotyping error, de novo marker mutations or missing data (Gerber *et al.* 2000; Morrisey and Wilson 2005; Marshall *et al.* 1998).

Estimating the total recruitment contribution of marine reserves

To estimate the proportion of total recruitment (P_{RT}) in our study area supplied by the six marine reserves, we assumed that all adult *P. maculatus* and *L. carponotatus* within the reserves had an equal probability of contributing to local recruitment and that our juvenile sample represented a random sample of all juveniles in the study area at the time of sampling. Since we sampled only a fraction of the reproductive adults within focal reserves, the observed proportion of assigned juveniles (n_A/ N_{JS}) represents only a fraction of the total progeny of focal reserves (P_{RS}). An estimate of the proportion of missed assignments can be calculated based on the probability that neither parent of unassigned juveniles were sampled (Jamieson and Taylor 1997), or $(1\text{-}P_{AS})^2$, where P_{AS} is the proportion of the

population sampled. From the observed number of assigned juveniles (n_A) and the total number of juveniles sampled (N_{JS}), we then calculated the proportion of the juveniles that we would have expected to assign to the three focal reserves had we sampled 100% of the population:

$$P_{RS} = \frac{n_A}{N_{JS}} \times \frac{1}{1-(1-P_{AS})^2} \quad (eq.1)$$

Where:

P_{RS} = Proportion of recruitment provided by focal reserves in the Keppel islands

P_{AS} = Proportion of adults sampled in focal reserves

n_A = Number of assigned juveniles

N_{JS} = Total number of juveniles sampled

Assuming mean fecundity per unit area of reef was equal in both sampled (A_{FR}) and unsampled reserves (A_{TR}), we then accounted for the additional reproductive contribution to local recruitment from the three unsampled reserves:

$$P_{RT} = P_{RS} \times \frac{A_{TR}}{A_{FR}} \quad (eq.2)$$

Where:

P_{RT} = Proportion of total recruitment from all reserves in the Keppel islands

A_{TR} = Total reef area within all reserves in the Keppel islands

A_{FR} = Total reef area within focal (sampled) reserves

RESULTS

Dispersal trajectories from reserves

Our study revealed that adult fishes in reserves exported a significant proportion of their offspring to fished areas outside reserve boundaries. We identified 58

juvenile coral trout (Figure 2; Table 2) and 74 juvenile stripey snapper (Figure 2; Table 3) as the progeny of adults sampled within the three focal reserves. Overall, 83% (48 of 58) of assigned coral trout juveniles and 55% (41 of 74) of assigned stripey snapper juveniles were collected from reefs that were open to fishing, representing a clear demonstration of larval export from reserves (Figure 2A, B). For coral trout, 7% (4 of 58) of assigned juveniles were retained in natal reserves (self-recruitment) and 10% (6 of 58) were exchanged among reserves, while for stripey snapper, 22% (16 of 74) were retained in natal reserves and 23% (17 of 74) were exchanged between reserves (Figure 2C, D). Hence, for these two species, adults in reserve populations were not only exporting substantial numbers of offspring to nearby fished areas, they were also contributing to population replenishment within the reserve network.

The observed frequency distribution of dispersal distances within 30km of sampled reserves was remarkably similar for the two species (Figure 3A, B). Approximately 30% of assigned juveniles were collected within 1-2 km of their parents, and it is evident that the different modes in dispersal distance reflected the geographic distance between reefs (Figure 3C). For both species, the shortest dispersal mode (1-2km) was predominantly associated with an area of retention at the largest of the islands (Great Keppel), while longer dispersal modes were associated with dispersal among islands (Figure 2). Across the entire 1,000 km^2 sampling area, over 90% of reefs that are open to fishing were within the mean observed dispersal range of both species, suggesting that the spacing of reserves is small enough to benefit most fished areas (Figure 3C). Clearly, successful dispersal may also have extended to unsampled reefs beyond the Keppel Island group, and the full spatial extent of the benefits of larval export from reserves remains to be determined.

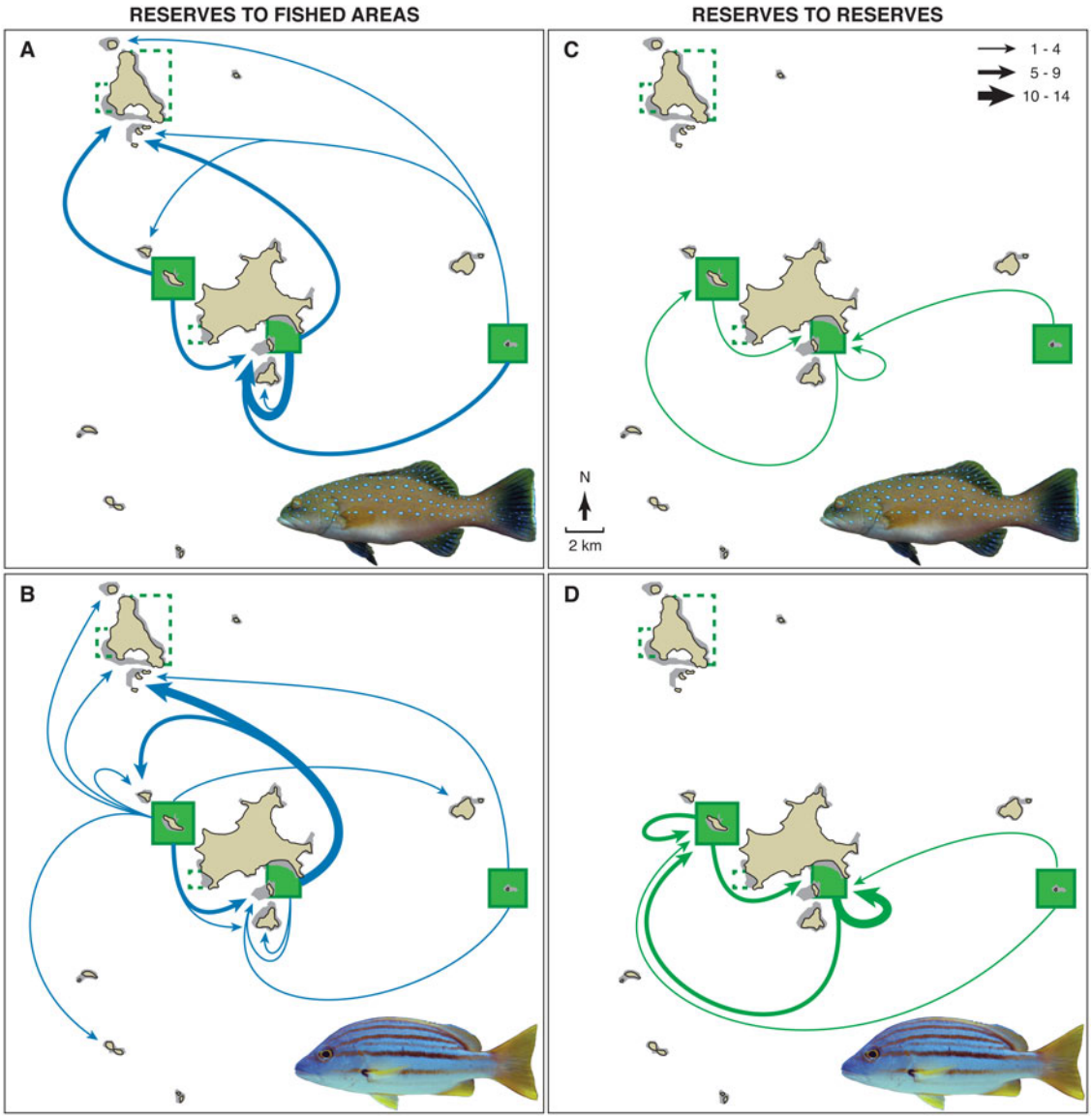

Figure 3 Realised dispersal patterns of juvenile fish from a network of marine reserves. The three focal marine reserves (green boxes) were an important source of juvenile recruitment for local fished areas. 48 juvenile *P. maculatus* (A) and 41 juvenile *L. carponotatus* (B) that had recruited to fished areas were assigned to adults from one of three focal reserves (see also Table 2 and Table 3). Coral reef areas are represented in grey, and arrow thickness is relative to the number of juveniles that were assigned to each focal reserve. Local retention within focal reserves and connectivity between reserves (dotted green boxes) also made an important contribution to juvenile recruitment in reserves. 10 juvenile *P. maculatus* (C) and 33 juvenile *L. carponotatus* (D) that had recruited in reserves were assigned to adults from one of three focal reserves (see also Table 2 and Table 3).

Table 2 Number of juvenile *P. maculatus* that were assigned to each of the three source populations within marine reserves. All assignments were to a single parent.

	Source populations			
Plectropomus maculatus	**Clam Bay & Halfway Is. (East) N = 272**	**Middle Is. N = 124**	**Egg Rock N = 70**	**Total N= 466**
Larval export from reserves				
Halfway Is. (West)	13	6	4	23
North Keppel Is.	5	8	4	17
Humpy Is.	4	-	2	6
Corroboree Is.	-	-	1	1
Miall Is.	-	-	1	1
Larval retention and exchange between reserves				
Clam Bay & Halfway Is. (East)	4	1	2	7
Middle Is.	3	-	-	3
Total	29	15	14	58

N = Number of adults sampled

Table 3 Number of juvenile *L. carponotatus* that were assigned to each of the three source populations within marine reserves. Three juvenile *L. carponotatus* were assigned to both parents (indicated in brackets); all other assignments were to a single parents.

