Estimating dispersal and population connectivity for temperate reef fishes at multiple spatial scales

Thesis submitted by
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Statement on the Contribution of Others and Declaration on Ethics

Drafts of the thesis were revised by Mike Kingsford, Tim Glasby, Michael Gillings, Heather Patterson, and Vanessa Miller-Sims. Development of microsatellite markers and resulting publications (Curley & Gillings 2004, Curley & Gillings 2006) resulted from collaborations with Michael Gillings at Macquarie University who provided technical expertise, support with laboratory work, and assisted with final interpretation and writing up of results.

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The research presented and reported in this thesis was conducted within the guidelines for research ethics outlined in the *National statement on Ethics Conduct in Research Involving Humans* (1999), the *Joint NHMRC/AVCC Statement and Guidelines on Research Practice* (1997), the *James Cook University Policy on Experimentation Ethics (Standard Practices and Guidelines)* (2001), and the *James Cook University Statement and Guidelines on Research Practice* (2001). The proposed research methodology received clearance from the James Cook University Experimentation Ethics Review Committee (approval number A827).

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'The relentless fury of the Sea
Knows none that it should fear,
A sullen thought is sacrifice
And in its rage will disappear'
MTB

Abstract

Knowledge of scales of dispersal and levels of population connectivity is critical for understanding population dynamics and effective management of reef fishes. These processes are important for effective design of Marine Protected Areas (MPAs) particularly if they are to generate 'spillover' and 'recruitment effects'. Despite this, empirical data across appropriate spatial and temporal scales are limited. This is the first study to focus on dispersal and population connectivity for temperate reef fishes in central NSW, Australia, at scales relevant to the implementation of MPAs (100's m - 100's km). The study provides: (1) empirical data on the localised benefits of small MPAs relative to the mobility of exploited reef fishes; (2) baseline data on the utility of different methods (microsatellite markers and otolith chemistry) for determining levels of population connectivity, and potential scales of benefits of MPAs to unprotected areas. Work on microsatellite markers compared population genetic structure in species which span the post-settlement dispersal potentials of reef fishes in this region, and provides a benchmark for understanding general mechanisms which govern gene flow, and population connectivity, in central NSW.

The response of exploited reef fishes to the establishment of small MPAs (≤ 0.2 km^2), was investigated relative to knowledge of post-settlement movement. Two established MPAs were surveyed: Cabbage Tree Bay (CTB) a 2.5 year old 'no-take' MPA, and Gordon's Bay (GB) a 12.5 year old MPA closed to spear fishing only. Abundances and sizes of four 'sedentary' and three 'mobile' fishes within each MPA were compared with three control locations at six times over two years. Temporal variation in abundances suggested that MPAs did not encompass the movement of most species, with the exception of two 'sedentary' species (*Cheilodactylus fuscus* and *Achoerodus viridis*). However, generalizations could not be made between estimated mobility, duration of protection and MPA response. Densities of legal-sized *C. fuscus* were 2.8-times higher and fish were larger within GB relative to controls. Legal *C. fuscus* were more abundant in shallow areas of GB indicating that spear fishing influences local depth distributions. Surprisingly, mean densities of legal-sized 'mobile' *Acanthopagrus australis* were 2.6-times higher in CTB relative to controls, with a similar trend for GB, and for *Girella tricuspidata* in CTB. Response of 'mobile' species to protection was indicative of pre-existing differences between MPAs and controls, immigration rather than recruitment of fish, and/or intraspecific variation in movement. The lack of detectable effect for all other species and differential response between MPAs were attributed to mobility relative to the scale of MPAs, inadequate protection of habitats or depths, population recovery time, and partial

protection versus 'no-take' status of MPAs. Overall results emphasise that small MPAs can have significant ecological value, even for highly mobile species. Importantly, as MPAs become smaller their location relative to habitat and depth, local aggregations, recruitment 'hotspots', adjacent habitats, and existing fishing pressure is critical in determining responses and rates of recovery.

Microsatellite markers were developed to provide information on population connectivity at scales ≤ 400 km for reef fishes with low (*Parma microlepis*) and high post-settlement dispersal capabilities (*G. tricuspidata*). It was hypothesized that *P. microlepis* would exhibit spatial genetic structure and a significant pattern of isolation-by-distance (IBD) at these scales, whereas *G. tricuspidata* would not. Genetic differentiation at seven microsatellite loci in *P. microlepis*, and six loci in *G. tricuspidata* were examined across multiple spatial scales. *P. microlepis* was collected from; sites (separated by 1-2 km), nested within locations (separated by 10-50 km), nested within three regions (separated by 70-80 km). *G. tricuspidata* were collected from a subset of the locations sampled for *P. microlepis.* This included five locations (separated by 50-60 km) spanning three sampling regions (separated by 70-100 km). There was no evidence that post-settlement dispersal capabilities influenced genetic structure. Broad-scale genetic homogeneity and lack of IBD was well supported for both species. The proportion of the total genetic variation attributable to differences among sampling regions, locations or sites was effectively zero (e.g. $\Phi_{PT} \leq 0.003$ and $R_{ST} \leq 0.004$). The geographic distribution of genetic diversity and the high polymorphism (*P. microlepis*, H_E 0.21-0.95; *G. tricuspidata*, H_E 0.65-0.97) was indicative of high mutation rates, large effective population sizes, and high rates of gene flow. Genetic homogeneity for fishes and invertebrates in central NSW suggests that gene flow important to genetic structure is driven by factors influencing pre-settlement dispersal such as the East Australian Current (EAC) and habitat continuity. Thus, genetic homogeneity is likely in other exploited reef fishes in this region which have similar pre-settlement durations (≥ 2 weeks). Scales of genetic homogeneity may not reflect demographically relevant dispersal distances. However, it does imply that populations of *P. microlepis* and *G. tricuspidata* are well connected from an evolutionary perspective and have large effective population sizes. This reduces the genetic risks associated with natural or anthropogenic declines in local populations. Furthermore, genetic diversity across spatial scales ≤ 400 km could be conserved within small MPAs as 99-100% of the total genetic variation for both species was represented within 1-2 km of reef. Future studies using genetics to determine population connectivity of reef fishes in central NSW should focus on species with very low

dispersal capabilities, small population sizes, short life spans, and whose habitats are rare or patchily distributed along-shore.

The use of otolith chemistry as a natural tag requires the presence of differences in the aquatic environment that translate into differences in otolith chemistry. Consequently, most studies focus on populations distributed across large environmental gradients and spatial scales. This study examined spatial variation in otolith chemistry of the territorial damselfish *P. microlepis* at fine spatial scales in an exclusively marine environment. Solution-based inductively coupled plasma-mass spectrometry was used to measure the integrated otolith chemistry of individual fish, reflective of average environmental differences among regions (separated by 70-80 km), locations within regions (separated by 10-50 km), and between sites within locations (separated by 1-2 km). Mean concentrations of Sr/Ca, Ba/Ca, Mg/Ca, Mn/Ca, Cu/Ca, and Zn/Ca and multi-element signatures varied among regions, locations and sites. Fine scale differences accounted for the majority of the variability in the data and there was a trend for unique chemistries at some sites and locations. Multi-element signatures were good spatial discriminators, with 75-80% of fish correctly classified to the regions in which they were collected. It was difficult to establish simple causal relationships for variation in individual elements. However, regional multi-element signatures were highly correlated with the behaviour of the EAC which delivers water masses varying in chemistry, temperature and salinity to the different regions. Results demonstrate that the magnitude of environmental variability within open coastal regions such as central NSW facilitates the use of otolith chemistry for determining population connectivity of reef fishes at scales < 100's km.

The thesis provides clear implications for management of reef fishes in central NSW, testable hypotheses, and priorities for future research. Overall results demonstrate the ecological value of small MPAs for protecting reef fishes of varying mobility, as well as population genetic diversity representative of broader-spatial scales. The determination of scales of 'spillover' of eggs, larvae and adults remains the greatest challenge. This study suggests that levels of gene flow will limit the utility of microsatellite markers for providing information on population connectivity for most reef fishes in central NSW. Given this, a combination of otolith chemistry, artificial tags, and modelling are the most promising techniques for future studies. Such studies should focus on species which demonstrated localised responses to MPA (e.g. *C. fuscus*, *G. tricuspidata*, and *A. australis*).

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Chapter 1: General Introduction

1.1. Introduction

Dispersal is an important life history trait. It influences the distribution and abundance of organisms, the dynamics and persistence of populations, assemblage structure, the level of gene flow between populations, local adaptation, speciation and the evolution of life-history traits (Slatkin 1987, Dieckmann et al. 1999). The relative importance of dispersal in contributing to ecological and evolutionary processes is determined by the species and the scale at which populations are defined and measured (Kingsford et al. 1998). For example, movement of individuals at small spatial scales may have an important influence on local population dynamics, while the rate of exchange of individuals across larger spatial scales determines whether local populations function as 'almost closed' or as a metapopulation (Sale 2004). Knowledge of dispersal across a range of spatial and temporal scales is, therefore, fundamental to a comprehensive understanding of population dynamics and for effective management of species. Although different models have been proposed to explain dispersal, we still have a poor understanding of the level of dispersal for many organisms and the relative benefits of dispersing in different ways (Dieckmann et al. 1999).

1.2. Management of reef fishes using Marine Protected Areas

Reef fishes are conspicuous faunal components of reef assemblages. Many species are harvested for food, sport and bait (Kingsford et al. 1991), leading to flow on effects for other organisms since fishes influence reef dynamics through herbivory, predation, feeding, excretion, and their role as prey (Kingsford 1998). Reef fishes have traditionally been managed across broad spatial scales (100 – 1000's kilometres) through the application of catch quotas and size restrictions (Bohnsack 1998). Recently, there has been a growing trend to manage reef fishes across smaller spatial scales (100-1000's metres) using Marine Protected Areas (MPAs) (Man et al. 1995, Jennings et al. 1996, Russ & Alcala 1996, Lauck et al. 1998, Westera et al. 2003). There has been considerable discussion on the importance of incorporating the ecology and life history traits of fishes into the design of MPAs (Dugan & Davis 1993, McNeill 1994, Gerber et al. 2002, Roberts et al. 2003), with a consequent increase in studies which collect and interpret data in this context (McNeill & Fairweather 1993, Curley et al. 2002, Griffiths

& Wilke 2002, Starr et al. 2002, Hoffman et al. 2005, Gladstone 2007). In practice, however, the location, size, and spacing of MPAs is primarily based on socio-economic factors rather than the conservation requirements of marine organisms (McNeill 1994).

Dispersal of fishes influences the effect of MPAs across a range of spatial scales. Significant reduction in fishing mortality inside MPAs often leads to significantly higher densities, mean size/age, and biomass of exploited species inside MPAs (Russ 2002). These changes may generate a 'spillover effect' due to the net export of post-settlement fishes to unprotected areas (Russ 2002, Russ et al. 2003). Higher production of propagules (eggs and larvae) of target species per unit inside MPAs could also result in a 'recruitment effect' due to the net export of propagules and enhanced supply of recruits to unprotected areas (Russ 2002). These effects and the spatial scales over which they occur will be influenced by several factors including the spatial and temporal scales at which fishes move throughout their life cycle. For example, within-MPA effects, and 'spillover' and 'recruitment effects' will be influenced by the degree to which adult populations are protected and hence the mobility of fishes relative to the size of the MPA (Kramer & Chapman 1999, Gerber et al. 2002). The spatial extent of the ‘recruitment effect’ will be influenced by the distances over which propagules disperse (Russ 2002, Shanks et al. 2003).

Empirical data on the effectiveness of individual MPAs may not be available for many decades, or may be logistically difficult to collect (Russ 2002). It is important, therefore, that the initial design of MPAs and evaluation of their effectiveness is based on the best available ecological information. Despite the importance of dispersal on the effectiveness of MPAs for protection of reef fishes, data on dispersal of fishes across appropriate spatial and temporal scales are limited, particularly in temperate regions. *The overall objective of this thesis was to provide data on the dispersal of temperate reef fishes across spatial scales relevant to the design of MPAs (hundreds of metres to hundreds of kilometres)*.

1.3. Dispersal and population connectivity of reef fishes

Reef fishes generally have a bipartite life cycle. Juveniles and adults are relatively sedentary and closely associated with the benthos (Sale 2004). Most spawn demersal or planktonic eggs which develop in the water column, while few species lack a pelagic

phase altogether (Leis 1991). The pelagic pre-settlement phase can last weeks to months before settlement back into benthic habitats (Sale 2004). Like many marine organisms, reef fish populations can be viewed in a metapopulation context, consisting of discrete local populations which occupy spatially separated patches of habitat or reefs (Holbrook et al. 1994, Hanski 1998). Interaction between local populations occurs via the exchange of propagules or the movement of juveniles and adults (Holbrook et al. 1994). The rate of exchange of individuals among local populations, often referred to as the level of population connectivity (Cowen et al. 2000), is determined by the species, the physical environment and the spatial and temporal scales at which investigations are made (James et al. 2002, Mora & Sale 2002). For example, populations may be completely open across scales of hundreds of metres and closed at broader spatial scales.

The level of population connectivity in reef fishes is often measured as the flux of successful propagules among local populations (Sale 2004). In this context, reef fish populations have been traditionally viewed as 'open' and connected over large spatial scales due to the potential for passive transport of pre-settlement fish by ocean currents (Leis 2002). Recent evidence suggests that populations of reef fishes may be more closed than previously thought and traditional hypotheses on the selective advantages of long-distance dispersal during the pre-settlement phase have been challenged (Cowen et al. 2000, Strathmann et al. 2002). Evidence for retention of pre-settlement fish near natal areas has been derived from an improved understanding of larval behaviour and ecology (Leis et al. 1996, Atema et al. 2002, Kingsford et al. 2002, Leis 2002, Paris & Cowen 2004), more sophisticated modelling techniques (Paris et al. 2005) and empirical data (Jones et al. 1999, Swearer et al. 1999, Taylor & Hellberg 2003, Almany et al. 2007, Gerlach et al. 2007). It has been demonstrated that late-stage larvae of many species are not passive but have strong sensory and swimming capabilities which could be used in conjunction with oceanographic features such as eddies and local currents to remain near natal reefs. Despite advances in our knowledge of these processes, it is still difficult to make generalizations on levels of pre-settlement connectivity (James et al. 2002, Mora & Sale 2002). Empirical studies are rare and often inconclusive due to logistical limitations of the methods (Mora & Sale 2002), and the conclusions vary among species and geographical locations (Leis 2002).

The magnitude of post-settlement movement by reef fishes may vary from metres to hundreds of kilometres (Samoilys 1997, Griffiths & Wilke 2002, Starr et al. 2002, Annese & Kingsford 2005, Popple & Hunte 2005). Fish may be site attached, move among habitats within a reef, among reefs or between reef and non-reef habitats (e.g. estuaries to open coast) (Morrison 1990, Gillanders & Kingsford 1996, Edgar et al. 2004a). Movements may vary diurnally and seasonally (Kingsford & MacDiarmid 1988, Lowry & Suthers 1998, Annese & Kingsford 2005, Popple & Hunte 2005) and with ontogenetic development (Morrison 1990, Gillanders & Kingsford 1996). For example, species may move from shallow water to deeper areas of reef (Lowry & Suthers 1998) or from estuaries to coastal reefs as they increase in size (Gillanders & Kingsford 1996).

Post-settlement movement of fishes has received much less attention in the MPA literature, particularly with respect to population connectivity (Powell & Bjork 1995, Zeller & Russ 1998, Kramer & Chapman 1999, Griffiths & Wilke 2002, Edgar et al. 2004a, Egli & Babcock 2004). This is presumably due to the focus on coral reef species in which connectivity is primarily achieved via dispersal of propagules rather than post-settlement movement (Sale 2004). Although site attachment is also common in many temperate reef fishes (Barrett 1995, Starr et al. 2002, Edgar et al. 2004a) some species are capable of moving tens to hundreds of kilometres alongshore, crossing non-reef habitat, (Gray et al. 2000, Griffiths & Wilke 2002), or migrating between estuaries and reef environments (Morrison 1990, Gillanders 1997). In these species post-settlement movement may have an important influence on local population dynamics, population connectivity and the effect of MPAs.

1.4. Methods for measuring dispersal and population connectivity

Several methods can be used to estimate dispersal and population connectivity of reef fishes. These include direct methods such as artificial tags and indirect methods such as investigation of spatial and temporal changes in abundances, otolith chemistry, and genetic markers. Each method has inherent limitations, and their efficacy varies across different spatial and temporal scales, and between species. A multidisciplinary approach incorporating several of these methods will provide a more comprehensive understanding of dispersal and connectivity (e.g. Gillanders & Kingsford 1996, Gillanders 1997, Bastow et al. 2002, Moran et al. 2003).

1.4.1. Artificial Tags

The only direct method of measuring movement is to observe individually recognized or tagged fish moving from one place to another (Gillanders 1997). This may be achieved by tagging individuals and 're-capturing' them, or by following individuals in real time. Tagging methods vary according to the phase of lifecycle under study and the size of fishes. Common methods for marking post-settlement fishes include: external tags such as T-bar and dart tags (Barrett 1995, Griffiths & Wilke 2002, Sumpton et al. 2003, Edgar et al. 2004a), thermal marking (Samoilys 1997, Zeller & Russ 1998, Egli & Babcock 2004), internal tags such as coded wire tags and visible implant fluorescent tags (Annese & Kingsford 2005), and telemetry techniques (Zeller 1998, Starr et al. 2002, Egli & Babcock 2004, Popple & Hunte 2005). Larval and juvenile fishes are often batch-tagged through incorporation of fluorescent chemicals, trace and micro-elements, radioactive isotopes or thermally induced marks into calcified tissues (Jones et al. 1999, Thorrold et al. 2002, Niva et al. 2005).

Tagging can be used to assess dispersal of pre- and post-settlement fishes across a range of spatial and temporal scales. For instance, tagging has been used to determine short and long-term movement of fishes (Barrett 1995, Starr et al. 2002), the contribution of juveniles inhabiting estuaries to coastal adult populations (Sumpton et al. 2003), and movement of fishes in and out of MPAs to provide evidence for the 'spillover effect' (Zeller et al. 2003). Larval fishes have been tagged using antibiotics to investigate the retention of larvae in their natal areas (Jones et al. 1999).

The use of tagging to determine levels of dispersal has limitations, many of which are specific to the individual tagging procedure (see reviews by; Gillanders et al. 2003, Heupel et al. 2006). For example, although external tags are generally economical, easy to apply and allow large number of fish to be tagged (Zeller & Russ 2000), loss rates are often high (Bergman et al. 1992) and tag-retention and tag-induced mortality must be assessed for individual species (Samoilys 1997, Zeller & Russ 1998, Gillanders et al. 2003). Furthermore, it is often difficult to capture, tag and release an adequate number of fish in order to get even low recaptures, as relocation of individuals may be unpredictable and occur almost instantaneously (Denson et al. 2002, Gillanders et al. 2003). Re-capture data may also be confounded by the spatial and temporal distribution of recapture efforts (Denson et al. 2002, Gillanders et al. 2003). Variation in levels of

reporting of tagged fish must be assessed when relying on recapture by recreational or commercial fishers (Denson et al. 2002). Finally, it is assumed that the movements of tagged individuals are representative of untagged fishes. Tagging, however, may have sub-lethal effects which alter the behaviour of fishes (Kingsford 1998) and such effects can be difficult to assess in the field.

1.4.2. Spatial and temporal variation in abundance

Measuring variation in the abundance of fishes over time relative to the size of a study area can be used to infer scales of movement. For example, Kingsford (2002) monitored the abundance of several exploited fish species on temperate rocky reefs over a one year period. Most species were found to vary in abundance among sampling times and it was suggested that this was due to movement at spatial scales greater than the sampling area (> 1 km). Temporal and spatial changes in abundance, and distribution of size classes can also be used to estimate movement within and among habitats including movement from shallow to deeper areas of reef with age, diurnal movements, and ontogenetic movements from estuaries to coastal environments (Morrison 1990, Gillanders 1997, Gillanders et al. 2003, Annese & Kingsford 2005). The response of exploited fishes to the implementation of MPAs may also be used to infer mobility. An increase in the abundance and/or size of fish inside MPAs suggests that a proportion of movements which expose fish to fishing mortality are contained within the MPA (Kramer & Chapman 1999). In theory, surveys conducted within MPAs, also allow human-induced mortality to be eliminated as an alternative model to explain temporal variation in abundances.

Patterns of abundance of reef fishes are generally monitored using underwater visual sampling (Mapstone & Ayling 1998, Curley et al. 2002, Kingsford 2002). This method is non-destructive, has few logistical requirements, allows for high levels of replication, and provides the ability to survey multiple species and associated variables simultaneously (e.g. habitats, depths) (Denny & Babcock 2004). Measurement of dispersal, however, is generally limited to post-settlement fishes, across fine spatial scales of hundreds of metres to kilometers, and within relatively short time periods of months to a few years (but see > 10 yr studies; Choat et al. 1988, Holbrook et al. 1994). This method is also subject to inter-observer variability and logistical limitations including safe diving depths (< 30 m) and ocean conditions (Denny and Babcock 2004).