	Source populations			
Lutjanus carponotatus	**Clam Bay & Halfway Is. (East) N = 582**	**Middle Is. N = 458**	**Egg Rock N = 114**	**Total N = 1154**
Larval export from reserves				
North Keppel Is.	10	4	1	15
Halfway Is. (West)	4	6	3	13
Miall Is.	6	1	-	7
Humpy Is.	2	1	-	3
Barren Is.	-	1	-	1
Corroboree Is.	-	1	-	1
Wedge Is.	-	1	-	1
Larval retention and exchange between reserves				
Clam Bay & Halfway Is. (East)	11	8	2	21
Middle Is.	6 (3)	5	1	12
Total	39	28	7	74

N = Number of adults sampled

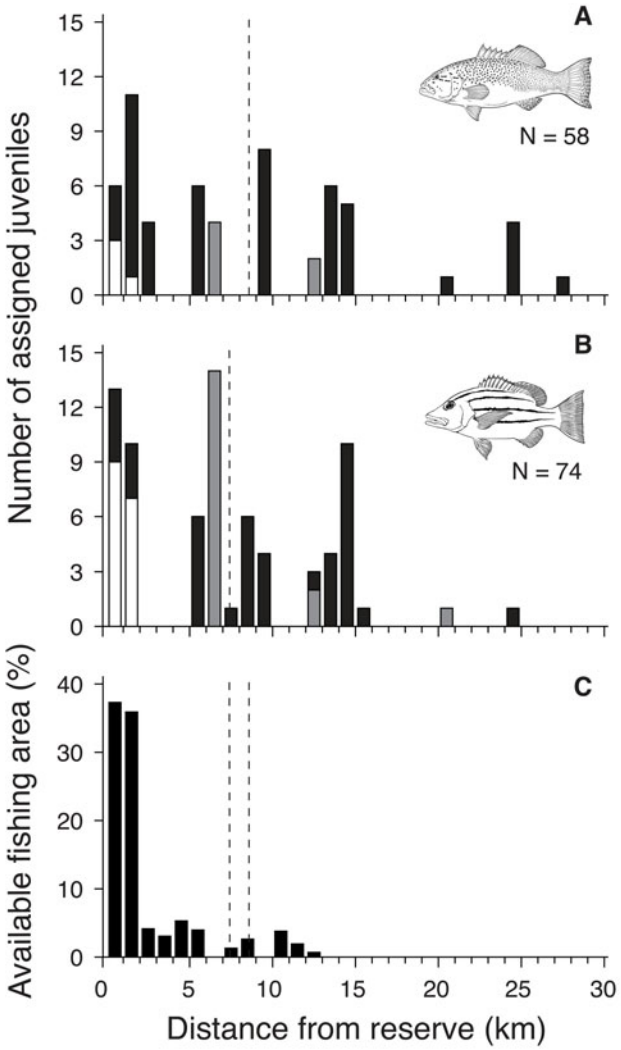

Figure 4 Dispersal distance of assigned juvenile from natal reserves. The frequency distributions of realised dispersal distances indicate that within the Keppel Islands, assigned juvenile *P. maculatus* (A) and *L. carponotatus* (B) were collected between 100's of meters and 28 km from the location where their parents were sampled (average observed dispersal distance is indicated by the dashed vertical lines: 8.6 km ± 1.0 km SE for *P. maculatus* and 7.4 km ± 0.6 km SE for *L. carponotatus*). Each histogram bar is divided according to the number of assigned juveniles that returned to natal reserves (white), the number that dispersed from one reserve to another reserve (gray) and the number that dispersed from reserves to fished areas (black). (C) The distribution of available reef area open to fishing that surrounds each of the six marine reserves is within the mean dispersal range of both species (dashed vertical lines).

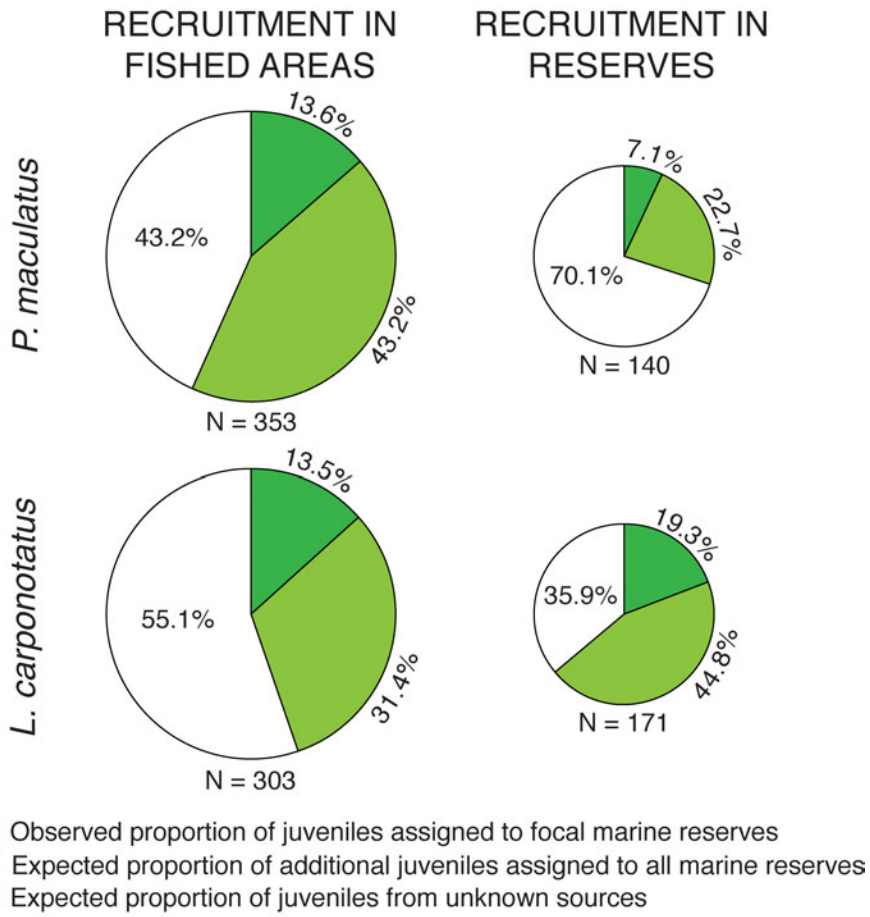

Figure 5 Local recruitment contribution from the reserve network. Observed (dark green) and estimated (light green) contribution of six marine reserves to local recruitment of coral trout and stripey snapper in fished and protected areas of the Keppel Islands. Proportions are based on the number of assigned juveniles relative to the total number of juveniles (*N*) collected in reserves and fished area. The estimated proportion of additional recruitment accounts for both unsampled adults in the three focal reserves and the three unsampled reserves. Pie charts are scaled relative to the size of available coral reef habitat in reserves (28%) and fished areas (72%).

Recruitment contribution of the reserve network

We estimated that the six reserves in the Keppel Islands, which represent ~28% of the reef habitat in the Keppel Islands, supplied ~50% of the total recruitment in the region. This estimate accounted for both the expected contribution of unsampled adults in the three focal reserves and the additional reef area within the three other unsampled reserves. We assigned 11.8% (58 of 493) of juvenile coral trout and 15.6% (74 of 474) of juvenile stripey snapper to known parents in the focal reserves. However, given that we sampled ~26.9% of adult coral trout

and ~35.7% of adult stripey snapper within the focal reserves, a significant number of the unassigned juveniles would have been the progeny of unsampled adults within those reserves. Taking these additional adult fish into consideration, we estimate that the three focal reserves accounted for ~25% of juvenile coral trout and ~27% of juvenile stripey snapper in our samples. Furthermore, if we also consider that the three focal reserves represented ~51.3% of total coral reef habitat within all six reserves, and assume that the three unsampled reserves made the same proportional contribution to recruitment per unit area as the sampled reserves, then the six reserves would have accounted for ~49% of coral trout and ~52% of stripey snapper recruitment in the region (see eq. 1 & 2).

The contribution of reserves to total regional recruitment for the two species was further subdivided to estimate the relative contribution to both fished areas and reserves (Figure 4). Applying the same calculations as above, our findings indicate that adults in reserves were making a large contribution to the replenishment of populations on both reserve and fished reefs in the Keppel Islands. Of the 353 juvenile coral trout and 303 juvenile stripey snapper that were collected from reefs that are open to fishing, we estimate that the six reserves accounted for ~57% of coral trout and ~46% of stripey snapper recruitment (Figure 4). Similarly, we estimate that within reserves, 29% of coral trout recruitment and 62% of stripey snapper recruitment was supplied through self-recruitment to natal reserves or through larval exchange between reserves (Figure 4). The remaining juveniles are likely to be the progeny of unsampled adults within fished areas of the Keppel Island group or immigrants from distant reefs outside the island group.

DISCUSSION

Our study confirms that effective reserve networks can provide a significant source of recruitment to populations in both fished and protected areas on a regional scale. Not only were adults in reserves exporting a high proportion of their offspring to adjacent fished areas, there was also significant larval retention within natal reserves and connectivity among neighbouring reserves.

Furthermore, the proportion of observed dispersal trajectories less than 30km is consistent with recent studies demonstrating that coral reef fish larvae may disperse relatively short distances despite spending several weeks in the pelagic environment (Jones *et al.* 2009).

The estimate that reserves contribute about half of the total recruitment in the Keppel Islands is clearly important, given that only 28% of reef area in the region is protected. This represents a ~1.8 fold increase in recruitment over that expected based simply on the area of reef within reserves. The significant role of reserves as sources of juvenile recruits in both fished areas and in reserves is likely due to the approximately two-fold greater adult biomass inside reserves (Figure 2), the larger average adult size inside reserves, and as a consequence, greater per capita and per unit area fecundity relative to adjacent fished populations (Evans *et al.* 2008).

Theoretical considerations highlight the importance of both self-recruitment and connectivity in ensuring metapopulation persistence in reserve networks (Botsford *et al.* 2003; Hastings and Botsford 2006; Steneck *et al.* 2009; Almany *et al.* 2009). It is therefore encouraging that our study documented both self-recruitment to natal reefs and connectivity among reserves. Although the observed magnitude of the reserve contribution to recruitment in fished areas is consistent with modelling scenarios (Pelc *et al.* 2010), the magnitude of larval supply from reserves may not be sufficient to offset a substantial increase in fishing pressure outside reserves (Hilborn *et al.* 2004). We recommend that reserves continue to be coupled with traditional harvest restrictions including fish size and catch limits, and seasonal spawning closures (McCook *et al.* 2010) to ensure that current yields for these fishes are sustainable.

While the proportion of coral reef habitat in our GBR study area designated as no-take reserve is comparatively high in global terms (Mora *et al.* 2006; Wood *et al.* 2008; McCook *et al.* 2010), it is important to note that our results clearly demonstrate that reserves can provide significant fishery and conservation benefits on a scale as small as 10 km. In many places where people rely heavily on coral reefs for their livelihoods, this scale is typical of reef tenure areas and the only scale at which marine reserves can realistically be applied (McCook *et al.* 2009). The fact that stakeholder communities can directly benefit from a source of

recruitment from their local reserves is the strongest support yet that reserve networks can be an effective tool for sustaining future generations of both fish and fishers.

CHAPTER 5 – CONSISTENT SPATIAL, TEMPORAL AND ONTOGENETIC PATTERNS IN THE RECRUITMENT OF JUVENILE FISHES SOURCED FROM MARINE RESERVES

ABSTRACT

With time, no-take marine reserves typically result in greater densities and larger sizes of targeted fishery species within their boundaries. Consequently, they generate a significant source of juvenile recruitment to adjacent fished areas and other reserves. The benefits of networks of marine reserves are greatest if the supply of juveniles is consistent with the growth of populations in reserves. However, little is known of the spatial and temporal patterns of larval supply from reserves, or the relationship between adult body size and reproductive success of any fish species. Here, I investigate spatial and temporal recruitment dynamics of coral trout (*Plectropomus maculatus*) and stripey snapper (*Lutjanus carponotatus*) throughout the Keppel Island group, a 1,000 km^2 archipelago of the Great Barrier Reef, Australia. During the 15-month study period, three juvenile cohorts of both species recruited to the island group of which a large proportion were known to have originated from local no-take marine reserves. I show that cohorts were genetically homogeneous, spatially consistent and largely locally sourced throughout the duration of the study. Although adult fish of all mature size classes contributed to local recruitment, I show for the first time that larger individuals were responsible for a greater proportion of local recruitment. Based on the relative abundance and size distributions of reproductively mature fish in reserves and fished areas, I conclude that reserves made a two-fold greater contribution to recruitment. These findings emphasise the value of combining current fisheries management measures with networks of no-take marine reserves to sustain juvenile recruitment.

INTRODUCTION

It is now widely appreciated that for many large, exploited fishes, establishing no-take marine reserves can lead to higher densities and larger size of fish in protected areas (Halpern *et al.* 2003; Russ *et al.* 2008; Lester *et al*. 2009; Babcock *et al.* 2010). As a major source of juvenile fish (**Chapter 4**), reserves play an important role in the long–term conservation of fish populations within reserves and in sustaining fish stocks in areas open to fishing (Pauly *et al.* 2002; Gell and Roberts 2003; Lubchenco *et al.* 2003; Gaines *et al.* 2010). Networks of marine reserve continue to be designed and implemented on the basis that juveniles sourced from reserves can be retained in natal reserves (Almany *et al.* 2007; **Chapter 4**), exported to fished areas (Christie *et al.* 2010a; **Chapter 4**) and exchanged between reserves (Planes *et al.* 2009; **Chapter 4**). However, the effectiveness of reserves as sources of recruitment assumes that they are located at important source sites, and the supply of juveniles is consistent with the growth of populations in reserves. Some empirical evidence is accumulating that patterns of larval retention and connectivity in small reef fishes may be persistent over time (Berumen *et al.* 2012; Saenz-Agudelo *et al.* 2012). However, virtually nothing is known of the relationship between the demographic distribution of source populations and the spatial or temporal patterns of larval supply.

Coral reef fish populations are distributed over a patchy seascape, and it is now recognised that populations on individual reefs are maintained by both self-recruitment and larval connectivity between reefs (Almany *et al.* 2009; Jones *et al.* 2009). Optimizing spatial management systems for both conservation and fishery management objectives require that reserves are placed at important source sites (Jones *et al* 2007; 2009) and that the recruitment contribution of reserves persists over time (Steneck and Wilson 2010). Juvenile recruitment is a major driver of reef fish dynamics (Doherty and Williams 1988; Doherty 2002), though the successful dispersal and recruitment (settlement to the reef) of pelagic larvae are reliant on the reproductive success of adult fishes (Robertson 1988; Meekan *et al.* 1993; Marshall and Morgan 2011). However, relatively little is known of spatial origin of larval cohorts that regulate population dynamics in coral reef fish populations. Recruitment in reef fishes is inherently variable (Fogarty *et al.* 1991*;* Caley *et al.*

1996; Doherty 2002; Armsworth 2002), and therefore the source and identity of adults contributing to recruitment may also be highly variable (Hedgecock 1994; Hedgecock and Pudovkin 2011).

Few studies have investigated parental and genetic variation in the composition of successive juvenile cohorts in coral reef fishes. Recruitment that is driven by only a few successfully breeding individuals would result in high variance in reproductive success, otherwise known as 'sweepstakes reproductive success' (Hedgecock 1994; Hedgecock and Pudovkin 2011). In theory, large variance in reproductive success would lead to temporal genetic differentiation between cohorts of recruits and the breeding population. A number of studies have documented lower allelic diversity between recruits and adults (Hegdecock 1994; Flowers *et al.* 2002), reduced effective breeding population sizes (Hedgecock 1994; Turner et 2002; Hedgecock et al 2007; Lee and Boulding 2007; Liu and Ely 2009), higher relatedness within cohorts (Planes and Lenfant 2002), or high variance between cohorts of newly settle recruits (Hedgecock 1994; Flowers *et al.* 2002; Christie *et al.* 2010b). However, few studies have examined the contribution of known adults in marine reserves to multiple cohorts of juveniles recruiting to the population.

Since fecundity in reef fishes increases with body length, it is generally assumed that larger fish make a disproportionate contribution to juvenile recruitment (Jennings 2001; Sadovy 2001). Because marine reserves allow fishes to reach larger sizes (Halpern *et al.* 2003; Russ *et al.* 2008; Lester *et al*. 2009; Babcock *et al.* 2010), they may provide benefits above and beyond the simple increases in fish density. However, whether larger adults in marine reserves account for a great proportion of total recruitment than smaller adults remains unknown. Furthermore size selective targeting of larger fishes may have a disproportionate impact on juvenile recruitment (Sadovy 2001; Anderson *et al.* 2008; Fenberg and Roy 2008). Fisheries management strategies often include a minimum legal length limit that is usually set above the size at maturity. The objective is to ensure that a proportion of the spawning stock biomass is protected from fishery mortality, and assumes that adults below the legal catch size limit also contribute to recruitment. However, the relative contribution to recruitment of adults from above and below the size limit has never been established.

The Coral Reef Fin Fish Fishery (CRFFF) on the Great Barrier Reef (GBR) arguably employs one of the world's most comprehensive fisheries management strategies. It includes limits on the number of commercial licenses, fishing capacity (boat size, number of dories and number of fishers), individual catch quotas for commercial fishers, minimum size limits, possession limits for recreational fishers, and seasonal spawning closures (focused on coral trout). In addition, the GBR Marine Park is managed using multiple-use zoning plan that includes a network of no-take areas covering approximately 33% of the coral reef area. All extractive activities (primarily fishing) are thus restricted to 67% of all available reef habitat in the Marine Park. The no-take reserves are the only means to protect the full demographic length and age distribution of adult fishes and this may have an important influence on shaping local demographic processes of coral reef fishes.

The paucity of dispersal and connectivity information has hindered efforts to effectively design networks of no-take reserves, and subsequently, to evaluate the effectiveness of reserves in meeting conservation objectives. Recent applications of genetic parentage analysis have shown that it is possible to match newly recruited juvenile individuals to their parents, and thus the relationship between adult body length (and/or age) and the successful supply of juveniles to populations can now be addressed. Here, I examine the spatial and temporal patterns of recruitment and their relationship with adult body size for the bar-cheek coral trout (*Plectropomus maculatus*) and the stripey snapper (*Lutjanus carponotatus*), two important fisheries species in the Indo-Pacific region. Focusing on three marine reserves as a source of recruits for each of the species, I examine the following questions: (i) Are the observed spatial and temporal patterns of recruitment derived from adults in reserves persistent over time? (ii) Does the genetic composition of multiple cohorts indicate consistent patterns of larval supply? (iii) Do larger adult fishes account for a greater proportion of local recruitment (settlement) than smaller adult fish? (iv) Do fish below the legal length limit contribute to local recruitment? (v) How important are the more abundant and larger fish in reserves to local recruitment? These questions were addressed by applying genetic parentage to relate juveniles collected throughout the Keppel Island group, a 1,000 km^2 archipelago of the Great Barrier Reef, Australia.