In addition, the indirect nature of this method means that alternative models such as mortality, recruitment, habitat selection, or the presence of different individuals at different sampling times must be rejected before movement is accepted as explanation for observed patterns (Gillanders 1997).

1.4.3. Otolith chemistry

Otoliths are located in the inner ear of teleost fish and function in balance and hearing (Campana 1999). They are composed primarily of calcium carbonate with other elements present at minor (> 100 ppm) and trace (< 100 ppm) levels (e.g. Ba, Sr, Mg, Mn) (Campana 1999). Otoliths form prior to hatching and grow continuously throughout the life of a fish, are acellular and metabolically inert (Campana 1999). Elements or compounds accreted onto the surface of the otolith are not resorbed or reworked but are permanently retained so that the entire elemental life-history of the fish is preserved in chronological order (Campana 1999). Otolith chemistry is primarily influenced by the chemical and physical properties of the aquatic environment (e.g. ambient water chemistry, salinity and temperature) (Elsdon & Gillanders 2003a, Dorval et al. 2007). The type, concentration and combination of elements (multi-element signature) in otoliths of individuals from geographically distinct stocks will, therefore, reflect spatial and temporal scales of environmental variability (Bastow et al. 2002, Elsdon & Gillanders 2003a).

Differences in the elemental composition of otoliths have been used to determine the level of population connectivity across a range of spatial and temporal scales, among different habitats, and at different phases in the life-history of fishes. For example, otolith chemistry has been used to determine the distance that fish move from recruitment estuaries to the open coast, and the number of source estuaries and proportion of recruits that contribute to maintaining coastal populations (Gillanders & Kingsford 1996, Gillanders 2002a, Fowler et al. 2005). Differences in otolith chemistry have also been used as a natural tag to track larval dispersal in coastal and oceanographic waters (Swearer et al. 1999, Patterson et al. 2004, Sandin et al. 2005).

Otolith chemistry can provide information on dispersal that is not possible using alternative techniques (Campana 1999). This approach overcomes fundamental disadvantages associated with conventional tagging methods such as the need to tag

large number of individuals in order to get meaningful sample sizes, difficulties in tagging larvae and juveniles, high rates of mortality in early life history, and sub-lethal effects (Gillanders 2002a). A number of criteria must be met before applying otoliths as natural tags. First and foremost, there must be differences in the aquatic environment that translate into differences in otolith chemistry in the target species at the spatial scales of interest (Begg et al. 1999, Fowler et al. 2005). Temporal variability in elemental composition of otoliths within each geographic location should also be assessed, as such differences may confound spatial comparisons (Campana 1999, Gillanders & Kingsford 2000, Gillanders 2002a). Finally, although differences in otolith chemistry among groups of fish indicates prolonged separation in different environments, it is not direct evidence for reproductive or genetic isolation (Begg et al. 1999).

1.4.4. Genetic Markers

Natural selection and genetic drift due to finite population size result in the accumulation of unique mutations within local populations that have been separated for a sufficient period of time (Slatkin 1987, Bernardi et al. 2001). This process of genetic differentiation is opposed by gene flow resulting from the dispersal of gametes, individuals or groups of individuals among local populations (Slatkin 1987). The level of genetic differentiation among local populations may, therefore, be used as an indirect measure of past gene flow and levels of dispersal among local populations (Slatkin 1987, Bohonak 1999). Unlike direct measurements of dispersal, estimates based on gene frequencies are averaged over long time periods (Slatkin 1987).

Genetic differentiation among local populations is measured using genetic markers. Genetic markers are sections of DNA found at specific positions on the genome which vary between homologous chromosomes within and among individuals. Several types of polymorphic markers are used for population genetics (e.g. allozymes, mitochondrial DNA (mtDNA), microsatellites) and no one marker is superior for all applications (see review by Ferguson & Danzmann 1998). To be useful for addressing questions related to population connectivity markers must 'drift' distinctively among populations at time scales that are relevant to population demography (Shulman 1998, Mora & Sale 2002). Markers such as allozymes and mtDNA are not very sensitive to drift, particularly in large populations over short time periods (Mora and Sale 2002). Consequently they are

thought to be most informative for investigating processes operating at evolutionary time scales (Shulman 1998, Mora & Sale 2002). In addition, allozymes are prone to selective forces (Bernal-Ramirez et al. 2003). Mitochondrial DNA is maternally inherited, limiting its ability to provide information on the male's genetic contribution to the population (Ferguson & Danzmann 1998), which is of concern when males and females have different dispersal strategies. Microsatellites are the most recently developed genetic markers. They consist of repeats of short sequences (1-6 base pairs) of DNA, are common in the eukaryote genome, have high levels of polymorphism, high rates of mutation, are bi-parentally inherited, and are probably neutral to selection (Mora & Sale 2002, Whittaker et al. 2003). These characteristics make microsatellites suitable for investigating population connectivity at ecologically relevant time scales.

It has been assumed that marine fishes should exhibit low levels of genetic differentiation due to large population sizes and their ability for large-scale dispersal (Ward et al. 1994). While some species have been found to be genetically homogeneous at spatial scales of kilometres to thousands of kilometres (Doherty et al. 1995, Dudgeon et al. 2000, Bernardi et al. 2001, Van Herwerden et al. 2003), others with and without a pelagic pre-settlement phase have shown genetic differentiation (Doherty et al. 1995, Bernardi 2000, Riginos & Nachman 2001, Rhodes et al. 2003, Taylor & Hellberg 2003, Hoffman et al. 2005, Bay et al. 2006, Gerlach et al. 2007). Consequently, genetic markers may offer a valid alternative for measuring population connectivity at spatial scales relevant to management techniques such as MPAs (Palumbi 2003). Furthermore, studies which do not investigate fine-scale variation may confound comparisons made across larger spatial scales leading to inaccurate conclusions regarding levels of connectivity (Doherty et al. 1995).

Although population genetics can be informative for understanding connectivity, it may be of limited use, even when exchange of individuals is minimal (Begg et al. 1999). In theory, only a small amount of gene flow (≥ 5 effective migrants per generation, (Shulman 1998) is required for ecologically distinct populations to be genetically homogeneous (Mora & Sale 2002). Therefore, genetic similarity between populations may suggest populations are largely 'open' with high exchange of individuals or largely 'closed' with the exchange of few individuals (Mora & Sale 2002). In addition, it is still unclear how life-history traits influence dispersal, gene flow and genetic structure

(Hoffman et al. 2005). For example, high dispersal potential does not necessarily equate to high gene flow or homogeneous structuring of populations (Avise 1998). Data on population genetics of reef fishes which tests *a priori* hypotheses are required to improve our understanding of these processes.

1.5. Outline of thesis

The broad objectives of this thesis were to use a multidisciplinary approach to:

1) Investigate the range of distances over which temperate reef fishes are moving with particular emphasis on adult mobility and the effect of MPAs at fine spatial scales.
2) Determine how scales of dispersal influence population connectivity in terms of exchange of pre- and post-settlement fishes.
3) Gain insight into the most viable methods for measuring dispersal and population connectivity across different spatial and temporal scales and for different species of rocky reef fishes in central New South Wales (NSW), Australia.

The first part of the project was to identify the range of spatial scales over which temperate rocky reef fishes were moving, throughout their life cycle. I focused on species that were relatively abundant on reefs in NSW and, with the exception of *Parma microlepis,* were subject to harvesting. Scientific data and anecdotal evidence were used to place fishes into one of two categories according to the mobility of adults (Fig. 1.1; Table 1.1). The first category was 'sedentary', which included species where adults were considered to move at scales less than kilometres. The second category included fish that were thought to move across larger spatial scales. This information was used to select appropriate species with which to test specific hypotheses.

Specific aims and hypotheses were as follows.

1) To test current knowledge on the mobility of exploited reef fishes in NSW relative to their response to small pre-existing MPAs. It was hypothesized that the abundance and size of legal-sized fishes that were assumed to moved at scales < kilometres would be greater inside MPAs than in adjacent fished areas, and that abundances within individual locations would be similar among sampling times. In contrast, the abundance and size of legal-sized species which move across larger spatial scales should not be

influenced by protection, and they should exhibit greater variation in abundance among sampling times at all locations. Hypotheses were tested by measuring temporal patterns of abundance of selected fishes inside and outside MPAs.

2) To investigate the spatial scales over which there is variation in the otolith chemistry of rocky reef fishes along a temperate coastline and determine if the magnitude of differences was sufficient to allow discrimination of fish collected from different sections of the coast. The temperate damselfish *P. microlepis* was used as a model species as it has limited post-settlement movement. A lack of variation in otolith chemistry of *P. microlepis* across different spatial scales would indicate limited environmental variability rather than the exchange of individuals among locations.

3) To examine the influence of post-settlement movement on the degree of population connectivity across a hierarchy of spatial scales (kilometres, tens of kilometres, and > 60 km). It was predicted that population connectivity would be greater for fishes that were capable of large-scale post-settlement movements than for sedentary species. Hypotheses were tested using two species that were representative of the lowest and highest dispersal capabilities of reef fishes in central New South Wales (NSW), Australia (Table 1.1). These included *P. microlepis* which exhibits limited post-settlement movement (< 100 m^2), and is restricted to reef habitats, and *Girella tricuspidata* which is capable of moving hundreds of kilometres and across sandy habitats. Fishes were sampled across the same geographic region, and population connectivity was measured using microsatellite markers.

Photos: B. Curley, M. Gillings.

Fig. 1.1. Temperate reef fishes used to test hypotheses in thesis. 'Sedentary' species: a) *Parma microlepis*, b) *Achoerodus viridis*, c) *Cheilodactylus fuscus*, d) Monacanthidae (e.g. *Meuschenia trachylepis*), e) *Girella elevata.* 'Mobile' species: f) *Kyphosus sydneyanus*, g) Sparidae (e.g. *Acanthopagrus australis*), h) *Girella tricuspidata*.

Table 1.1. Summary of dispersal capabilities of common temperate reef fishes in NSW based on empirical data and anecdotal information. Methods used to estimate dispersal are given in brackets.

a) Sedentary adults (movement < km's)

Species (Sources)	Harvesting on coastal reefs/ Legal size limits in NSW (TL; total length)	Demersal eggs	Pelagic eggs	Pelagic larval duration (PLD) & recruitment habitat	Scale of post-settlement dispersal and movement among habitats
POMACENTRIDAE ***Parma microlepis*** **(White Ear)** (Coleman 1974, Lawler 1974, Moran & Sale 1977, Tzioumis & Kingsford 1995, Tzioumis & Kingsford 1999, Kingsford & Gillanders 2000, Curley et al. 2002)	Non-target species.	X		2-4 weeks (estimates indirect, based on spawning season and timing of settlement). Recruits to rocky reefs.	Territorial with limited post-settlement movement < 100 m^2 (abundance, otolith chemistry). Juveniles and adults move within reef habitat (abundance).
CHEILODACTYLIDAE ***Cheilodactylus fuscus*** **(Red Morwong)** (Lincoln Smith et al. 1989, Cole et al. 1990, Lockett & Suthers 1998, Lowry & Cappo 1999, Curley et al. 2002)	Heavily targeted by inexperienced and experienced recreational spear fishers. 250 mm TL		X	9-12 months (based on studies of other species of Cheilodactylidae). Recruits to rocky reefs.	Fish > 16 cm fork length have a home range of 1865 ± 268 m^2 during the day, 3639 ± 416 m^2 at night. Demonstrated homing behaviour 200-900 m from point of capture, crossing sandy habitat (tagging, abundance). Related temperate species *C. spectabilis* responded to protection within a 5.2 km^2 Marine Protected Area. Juveniles and adults move within reef habitat (abundance)
LABRIDAE ***Achoerodus viridis*** **(Eastern Blue Groper),** (Kingsford et al. 1991, Barrett 1995, Gillanders 1997, Edgar & Barrett 1999, Gillanders 1999, Curley et al. 2002, Edgar et al. 2004a)	Few taken by recreational line fishers. Protected from spearing in NSW since the 1970's. No legal size limit.		X	1-2 months. Recruits to estuarine habitats and coastal rocky reefs.	Could be territorial, based on observations by local SCUBA divers who feed the same individuals at specific sites (anecdotal). Other temperate labrids such as *Pictilabrus laticlavius* and *Notolabrus tetricus* move distances < 100 m, and *Notolabrus fucicola* > 150 m (tagging) and have responded to protection within a 7 km^2 Marine Protected Area. Juveniles that recruit to estuarine habitats move to the open coast as they increase in size (abundance, otolith chemistry). Adults move within reef habitat (abundance).

Table 1.1. cont.

a) Sedentary adults (movement < km's) cont.

Species (Sources)	Harvesting on coastal reefs/ Legal size limits in NSW (TL; total length)	Demersal eggs	Pelagic eggs	Pelagic larval duration (PLD) & recruitment habitat	Scale of post-settlement dispersal and movement among habitats
MONACANTHIDAE *Eubalichthys bucephalus* *Meuschenia trachylepis* *Scobinichthys granulatus* *Meuschenia flavolineata* *Acanthaluteres vitteger* *Nelusetta ayraudi* *Meuschenia freycineti* *Eubalichthys mosaicus* (Thomson 1959, Kingsford & Millicich 1987, Barrett 1995, Hutchins 1999, Edgar et al. 2004a)	Taken by recreational line and spear fishers. No legal size limit		X	PLD generally unknown. 3-9 weeks recorded for the temperate monacanthid, *Parika scaber* in New Zealand. Recruit to estuaries or coastal reefs.	*A. vitteger* has a home range of < 2500 m^2 (tagging) and have responded to protection within a 7 km^2 Marine Protected Area. *M. australis* and *M. freycineti* generally move < 100 m (tagging). Juveniles which recruit to estuarine habitats may move to the open coast as they increase in size or stay within estuaries. Adults move within reef habitat (abundance, tagging).
GIRELLIDAE ***Girella elevata*** **(Rock Blackfish)** (Wilson 1984, Kingsford et al. 1991, Griffiths 2003)	Important recreational species in NSW, taken by line and spear. 300 mm TL		X	PLD unknown. Recruits to shallow rocky reefs and intertidal pools adjacent to reefs.	Thought to be territorial as the longest distance travelled by tagged individuals was 500 m (M. Aston Pers. Comm.). However, temporal patterns of abundance suggest that they move at scales > 1 km. Juveniles and adults move within reef habitat.

Table 1.1. cont.

b) Mobile adults (movement > 10's of km's)

Species (Sources)	Harvesting/ Legal size limits in NSW	Demersal eggs	Pelagic eggs	Pelagic larval duration (PLD) & recruitment habitat	Scale of post-settlement dispersal
Girella tricuspidata **(Luderick)** (Middleton et al. 1984, Morrison 1990, Kingsford et al. 1991, McNeill et al. 1992, Kailola et al. 1993, West 1993, Gray et al. 2000, Kingsford 2002)	Important recreational species in NSW, taken primarily by line but also by spear. Harvested by commercial ocean and estuarine haul fisheries. 250 mm TL		X	1 month (based on similar species, Sparidae, T. Trnski Pers. Comm.). Most recruit to estuaries with few observed within intertidal pools on coastal reefs.	Move at scales > 1 km on coastal reefs (abundance). Known to undertake annual spawning migrations. Individuals inhabiting estuaries are capable of travelling > 150 km between estuaries, and along the NSW coastline (tagging). Juveniles may stay within estuaries as they increase in size or move to the open coast (abundance).
SPARIDAE ***Acanthopagrus australis*** **(Yellow-fin Bream)** ***Rhabdosargus sarba*** **(Tarwhine)** (Pollock et al. 1983, Pollock 1984, Kingsford et al. 1991, Kingsford 2002)	Important recreational species, taken by line and spear. *A. australis* 250 mm TL *R. sarba* 200 mm TL		X	1 month for *A. australis*. *R. sarba* thought to be similar (K. Smith, Pers. Comm.). Most recruit to estuaries.	Move at scales > 1 km (abundance). Undertake annual spawning migrations along the NSW coast. Juveniles may stay within estuaries as they increase in size or move to the open coast (abundance).
KYPHOSIDAE ***Kyphosus sydneyanus*** **(Silver Drummer)** (Eristhee & Oxenford 2001, Kingsford 2002)	Taken by recreational line and spear fishers. No legal size limit		X	Unknown.	Move at scales > 1 km on coastal reefs (abundance). Related species *K. sectatrix* has a home range of 30,000-40,000 m^2 (tagging). Post-settlement movement restricted to reef habitat (anecdotal).

Chapter 2: Movement of exploited temperate reef fishes and their response to small Marine Protected Areas

2.1. Introduction

Knowledge of the spatial and temporal scales over which reef fishes move is critical for understanding population dynamics, and for the implementation of appropriate management strategies. Mobility is particularly important in determining the response of reef fishes to Marine Protected Areas (MPAs) (Gerber et al. 2002, Halpern 2003, Palumbi 2004). Reductions in fishing mortality inside MPAs can result in an increase in density, mean size/age, and biomass of exploited species (within-MPA effects), and in turn the net export of post-settlement fishes and propagules to unprotected areas (Russ 2002, Russ et al. 2003, Willis et al. 2003). The magnitude of within-MPA, 'spillover', and 'recruitment effects', however, will be influenced by the degree to which adult populations are protected and hence the mobility of fishes relative to the size of individual MPAs (Kramer & Chapman 1999, Cole et al. 2000, Gerber et al. 2002, Palumbi 2004).

Despite the importance of fish movement for expected benefits of MPAs, empirical data across appropriate spatial and temporal scales are limited for most species (Palumbi 2004). In general, the magnitude of post-settlement movement by reef fishes can vary from metres to hundreds of kilometres (Samoilys 1997, Griffiths & Wilke 2002, Starr et al. 2002, Annese & Kingsford 2005, Popple & Hunte 2005). Fishes may be site attached, move among habitats within a reef, among reefs, or among reef and non-reef habitats (e.g. estuaries to open coast) (Morrison 1990, Gillanders & Kingsford 1996, Gillanders 1997, Edgar et al. 2004a). Movements may vary diurnally, seasonally, and with size and age (Morrison 1990, Gillanders & Kingsford 1996, Lowry & Suthers 1998). The consequences of such movements relative to the design of MPAs and other ecological and social factors (e.g. fishing pressure), are poorly understood. For example, mobile species may respond to protection even within small MPAs due to intraspecific variation in mobility, aggregative behaviour, and high levels of external fishing pressure (Roberts & Hawkins 1997, Roberts et al. 2001, Willis et al. 2003).

Meta-analyses of globally derived MPA studies, have concluded that the relative magnitude of within-MPA effects and rates of recovery are independent of MPA size (Côté et al. 2001, Halpern 2003). These generalizations have been criticized as many studies included in analyses were based on once only spatial comparisons of MPAs of different sizes and ages, and are often confounded by variation in habitat type, poaching histories, and the life history of target organisms (Russ et al. 2005, Barrett et al. 2007). In addition, only a small proportion of studies have investigated the utility of very small MPAs (Roberts & Hawkins 1997, Côté et al. 2001, Halpern 2003, Russ et al. 2005, Floeter et al. 2006). For example, 25% of the MPAs reviewed by Halpern *et al.* 2003 were ≤ 1 km^2. Consequently, proposed effects of MPAs may not be universal and will not apply to all species, locations, or MPA designs; therefore independent tests of expected functions of individual MPAs remain imperative (Denny et al. 2004).

Small MPAs have been widely implemented in NSW, Australia. However, there are no published data on the ecological value of these MPAs for protecting reef fishes, and scientific and social justifications for their existence and design are poor. Although MPAs in NSW are not specifically designed to protect exploited reef fishes, they are advocated to fulfill this role (Marine Protected Areas 2006). It is also likely that reef fishes will generate the strongest differences between MPAs and controls as many species are subject to intense fishing pressure (Lincoln Smith et al. 1989, Kingsford et al. 1991, Palumbi 2004). Knowledge of the mobility of most exploited fishes in NSW is poor and it is difficult to predict the responses of species to protection. The limited movement of temperate reef fishes in other regions suggests that small (< 1 km^2) MPAs should be beneficial (Moran & Sale 1977, Barrett 1995, Starr et al. 2002, Edgar et al. 2004a). In contrast, many species are capable of moving tens to hundreds of kilometres alongshore, crossing non-reef habitat, or migrating between estuaries and reef environments (Morrison 1990, Gillanders 1997, Griffiths & Wilke 2002) and are unlikely to benefit from small MPAs. Species-specific data on the mobility of reef fishes in NSW relative to their response to small MPAs are required to test these assumptions.

Quantitative studies of the movement of reef fishes are logistically challenging. Different methods have inherent limitations and vary in utility according to the species and spatial and temporal scales of interest. Temporal and spatial changes in abundance

and size of fishes relative to the size of a sampling area can be used to infer movement (Morrison 1990, Holbrook et al. 1994, Gillanders 1997, Annese & Kingsford 2005). Patterns of abundance of reef fishes are often monitored using underwater visual counts (Gillanders & Kingsford 1998, Curley et al. 2002, Kingsford 2002). Unlike alternative methods for estimating movement (e.g. artificial and natural tags), visual counts are non-destructive, have few logistical requirements, allow high levels of replication, and the ability to survey multiple species and associated variables simultaneously (e.g. habitat, depth; Denny & Babcock 2004). This method is, therefore, useful for providing rapid information, on the movement of multiple reef fish species across small spatial and temporal scales in areas where invasive methods are inappropriate (e.g. MPAs). The effects of different sized MPAs can also be used to infer the mobility of exploited species. An increase in the abundance and/or size of fish inside MPAs suggests that a proportion of movements which expose fish to fishing mortality are contained within the MPA (Kramer & Chapman 1999). In theory, surveys conducted within MPAs, also allow human-induced mortality to be eliminated as an alternative model to explain temporal variation in abundances.

The objective of this study was to use spatial and temporal variation in abundances to test current knowledge on the mobility of exploited reef fishes in NSW relative to their response to small MPAs. Two pre-existing MPAs, ≤ 0.2 km^2 were examined. It was hypothesized that the abundance and size of legal-sized fishes that were assumed to move at scales < kilometres would be greater inside MPAs than in adjacent fished areas, and that abundances within individual locations would be similar among sampling times. In contrast, the abundance and size of legal-sized species which move across larger spatial scales should not be influenced by protection, and should exhibit great variation in abundance among sampling times at all locations. It was also predicted that effects would differ between the two MPAs given that one was recently established and 'no-take' and the other was much older and closed only to spear fishing.

2.2. Methods

2.2.1. Study areas and experimental design

Two MPAs located in Sydney, New South Wales were sampled; Cabbage Tree Bay (CTB, MPA 1), and Gordon's Bay (GB, MPA 2). These MPAs are similar in size, and

contain similar reef habitats (e.g. urchin-grazed barrens, Ecklonia forest; Underwood et al. 1991) over similar depths (≤ 12 m). They differ, however, in the degree and duration of protection provided for reef fishes. CTB is 0.2 km^2 and is a 'no-take' MPA and has been closed to all methods of fishing since March 2002. GB is 0.1 km^2 and has been closed to spear fishing since January 1992. GB was extended in March 2002 (eight months prior to the start of this study) to form the Bronte-Coogee Aquatic Reserve which is 0.43 km^2. Spear fishing is prohibited within most of this new MPA, and invertebrates and the Eastern Blue Groper *Achoerodus viridis* may not be taken by any method. At the completion of this survey GB had been established for 12.5 years, while CTB had been protected for 2.5 years.

Because baseline data were not available for either MPA, hypotheses were tested using spatial comparisons of MPAs versus controls. An asymmetrical design (one MPA location vs. three control locations) was used to overcome problems of spatial confounding (Underwood 1993, Glasby 1997). Abundances and sizes of selected fishes inside each MPA were compared to those at three control locations: Toowoon Bay, Terrigal and Long Bay (Fig. 2.1*a*). Control locations were chosen to encompass similar types of fish assemblages, reef habitats, depths, and wave exposures as MPAs. MPAs and controls were located in highly urbanized areas and had easy land access for line and spear fishers. The number of fishers and the methods used (line or spear) were sampled opportunistically at all locations over the survey period. This was done to ensure that controls and MPAs were representative of fished versus unfished areas rather than as a measure of relative fishing pressure which would require intensive sampling (see Kingsford et al. 1991). No spear fishers were observed within either MPA. The mean numbers of line fishers observed per day within MPAs were: CTB (0.3 ± 0.1; n = 6), GB (0.9 ± 0.1; n = 14). Although two line fishers were observed at CTB in a single day they were present for < 1 hour and were presumably informed of the 'no-take' status by members of the public. All control locations were subject to line and spear fishing; Toowoon Bay (line = 1.3 ± 0.1; spear = 0.1 ± 0.0; n = 14), Terrigal (line = 5.0 ± 0.2; spear = 1.6 ± 0.1; n = 16), and Long Bay (line = 1.1 ± 0.1; spear = 0.3 ± 0.1; n = 11).

At each MPA and control location, two sites (separated by hundreds of metres) were sampled (Fig. 2.1*b*). Replication at this spatial scale was required as a previous study

found that most variation in abundance of reef fishes in central NSW occurred at scales of hundreds of metres rather than at kilometres or tens of kilometres alongshore (Curley et al. 2002). Sampling within each site was stratified by depth (≤ 3.5 m and 4-12 m) as most target fishes exhibit depth-related patterns of abundance (Gillanders 1997, Curley et al. 2002, Kingsford 2002). Underwater visual counts (UVCs) using SCUBA were used to sample fishes in five replicate 40 x 5-m belt transects within each depth at each site (Fig. 2.1b). The choice of sampling unit and number of replicates was based on a pilot study which compared the relative accuracy and precision of 20, 40, 60 and 80 x 5-m transects for estimating abundances of the target species (Curley unpublished data). All locations were surveyed six times by a single observer during the period November 2002 to November 2004. Sampling months and seasons are given in Fig. 2.2-2.9.

Several species that were subject to harvesting and with varying degrees of mobility were counted. Empirical data and anecdotal evidence was used to separate fishes into two mobility categories (Table 2.1). Fishes that moved at scales less than the area protected by the MPAs (< kilometres) were classed as 'sedentary' and included *Cheilodactylus fuscus* (Red Morwong), *Achoerodus viridis* (Eastern Blue Groper), Monacanthidae (Leatherjackets), and *Girella elevata* (Rock Blackfish). Fishes that moved across larger spatial scales were classed as 'mobile' and included *Girella tricuspidata* (Luderick), *Kyphosus sydneyanus* (Silver Drummer) and Sparidae (*Acanthopagrus australis* (Yellow-fin Bream) and *Rhabdosargus sarba* (Tarwhine)). All species may be legally harvested by recreational fishers in NSW using line or spear, with the exception of *A. viridis* which may be taken by line only (Table 2.1). Standard lengths (SL) of all fishes were estimated to the nearest 50 mm during UVCs to allow patterns of abundance of fish above and below the legal size limits to be assessed and to determine if MPA effects could be attributed to spatial variation in recruitment among MPAs and controls. Size estimates were based on comparison of observed fish length with a ruler attached to a dive slate.

Habitat-related patterns of abundance have been documented for some of the target species e.g. *C. fuscus* (Curley *et al*. 2002). The occurrence of different types of reef habitat at each location was, therefore, quantified to determine whether variation in the abundance of fishes among MPA and control locations could be attributed to differences in habitat assemblages. Subtidal habitats were divided into six categories

(Table 2.2). The habitat type directly below the diver was recorded every four fin-kicks along each transect concurrent to fish counts, during sampling times 2-6.

2.2.2. Data analysis

A four-factor ANOVA was used to compare abundances of fishes among MPA and control locations, sites, depths and times of sampling. Factors in analyses included: time (orthogonal and random, six levels), location (orthogonal and random, four levels), sites (nested in location and random, two levels), and depth (orthogonal and fixed, two levels). It was considered inappropriate to combine CTB and GB in a single analysis due to differences in degree and duration of protection. Location was, therefore, partitioned into a comparison between each MPA location and the average of the three control locations, and a comparison of controls (Glasby 1997). MPAs were found to be statistically different from controls if variation in abundance of fish among the MPA and controls was greater than the variation among control locations. After analyses, terms that were non-significant at $P \geq 0.25$ were eliminated or pooled to enable tests of terms that could not be tested using the original design, or to create more powerful tests which had a greater number of degrees of freedom for the denominator of *F*-tests. An example of the complete asymmetrical ANOVA is given in Table 2.3. The significance of *F*-ratios was presented for the most relevant levels of the analyses for each species and full analyses included in Appendix A.

Most species were separated into two size classes for analyses, legal (the smallest size which can be legally retained by fishers in NSW), and sub-legal. A total of 21 *R. sarba* were counted across all sampling locations over the 2-year sampling period and were, therefore, excluded from analyses. *A. viridis*, monacanthids and *K. sydneyanus* were divided into small (≤ 150 mm SL) and large (≥ 200 mm SL) fish for analyses as there are currently no legal size limits for these species in NSW. All species of monacanthids were pooled for analyses due to low numbers of individual species counted. The total number (small ≤ 150, and large ≥ 200 mm SL) recorded throughout the sampling period included: *Eubalichthys bucephalus* (Black Reef Leatherjacket; 0, 21), *Meuschenia trachylepis* (Yellow-finned Leatherjacket; 17, 38), *Scobinichthys granulatus* (Rough Leatherjacket; 2, 34), *Meuschenia flavolineata* (Yellow-stripe Leatherjacket; 5, 7), *Acanthaluteres vitteger* (Toothbrush Leatherjacket; 39, 3), *Nelussetta ayraudi* (Chinaman Leatherjacket; 34, 0), *Meuschenia freycineti* (Six-spine Leatherjacket; 3,

19), *Eubalichthys mosaicus* (Mosaic Leatherjacket; 0, 5), unidentified (7, 9). *Cheilodactylus fuscus* < 75 mm SL were excluded from analyses of abundances as they were often overlooked in UVCs (Curley unpublished data).

Heterogeneity of variances was tested prior to ANOVA using Cochran's *C* Test (Winer et al. 1991). Variances were heterogeneous for all species ($P < 0.05$), therefore, data were transformed to $\ln(x+1)$. Analyses were performed if data remained heterogeneous following transformation as ANOVA is generally robust to this assumption particularly for balanced experiments with relatively large sample sizes (Underwood 1997). Alpha was not adjusted to account for heterogeneous variances as the increased risk of erroneous rejection of the null hypothesis (Type I error) was considered acceptable when assessing potential environmental effects associated with MPAs (Underwood 1997).

Size frequency data for each species were graphed at the level of location for each sampling time, and for all sampling times pooled. A single-factor ANOVA was used to compare the mean size of fishes among MPA and control locations. Mean size of fish was calculated for each location for each sampling time and these values were used as replicates in ANOVA. The number of replicates for each species was equal to the maximum number of sampling times in which fish were present at all locations. When significant differences were detected, means were compared using Student-Newman-Keuls (SNK) tests. Variances were heterogeneous for *A. viridis* and *A. australis* data ($P < 0.05$). *A. viridis* data was $\ln(x+1)$ transformed to remove heterogeneity. Variances for *A. australis* were still heterogeneous following transformation; therefore, raw data were used in analyses.

The percentage of each habitat type that occurred at each site and depth was calculated by averaging data across replicate transects and data was pooled at the level of location. Percentage data were arcsine transformed to avoid dominance of common habitat types and to allow greater contribution from rarer habitats (Sokal & Rohlf 1995). Habitat-type by sample matrices were converted to dissimilarity matrices using the Bray-Curtis coefficient. Semi-parametric permutational multivariate analysis of variance (Anderson 2005) was then used to test for significant differences in habitat assemblages among depths and locations for each sampling time. Factors in analyses included location

(orthogonal and random, five levels), and depth (orthogonal and fixed, two levels). Between group similarities (SIMPER) was used to identify the major habitat types contributing to dissimilarities detected (PRIMER; Clarke 1993) and non-metric multidimensional scaling (MDS) was used to visualise multivariate patterns.

2.3. Results

Full ANOVA, size frequencies for each sampling time and graphs of densities of sub-legal and small fishes are provided in Appendix A.

2.3.1. Temporal variation in legal and large fishes

Two of the four species classed as 'sedentary' exhibited minimal temporal variation as predicted. Densities of legal *C. fuscus* and large *A. viridis* did not vary markedly over time and relative differences in abundances among locations were persistent (Fig. 2.2 & 2.3; Table 2.4). Patterns of temporal variation for legal *C. fuscus*, however, depended on the site and depth considered (Table 2.3). The remaining 'sedentary' species, (monacanthids and *G. elevata*), and all 'mobile' fishes (*G. tricuspidata*, *A. australis*, *K. sydneyanus*) exhibited substantial temporal variation in abundances at MPA and control locations (Fig. 2.4-2.8). This was often stochastic, for example, large schools of *G. tricuspidata* were generally observed during the winter months (Fig. 2.6). Despite these trends, the factor 'time' was not statistically significant in ANOVA for these species, with the exception of *G. elevata* (Table 2.4). This is probably due to the low number of fishes encountered (e.g. *A. viridis*, *K. sydneyanus*), and/or large standard errors (> 60% of mean) associated with counts of schooling species (e.g. *G. tricuspidata*).

2.3.2. Cabbage Tree Bay (MPA1)

There was little evidence of MPA effects within CTB for the four species classed as 'sedentary'. Large *A. viridis* (≥ 200 mm SL) and legal *G. elevata* (≥ 250 mm SL) occurred in similar densities within CTB and controls (Fig. 2.3 & 2.5; Table 2.4) and legal *C. fuscus* (≥ 200 mm) and large monacanthids (≥ 200 mm SL) were often found in lower densities inside CTB relative to controls (Fig. 2.2 & 2.4; Table 2.4). Comparisons for monacanthids could not be tested formally as terms requiring elimination in ANOVA were significant at $P \leq 0.25$ (Appendix A, Table A.2). No consistent trends for an increase in the abundance of 'sedentary' fishes were observed over the two-year

sampling period. The mean size of *C. fuscus*, monacanthids, *G. elevata* within CTB was similar to controls (Fig. 2.9*a*, *c* & *d*). Although the mean size of *A. viridis* varied among locations this was not correlated with protection (Fig. 2.9*b*). There was little evidence of an increase in the size of *A. viridis* or monacanthids in CTB over time (Appendix A; Fig. A.2 & A.3). In contrast, there was a slight trend for an increase in the size of *C. fuscus* and *G. elevata* within CTB (Appendix A; Fig. A.1 & A.4). For example, *C. fuscus* ≥ 300 mm SL were only recorded within CTB during sampling times 2-6.

In contrast two of the three species classed as 'mobile' showed evidence of MPA effects within CTB. Densities of legal *A. australis* (≥ 200 mm SL) were 2.6-times higher inside CTB when compared to control locations (Fig. 2.7; Table 2.4, $P = 0.007$). This pattern was particularly clear over sampling times 4 – 6 (corresponding to 18-32 months of protection) where 77% of *A. australis* counted were found in MPAs. Size frequencies of *A. australis* were similar for CTB and controls with most individuals being 200-250 mm SL and significant differences in mean size occurred between control locations only (Fig. 2.9*f*). Fish ≥ 300 mm SL were only observed in the last four sampling periods within CTB, and may indicate a preliminary size-related effect (Appendix A, Fig. A.6). Similar trends were observed for *G. tricuspidata*. Legal fish (≥ 200 mm SL) were often more abundant within CTB than at control locations, although this pattern depended on the site, depth and time of sampling considered (Fig. 2.6; Table 2.4; Appendix A, Table A.4). The effect of CTB could not be tested statistically, as terms requiring elimination were significant at $P \leq 0.25$. Large schools of *G. tricuspidata* generally consisted of individuals between 250-350 mm SL. These size classes were grouped together in size frequency analyses as accurate counts of individual classes were not possible. Size frequencies and mean sizes of *G. tricuspidata* were similar for MPA and controls over the entire sampling period (Fig. 2.9*e*; Appendix A; Fig. A.5). The remaining 'mobile species' legal *K. sydneyanus* (≥ 200 mm SL) were generally observed in low numbers across all sampling locations and times (Fig. 2.8; Table 2.4). Although significant differences in mean sizes occurred between control locations only (Fig. 2.9*g*), fish ≥ 250 mm SL were most frequently observed within CTB (Appendix A; Fig. A.7).

2.3.3 Gordon's Bay (MPA2)

Cheilodactylus fuscus was the only species of the four classed as 'sedentary' that exhibited within-MPA effects. Densities of legal *C. fuscus* (≥ 200 mm) were 2.8-times

higher within GB, when compared to average densities found at controls (Fig. 2.2; Table 2.3, $P = 0.001$). This pattern was consistent for each sampling time and for shallow and deep areas of the reef (Table 2.3). In general, fish were 20% larger within GB relative to controls; although mean sizes were only found to be significantly higher than at Terrigal and CTB (Fig. 2.9*a*). In addition, GB was the only location in which individuals ≥ 450 mm were recorded. Densities of large *A. viridis* and monacanthids, and legal *G. elevata* within GB were similar to controls throughout the two year study (Fig. 2.3-2.5; Table 2.4). The mean size of these species within GB was generally similar to controls (Fig. 2.9*c* & *d*). Although the mean size of *A. viridis* varied among locations this was not correlated with protection (Fig. 2.9*b*). For example, GB and Long Bay had a higher proportion of fish ≤ 200 mm SL relative to other locations.

One of the 'mobile' species, legal *A. australis*, was often more abundant within GB relative to controls (Fig. 2.7). Differences were non-significant and were only apparent when comparing shallow areas of reef (Table 2.4). Size frequencies were similar for MPAs and controls with most fishes being 200-250 mm SL, and significant differences in the mean size of fish occurred between control locations only (Fig. 2.9*f*). The largest fishes in the study were, however, recorded within GB. In contrast, GB had consistently low densities, and similar mean sizes of *G. tricuspidata* relative to controls (Fig. 2.6; Table 2.4). The remaining 'mobile species' legal *K. sydneyanus* were generally observed in low numbers across all sampling locations and times; significant differences in mean sizes occurred between control locations only (Fig. 2.8; 2.9*g*).

2.3.4. Spatial and temporal variation in sub-legal and small fishes

There was no evidence that MPA effects for *C. fuscus*, *A. australis* and *G. tricuspidata* were a consequence of higher levels of recruitment inside MPAs relative to controls. *C. fuscus* ≤ 50 mm SL, were found at all locations during the months of spring (Appendix A, Fig. A.1). Although significant differences in the abundance of sub-legal *C. fuscus* (100-150 mm SL) occurred between MPAs and controls, this pattern varied according to the depth and time considered (Table 2.3; Appendix A, Fig. A.8). Densities of sub-legal *C. fuscus* varied substantially among sampling times at all locations, however, differences depended on the site, time and depths compared. Sub-legal *A. australis* (≤ 150 mm SL) were extremely rare with only six individuals observed within transects over the two year sampling period; no sub-legal *G. tricuspidata* were

recorded in counts. This was expected as *A. australis* and *G. tricuspidata* generally recruits to estuarine habitats (Pollock et al. 1983, Hannan & Williams 1998).

There was no evidence that lack of MPA effect was due to limited recruitment of fish inside MPAs relative to controls. Small (≤ 150 mm SL) *A. viridis*, monacanthids and *K. sydneyanus* were generally uncommon at all locations (≤ 1 fish per 200 m^2) (Table 2.4; Appendix A, Fig. A.9, A.10 & A.12). This was not surprising given that *A. viridis* and monacanthids also recruit to estuarine habitats (Middleton et al. 1984, Gillanders & Kingsford 1996). In contrast, *G. elevata* recruit to intertidal pools adjacent to reefs (Griffiths 2003) and sub-legal fish (≤ 200 mm SL) were relatively abundant at MPA and controls (Table 2.4; Appendix A, Fig. A.11). Although, some locations had a larger number of recruits this was not related to protection. For example, high densities of small *A. viridis* were found in shallow water within GB relative to controls at some times (e.g. times 2 and 3) and small monacanthids were abundant in shallow and deep areas of reef in Long Bay at sampling times one and two (Appendix A, Fig. A.9 & A.10; Table A.1 & A.2). All small fish showed substantial temporal variation as expected, and high densities often occurred simultaneously at all locations (e.g. *G. elevata*, Times 1,2 and 5) (Appendix A, Fig. A.11). The effect of time was often non-significant in ANOVAs due to large variances and small numbers of fish recorded, and was often dependent on the location, site or depth considered (Table 2.4; Appendix A, Table A.1 – A.3 & A.5).

2.3.5. Variation in abundance of fishes among control locations, sites (with locations) and depths

Abundances often exhibited marked variation among controls, sites and depths and should be considered in future spatial comparisons of these species. Significant differences in the abundance of large *A. viridis* occurred among controls (Fig. 2.3; Table 2.4) similar but non-significant trends apparent for legal/large monacanthids, *G. elevata*, *G. tricuspidata*, *A. australis*, and *K. sydneyanus* and some small fishes (e.g. monacanthids) (Fig. 2.4-2.8; Appendix A, Fig. A.10). Significant differences in abundances of small/sub-legal and large/legal fishes also occurred between sampling sites within locations (Fig. 2.3-2.8; Table 2.4). This relationship often depended on the sampling time and depth examined (e.g. *G. elevata*, *G. tricuspidata*, *A. australis*;

Appendix A.1, Tables A.3 & A.4). Legal *C. fuscus* were found in similar abundances at controls and at sites within sampling locations (Fig. 2.2; Table 2.4).

Abundances of fishes also varied between depths. For example legal *C. fuscus* and large monacanthids were more abundant in deep (4-12 m) than in shallow (≤ 3.5 m) areas of reef (Fig. 2.2 & 2.4, Table 2.4). In contrast, other species such as *G. elevata* were generally only recorded in shallow areas of reef (Fig 2.5). Formal tests of depth-related patterns were often prevented as terms requiring elimination were significant at $P \leq 0.25$ (Appendix A.1, Tables A.1-A.4). Furthermore, relationships were complex due to the low numbers of fish observed at both depths at some sampling times (e.g. *K. sydneyanus*, small *A. viridis*, *C. fuscus* all sizes). Large *A. viridis* was the only species to exhibit consistently similar abundances in shallow and deep areas of reef (Fig. 2.3).