MATERIALS AND METHODS

Spatial and temporal patterns in local recruitment

This study was carried out in the Keppel Island group (23°10´ S, 150°57´ E) in the Great Barrier Reef Marine Park, Australia. Sampling of adult coral trout (*Plectropomus maculatus*) and stripey snapper (*Lutjanus carponotatus*) focused on four reefs within three no-take marine reserves in the Keppel Island group. A total of 439 mature adult coral trout and 1145 stripey snapper of known length were captured between November 2007 and February 2008. Sampling effort was broadly representative of the abundance of adult fish at each location. The majority of fish were obtained by hook and line fishing (using barbless hooks), though a subset of 86 coral trout from Clam bay were obtained with the use of tissue biopsy probes (Evans 2008). Each captured fish was measured to the nearest millimeter and a small section of the dorsal or anal fin was removed prior to the fish being released alive. The total length (*TL*) of individuals sampled with biopsy probes underwater was estimated to the nearest centimetre by trained observers. Tissue samples were preserved in 85% ethanol for subsequent genetic analyses.

Following the sampling of adult fishes, 491 juvenile coral trout and 465 juvenile stripey snapper were collected from 19 locations throughout the island group. As with the adults, the sampling effort and the resulting number of samples was broadly representative of the abundance of juvenile fish at each location. The total length (*TL*) and fork length (*FL*) of each juvenile fish was measured to the nearest millimeter. Juvenile tissue samples were stored in 85% ethanol for genetic analyses. Sagittal otoliths (ear bones) were removed for ageing analysis and to determine the approximate spawn date of each juvenile fish. The age distributions indicated three distinct juvenile cohorts in the samples.

Genetic composition and source of successive juvenile cohorts

All adult and juvenile coral trout and stripey snapper were genotyped with a panel of 11 and 13 microsatellite markers, respectively, as described in **Chapter 3**. In each juvenile cohort, observed genotypes were tested for departures from Hardy-Weinberg equilibrium due to heterozygote deficiency at each locus as implemented in FSTAT v. 2.9.3.2 (Goudet 1995). Significance of multiple *P*-values (α = 0.05) was assessed with strict Bonferroni correction applied for multiple comparisons ($P < 0.005$; Rice 1989). The number of alleles, Nei's unbiased expected and observed heterozygosity (Nei 1987), and Weir & Cockerham's (Weir and Cockerham 1984) estimator of the inbreeding coefficient F_{IS} were calculated in FSTAT for each locus in each cohort. The occurrence of null alleles and large allele drop-outs were assessed at each locus using MICROCHECKER v.2.2.3 (van Oosterhout 2004). Analyses of molecular variance between cohorts were performed in ARLEQUIN v3.11 (Excoffier *et al.* 2005). The program GENECLASS 2 (Piry *et al.* 2004) was used to estimate the likelihood that juveniles originated from a population other than the local populations in the Keppel island group. I used the Bayesian assignment method of Rannala and Mountain (1997) and 10,000 simulations with the Monte Carlo re-sampling method of Paetkau *et al.* (2004) and calculated the mean probability of assignment across 5 runs, using a threshold of 0.05 below which individuals were considered as likely immigrants.

For both coral trout and stripey snapper, parent-offspring relationships were identified in each cohort against the total pool of sampled adults to determine the recruitment contribution of reserves to successive cohorts. Parentage was assessed based on a maximum likelihood approach implemented in the software FaMoz (Gerber *et al.* 2003) as described in **Chapter 4**. The time of spawning for each assigned juvenile was then compared to the total pool of juveniles for each species.

Relative recruitment contribution of different size classes of adult fish

The contribution of adult fish to local recruitment was estimated based on the number of juvenile assignments to different size classes. The size frequency distributions of sampled and assigned adults in each population were compared using two-way Kolmogorov-Smirnov tests performed in R v 2.15.0 (R Development

Core Team) to determine the relative recruitment contribution of individual size classes. The size of first reproduction, where 50% of individuals reach maturity, is ~31.5 cm TL for coral trout, *P. maculatus* (Ferreira 1993), and ~20, cm TL for stripey snapper, *L. carponotatus* (Kritzer 2004). In Queensland, the minimum legal catch size for coral trout is 38 cm (TL) and for stripey snapper it is 25 cm (TL).

Relative recruitment contribution from no-take reserves and fished areas

To estimate the relative contribution of each size class to the overall juvenile recruitment (R_{Rec}) in the Keppel island group, I first measured the reproductive success of adult fish as the number of adults to which offspring were assigned to (N_A) relative to the number of adults sampled (N_S) in each size class. Based on the underwater visual census of fish population at 22 sites in fished and protected populations in the Keppel Island (see **Chapter 4** for details), I then estimated the proportion of adult fish of each size class in fished and protected populations (N_{Obs}/N_{Total}). Assuming that the per capita recruitment contribution was the same in both fished areas and reserves, we then calculated the relative recruitment contribution from each size class of adults in fished areas and reserves.

$$\boldsymbol{R_{Rec}} = \frac{\boldsymbol{N_A}}{\boldsymbol{N_S}} \times \frac{\boldsymbol{N_{Obs}}}{\boldsymbol{N_{Total}}}$$

Where:

N_A = Number of assigned adults in individual size classes

N_S = Number of sampled adults in individual size classes

N_{Obs} = Number of observed adults in individual size classes in either fished of protected areas

N_{Total} = Total number of observed adults in both fished and protected areas

RESULTS

Spatial and temporal patterns in local recruitment

Juvenile coral trout and stripey snapper were collected throughout the island group from all locations with suitable recruitment habitat. For each species, samples were unevenly distributed with the majority of juveniles collected from three key recruitment locations ('hotspots'). Collectively, Clam Bay, Halfway Island and North Keppel Island represented 79% of sampled juvenile coral trout (Table 1) and 69% of sampled juvenile stripey snapper (Table 2). Other important areas of juvenile recruitment included Middle Island and Humpy Island, which together, represented 15% of sampled juvenile coral trout (Table 1) and 17% of sampled juvenile stripey snapper (Table 2). The remainder of each sample was distributed across 8 and 10 locations, respectively.

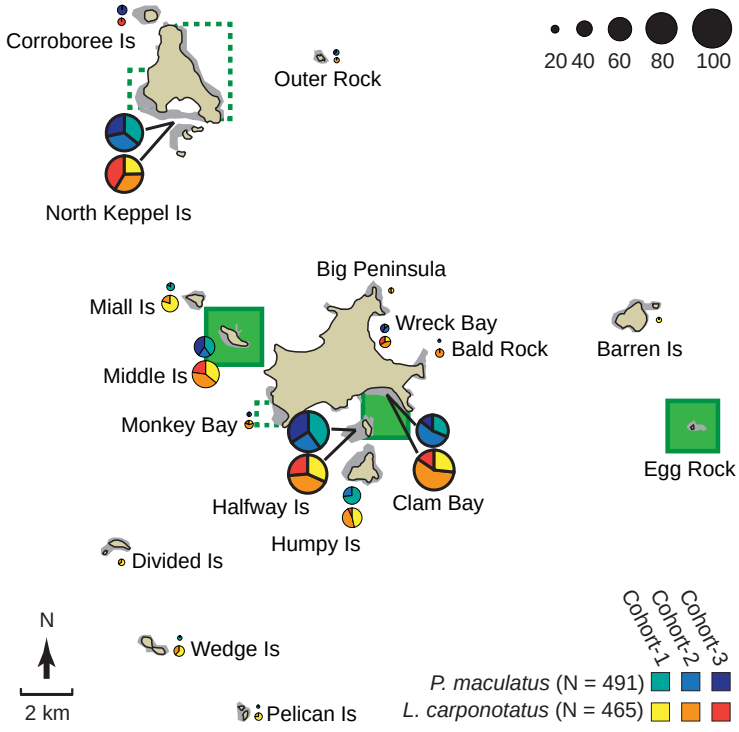

Figure 1 The Keppel Islands include six no-take marine reserves protecting 28% of coral reefs. Adult *P. maculatus* and *L. carponotatus* were sampled in three focal reserves represented by green boxes and juvenile fish of each species were collected in 19 locations throughout the island group. The size of pie charts is relative to the number of juveniles collected for each cohort and recruitment hotspots are represented in dark circles. Grey areas represent coral reefs and green dashed lines represent other reserves in the island group.

Based on the estimated time of spawning of each juvenile fish, three distinct recruitment cohorts were identified within the juvenile sample for both species. All major recruitment hotspots persisted throughout successive cohorts, thus spatial patterns of recruitment in the Keppel Islands were unevenly distributed between locations but temporally consistent within locations. The time and duration of spawning events varied between species. For coral trout, each cohort was generated over the course of 3 to 5 months with a peak in December 2007, May/June 2008 and September/October 2008 (Figure 2A). The average fork length of the 491 sampled juvenile coral trout was 153.2 mm ± 3.1 SE, which corresponded to an age of 183 days ± 4 SE. For stripey snapper, each cohort was generated over the course of 56 to 10 months with a peak in August/September 2007, February through to April 2008 and November 2008 (Figure 2B). The average fork length of the 465 sampled juvenile stripey snapper was 119.4 mm ± 2.4 SE, which corresponded to an age of 208 days ± 5 SE.

Table 1 Proportion of the total number of juvenile *P. maculatus* (N = 58) assigned to adults in four protected populations in the Keppel Island group.