2.3.6. Spatial variation of habitats

Differences in abundances of fishes between MPAs and controls could not be attributed to the occurrence of habitats. Habitats were similar among all locations regardless of the sampling time (Fig. 2.10*a*; Table 2.5). Significant differences were detected between depths at all locations and among times (Fig. 2.10*b*; Table 2.5). Three habitat types accounted for 72-97% of the overall dissimilarity between depths. Mixed algal habitat was more frequent in shallow (≤ 3.5 m) than in deep (4-12 m) areas of reef and accounted for 28-43% of the total variation between depths (Fig. 2.11; Table 2.6). Rock and macroalgal habitats occurred more frequently in deep areas of reef and accounted for 26-34% and 16-25% of depth-related variation respectively.

Table 2.1. Description of scales of movement and methods used to harvest reef fishes used in this study. Empirical data and anecdotal evidence were used to separate fishes into two categories 'sedentary' and 'mobile'.

a) Sedentary adults (movement < km's)

Species	Harvesting methods	Scale of adult movement
CHEILODACTYLIDAE		
Cheilodactylus fuscus **(Red Morwong)** (Lincoln Smith 1989, Lockett & Suthers 1998, Lowry & Cappo 1999, Curley et al. 2002)	Heavily targeted by spear fishers.	Fish > 16cm fork length have home range of 1865 ± 268 m^2 during day, 3639 ± 416 m^2 at night. Tagged fish demonstrated homing behaviour 200-900 m from point of capture, crossing sandy habitat. Related temperate species *C. spectabilis* responded to protection in 5.2 km^2 MPA.
LABRIDAE		
Achoerodus viridis **(Eastern Blue Groper),** (Kingsford et al. 1991, Barrett 1995, Gillanders 1997, Edgar & Barrett 1999, Gillanders 1999, Curley et al. 2002, Edgar et al. 2004a)	Few taken by line fishing, protected from spearing since 1970's.	Could be territorial based on observations by local SCUBA divers who feed same individuals. Other temperate labrids such as *Pictilabrus laticlavius* and *Notolabrus tetricus* move distances < 100 m, and *Notolabrus fucicola* > 150 m and have responded to protection by a 7 km^2 MPA.
MONACANTHIDAE		
(Kingsford & Millicich 1987, Barrett 1995, Hutchins 1999, Edgar et al. 2004a)	Taken by line and spear.	*A. vitteger* shown to have home ranges < 2500 m^2 and have responded to protection within a 7 km^2 MPA. Tagged *M. australis* and *M. freycineti* generally move < 100 m.
GIRELLIDAE		
Girella elevata **(Rock Blackfish)** (Wilson 1984, Kingsford et al. 1991)	Important recreational species in NSW, taken by line and spear.	Thought to be territorial , longest distance travelled by tagged fish was 500 m (M. Aston per. comm.); however, temporal patterns of abundance suggest that they move at scales > 1 km.

Table 2.1. cont.

b) Mobile adults (movement > 10's of km's)

Species	Harvesting methods	Scale of adult movement
GIRELLIDAE		
Girella tricuspidata **(Luderick)** (Morrison 1990, Kingsford et al. 1991, West 1993, Gray et al. 2000, Kingsford 2002)	Important recreational species in NSW, taken primarily by line but also by spear. Harvested by commercial ocean and estuarine haul fisheries.	Temporal changes in abundance suggest they move at scales > 1 km on coastal reefs and undertake annual spawning migrations. Tagged individuals that inhabit estuaries capable of travelling between estuaries, and along coast covering distances > 150 km.
SPARIDAE		
Acanthopagrus australis **(Yellow-fin Bream)** ***Rhabdosargus sarba*** **(Tarwhine)** (Pollock 1984, Kingsford et al. 1991, Kingsford 2002)	Important recreational species, taken by line and spear.	Temporal changes in abundances suggest they move at scales > 1 km. Known to undertake annual spawning migrations.
KYPHOSIDAE		
Kyphosus sydneyanus **(Silver Drummer)** (Eristhee & Oxenford 2001, Kingsford 2002)	Recreational species, taken by line and spear	Temporal changes in abundances suggest they move at scales > 1 km on coastal reefs. Related species *K. sectatrix* has a home range of 30,000-40,000 m^2 (tagging).

Table 2.2. Description of subtidal habitat categories and the depth ranges over which they were recorded.

Habitat	Depth (m)	Description
Rock	≤ 11.5	Urchin-grazed barrens or bare rock.
Rock with sparse algae	2.5 - 11.5	Urchin-grazed barrens or rock with very sparse algae including: *Ecklonia radiata*, *Phyllospora comosa*, geniculate corallines (e.g. *Amphiroa* and *Corallina* spp.), *Sargassum* spp., and brown turfing algae (e.g. dictyotalean algae).
Mixed algae	≤ 9	Substrate covered by mixture of algae including: *E. radiata*, *P. comosa*, geniculate corallines, *Sargassum* spp., and brown turfing algae.
Turf	≤ 6	Macroalgae absent. Geniculate corallines and brown turfing algae cover substratum.
Kelp	≤ 11.5	*E. radiata* and/or *P. comosa* most conspicuous organism, often forming a canopy.
Sand	2 - 7.5	Patch of sand within reef or on sand/reef interface.

Table 2.3. Example of complete asymmetrical ANOVA comparing the abundance of *C. fuscus* at two MPAs and three control locations. Repartitioned sources of variation are offset and shaded rows indicate the most important factors for testing for an effect of the MPAs. *F* vs., first number shown is the row number for term used as denominator in *F* test, second number is row for alternative test whose use is indicated in superscript next to the Mean Square estimate (MS). Data were ln(x+1) transformed. ns $P \geq 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, NT no valid test could be created because pooling was not possible.

				Cabbage Tree Bay		Gordon's Bay	
				Sub-legal	Legal	Sub-legal	Legal
		Size mm (SL)		100-150	≥ 200	100-150	≥ 200
Row	Source of variation	df	*F* vs.	MS	MS	MS	MS
1	Time	5	9	0.528	0.477	0.620	0.587
2	Location	3		0.230	1.131	0.393	9.024
3	MPA vs. Controls	1	4,5	0.042^{5}	2.012	0.530	25.692^{**5}
4	Among Controls	2	5	0.325	0.691	0.325^{NT}	0.691
5	Site(L)	4	12	0.849*	0.318	1.018**	0.361
6	Si(MPA)	1	12	0.042	0.677	0.719	0.847
7	Si(Controls)	3	12	1.118*	0.199	1.118**	0.199
8	Depth	1	16	0.740^{NT}	12.480^{NT}	0.896^{NT}	23.371**
9	T x L	15	12	0.343	0.272	0.329	0.346
10	T x (MPA vs. Controls)	5	11,12	0.261	0.062	0.218	0.281
11	T x Controls	10	12	0.384	0.378	0.384	0.378
12	T x Si(L)	20	28	0.267***	0.229	0.221*	0.261
13	T x Si(MPA)	5	28	0.230	0.141	0.044	0.269
14	T x Si(Controls)	15	28	0.280**	0.258	0.280**	0.258
15	T x D	5	22	0.270**	0.539*	0.445**	0.195
16	L x D	3	19	0.041	1.524*	0.014	0.225
17	(MPA vs. Controls) x D	1	18,22	0.121**	3.966	0.040*	0.069^{NT}
18	Controls x D	2	19	0.001	0.303	0.001	0.303
19	Si(L) x D	4	25	0.425	0.119	0.425	0.273
20	Si(MPA) x D	1	25	0.124	0.019	0.125	0.633
21	Si(Controls) x D	3	25	0.526	0.152	0.526	0.152
22	T x L x D	15	25	0.050	0.180	0.055	0.344
23	T x (MPA vs. Controls) x D	5	24,25	0.065^{25}	0.135^{25}	0.078^{25}	0.626^{25}
24	T x (Controls) x D	10	25	0.043	0.203	0.043	0.203
25	T x Si(L) x D	20	28	0.257**	0.272	0.247**	0.364
26	T x Si(MPA) x D	5	28	0.274*	0.225	0.231	0.592*
27	T x Si(Controls) x D	15	28	0.252**	0.288	0.252*	0.288
28	Residual	384		0.105	0.191	0.123	0.235

Table 2.4. Significance of *F*-ratios for most relevant levels of ANOVA when comparing the abundance of fish at two MPAs and three control locations. Full analyses for each species are given in Appendix A; Table A.1-A.5. ns $P \geq 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, NT no valid test could be created because pooling was not possible. Shaded rows indicate tests relevant to MPA effects.

	Sedentary (movement < km's)								**Mobile (movement > 10's km's)**			
	C. fuscus		***A.viridis***		**Monacanthidae**		***G. elevata***		***G. tricuspidata***	***A. australis***	***K. sydneyanus***	
	sub-legal	legal	small	large	small	large	sub-legal	legal	legal	legal	small	large
Size mm (SL)	100-150	≥ 200	≤ 150	≥ 200	≤ 150	≥ 200	≤ 200	≥ 250	≥ 200	≥ 200	≤ 150	≥ 200
Source of variation												
Cabbage Tree Bay												
MPA vs. Controls	ns	ns	ns	ns	ns	NT	ns	NT	NT	**	ns	NT
T x (MPA vs. Controls)	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns
(MPA vs. Controls) x D	**	ns	ns	NT	ns	ns	ns	NT	NT	ns	ns	NT
T x (MPA vs. Controls) x D	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Time (T)	ns	ns	ns	ns	ns	ns	***	ns	ns	ns	ns	Ns
Site	*	ns	*	ns	ns	**	*	**	**	ns	ns	ns
Among Controls	ns	ns	NT	*	NT	NT	ns	ns	ns	ns	ns	ns
Depth (D)	NT	NT	ns	NT	ns	**	NT	NT	*	ns	NT	NT
Gordon's Bay												
MPA vs. Controls	ns	**	ns	ns	ns	NT	ns	ns	ns	ns	ns	ns
T x (MPA vs. Controls)	ns	Ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns
(MPA vs. Controls) x D	*	NT	NT	ns	NT	ns	ns	ns	ns	*	ns	*
T x (MPA vs. Controls) x D	ns	ns	*	ns	*	ns	ns	ns	ns	ns	ns	ns
Time	ns	ns	ns	ns	*	ns	***	*	ns	ns	ns	ns
Among Controls	NT	ns	NT	NT	NT	NT	ns	ns	ns	NT	ns	ns
Site	**	ns	*	*	NT	NT	*	**	**	**	ns	ns
Depth	NT	**	NT	NT	NT	NT	NT	NT	*	ns	NT	NT

Interpretation of significant results for MPA effects: MPA vs. Controls = There is a difference between MPA and controls independent of times, sites or depths; T x (MPA vs. Controls) = Patterns found between MPA and controls are dependent on the time of sampling; (MPA vs. Controls) x D = The difference between MPA and controls is dependent on the depth considered and/or the effect of depth is dependent on the location; T x (MPA vs. Controls) x D = Time, locations (MPA and control) and depths are interdependent.

Table 2.5. Semi-parametric permutational ANOVA comparing habitat assemblages among locations and depths for sampling times 2-6. ns $P \geq 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

			Time 2	Time 3	Time 4	Time 5	Time 6
	df	*F* vs.	MS	MS	MS	MS	MS
Location	4	Res.	1824.342^{ns}	1002.846^{ns}	491.425^{ns}	1922.835^{ns}	2145.516^{ns}
Depth	1	L x D	12558.759**	9451.976*	13636.262**	7106.418*	7159.067*
L x D	4	Res.	598.641^{ns}	1089.893^{ns}	614.459^{ns}	1463.533^{ns}	1227.901^{ns}
Residual	10		1542.760	1509.575	831.663	1035.272	1815.992
Total	19						

Table 2.6. Habitat types contributing to greater than 15% of the average dissimilarity between depths at each sampling time 2-6**.** Average percentage occurrence of each habitat type in shallow (≤ 3.5 m) and deep (4-12 m) water is given.

	Time 2	Time 3	Time 4	Time 5	Time 6
Average dissimilarity	***68.02***	***61.41***	***59.37***	***57.98***	***63.03***
Algae mixture					
Shallow	64.73	47.39	57.25	45.01	46.57
Deep	16.65	14.13	10.30	9.12	13.48
% contribution	*39.29*	*28.00*	*42.60*	*35.59*	*33.20*
Rock					
Shallow	13.74	15.18	17.39	27.25	23.14
Deep	43.57	44.38	54.62	48.12	48.00
% contribution	*26.85*	*28.60*	*33.58*	*26.29*	*31.06*
Macroalgae					
Shallow	6.58	13.63	18.62	19.27	14.00
Deep	25.20	24.31	29.49	29.53	29.16
% contribution	*17.95*	*15.88*	*21.25*	*24.68*	*21.52*

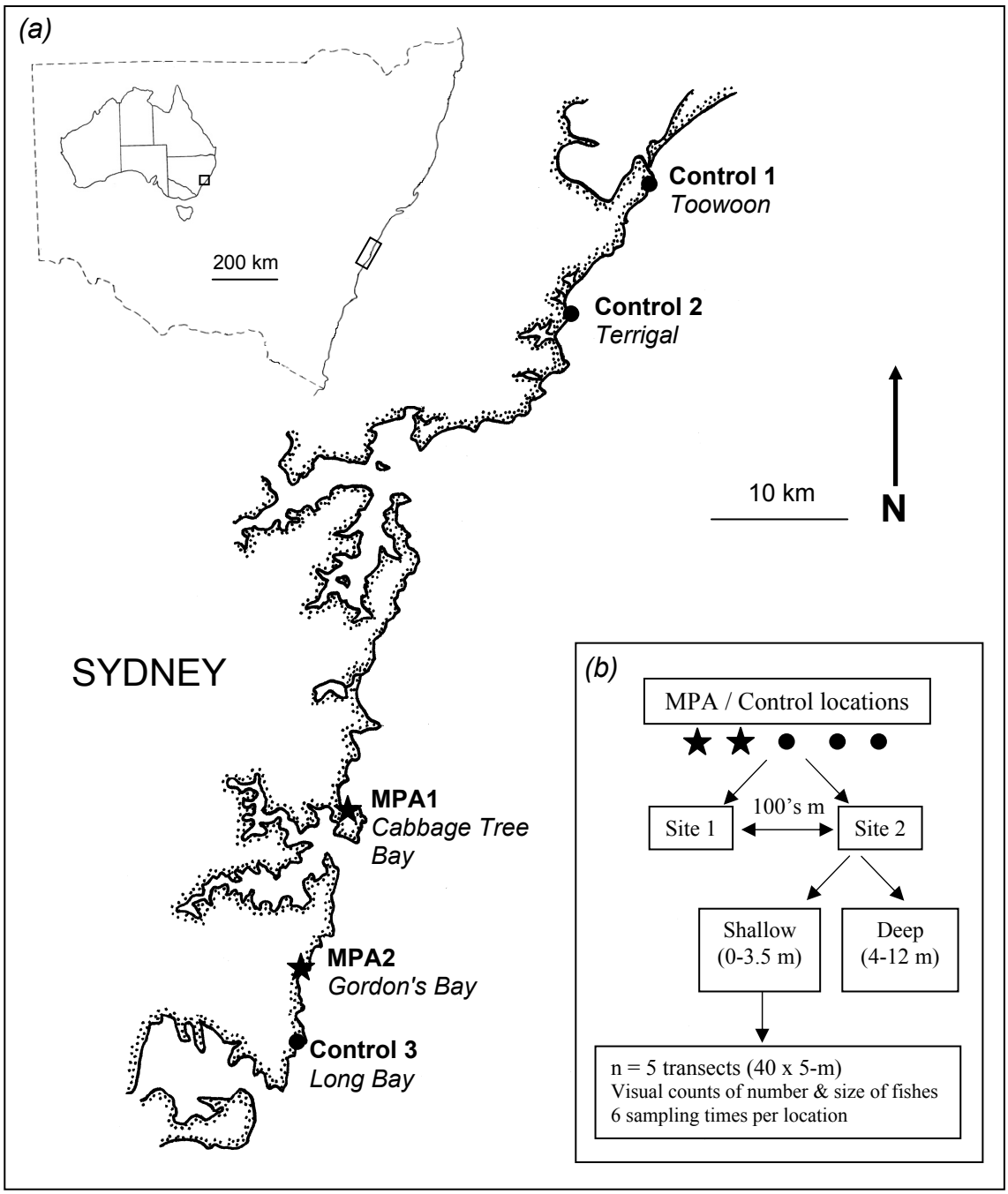

Fig. 2.1. ***(a)*** Sydney region of NSW, Australia showing position of two Marine Protected Areas (MPAs) (Gordon's Bay 33°92'S, 151°26'E; Cabbage Tree Bay 33°80'S, 151°29'E) and three control locations (Toowoon Bay 33°36'S, 151°50'E; Terrigal 33°45'S, 151°45'E; Long Bay 33°97'S, 151°25'E). ***(b)*** Sampling design used to estimate the abundance of fishes at MPA and control locations.

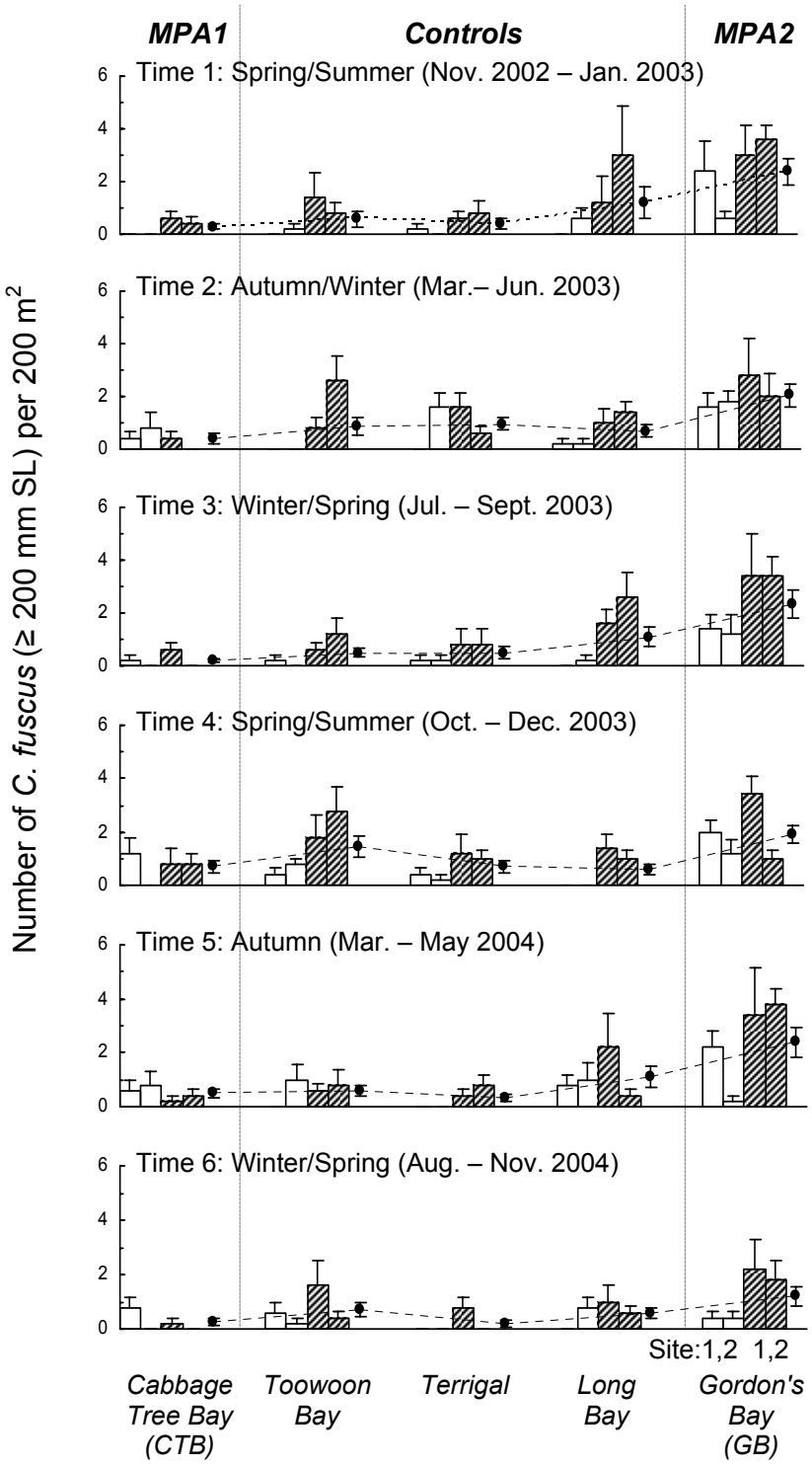

Fig. 2.2. Mean abundance (+ SE) of legal *C. fuscus* (≥ 200 mm standard length) in shallow (☐) and deep (▨) areas of reef in MPA and control locations at six sampling times. Abundances are shown for each of two sites (separated by 100's m) within each location. n = 5 replicates at each depth. ● = Mean abundance for location (± SE).

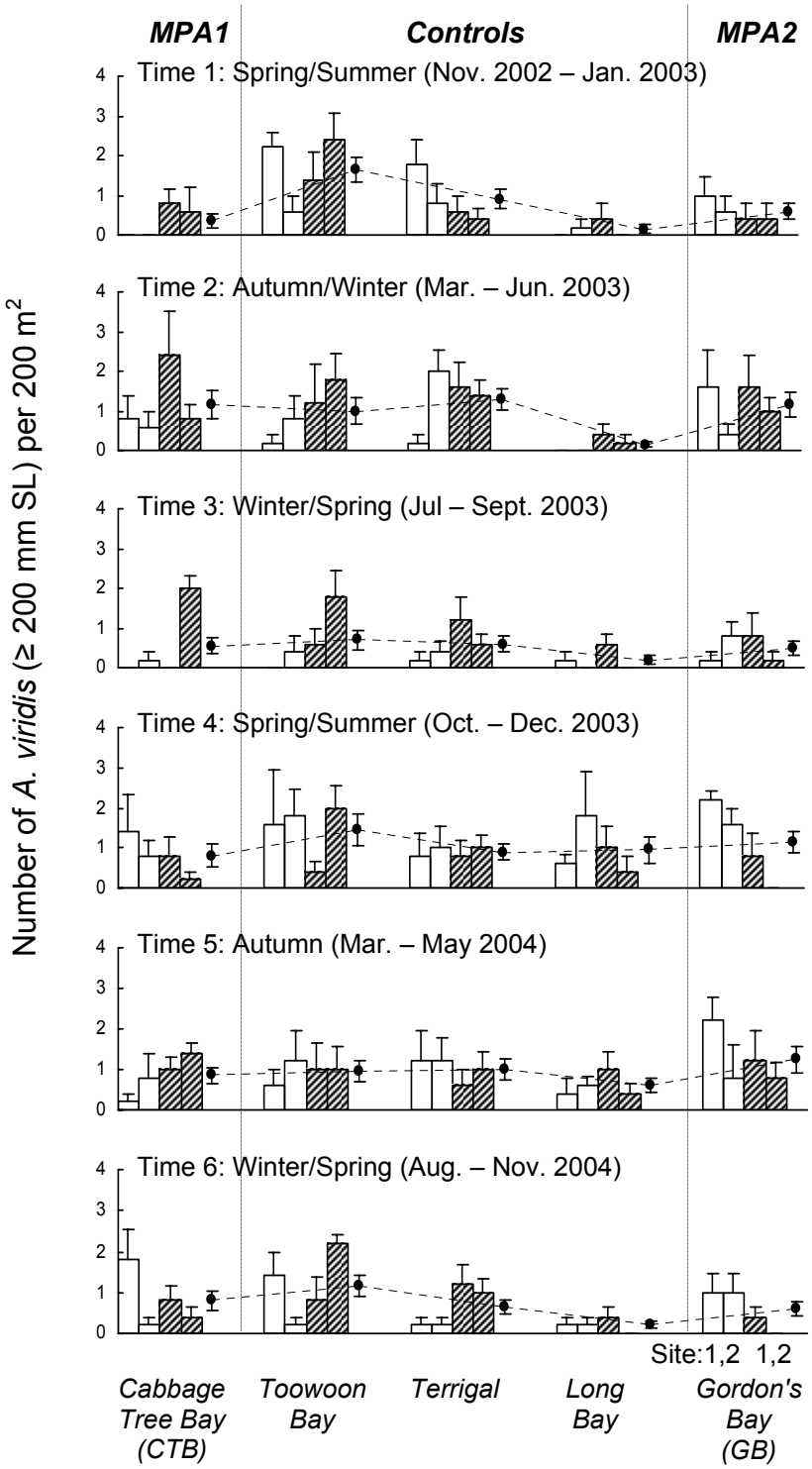

Fig. 2.3. Mean abundance (+ SE) of large *A. viridis* (≥ 200 mm standard length) in shallow (☐) and deep (▨) areas of reef in MPA and control locations at six sampling times. Abundances are shown for each of two sites (separated by 100's m) within each location. n = 5 replicates at each depth. ● = Mean abundance for location (± SE).

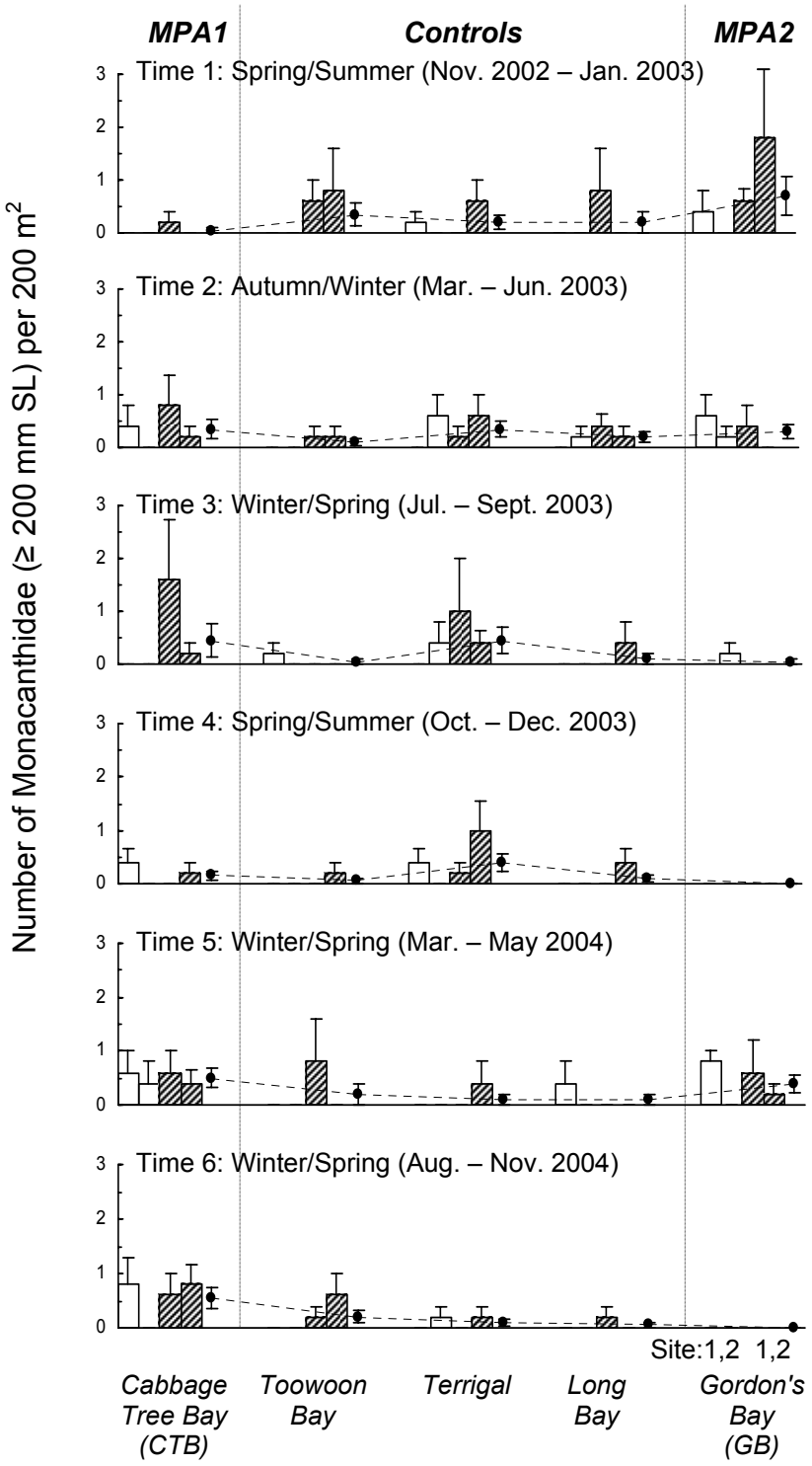

Fig. 2.4. Mean abundance (+ SE) of large Monacanthidae (≥ 200 mm standard length) in shallow (□) and deep (▨) areas of reef in MPA and control locations at six sampling times. Abundances are shown for each of two sites (separated by 100's m) within each location. n = 5 replicates at each depth. ● = Mean abundance for location (± SE).

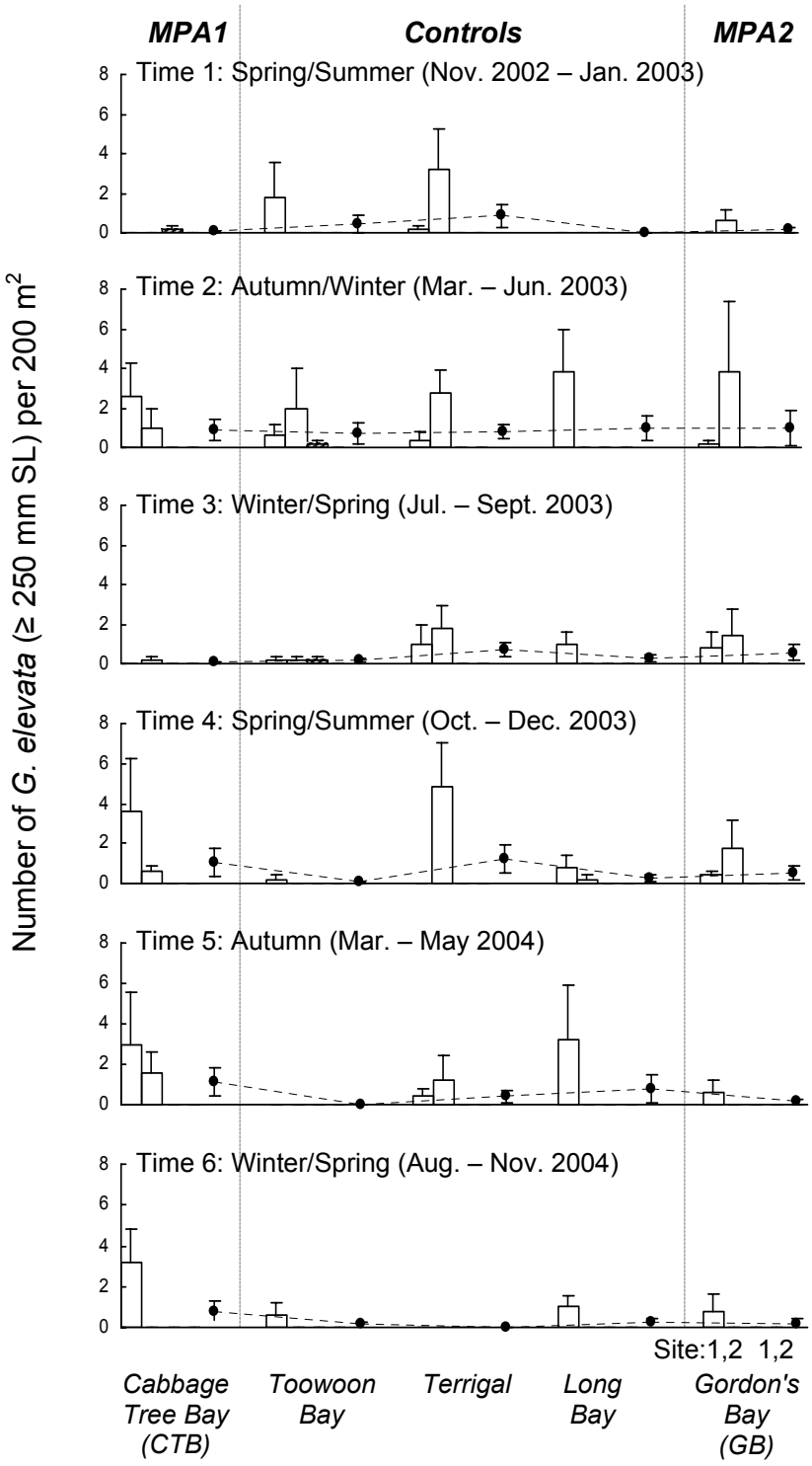

Fig. 2.5. Mean abundance (+ SE) of legal *G. elevata* (≥ 250 mm standard length) in shallow (☐) and deep (▨) areas of reef in MPA and control locations at six sampling times. Abundances are shown for each of two sites (separated by 100's m) within each location. n = 5 replicates at each depth. ● = Mean abundance for location (± SE).

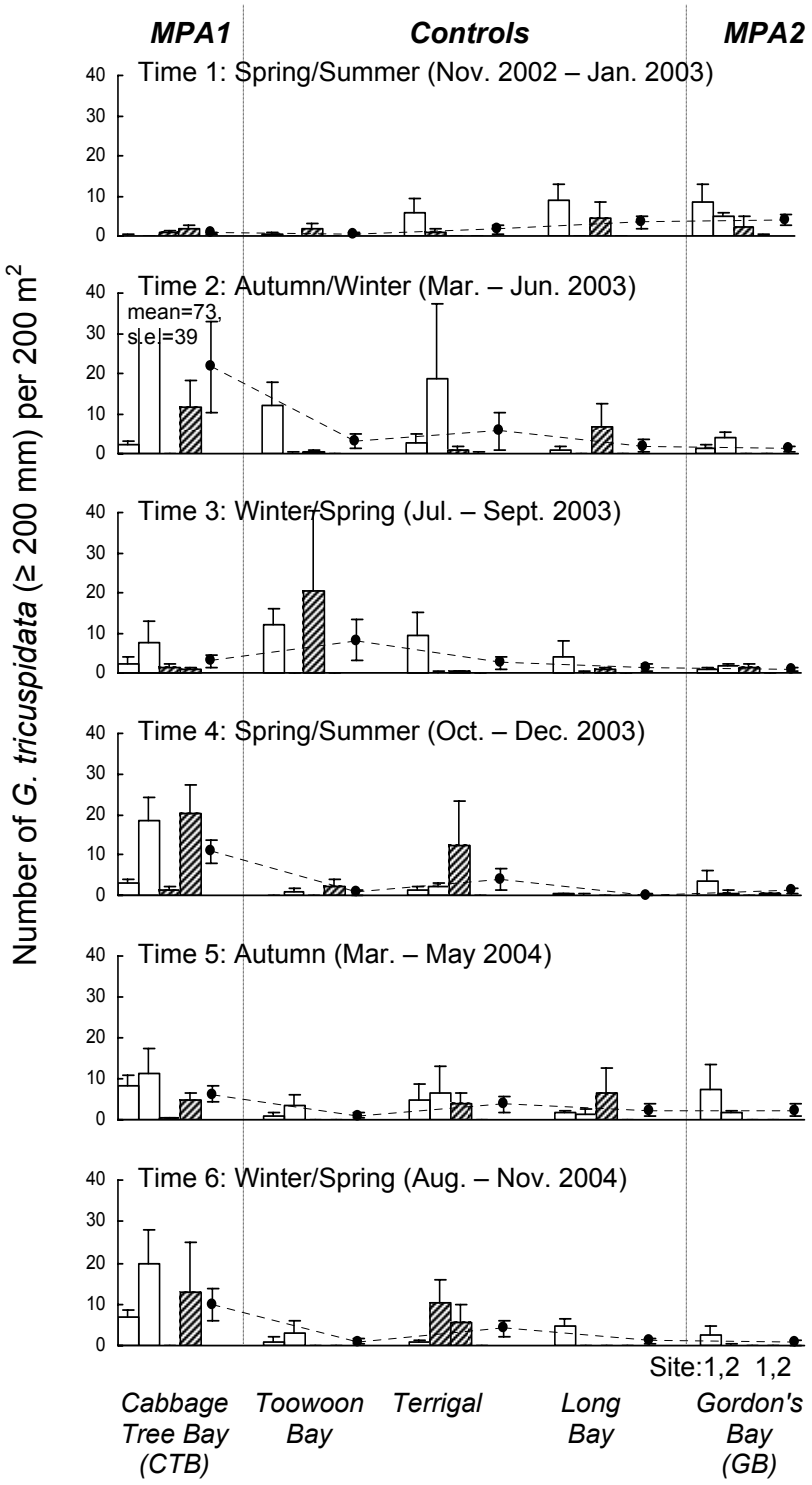

Fig. 2.6. Mean abundance (+ SE) of legal *G. tricuspidata* (≥ 200 mm standard length) in shallow (☐) and deep (▨) areas of reef in MPA and control locations at six sampling times. Abundances are shown for each of two sites (separated by 100's m) within each location. n = 5 replicates at each depth. ● = Mean abundance for location (± SE).

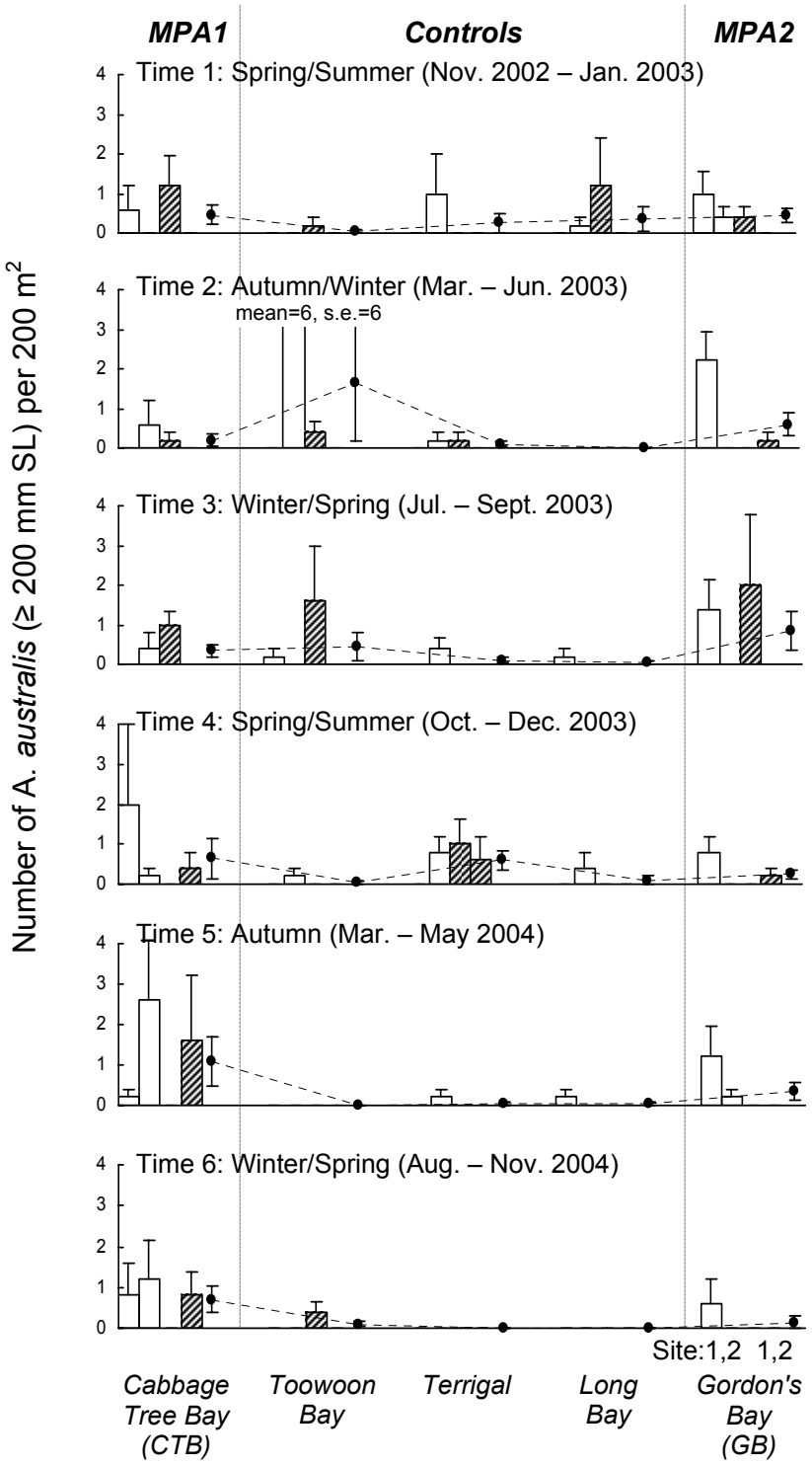

Fig. 2.7. Mean abundance (+ SE) of legal *A. australis* (≥ 200 mm standard length) in shallow (☐) and deep (▨) areas of reef in MPA and control locations at six sampling times. Abundances are shown for each of two sites (separated by 100's m) within each location. n = 5 replicates at each depth. ● = Mean abundance for location (± SE).