	Middle Is (N = 115)	**Halfway Is (N = 105)**	**Clam Bay (N = 153)**	**Egg Rock (N = 66)**	**Total**
Cohort 1	6.9	6.9	15.5	5.2	34.5
Cohort 2	12.1	3.4	8.6	15.5	39.6
Cohort 3	6.9	5.2	10.3	3.4	25.9
Total	25.9	15.5	34.5	24.1	

Table 2 Proportion of the total number of juvenile *L. carponotatus* (N = 74) assigned to adults in four protected populations in the Keppel Island group.

	Middle Is (N = 430)	**Halfway Is (N = 374)**	**Clam Bay (N = 165)**	**Egg Rock (N = 66)**	**Total**
Cohort 1	16.2	14.9	6.8	1.4	39.2
Cohort 2	13.5	16.2	8.1	5.4	43.2
Cohort 3	10.8	4.1	1.4	1.4	17.6
Total	40.5	35.2	16.2	8.1	

Using parentage analysis, 58 juvenile coral trout were assigned to 56 adults, and 74 juvenile stripey snapper were assigned to 77 adults resident in the three focal reserves. In each cohort, the number of assignments was proportional to the number of sampled juveniles. Overall, the parent, or parents of 11.3% ± 0.9% SE of juvenile coral trout (Figure 2C) and 15.0% ± 1.7% SE of juvenile stripey snappers (Figure 2D) were identified across all three cohorts. Across the three sampled cohorts, the greatest proportion (34.5%) of assigned juvenile coral trout were identified as the progeny of adults in the Clam Bay reserve. Similarly, adults at Middle Island accounted for 40.5% of assigned juvenile stripey snapper. However, relative to the number of adults sampled at each location, adult coral trout from Egg Rock generated nearly twice as many juvenile assignments than other locations. Across the three sampled cohorts, 21.2% of adults sampled at Egg Rock were identified as parents of assigned juvenile coral trout, compared to 11.6% ± 1.5% SE averaged across all other locations. In contrast, the relative recruitment contribution of adult stripey snapper was similar across all source populations with 6.8% ± 0.3% SE of sampled adults assigned as parents of juvenile fish.

Genetic composition and source of successive cohorts

The genetic composition of successive coral trout and stripey snapper recruitment cohorts suggests that the majority of juvenile fish were locally sourced with minimal immigration to the Keppel islands. Across all *P. maculatus* cohorts, the mean number of alleles and allelic richness per locus was 21.2 ± 0.5 SE and 20.4 ± 0.5, respectively, with average observed heterozygosity of 0.745 ± 0.004 SE and expected heterozygosity of 0.745 ± 0.004 SE (Table 3). Across all *L. carponotatus* cohorts, the mean number of alleles and allelic richness per locus was 15.7 ± 0.6 SE and 14.6 ± 0.1 SE, respectively, with average observed heterozygosity of 0.745 ± 0.003 SE and expected heterozygosity of 0.756 ± 0.006 SE (Table 4). No departure from Hardy-Weinberg expectations was detected after correcting for multiple comparisons in any of the cohorts or when samples were pooled for each species. Differences in genetic diversity between recruitment cohorts were low (F_{ST} < 0.001) and largely insignificant ($p > 0.1$). Only coral trout cohorts 2 and 3 were significantly different from one another (F_{ST} = 0.001, $p = 0.029$), though these differences were not confirmed by an analysis of molecular variance ($F_{ST} < 0.0001$,

p = 0.417). Furthermore, assignment tests identified 31 juvenile coral trout and 4 juvenile stripey snapper as unlikely to have originated from populations in the Keppel Island group, representing a likely immigration rate of 6.4% ± 0.9% per cohort for coral trout and 0.8% ± 0.2% per cohort for stripey snapper. The low genetic diversity between cohorts and low immigration rates indicate that the large majority of juvenile coral trout and stripey snapper in the Keppel islands were likely to be the progeny of local adult populations.

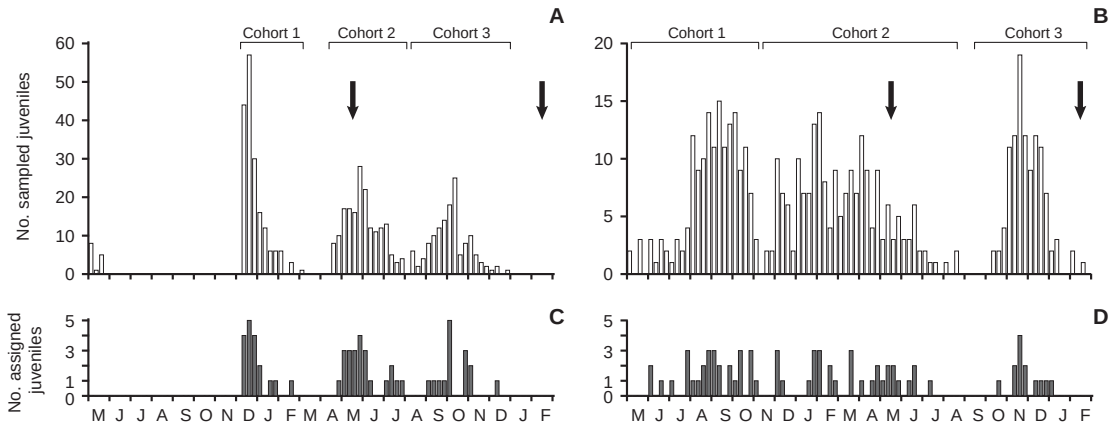

Figure 2 Time of spawn of sampled juvenile fish between May 2007 and February 2009. A total of 493 juvenile *P. maculatus* (**A**) and 465 juvenile *L. carponotatus* (**B**) were collected during two collection events in March 2008 and February 2009 (indicated by arrows). Time of spawning was inferred from daily ring counts of juvenile otoliths suggesting that three cohorts of each species settled during the study period. Parentage assignments identified 58 *P. maculatus* (**C**) and 74 *L. carponotatus* (**D**) as the progeny of adult fish collected in three focal reserves in the Keppel Islands. Time of spawning was inferred from daily ring counts of juvenile otoliths suggesting that three cohorts of each species settled during the study period.

Table 5 Number of juvenile *P. maculatus* in three successive recruitment cohorts in the Keppel Islands.

Location	Cohort			Grand Total
	1	2	3	
Bald Rock		1		1
Clam Bay	31	53	14	98
Corroboree Is			9	9
Divided Is	1			1
Halfway Is	64	41	54	159
Humpy Is	22	8		30
Miall Is	5	1		6
Middle Is	17	8	17	42
Monkey Bay		1	1	2
North Keppel Is	47	47	37	131
Outer Rocks		2	1	3
Wedge Is	2			2
Wreck Bay	1	3	3	7
Grand Total	190	165	136	491

Table 6 Number of juvenile *L. carponotatus* in three successive recruitment cohorts in the Keppel Islands.

Location	Cohort			Grand Total
	1	2	3	
Bald Rock		5		5
Barren Is	2			2
Big Penninsula	1	1		2
Clam Bay	31	67	18	116
Corroboree Is			4	4
Divided Is	3	1		4
Halfway Is	34	45	28	107
Humpy Is	13	13	2	28
Miall Is	16	4		20
Middle Is	19	22	12	53
Monkey Bay	1	3	1	5
North Keppel Is	24	33	40	97
Outer Rocks		2		2
Pelican Is	2	1		3
Wedge Is	5	3		8
Wreck Bay	2	4	3	9
Grand Total	153	204	108	465

Relative recruitment contribution of different size classes

Comparing the size frequency distributions of all sampled adults with adults that were assigned offspring revealed that larger individuals clearly generated a greater proportion of recruitment in the Keppel Islands. Overall, a third of all assigned juveniles were produced from the top quarter of the demographic size distribution suggesting that, where present, larger individuals tended to have greater reproductive success. For coral trout, 35.7% of assigned juvenile fish were the progeny of adults that were larger than 53 cm TL, which represented 27.6% of the total adult population sampled within reserves (Figure 3A). Comparatively, for stripey snapper, 36.4% of assigned juveniles were the progeny of adults larger than 35 cm TL, which represented 25.9% of the total adult population sampled in reserves (Figure 3B). Furthermore, 87.9% ± 8.3% SE of juvenile coral trout were assigned to adults larger than 38 cm TL, and 99.2% ± 0.8% SE of juvenile stripey snapper were assigned to adults larger than 25 cm TL, the respective legal catch lengths for these species in Queensland (Figure 3). However, it is notable that all individuals of reproductive size made some contribution to recruitment.

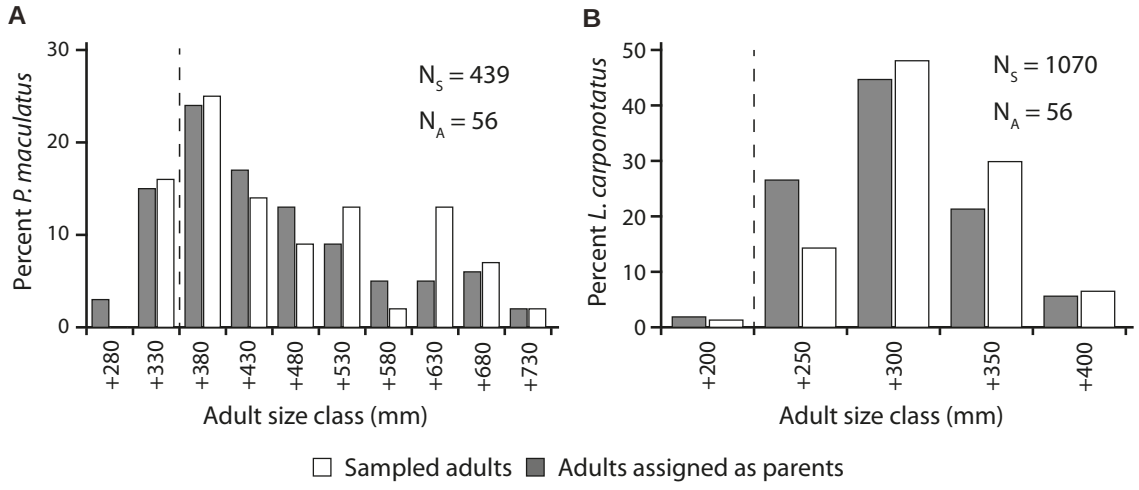

Figure 3 Overall length frequency distribution of sampled and assigned adults in populations of *P. maculatus* (**A**) and *L. carponotatus* (**B**) in no-take marine reserves of the Keppel Islands. For The number of sampled (N_S) and assigned (N_A) adults is shown for each location. Dashed lines represent the minimum legal catch length for each species.

The length-frequency distributions of sampled adults were significantly different amongst the four reefs in the three focal reserves (Figure 4; 2-way Kolmogorov-Smirnov test, $p < 0.05$). However, in all but one location, length-frequency distributions of assigned adults were not only distributed across all size classes but were also representative of the distribution of adults sampled (Figure 4; KS: $p > 0.1$). These results would suggest that the reproductive success of populations is influenced by the their demographic distribution. At Halfway Island, the distribution of assigned stripey snapper was significantly different from the distribution of sampled adults (Figure 4B-b; KS: $D = 0.25$, $p = 0.03$), clearly demonstrating that larger fish contributed disproportionately to local recruitment. This skew in reproductive success may also be observed at Egg rock (Figure 4A-d), where coral trout are much larger, which resulted in the greatest per-capita contribution to local recruitment relative to the number of individuals sampled.

Relative recruitment contribution protected and fished populations

Assuming that adult fish within both fished areas and reserves had equal probability of contributing to local recruitment, I estimated the relative recruitment contribution of fished and protected areas in the island group based on their overall demographic distributions. At the time of sampling, the abundance of mature coral trout and stripey snapper was 2-fold greater in reserves than in fished areas (Figure 5). Furthermore, the abundance of mature fish in reserves relative to fished areas increased for larger size classes. Based on the relative length-frequency distribution and the per capita recruitment contribution for each size class, I estimated that, for coral trout, a ~1.70-fold greater overall abundance of mature fish in reserves translated into an overall ~1.72-fold greater recruitment contribution to local reefs (Figure 5C). Similarly, for stripey snapper a ~1.90-fold greater abundance of mature fish in reserves translated into a ~1.99-fold greater recruitment contribution to local reefs (Figure 5D).

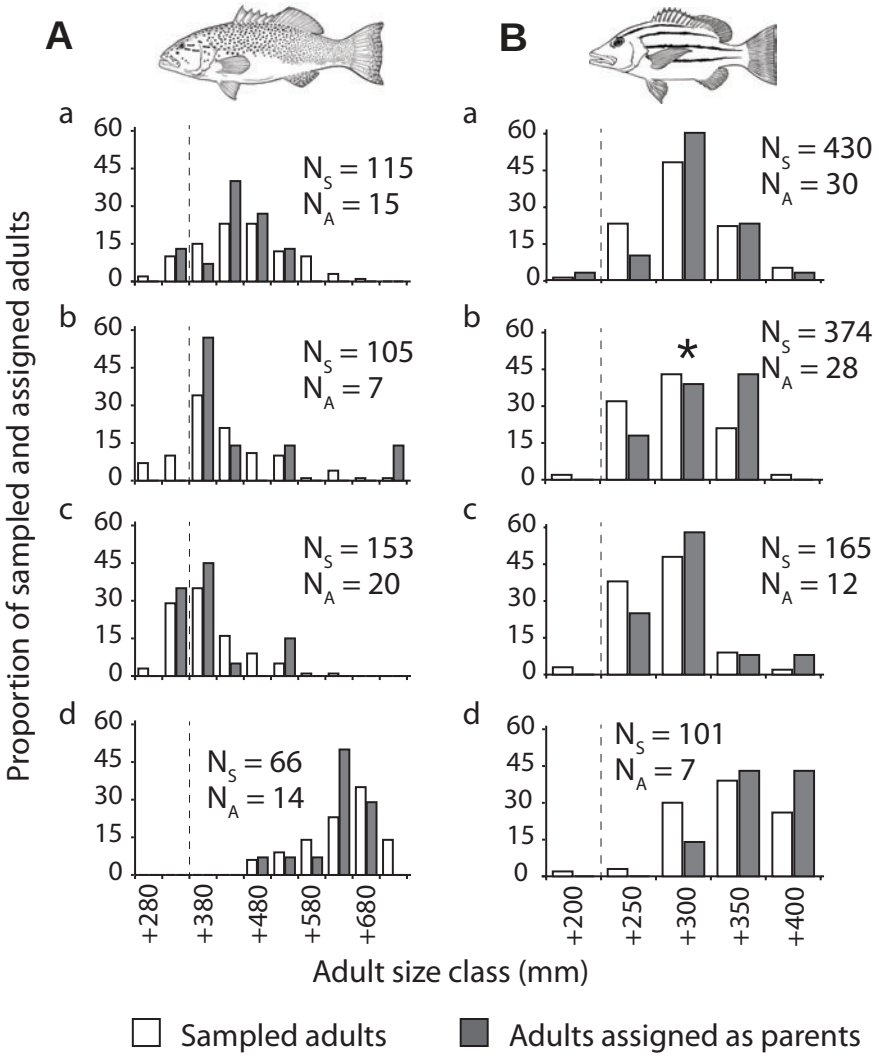

Figure 4 Length frequency distribution of sampled and assigned adults in populations of *P. maculatus* and *L. carponotatus* in no-take marine reserves of the Keppel Islands. Four populations of *P. maculatus* (**A**) and *L. carponotatus* (**B**) are represented in three reserves of the island group. The number of sampled (N_S) and assigned (N_A) adults is shown for each location. Dashed lines represent the minimum legal catch length for each species. Locations are (a) Middle island; (b) Halfway island; (c) Clam bay; (d) Egg rock.

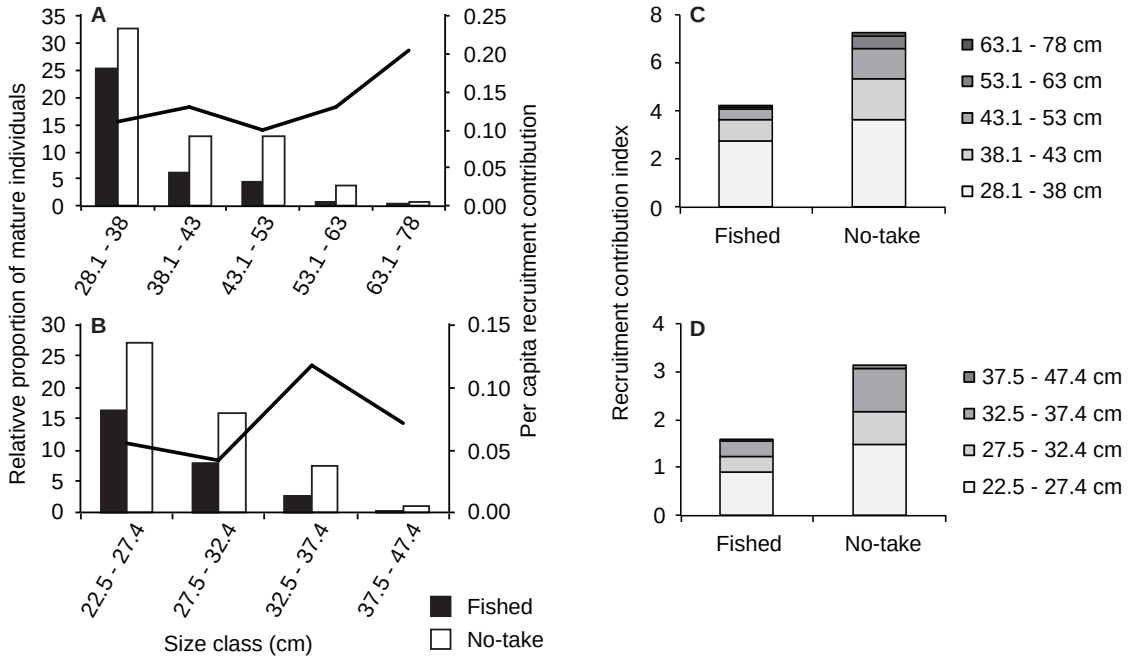

Figure 5 Relative reproductive success of mature size classes in fished and no-take areas. The per capita reproductive success was infered from the number of adults assigned as parents, and therefore known to have contributed to local recruitment. Assuming that the relative proportion of mature coral trout (**A**) and stripey snapper (**B**) were representative of the populations in fished an no-take areas in the Keppel Islands, I measured their relative recruitment contribution from the per capita reproductive success for individual size classes of *P. maculatus* (**C**) and *L. carponotatus* (**D**).