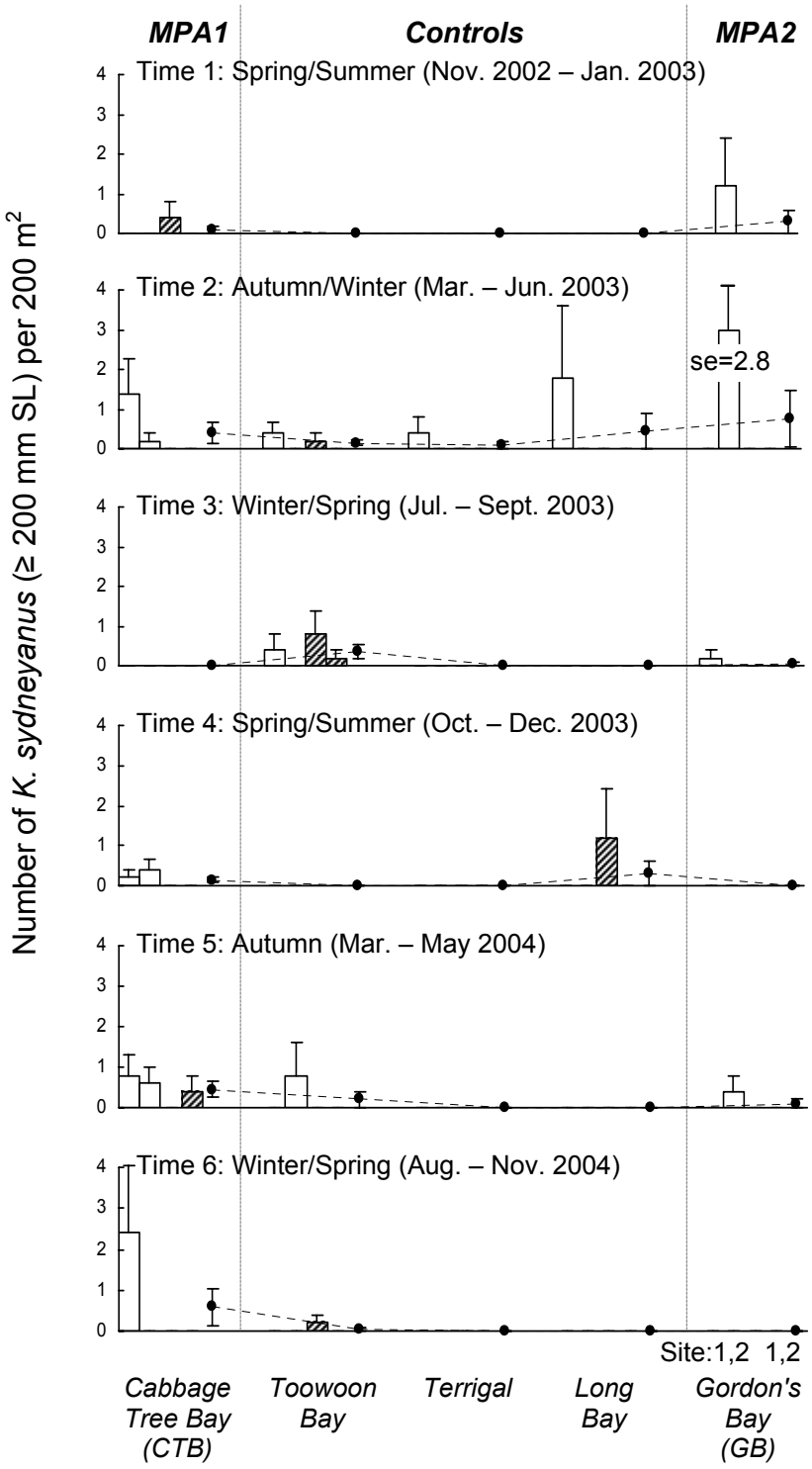

Fig. 2.8. Mean abundance (+ SE) of large *K. sydneyanus* (≥ 200 mm standard length) in shallow (☐) and deep (▨) areas of reef in MPA and control locations at six sampling times. Abundances are shown for each of two sites (separated by 100's m) within each location. n = 5 replicates at each depth. ● = Mean abundance for location (± SE).

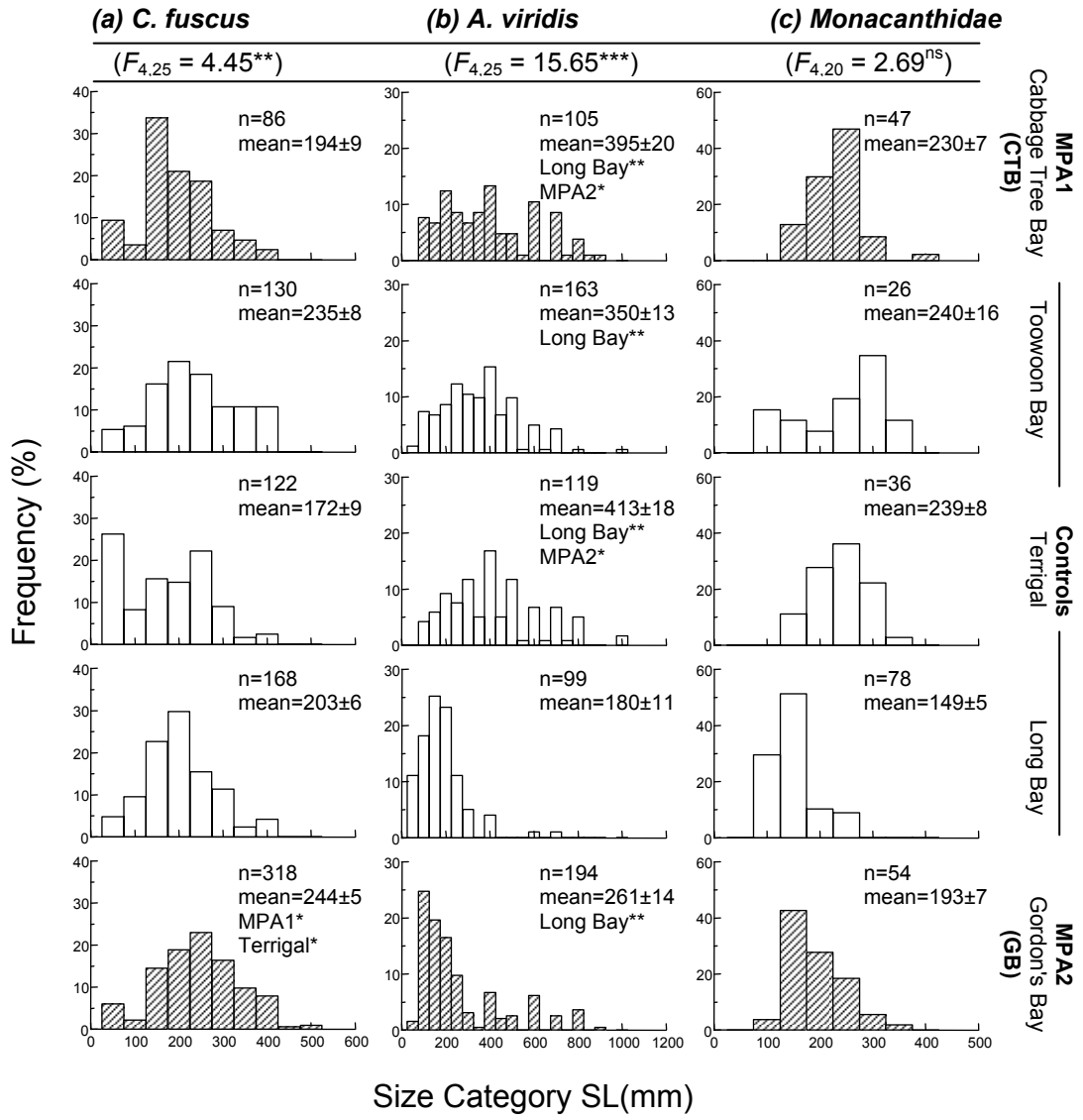

Fig. 2.9. Size frequency for exploited reef fishes at MPA and control locations for six sampling times pooled. *F*-ratios from ANOVA comparing mean size of fish among locations are given below each species name. Significantly higher mean sizes within a location are indicated below means values. For example, the mean size of *C. fuscus* at MPA2 was greater than MPA1 and Terrigal ($P < 0.05$); ns $P \geq 0.05$, *$P < 0.05$, **$P < 0.01$.

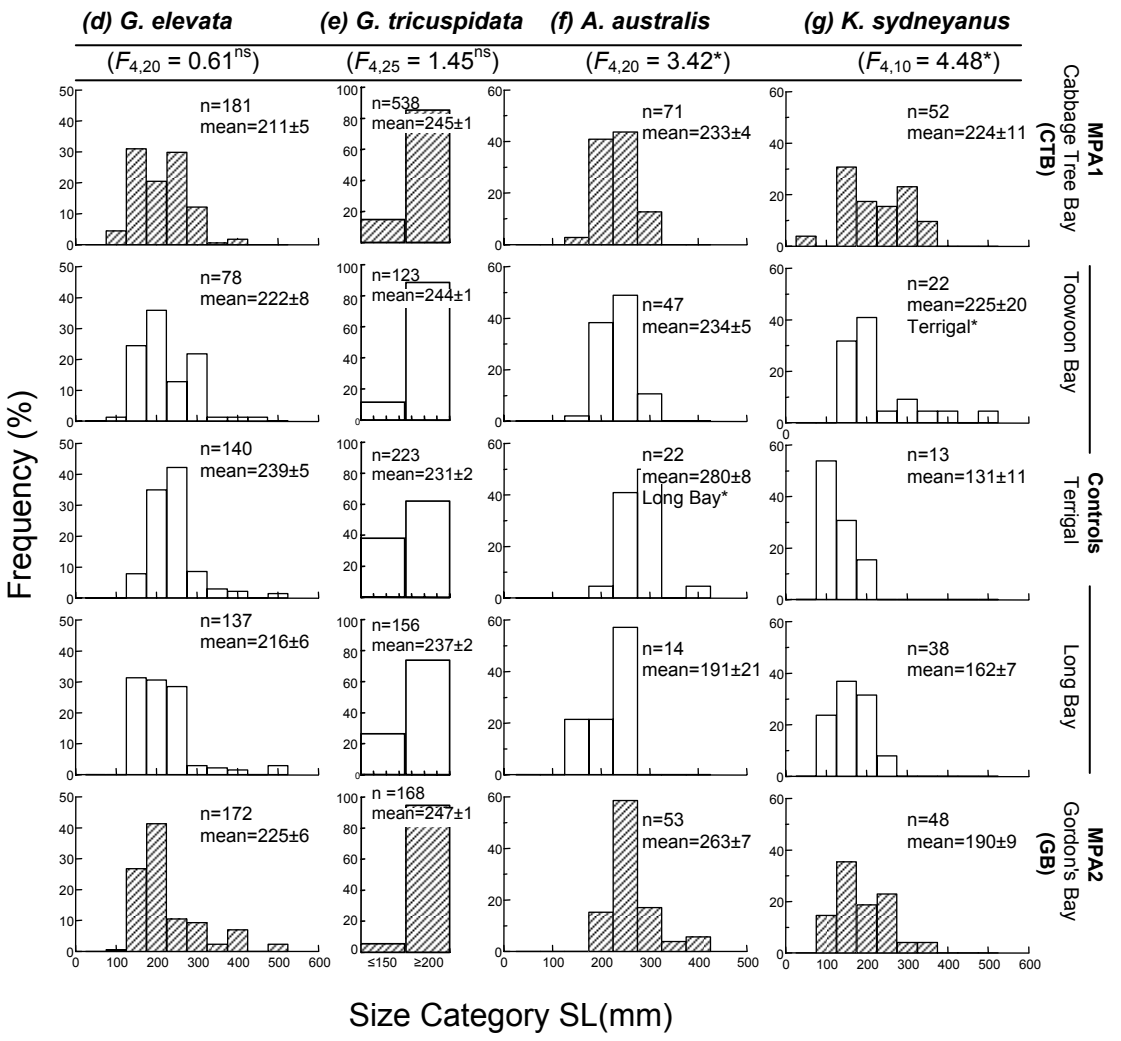
(d) G. elevata
(e) G. tricuspidata
(f) A. australis
(g) K. sydneyanus
($F_{4,20}$ = 0.61^{ns})
($F_{4,25}$ = 1.45^{ns})
($F_{4,20}$ = 3.42*)
($F_{4,10}$ = 4.48*)
n=181 mean=211±5
n=538 mean=245±1
n=71 mean=233±4
n=52 mean=224±11
MPA1 (CTB) Cabbage Tree Bay
n=78 mean=222±8
n=123 mean=244±1
n=47 mean=234±5
n=22 mean=225±20 Terrigal*
Toowoon Bay
n=140 mean=239±5
n=223 mean=231±2
n=22 mean=280±8 Long Bay*
n=13 mean=131±11
Controls Terrigal
n=137 mean=216±6
n=156 mean=237±2
n=14 mean=191±21
n=38 mean=162±7
Long Bay
n=172 mean=225±6
n =168 mean=247±1
n=53 mean=263±7
n=48 mean=190±9
MPA2 (GB) Gordon's Bay
Frequency (%)
≤150
≥200
Size Category SL(mm)

Fig. 2.9. cont.

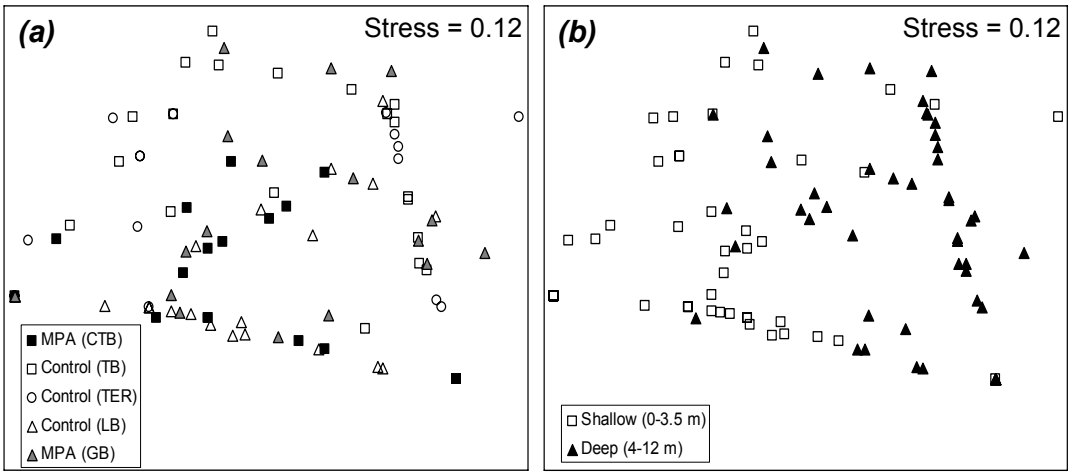

Fig. 2.10. Non-metric MDS ordinations comparing the composition and relative percentage of habitat types in ***(a)*** MPA (Cabbage Tree Bay (CTB), Gordon's Bay (GB)) and control locations (Toowoon Bay (TB), Terrigal (TER), Long Bay (LB)), and ***(b)*** shallow and deep areas of reef. Data from all five sampling times were included in ordinations. Stress values indicate how well the dissimilarity matrix was represented by the MDS and stress tends towards zero when data are perfectly represented (Clarke 1993).

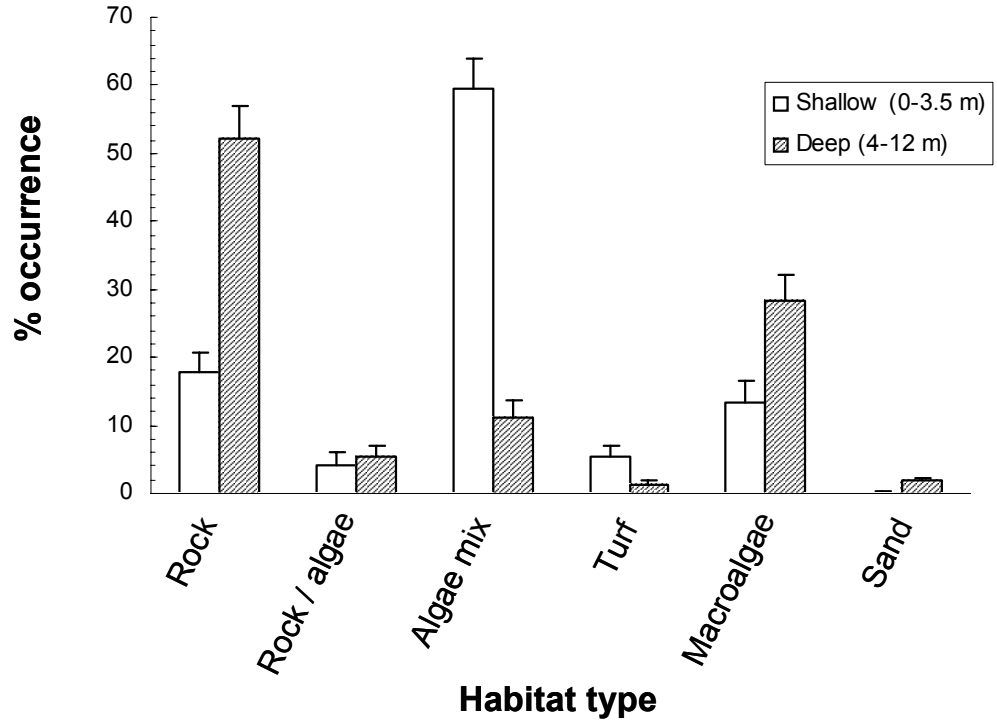

Fig. 2.11. Mean percentage occurrence (+ SE) of six habitat types in shallow (≤ 3.5 m) and deep (4-12 m) areas of reef. Data pooled for all times, locations, and sites (n = 50).

2.4. Discussion

Temporal variation in abundances suggested that five of the seven species examined moved at scales greater than the area encompassed by MPAs ≤ 0.2 km^2. Overall results, however, were in agreement with previous studies that failed to make generalizations between estimated mobility, duration of protection and the response of fishes to MPAs (Micheli et al. 2004, Palumbi 2004). MPA effects were detected for one 'sedentary' species (*C. fuscus*) within the 12.5 year old MPA, and two 'mobile' species (*A. australis* and *G. tricuspidata*) within the 2.5 year old MPA. The magnitude of effects detected for these species were consistent with meta-analyses which indicate that protection generally leads to doubling of densities and a 20-30% increase in the mean size of exploited organisms (*C. fuscus* only) relative to unprotected areas, irrespective of the size of MPAs (Halpern 2003).

Cheilodactylus fuscus was the only species of the four classed as 'sedentary' that exhibited a significant response to protection. *C. fuscus* is a large benthic carnivore, is highly targeted by spear fishers in NSW, has a small home range (day time 1865 ± 268 m^2), diver-neutral behaviour, is long-lived (> 40 yrs) and has a tendency to aggregate in relatively shallow water (Lincoln Smith et al. 1989, Lockett & Suthers 1998, Lowry 2003). It has been proposed that these traits would increase the susceptibility of local populations to spearing effects (Lowry 2003) which can alter the abundance, size and the depth distribution of fishes (Harmelin et al. 1995, Jouvenel & Pollard 2001). Results of this study strongly support this hypothesis. Legal-sized fish were 2.8-times more abundant and larger inside GB, where spearing is prohibited, relative to unprotected areas. Furthermore, the local depth distribution of *C. fuscus* varied among GB and controls, with higher densities of legal fish found in shallow areas of reef inside GB. This pattern is indicative of a spearing effect as shallow areas of reef are likely to be fished more intensively than deeper areas as they are more accessible to experienced and inexperienced divers. Similar trends for higher densities of *C. fuscus* inside MPAs have been reported for larger MPAs in central NSW (Pers. Comm. W. Gladstone), Jervis Bay and Solitary Islands Marine Parks (Pers. Comm. G. Edgar; N. Johnstone), and for a related species, *Cheilodactylus spectabilis*, in a New Zealand MPA (Cole et al. 1990). MPA effects, tagging studies and the limited temporal variation in abundance described here, suggests that a significant proportion of the movements of

C. fuscus are contained within relatively small MPAs, and that results of this study are unlikely to be an artifact of pre-existing differences between MPAs and controls. Despite strong evidence of MPA effects within GB, the mechanisms for recovery of *C. fuscus* (immigration or recruitment) could not be determined due to the lack of baseline data and long-term monitoring over the 12.5 years of protection.

In contrast, there was little evidence of MPA effects for *C. fuscus* within CTB. It is likely that the duration of protection for CTB (2.5 yrs) was inadequate to see detectable changes in the abundance of *C. fuscus* and other species, regardless of mobility. Although increases in the density of target species can be rapid (< 3 yrs; Halpern & Warner 2002), full recovery of a fished stock to a 'natural state' may take considerably longer (10-40 yrs; Russ & Alcala 2004, Barrett et al. 2007). Theoretical recovery rates depend on several factors including: initial population size, intrinsic rate of population increase, life-history characteristics, recruitment variation, reduction in fishing mortality, immigration rates, and local habitat quality (Jennings 2001, Denny et al. 2004). A previous study which involved the experimental removal of > 70% of large *C. fuscus* from a reef demonstrated that densities could return to pre-speared levels within 2-4 months. This recovery, however, was attributed to re-colonisation by fish from adjacent areas and is likely to be site dependent (Lowry & Suthers 2004). The absence of significant MPA effects for *C. fuscus* within CTB suggest that recovery at this site is dependent on recruitment, rather than immigration and, therefore, a time-lag is to be expected (Polunin & Roberts 1993, Denny et al. 2004). The trend for an increase in size of fish within CTB in the last two sampling periods (corresponding to 2.5 years of protection) may indicate the start of a detectable response as juvenile *C. fuscus* grow rapidly and may reach legal size in 2.5 years (Lowry 2003). Long-term monitoring will be required to test this model.

More uncertainty surrounds results for *A. australis* and *G. tricuspidata*. Densities of legal *A. australis* were 2.6-times higher inside CTB when compared to controls with similar trends observed for *G. tricuspidata* at some sampling times. Existing knowledge of mobility of these species suggests that the 0.2 km^2 area protected by CTB does not encompass the movements of these species. Large temporal variation in densities of these species on coastal reefs were detected in this study and have been reported previously (Kingsford 2002). In addition, estuarine populations of *G. tricuspidata* and

A. australis are known to undertake pre-spawning migrations along the NSW coast, with tagged individuals capable of travelling hundreds of kilometres (Gray et al. 2000). Several models or combinations may explain observed effects.

Effects of MPAs could only be inferred using spatial comparisons, as no 'before data' were available. It is possible, therefore, that differences in abundance and size of fishes between MPAs and controls were due to pre-existing differences rather than protection from harvesting (Edgar et al. 2004b). Several potentially confounding variables were controlled for in the sampling design (e.g. small-scale variation in abundance, depth, and accessibility to fishers, temporal variability due to episodic recruitment). In addition, differences could not be attributed to variation in habitat assemblages with most variation occurring between depths rather than among MPAs and controls. Alternative factors such as topographic complexity, wave exposure, variation in absolute depth among locations, and spatial variation in fishing effort however, could explain observed patterns (Jones 1988, Curley et al. 2002, Barrett et al. 2007). For example, large site-specific aggregations of *G. tricuspidata* were only found within CTB suggesting that this site has favourable habitat characteristics for this species.

Despite the potential for pre-existing differences, relatively mobile species may still respond to protection (Roberts et al. 2001, Apostolaki et al. 2002, Willis et al. 2003). Previous local fishing pressure, and reserve attributes, rather than mobility alone, are critical in determining the response of organisms to protection (Palumbi 2004). For example, strong responses by highly mobile species within small MPAs have been attributed to high levels of previous fishing pressure (Palumbi 2004). Furthermore, MPAs may provide protection at times when species are highly vulnerable to disturbance and mortality by fishing (e.g. aggregating near-shore; Barrett et al. 2007). This is a possible explanation for observed MPA effects for *A. australis* and *G. tricuspidata* within CTB, as both species are heavily targeted by recreational fishers in central NSW (Kingsford et al. 1991). Rapid recovery of local populations of *A. australis* and *G. tricuspidata* within CTB would be expected in this scenario as both species recruit to estuarine habitats (Pollock et al. 1983, Hannan & Williams 1998) such that recovery within coastal MPAs would be achieved through the immigration of fish from adjacent areas.

Alternatively, assumptions regarding the mobility of *A. australis* and *G. tricuspidata* may be inaccurate. For example, it was proposed that the sparid *Pagrus auratus* (Snapper) would not benefit from MPAs in New Zealand as they were thought to be highly mobile (Crossland 1976, Willis et al. 2003, Denny et al. 2004). However, the abundance of *P. auratus* was shown to increase inside MPAs following protection (Willis et al. 2000, Willis et al. 2003). Additional tagging studies demonstrated that this response could be explained by intraspecific variation in the movement of *P. auratus* with some fish displaying high site fidelity and others being migratory (Parsons et al. 2003, Egli & Babcock 2004). This bi-modal pattern of movement has been recorded for other temperate species (Attwood & Bennett 1994) and has been proposed for *G. tricuspidata* (Morrison 1990). This model is supported for *G. tricuspidata* as many tagged individuals were re-captured within the estuary in which they were released, while others travelled > 100 km among estuaries (Thomson 1959, West 1993, Gray et al. 2000). In addition, the only two fish recovered after tagging of 66 *G. tricuspidata* on coastal reefs were caught in the same 500 m section of reef 4.5 and 8 months after release (Curley unpublished data). It is unclear if these movements are representative of all individuals within the population and further studies are required using alternative methods such as ultrasonic tagging and otolith chemistry (Campana et al. 2000, Egli & Babcock 2004).

Lack of clear MPA effects for *A. australis, G. tricuspidata* and several other species within GB was predicted given the lower degree of protection offered. GB protects fish from spear fishing but not line fishing (with the exception of *A. viridis*), therefore, species that are often taken by line fishers (e.g. *A. australis*, *G. tricuspidata*, *G. elevata*) would not be expected to exhibit the same response to protection as species that are primarily targeted by spear fishers (e.g. *C. fuscus*). Partial closures are seen as a 'compromise' between fishing and protection and may result in benefits such as reduction in overall and incidental mortality (Denny & Babcock 2004). However, partial protection may also encourage fishing pressure within MPAs due to their unique locations and the perception that there will be more fish inside MPAs relative to unprotected areas (Westera et al. 2003, Denny & Babcock 2004, Denny et al. 2004, Shears et al. 2006). Data on relative fishing pressure did not support this scenario with the number of line fishers observed at GB being less or similar to the number found at unprotected locations. Despite the partial protection status, there was a trend for higher

densities of *A. australis* in GB relative to controls and the largest *A. australis* were recorded at this location. The closure of GB to all methods of fishing would allow the effects of line fishing on species such as *A. australis* to be investigated. This study could act as a baseline for such studies and allow for more conclusive comparisons with CTB, which are currently confounded by differences in the level of protection between CTB and GB.

The apparent lack of MPA effect observed for *A. viridis*, Monacanthidae, *G. elevata*, *K. sydneyanus* in either MPA could be explained by several models. According to the initial hypotheses the most likely explanation is that fish are moving at scales greater than MPAs and protection is inadequate to cause a detectable effect. This model is supported by observed temporal variation in abundances of *Monacanthidae*, *G. elevata*, and *K. sydneyanus*. Inadequate size of MPAs relative to the mobility of fishes is thought to be the major factor impeding recovery in other small temperate MPAs (Barrett et al. 2007). Mobility in this study, however, was inferred by temporal variation in abundances. As such alternative models such as mortality, habitat selection, or the presence of different individuals at different sampling times cannot be rejected (Gillanders 1997).

There was no evidence that the lack of response to protection was due to limited recruitment of fish inside MPAs relative to controls. Densities of small and sub-legal fishes at both MPAs were similar to the range found at controls. Specific locations, however, appeared more favourable for recruitment in this and previous studies (Curley et al. 2002). Spatial variation in recruitment should, therefore, be considered in the selection of sites for MPAs in the future, as higher levels of recruitment may lead to faster recovery of local populations following protection. Lack of observed effects may also be due to the failure to protect appropriate habitat types or an adequate amount of habitat within MPA boundaries (Gell & Roberts 2003, Barrett et al. 2007). The home range of a fish may be influenced by a number of factors such as diversity and abundance of prey items, variation in habitat quality or characteristics, reproduction, and habitat requirements may vary with size and age (Choat & Ayling 1987, Matthews 1990, Gillanders & Kingsford 1998, Lockett & Suthers 1998, Lowry & Suthers 1998, McCormick 1998). In addition, although the home range of fish may be large, movements may be restricted to a small number of preferred sites (Eristhee & Oxenford

2001). For example adjacent schools of large *G. elevata* may be separated by hundreds of metres to kilometres and are often associated with wave exposed areas and deep holes adjacent to rock platforms (Curley unpublished data). Small, randomly placed MPAs like CTB and GB are unlikely to include preferred aggregations sites or a representative range of habitats and depths (Curley et al. 2002). Real-time tagging techniques (e.g. acoustic telemetry) will provide more accurate knowledge of habitat use and ecologically relevant placement of MPAs (Eristhee & Oxenford 2001, Popple & Hunte 2005).

It is also possible that fishing may not have a discernible impact on abundance and size structure of some exploited species at the spatial scales examined. Lack of empirical data on pre-fished populations, and the impacts of fishing makes interpretations difficult (Barrett et al. 2007). This scenario is likely for *A. viridis* as temporal variation in abundance of this species supported the hypothesis that it was relatively sedentary and should show a MPA effect. In addition it is thought that the effects of fishing on the occurrence of large *A. viridis* is probably minimal as they are rarely taken by recreational fishers and have been protected in all coastal areas from spear fishing since the 1970's (Kingsford et al. 1991, Gillanders 1999). Poaching is a valid alternative to explain the lack of response to protection for some fishes (Russ & Alcala 1989, Jennings & Polunin 1996). Although no data was available on how well fishing restrictions in MPAs were enforced, poaching was not considered to be a major factor impeding recovery as both MPAs are located in highly urbanized areas and are often policed by the general public (Pers. Obs.). For example, the two line fishers observed at CTB were present for < 1hr.

2.4.1. Conclusion

In summary, very small MPAs in central NSW are not large enough to encompass the average movements of commonly exploited reef fishes or to contain the range of habitat types and depths required throughout their life histories (see Curley et al. 2002, Kingsford 2002). Despite this, there was strong evidence that small MPAs are effective for protecting extremely sedentary species (e.g. *C. fuscus*), and may benefit more mobile species if they are strategically placed. These results are significant as small MPAs may be the only option in some parts of central NSW where reefs are regularly intercepted by beaches (i.e. 60 % of reefs in central NSW are < 2 km in length; Curley

et al. 2002). Overall results, also support the consensus that responses to protection is species-specific, slow, complex and varies with MPA design and local geography (Russ & Alcala 2004, Barrett et al. 2007). This necessitates the collection of empirical data on individual MPAs rather than reliance on generalities obtained through the study of MPAs in other geographic areas. Long-term studies of MPA effects and direct methods for measuring magnitude of movement of fishes are also required to provide a more comprehensive understanding of movement and ecologically relevant sizes for MPAs (Gillanders & Kingsford 1996, Gillanders 1997, Bastow et al. 2002, Moran et al. 2003). Although small MPAs do benefit some species within their boundaries they may not provide significant export functions (Halpern 2003). Further studies which investigate scales of population connectivity for reef fishes in central NSW will be required to determine potential benefits of MPAs to unprotected areas.

Chapter 3: Along-shore variation in otolith chemistry of the temperate damselfish *Parma microlepis*

3.1. Introduction

Knowledge of dispersal and connectivity among reef fish populations is important for understanding population dynamics and ecologically relevant management decisions (e.g. design of marine protected areas, stock delineation; Gillanders 2002a). Quantifying dispersal of reef fishes, however, remains challenging and for most species dispersal is poorly understood. Although numerous methods are available for measuring movement, the utility of these techniques will be determined by: the spatial and temporal scales, geographic region, life-history stage, and ecology of the target species of interest (see comparison of methods in Chapter 1). For example, the use of genetics for estimating levels of population connectivity in marine fishes is often limited to large spatial scales, due to large population sizes which experience minimal genetic drift, and the potential to disperse and spread genes over large geographic distances (Ward et al. 1994).

Differences in the elemental composition of otoliths have been used to determine dispersal across a range of spatial and temporal scales, among different habitats, and at different phases in the life-history of fishes (Campana et al. 1994, Gillanders & Kingsford 1996, Swearer et al. 1999, Campana et al. 2000, Thorrold et al. 2001, Rooker et al. 2003, Swearer et al. 2003, Patterson et al. 2004, Fowler et al. 2005, Patterson et al. 2005, Sandin et al. 2005). This approach overcomes fundamental disadvantages associated with conventional tagging methods such as the need to tag large number of individuals in order to get meaningful sample sizes, difficulties in tagging larvae and juveniles, high rates of mortality in early life, and sub-lethal effects (Gillanders 2002a). Furthermore, otolith chemistry can give insight into population connectivity when levels of mixing prevent genetic differentiation (Thorrold et al. 2001, Rooker et al. 2003).

Otoliths are composed primarily of calcium carbonate with other elements present at minor (> 100 ppm) and trace (< 100 ppm) levels (e.g. Ba, Sr, Mg, Mn; Campana 1999). Otoliths form prior to hatching and grow continuously throughout the life of a fish, are acellular and metabolically inert (Campana 1999). Therefore, elements or compounds accreted onto the surface of the otolith are not resorbed or reworked but are permanently

retained so that the entire elemental life-history of the fish is preserved in chronological order (Campana 1999). Otolith chemistry is primarily influenced by the chemical and physical properties of the aquatic environment (e.g. ambient water chemistry, salinity and temperature; Elsdon & Gillanders 2003a, Dorval et al. 2007). Consequently, the type, concentration and combination of elements (multi-element signature) in otoliths of individuals from geographically distinct stocks will reflect spatial and temporal scales of environmental variability (Bastow et al. 2002, Elsdon & Gillanders 2003a).

The basic requirement when using otolith chemistry as a natural tag is the presence of differences in the aquatic environment that translate into differences in otolith chemistry in the target species at the spatial scales of interest (Begg et al. 1999, Fowler et al. 2005, Chittaro et al. 2006). The use of otolith chemistry to determine population connectivity of marine fishes has primarily focused on variability within and among estuaries, between estuarine and coastal environments, and/or across large spatial scales (Thorrold et al. 1998, Gillanders & Kingsford 2000, Forrester & Swearer 2002, Swearer et al. 2003, Dorval et al. 2005, Fowler et al. 2005). The application of otolith chemistry to exclusively marine environments has received comparatively less attention (Ashford et al. 2005), particularly at fine spatial scales. This imbalance is due to the greater probability of environmental variation within and between estuarine and coastal habitats or across large spatial scales (Kingsford & Gillanders 2000, Swearer et al. 2003).

Despite the potential for homogeneity of water masses in marine environments, differences in the chemistry of pre- and post-settlement portions of otoliths of oceanic and coral reef fishes have been detected at scales of tens of metres to thousands of kilometres in exclusively marine environments (Patterson et al. 1999, Swearer et al. 1999, Ashford et al. 2005, Patterson et al. 2005, Patterson & Swearer 2007). Several studies have also focused on temperate coastal reef fishes (Dove & Kingsford 1998, Miller et al. 2005, Warner et al. 2005), however, most have limited spatial replication comparing less than six locations (but see Dove & Kingsford 1998). Few studies have examined variation in otolith chemistry along temperate coastlines at multiple spatial scales < 100's km. The application of otolith chemistry to determine population connectivity within such environments is feasible given along-shore differences in near-shore water masses resulting from variation in river inputs, sewage, industrial effluents, and upwelling intensity (Warner et al. 2005).

The broad objectives of this study were to: (1) investigate the spatial scales over which there is variation in the otolith chemistry of rocky reef fishes along a temperate coastline; (2) determine if the magnitude of differences was sufficient to allow discrimination of fish collected from different sections of the coast. The temperate damselfish *Parma microlepis* was used as a model species as it is locally abundant, and territorial with limited post-settlement movement (< 100 m^2; (Moran & Sale 1977, Kingsford & Gillanders 2000, Gillanders 2001, Curley et al. 2002). A lack of variation for *P. microlepis* across different spatial scales, therefore, would indicate limited environmental variability rather than the exchange of individuals among locations. The study was conducted in central New South Wales (NSW), Australia. This region is home to approximately one third of the total Australian population (Birch 2000), and is subject to a wide range of anthropogenic impacts such as pollutants from urban, industrial, agricultural, and recreational activities (e.g. storm water, sewage, pesticides, boating). These impacts, in conjunction with natural variation due to oceanographic currents, upwelling, and riverine discharge, estuaries and coastal lagoons, influence the physical and chemical properties of water masses. It was predicted, therefore, that otolith chemistry would vary across a variety of spatial and temporal scales.

3.2. Methods

3.2.1. Sample collection and age determination

Spatial variation in the elemental composition of *P. microlepis* otoliths was examined at multiple spatial scales along the coast of NSW, Australia, using a hierarchical design. Samples were collected from each of three regions separated by 70-80 km (Port Stephens, Sydney, and Jervis Bay; Fig 3.1). Within each region I sampled fish at four locations separated by 10-50 kilometres. Five fish were collected from each of two sites (separated by 1-2 km) within each location. Previous studies have indicated that this sample size can resolve differences in otolith chemistry of *P. microlepis* at relatively fine-spatial scales in NSW (Kingsford & Gillanders 2000; Dove & Kingsford 1998). Fish were collected using a handspear at depths < 5 m, as depth-related patterns in elemental composition have been demonstrated for this species and could confound spatial comparisons (Kingsford & Gillanders 2000). It was not possible to collect *P. microlepis* at all proposed sites in Port Stephens as fish could not be found north of

location 1. Samples were kept on ice after collection and then frozen until processed. Standard lengths (SL) of fish were measured and sagittal otoliths extracted, washed in Milli-Q H_2O, dried, and stored in eppendorf tubes.

Variation in the age of a fish and hence temporal exposure to the environment could potentially confound spatial comparisons. Previous studies found no significant relationship between otolith chemistry and age (1-35 yrs) of *P. microlepis* collected from estuaries in Sydney (Kingsford & Gillanders 2000). In contrast significant differences have been detected between adult (< 37 yrs) and juvenile (< 3 yrs) *P. microlepis* collected from the same locations on the open coast (Gillanders 2001). The age of each specimen was, therefore, estimated using one of the sagittal otoliths. Otoliths were mounted on the edge of a microscope slide with Crystalbond thermoplastic and a transverse-section through the core was made by using a diamond grinding wheel. Opaque increments, formed on an annual basis (Tzioumis & Kingsford 1999), were enumerated using transmitted light. Increments were counted on three occasions and mean values were used if counts differed.

3.2.2. Sample preparation and analysis

The remaining otolith from each individual was weighed to the nearest 0.00001 g and used in subsequent analyses of relationships between otolith weight and fish length. All subsequent preparation of samples was done inside a Class-100 laminar flow cabinet (AS 1807). Otoliths were cleaned in 1% HNO_3 (65% Merk Suprapure®) for 5-10 s, rinsed three times in Milli-Q H_2O and placed in acid washed polyethylene tubes until completely dry. Acid washed otoliths were then re-weighed and dissolved in 500 µl of 20% HNO_3. Each sample was made up to a final volume of 5 ml with Milli-Q H_2O. Two samples which were > 0.035 g were made up to 8 ml. For trace element analyses a 1 or 2 ml sub-sample of this stock solution was taken depending on the initial otolith weights (0.005-0.020 g = 2 ml) and (0.020-0.035 g = 1 ml), placed in a new tube and made up to a final volume of 10 ml using a dilution solution. Separate samples were prepared for analyses of Sr and Ca containing 100 µl of stock solution and 9.9 ml of dilution solution. Dilution solutions contained internal standards (10 ppm Ga and In), HNO_3 (65% Merk Suprapure®), and Mill-Q H_2O. Blank samples were prepared in a similar way, but without otolith solution and were used for blank corrections and to calculate limits of detection (LOD).

Solution-based inductively coupled plasma-mass spectrometry (ICP-MS) was used to measure the integrated otolith chemistry across the entire life time of an individual fish (Campana 2000). This approach was taken as I was primarily interested in the detection of average environmental differences among locations. All analyses were conducted at the Advanced Analytical Centre, James Cook University, using a Varian Ultra Mass 700 ICP-MS. Calibration solutions consisted of commercially available multi-element standards (Alpha Resources, Sydney). The National Institute for Environmental Studies Certified Reference Material No. 22, fish otolith, was used as a quality control standard to monitor instrument drift. The ICP-MS was recalibrated during analyses if recovery of the internal standards (Ga and In) in the reference sample deviated by 30% of their initial values.

Preliminary analyses indicated that Sr, Ca, Ba, Mn, Mg, Cu, Zn and Pb were detectable in otolith solutions. ^{88}Sr, ^{43}Ca, ^{137}Ba, ^{55}Mn, ^{25}Mg, ^{65}Cu, and ^{66}Zn were chosen for analyses as they generally occurred in concentrations greater than the LODs, and had acceptable levels of precision. LODs were calculated as the mean blank value plus three times the standard deviation (3σ) in ppb: ^{88}Sr 0.236, ^{43}Ca 51.115, ^{137}Ba 0.082, ^{55}Mn 0.085, ^{25}Mg 2.926, ^{65}Cu 0.357, ^{66}Zn 1.589. Percent relative standard deviation (%RSD) values were used as a measure of instrument precision for each element and values were considered unreliable if variation in replicate measurements within otolith samples exceeded 10% (n = 3). The concentration of trace elements were expressed relative to the concentration of Ca (μmol element: mol Ca) to reduce the influence of systematic errors. One erroneous replicate from each of the Mg and Zn data sets was replaced with the average value found within the collection site (n = 4) and degrees of freedom in statistical analyses were reduced accordingly (Underwood 1997).