DISCUSSION

This study provides a unique insight into the spatial, temporal and ontogenetic patterns of recruitment for two commercially and recreationally important fish species. Our findings suggest that the supply of juveniles from the focal reserves was consistent over three successive cohorts of both species, with most recruits settling at back reef "recruitment hotspots". Successive cohorts were genetically homogeneous, suggesting that the adult source population supplying most of the juvenile recruitment was consistent and likely to be largely local. Furthermore genetic parentage demonstrated that larger adults in reserves generated the greatest proportion of local recruitment than smaller adults. Although catch length limits were effective at protecting a proportion of the reproductively active individuals in the population, individuals above the minimum catch length

supplied the vast majority of local recruitment. Our results indicate that reserves in the Keppel islands provide a refuge to spawning stocks, which provide regular and consistent recruitment to the island group.

Consistent spatial recruitment patterns

This study provides the first empirical assessment of dispersal and recruitment patterns for two important fisheries species of the GBR at the scale of a single island archipelago. Overall, observed patterns of larval dispersal were spatially consistent between species and among successive cohorts. Juvenile recruitment of both coral trout and stripey snapper was concentrated in the same three recruitment hotspots: Clam Bay, the western side of Halfway island, and the southern side of North Keppel island. These are all sheltered locations, with reef flat habitat and areas that are dominated by small patch reefs amongst rubble and sand. Other work has identified patchy reef habitats to be the preferred recruitment habitat for coral trout and stripey snapper (Wen *et al.* 2013a, 2013b). While these recruitment hotspots are clearly important for the replenishment of local populations, smaller numbers of juveniles were found and collected wherever suitable habitat existed throughout the island group. The spatial distribution of recruitment and the habitat preferences of juveniles of both species suggest that sustained replenishment of these populations is limited by both the supply of juvenile fish and the availability of suitable recruitment habitat.

Spawning activity for coral trout and stripey snapper, as indicated by the back calculation of juvenile fish ages, was recorded across three periods that each lasted several weeks. These observations are consistent with spawning periods from September to December previously described for these species at other inshore island groups of the central and northern GBR (Ferreira 1993; Kritzer 2004). For most coral reef fish, spawning is triggered by increases in water temperatures, typically during the warmer austral summer. However, in this study, spawning activity was also recorded during the austral winter. While the reproductive stage of sampled female and male individuals was not directly assessed, the juvenile fish ageing results combined with the parentage analyses clearly confirms that coral trout and stripey snapper in the Keppel Islands were generating local recruitment during both summer and winter. Queensland fishery

spawning closures that are in effect for 5 days around the new moons in October and November may therefore be insufficient to protect periodic spawning activity in the Keppel Islands and additional spawning closures in April, March and June may be beneficial for long-term population persistence and enhance local fisheries.

Temporal trajectories of juvenile recruitment

No genetic differences were identified between juvenile cohorts and the adult population, suggesting that all cohorts were primarily sourced from local populations. In addition, local marine reserves were projected to have generated the majority of juvenile recruitment across successive cohorts (see **Chapter 4**). While regular immigration from other inshore and mid-shelf reefs is possible, only a small number of individuals in each cohort were genetically distinguishable from the local populations and may also be the progeny of resident immigrant adults. It is clear from these results that local demographic processes and persistent self-recruitment at the scale of the island group are the most important factors regulating the replenishment of local populations.

The high level of local recruitment and the temporal stability of recruitment patterns observed during the course of this study indicate that the processes that drive the replenishment of populations at the scale of the island group are temporally persistent, at least over the 15-month duration of this study. Other studies have made similar observations for species with distinct reproductive strategies and at similar scales (Berumen *et al*. 2012; Saenz-Agudelo *et al.* 2012). However, at larger spatial scales, these same studies and others (Hogan *et al.* 2012; Christie *et al*. 2010b) also noted strong fluctuations in connectivity patterns. The scale at which these processes remain stable, and the intensity of fluctuations over larger distances has important implications for the management and conservation of coral reef fishes and the design of marine reserve networks. Assessing connectivity patterns over increasingly large spatial and temporal scales will be essential to provide a mechanistic understanding of larval connectivity for fisheries management and conservation planning.

Bigger fish provide greater contribution to local recruitment

Our results demonstrate that the contribution of coral trout and stripey snapper to

local recruitment increased with the body size of individuals. However, the relationship between size and reproductive success for either coral trout or stripey snapper varied amongst locations, suggesting that differences in the reproductive strategies may influence local recruitment patterns. Coral trout (*P. maculatus*) generally first mature as females and change sex to males later in life (protogynous hermaphroditism), although initial males are also known to occur (Ferreira 1993). In contrast, the sex of stripey snapper (*L. carponotatus*) is genetically predetermined (gonochorism) and individuals remain male or female throughout their lives. Male stripey snapper have been shown to grow faster and larger than females (Newman *et al.* 2000; Kritzer 2004), thus the patterns observed here may be partly attributed to a sex-biased differential in reproductive success (lottery polygyny; Nunney 1993). However, we do not know the sex of individual fish or whether the sex-ratio differed between the three focal reserves. Nevertheless, these are important factors that need to be considered in connectivity research and in the management of coral reef fisheries.

Of particular interest was the demographic distribution of sampled adults at Egg Rock, where mature coral trout and stripey snapper were on average much larger than in other reserves in the island group. While this translated into a greater recruitment contribution for coral trout, it was not significantly different from other reserves in the island group for stripey snapper. Since coral trout larger than 63 cm were likely to have been male (Ferreira 1993), the larger recruitment contribution of Egg Rock could be associated with a greater reproductive success of male individuals through some individuals spawning with several females. Alternatively, the larger mean length and greater abundance of large coral trout may increase the size of sex change from male to female. As large females are known to produce exponentially more eggs than smaller females, the overall reproductive output may increase several times overs. In contrast stripey snapper at Egg Rock did not make a disproportionately higher contribution to local recruitment than other reserves in the island group.

Legal catch size limits

Many fisheries impose a minimum legal size limit to allow individuals to reproduce at least once before they enter the fishery (Berkeley *et al.* 2004). However,

whether or not fish at this early stage of maturation actually contribute to recruitment has never been demonstrated. Here we show, that at least for coral trout, smaller individuals are making a sizeable contribution. However, overall, individuals above the legal size contributed the majority of local recruitment in the island group. Clearly, it is the populations in reserves, with full size and age distributions, which were essential in generating a regular supply of juvenile fishes to the Keppel Island group.

The role of marine reserves in fisheries management

Reproductive productivity is intrinsically linked to adult fish body size at an individual level, and spawning stock biomass at the scale of populations (Evans *et al.* 2008). Results presented here demonstrate that the size-distribution of local populations was an important factor in determining reproductive output from source populations. Clearly, the larger density and biomass of both coral trout and stripey snapper in reserves were responsible for generating the majority of local recruitment over three successive cohorts (see also **Chapter 4**). However, large differences in the abundance of smaller size-classes with low per capita reproductive success did not necessarily yield greater recruitment contributions. It appears that the accumulation of larger and older size classes of target fish in reserves was largely responsible for the approximately two-fold difference in the recruitment contribution of reserves relative to fished areas. The accumulation of larger and older size classes of target fish in reserves is clearly an important factor in generating a regular supply of juvenile fish in both fished and reserve areas of the island group and an important safeguard against recruitment overfishing in these populations.

Given the scarcity of information regarding source-sink dynamics of coral reef fish populations, the design and implementation of networks of marine reserves have been largely based on theoretical predictions of connectivity (McCook *et al.* 2010; Pelc *et al.* 2010). To fulfill both conservation and fishery objectives, a reserve network should ensure regular recruitment to both protected and fished areas. While it remains difficult to identify a general pattern of dispersal in the Keppel Islands, or determine whether reserves have altered source-sink dynamics, it is clear that the greater density and larger size of fish in reserves

represent important sources of juvenile fish for the island group. Critically, all recruitment 'hotspots' benefited from a regular supply of juvenile fish from multiple sources, thus enhancing the resilience of fish populations and fulfilling key objectives of the reserve network.

CONCLUSION

Understanding the relationship between the reproductive potential of adults, the dispersal trajectories of larvae, and the formation of cohorts that will subsequently join the fishable population is central for the effective management of coral reef fisheries, yet the processes that influence the success of any particular life history stage on another remain poorly understood. No-take marine reserves are currently the only management strategy that protects the full demographic structure of coral reef fish populations and should be considered an integral component of fisheries management strategies to fulfill both fishery and conservation objectives. Traditional fishery management approaches have not always been successful in preventing the decline of fish stocks (Pauly *et al.* 1998; Watling and Norse 1998; Jackson *et al.* 2001; Myers and Worm 2003; Beddington *et al.* 2007), and a more concerted effort that incorporates ecosystem processes is required (Roberts *et al.* 2005). Since there appears to be little insurance that distant populations can effectively replenish local populations in the Keppel Islands, reserves provide a critical safeguard against local recruitment failure.

CHAPTER 6 - GENERAL DISCUSSION

In this thesis, I have used genetic parentage analysis to resolve patterns of larval dispersal for two important fishery species in a network of no-take marine reserves within the Great Barrier Reef Marine Park (GBRMP), Australia. This required, in the first instance, an assessment of the suitability of different approaches to parentage analysis in large natural populations of coral reef fish, where complete sampling of adult populations was not possible. In **Chapter 2**, I measured the accuracy of three distinct approaches to parentage analysis where I found that the number and allelic diversity of microsatellite loci were the most important factors in identifying true parent-offspring pairs. These principles were applied in **Chapter 3** for the development of novel sets of microsatellite loci for *Plectropomus maculatus* and *Lutjanus carponotatus*, two significant fisheries species of the GBRMP. **Chapter 4** describes the first application of parentage analysis to identify the dispersal trajectory of commercially and recreationally targeted fish species from a network of no-take marine reserves in the Keppel Island group. The results provide the first conclusive evidence that adequately protected reserve networks can make a significant contribution to the replenishment of populations on both protected and fished reefs up to 30km from reserves. **Chapter 5** demonstrates that these reserves were a regular source of juvenile fish to the island group, where larger fish inside reserves made a disproportionate contribution to recruitment over three successive cohorts spanning 15 months. This thesis represents an important case study, which fills a major knowledge gap that has impeded wider acceptance of marine reserve networks as an effective strategy for biodiversity conservation and fisheries management.

INDIVIDUAL LEVEL GENETIC ANALYSES FOR CONNECTIVITY RESEARCH

Individual level genetic analyses, such as parentage analysis, have become an important tool in connectivity research. In **Chapter 2**, I consider some of the applications of parentage analysis, review different approaches to identify parent-offspring pairs, and identify key principles to maximise the accuracy of parentage studies in natural populations. The simulation framework demonstrates the capacity of parentage analysis to investigate ecological processes with high levels of accuracy. While incomplete sampling is considered an important limiting factor in likelihood-based parentage analysis, increasing the number and diversity of loci can reduce the susceptibility of these approaches to negligible levels. Nevertheless, obtaining large numbers of individuals remains an important challenge in applying individual level genetics in the marine environment. For most parentage studies, only ~10-15% of juveniles are assigned, suggesting that a large majority of parents remain unknown. Obtaining larger sample sizes of candidate parents obviously increases the number of possible assignments, but more importantly it offers a greater potential of identifying the full spatial scale of dispersal and demographic connectivity for any given species.

Since the inception of this thesis, methods to isolate microsatellite loci for non-model organisms have become faster and cheaper. Using available technologies, I characterise a novel suite of microsatellite loci for multiplex PCRs in *P. maculatus* and *L. carponotatus* (**Chapter 3**). For both focal species, several loci showed very high levels of polymorphism (> 30 alleles per locus), much higher than those simulated in **Chapter 2**. This allowed for fewer loci to be used in the parentage analyses and a greater number of individuals to be processed without compromising the accuracy of assignments. However, recent technical advances in the isolation of molecular markers, notably 454 sequencing, and the high-throughput screening of multi-locus genotypes allow for a much greater resolution of genealogical relationships among individuals. Parent-offspring pairs are the simplest and easiest of these relationships to identify since they share half of their genetic information and generations are easily distinguished. However, with greater genetic resolutions, full pedigree relationships within populations are not unfeasible. Individual level genetic analyses allow us to investigate demographic

processes, such as mating behaviour and reproductive success or 'intraspecific community structure' through kin-association, that have remained largely unknown for coral reef fishes. Simulations, as presented in **Chapter 2**, provide a simple framework within which to test these applications and identify the limitations of different genetic analyses.

DEMOGRAPHIC CONNECTIVITY AND THE DESIGN OF MARINE RESERVE NETWORKS

Recent advances in our understanding of the ontogenetic development of marine larvae indicate that they have the morphological and sensory aptitude to influence their own dispersal (Kingsford *et al.* 2002; Leis 2006). Larvae are capable of using both auditory (Simpson *et al.* 2005; Montgomery *et al.* 2006) and olfactory cues (Atema *et al.* 2002; Gerlach *et al.* 2007; Dixson *et al.* 2008) to identify suitable recruitment habitat (Lechini *et al.* 2005). Even after pelagic phases lasting days to weeks, some larvae are able to settle close to home (Jones *et al.* 1999; Swearer *et al.* 1999; Jones *et al.* 2005; Almany *et al.* 2007; Planes *et al.* 2009; White *et al.* 2010; Saenz-Agudelo *et al.* 2011, 2012), while others disperse to more distant reefs (Planes *et al.* 2009; Christie *et al.* 2010a). While these critical milestones have shaped current paradigms of population dynamics and connectivity of marine populations, there remains a lack of robust information with which to inform management strategies and achieve conservation goals (Willis *et al.* 2003; McCook *et al.* 2009).

As the main driver of connectivity in coral reef ecosystems, understanding the spatial scale of larval dispersal is essential to understanding how populations are regulated, but also to balance conservation and fishery objectives in the design of marine reserve networks. Marine reserves continue to be advocated and implemented to conserve biodiversity, restore degraded habitats, protect exploited populations, and in some cases, to supplement fisheries. However, the extent to which larval offspring are exported and the relative contribution of reserves to recruitment in fished and protected populations are unknown. **Chapter 4** and **Chapter 5** address these critical knowledge gaps that have so far limited our appreciation of the wider benefits of marine reserve networks. Results

from **Chapter 4** demonstrate that it is possible not only to investigate patterns of connectivity for large commercially important species, but also to measure the relative recruitment contribution of discreet populations. This study is the first to demonstrate an unequivocal link between adult populations of exploited reef fishes in reserves and juvenile fish within local reserves and fished areas. No-take marine reserves were shown to be providing an important source of local recruitment within the Keppel Islands, and it was evident that local management measures were directly benefiting local stakeholders.

It is likely that the disproportionate contribution of reserves to local recruitment observed in **Chapter 4** was largely due to a two-fold greater adult biomass in reserves. In theory, the larger average adult size inside reserves can dramatically increase the per capita and per unit area fecundity of reserves relative to adjacent fished areas (Evans *et al.* 2008). However the relationship between reproductive potential and the formation of juvenile cohorts that will subsequently join the fishable population remains unclear. **Chapter 5** is the first study to investigate this relationship and measure the relative reproductive success of individual adult size classes for large commercially exploited reef fishes. One of the main assumptions of size-selective fisheries that impose a minimum catch-size limit, is that mature adults will reproduce at least once before they reach the minimum legal length limit. Although it is assumed to occur, the degree to which sub-legal size fishes actually contribute to the replenishment of populations has not been empirically demonstrated and is generally not a realistic consideration of fishery management practices. It was encouraging that this study identified sub-legal sized coral trout and stripey snapper that had contributed to local recruitment, even though a large majority of assigned recruits were identified as the progeny of fish that were above the legal catch size. In addition, this study demonstrated that the large majority of recruitment in the Keppel Island group is generated from local populations, either protected or fished, with limited supply from populations further afield. This pattern persisted through three successive cohorts spanning a 15-month period. As a major and consistent source of local recruitment, reserves in the Keppel Islands represent an important safeguard that may complement current fishery management practices in the region.

FUTURE DIRECTIONS IN CONNECTIVITY RESEARCH

Otolith microchemistry, genetics and biophysical modelling have all become commonly employed tools in connectivity research. While each approach has received a great deal of attention, the rapid progress and technological innovations in the field of genetics make it an attractive option for future research. Recent technical advances in the identification and isolation of molecular markers have facilitated the development of large marker sets for non-model organisms. Furthermore, improved PCR chemistry and new genotyping technologies now allow high throughput screening of multi-locus genotyping at minimal cost. These advances not only broaden the scope of species that can be investigated and the number of samples that can be processed, but also opens the possibility of new applications and new questions to be explored. Ultimately, the goal of connectivity research for the next decade will be in providing empirical measures of connectivity to refine and validate indirect measures of connectivity, such as biophysical models, and generate a mechanistic understanding of larval dispersal and connectivity in coral reef fishes.

No study to date has been able to fully explain recruitment patterns and dynamics in coral reef fishes. There are always a large proportion of juveniles for which no source populations can be attributed. Characterising the full dispersal kernel and understanding the mechanisms that favour long distance dispersal over local retention remain major priorities in the field. These will require expanding both the spatial and temporal scale of field and laboratory studies to identify the factors affecting larval quality and dispersal potential. While these are important questions to address, more pressing questions remain regarding the role of marine reserves in achieving global conservation and fisheries objectives. While it is clear from this thesis that marine reserves can provide an important recruitment benefit to neighbouring fished areas, whether this represents a net increase in local fisheries output remains to be determined. Identifying whether reserves can offset displaced fishing effort will require careful consideration of ecological and socio-economic factors, notably catch effort, but also natural fluctuations in population sizes. Densities of top predators such as coral trout fluctuate in response to other factors, including habitat degradation (Williamson in prep). Hence, the

effectiveness of reserves is contingent on both biological and physical attributes of the reserve network that remain poorly constrained. Unlocking the full potential of marine reserves will require a more concerted effort that accounts for all these factors.

CONCLUDING REMARKS

Coral reefs ecosystems are facing an extensive array of cumulative impacts from both acute and chronic disturbances that, for the most part, can be attributed to human influence on the environment (Pandolfi *et al.* 2003; Hoegh-Guldberg *et al.* 2007; De'ath *et al.* 2012). While no-take marine reserves are unlikely to provide any protection from acute impacts such as crown of thorn outbreaks, coral bleaching, storm damage or flood plumes, they can reduce some of the chronic stressors such as commercial and recreational fishing, and provide refuges that enhance population recovery. This thesis represents a critical assessment of the effectiveness of marine reserves in generating larval subsidies to neighbouring fished areas through larval export. It is the first attempt at correlating the increased fish biomass in reserves, reproductive output, and recruitment dynamics for exploited fish species in both space and time. It demonstrates that marine reserves are not only critical conservation tools, but can also complement existing fisheries management practices to boost the sustainability of exploited fish populations.

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APPENDIX - PUBLICATIONS ARISING FROM THIS THESIS

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., Berumen M. L., Jones G. P. (2012). Larval export from marine reserves and the recruitment benefit for fish and fisheries. *Current Biology*, **22**, 1023-1028.

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