3.2.3. Statistical analyses

Differences in the size of fish/otolith weight as well as age may confound spatial comparisons of otolith chemistry. Fish collected in this study ranged in size from 91-137 mm SL and < 1 to 35 years of age, although 89% of fish were ≤ 6 years old. Distributions of size and age varied among sites, locations and regions (Fig. 3.2). Otolith weight was significantly correlated with fish length (Pearson's $r = 0.77$, n = 105) and, therefore, was used as a proxy for size in further analyses. The

relationship between otolith weight, age, and the concentration of individual elements was tested using linear regression. Regressions were performed on the entire data set, as sample sizes for sites and locations were considered too small for meaningful analyses. When significant relationships were detected, regressions were analysed for individual regions to test generality of results. No significant relationships were detected for Ba/Ca or Mn/Ca. Concentrations of Sr/Ca, Mg/Ca, and Zn/Ca were significantly correlated with otolith weight and age ($P < 0.01$) and Cu/Ca was correlated with otolith weight only ($P < 0.05$). 49% and 56% of the total variation in Sr/Ca, and 14% and 24% of the total variation in Mg/Ca was explained by age and otolith weight respectively. These relationships were consistent within each sampling region. Otolith weight was positively correlated with age (Pearson's $r = 0.81$ n = 105). Consequently, the effect of age and otolith weight was removed from Sr/Ca and Mg/Ca data by subtracting the slope of the regression relationship multiplied by otolith weight from the original values (example; Fig. 3.3). Data for Zn and Cu were not de-trended in this manner as ≤ 0.07% of the variation in concentration of these elements was explained by otolith weight and age, and relationships were not consistent for all sampling regions.

Univariate and multivariate methods were used to test hypotheses for individual elements and multi-element signatures. A three-factor nested analyses of variance (ANOVA) was used to examine variation in the concentrations of individual elements across multiple spatial scales (regions, locations, and sites). Two different designs were used to avoid problems associated with unbalanced data due to missing sites; the first design compared two regions (Sydney and Jervis Bay) using all four locations within each region while the second design compared three regions (Port Stephens, Sydney, Jervis Bay) using only two locations within each region (locations 2 and 3). All factors were treated as random. Cochran's *C* tests were used to test homogeneity of variances. Data that were heterogeneous ($P < 0.05$) were $\ln(x+1)$ transformed. Analyses were still performed if data remained heterogeneous following transformation as ANOVA is robust to departures from this assumption (Underwood 1997). When significant differences were detected, means were compared using Student-Newman-Keuls (SNK) tests. The variance components at each level of variation were estimated from each ANOVA model using untransformed data.

A multivariate approach was used to examine differences in multi-element signatures across different spatial scales and to test discriminating power. All data were ln(x+1) transformed to allow greater contribution from rarer elements. Permutational multivariate analysis of variance (PERMANOVA; Anderson 2005) was used to test for significant differences in multi-element signatures among regions, locations and sites. Distance matrices were obtained by calculating Euclidean distances between each pair of samples. Data was analysed using the same designs described for univariate ANOVA and permutation of raw data was used due to relatively small samples sizes (Anderson 2005). Pair-wise *a posteriori* comparisons using a multivariate version of the *t*-statistic (based on distances) where conducted when significant differences were detected (Anderson 2005).

Discriminant function analyses (DFA) and jackknife cross-validation were used to determine if multi-element signatures could be used to reliably distinguish among individuals collected from different regions and which elements were the most useful discriminators. Quadratic DFA were used as Box's *M*- test showed that covariance matrices were heterogeneous ($P < 0.001$). Comparisons were done for the factor 'region' only due to small sample sizes at finer spatial scales, and all 21 sites were included in analyses as equal sample sizes are not required for DFA. Patterns within and among regions were visualised by plotting the first two canonical variates and discriminant function coefficients were used to assess the relative contributions of different elements to discrimination among regions.

3.3. Results

Concentrations of Sr/Ca, Mn/Ca and Zn/Ca varied significantly among regions with differences accounting for 27.7-41.1% of the total variability in these elements (Fig. 3.4; Table 3.1 & 3.2). Mean concentrations of Sr/Ca were significantly higher in the Sydney region than in Jervis Bay and concentrations of Mn/Ca were significantly higher in Port Stephens than in Jervis Bay, (Fig. 3.4; SNK tests; $P < 0.05$). Zn/Ca concentrations were 2.6 and 3.1 times lower in Jervis Bay when compared to Port Stephens and Sydney (Fig. 3.4; SNK tests $P < 0.05$). Despite a strong trend for higher concentrations of Mg/Ca in the Port Stephens region, no significant regional differences were detected (Fig. 3.4). This was probably due to the large variation in Mg/Ca

between locations 2 and 3 within Port Stephens. Concentrations of Ba/Ca and Cu/Ca were similar among sampling regions.

Fine-scale differences accounted for the majority of the total variability in the concentration of individual elements (Fig. 3.4; Table 3.1 & 3.2). Significant differences in the concentrations of Cu/Ca and Zn/Ca were detected among locations (within regions) separated by 10-50 km (e.g. Jervis Bay L1 versus L4). Concentrations of Sr/Ca, Ba/Ca and Mn/Ca varied significantly among sites (within locations) separated by 1-2 km of coastline. Of particular note was the high Ba/Ca concentration at site 1, location 2, in Port Stephen which was approximately three times higher than the average concentrations of Ba/Ca at all other sites (Fig. 3.4). There was also a trend for higher concentrations of Cu/Ca at locations 3 and 4 in Jervis Bay.

Multi-element signatures of otoliths varied among regions, locations and sites (Table 3.3). The two-region PERMANOVA and pair-wise *a posteriori* comparisons of the three-region analyses showed that differences occurred between Sydney and Jervis Bay only (two-region $P < 0.01$, three-region $P < 0.05$). DFA indicated that there was significant separation in elemental signatures by region (Pillai's trace, $F_{12, 194} = 14.303$, $P < 0.001$). The canonical variates plot illustrated a clear separation between Port Stephens and Jervis Bay; however, 95% confidence ellipses around mean values of both regions partially overlapped with Sydney (Fig. 3.5). A trend for unique signatures at some locations was also apparent (e.g. Jervis Bay, L4). The first and second canonical discrimination functions accounted for 75.9% and 24.1% of the total dispersion, respectively. Standardized discriminant function coefficients indicated that Mg/Ca was most important for discriminating among regions in the first function followed by Cu/Ca, Sr/Ca, Zn/Ca and Mn/Ca (Fig. 3.5). Zn/Ca and Sr/Ca were most important in the second component of the discriminant function while Cu/Ca, Mg/Ca and Mn/Ca were of similar value. Ba/Ca was the least important element in both functions. Using jackknife cross-validation 76% of fish were correctly classified to Port Stephens, 75% to Sydney and 80% to Jervis Bay (Table 3.4).

Table 3.1. ANOVA comparing elemental ratios in the otoliths of *P. microlepis*.

(a) Two-region analyses (Sydney, Jervis Bay), 4 locations, 2 sites
(b) Three-region analyses (Port Stephens, Sydney, Jervis Bay), 2 locations, 2 sites.
ns $P \geq 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Source	df	*F* vs.	Sr/Ca	Ba/Ca	Mg/Ca	Mn/Ca	Cu/Ca	Zn/Ca
			MS	MS	MS	MS	MS	MS
(a) Two regions (Sydney, Jervis Bay)								
Region	1	Location	3523895*	0.606^{ns}	99.402^{ns}	0.752^{ns}	0.093^{ns}	12.850**
Location (Region)	6	Site	467436^{ns}	0.463^{ns}	76.412^{ns}	0.414^{ns}	0.110***	0.868*
Site (Location(Region))	8	Residual	654706***	0.363***	55.423^{ns}	0.119*	0.007^{ns}	0.197^{ns}
Residual	64		109527	0.090	27.085	0.044	0.009	0.408
Transformation			none	$\ln(x+1)^{ns}$	none	$\ln(x+1)$*	$\ln(x+1)^{ns}$	$\ln(x+1)^{ns}$
(b) Three regions (Port Stephens, Sydney, Jervis Bay)								
Region	2	Location	344483^{ns}	0.2389^{ns}	1367.233^{ns}	1.542*	0.232^{ns}	92.689*
Location (Region)	3	Site	515162^{ns}	1.2492^{ns}	260.529^{ns}	0.136^{ns}	0.252**	3.931^{ns}
Site (Location(Region))	6	Residual	159290^{ns}	0.7062***	58.360^{ns}	0.279***	0.014^{ns}	10.485^{ns}
Residual	48		117719	0.0846	32.339	0.054	0.026	6.879
Transformation			none	$\ln(x+1)^{ns}$	none	$\ln(x+1)^{ns}$	none	none

[+] One erroneous replicate from each of the Mg and Zn data was replaced with the mean concentration found at that site (n = 4) (two-region analyses only). Degrees of freedom were reduced accordingly.

Table 3.2. Percentage of variation attributed to each factor in ANOVA performed on untransformed data (a) Comparison among two regions and (b) Comparison among three regions.

Source	Sr/Ca	Ba/Ca	Mg/Ca	Mn/Ca	Cu/Ca	Zn/Ca
(a) Two regions (Sydney, Jervis Bay)						
Region	27.7	0.0	1.6	11.0	0.0	35.4
Location	0.0	8.1	5.9	26.8	55.3	11.9
Site	39.5	22.8	16.0	14.5	0.0	0.0
Residual	39.6	72.1	76.5	47.7	48.5	59.6
(b) Three regions (Port Stephens, Sydney, Jervis Bay)						
Region	0.0	0.0	48.9	41.1	0.0	39.0
Location	23.2	15.6	17.9	0.0	51.8	0.0
Site	5.4	60.1	4.6	29.3	0.0	6.3
Residual	76.9	44.0	28.6	38.3	55.6	60.4

Table 3.3. PERMANOVA for comparison of multi-element signatures (Sr, Ba, Mg, Mn, Cu, Zn ratioed to Ca) among regions, locations and sites. ns $P \geq 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Source	df	*F* vs.	MS
(a) Two regions (Sydney, Jervis Bay)[+]			
Region	1	Lo(Re)	14.8874**
Location(Re)	6	Si(RexLo)	2.0301*
Site(RexLo)	8	Res	0.8335ns
Residual	63		0.6029
(b) Three regions (Port Stephens, Sydney, Jervis Bay)			
Region	2	Lo(Re)	11.2907*
Location(Re)	3	Si(RexLo)	1.8302ns
Si(RexLo)	6	Res	1.9210***
Residual	48		0.5949

[+] One erroneous replicate from each of the Mg and Zn data was replaced with the mean concentration found at that site (n = 4) (two-region analyses only). Degrees of freedom were reduced accordingly.

Table 3.4. Number of *P. microlepis* correctly classified to the region in which they were collected using jackknife cross-validation procedure based on quadratic DFA of multi-element signatures.

	Predicted Region			
Actual Region	Port Stephens	Sydney	Jervis Bay	% correct
Port Stephens	**19**	5	1	76
Sydney	6	**30**	4	75
Jervis Bay	2	6	**32**	80

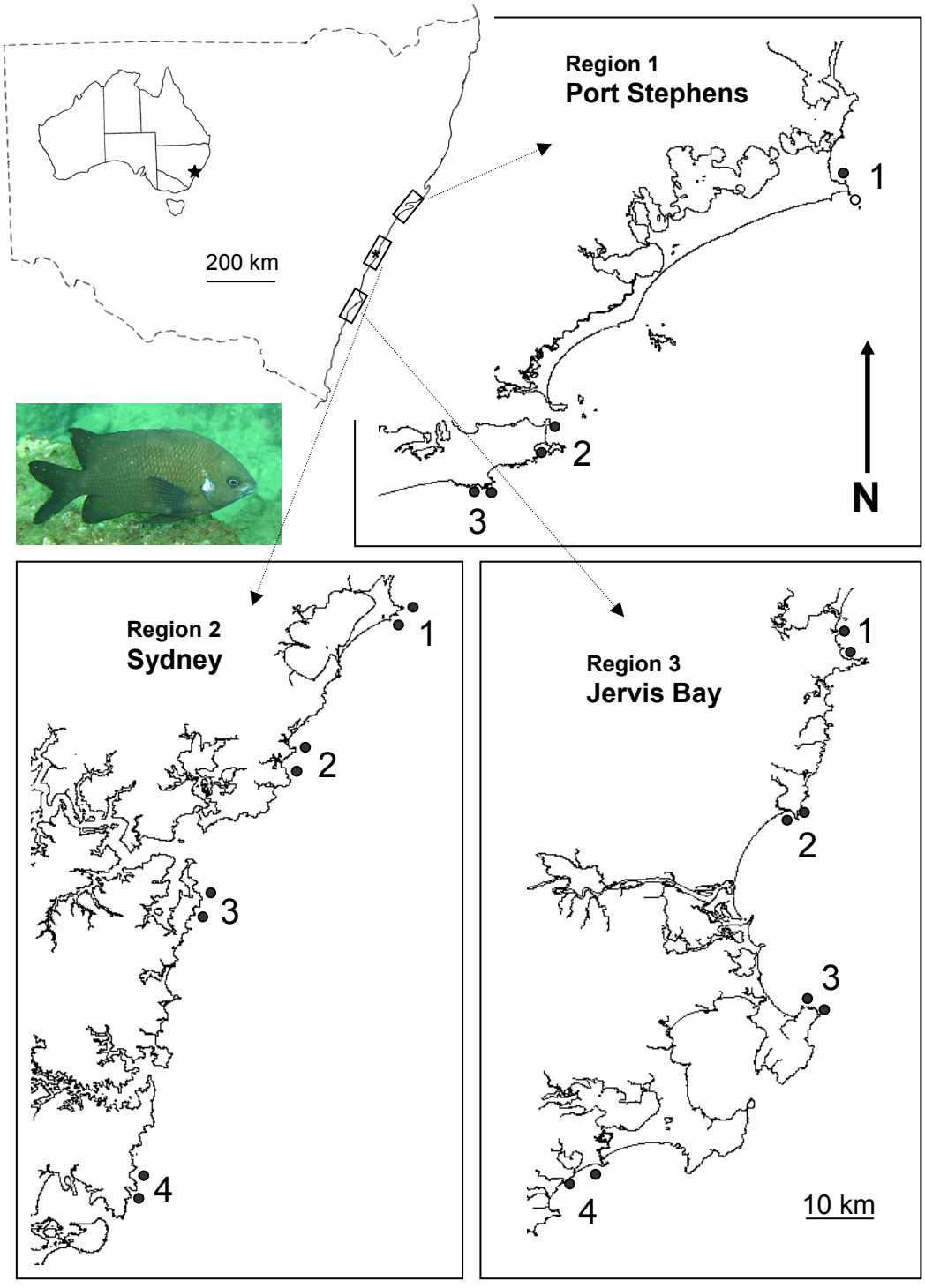

Fig. 3.1. The central coast of NSW, Australia, showing three sampling regions: Port Stephens, Sydney and Jervis Bay. Within each region, the locations (1-4) and sites (within locations) at which *P. microlepis* were collected (●) are shown. Fish were not collected at this site (○). GPS co-ordinates for sites are given in Appendix B, Table B.1.

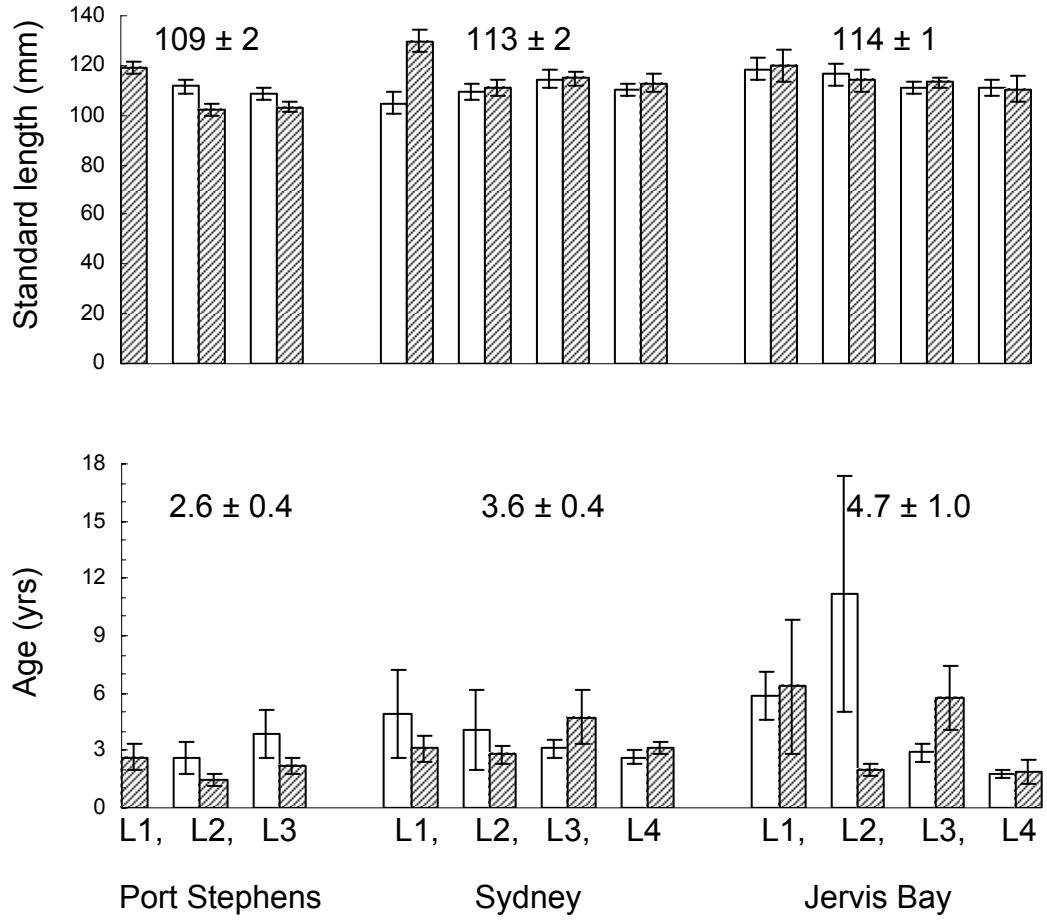

Fig. 3.2. Mean standard length and age (± SE) of *P. microlepis* at each of two sites within locations (L), across three sampling regions: Port Stephens, Sydney and Jervis Bay (n = 5, per site). Mean regional values (± SE) are shown above each graph.

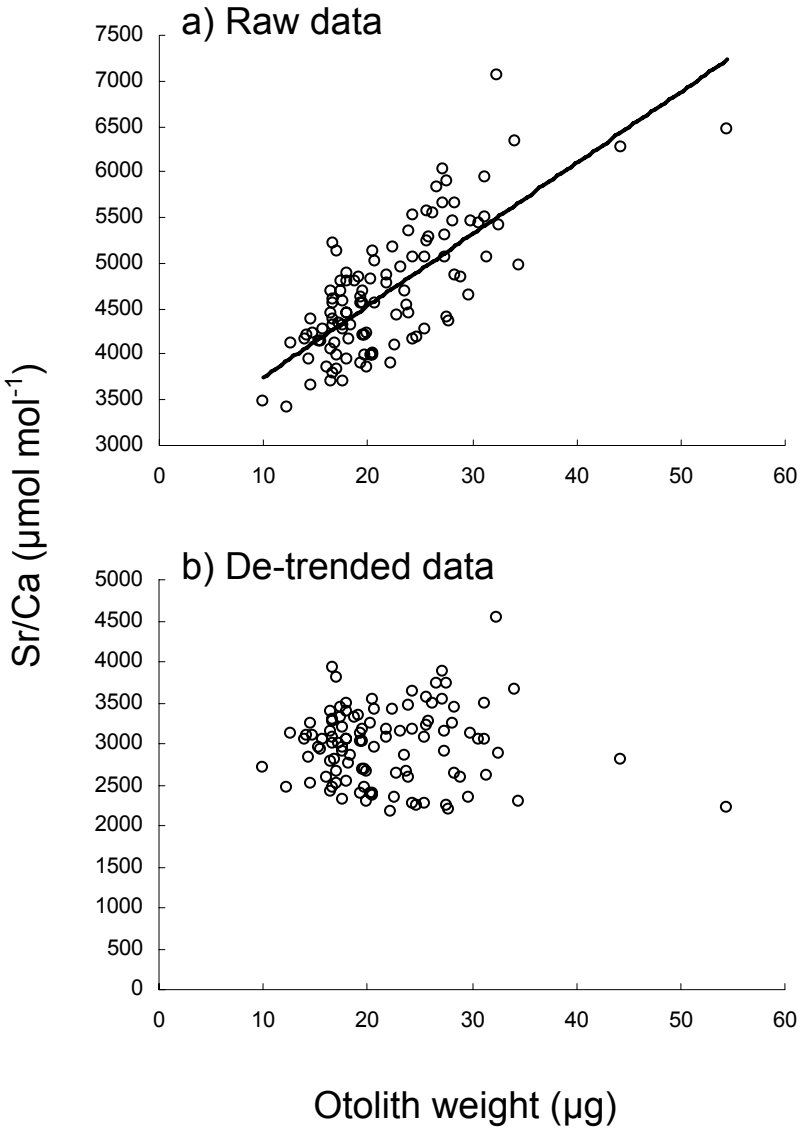

Fig. 3.3. Sr/Ca concentrations versus otolith weight for (a) raw and (b) de-trended data. An example of how the effect of otolith weight (used as a proxy for age and fish length) was removed by subtracting the slope of the regression relationship multiplied by otolith weight from the elemental data.

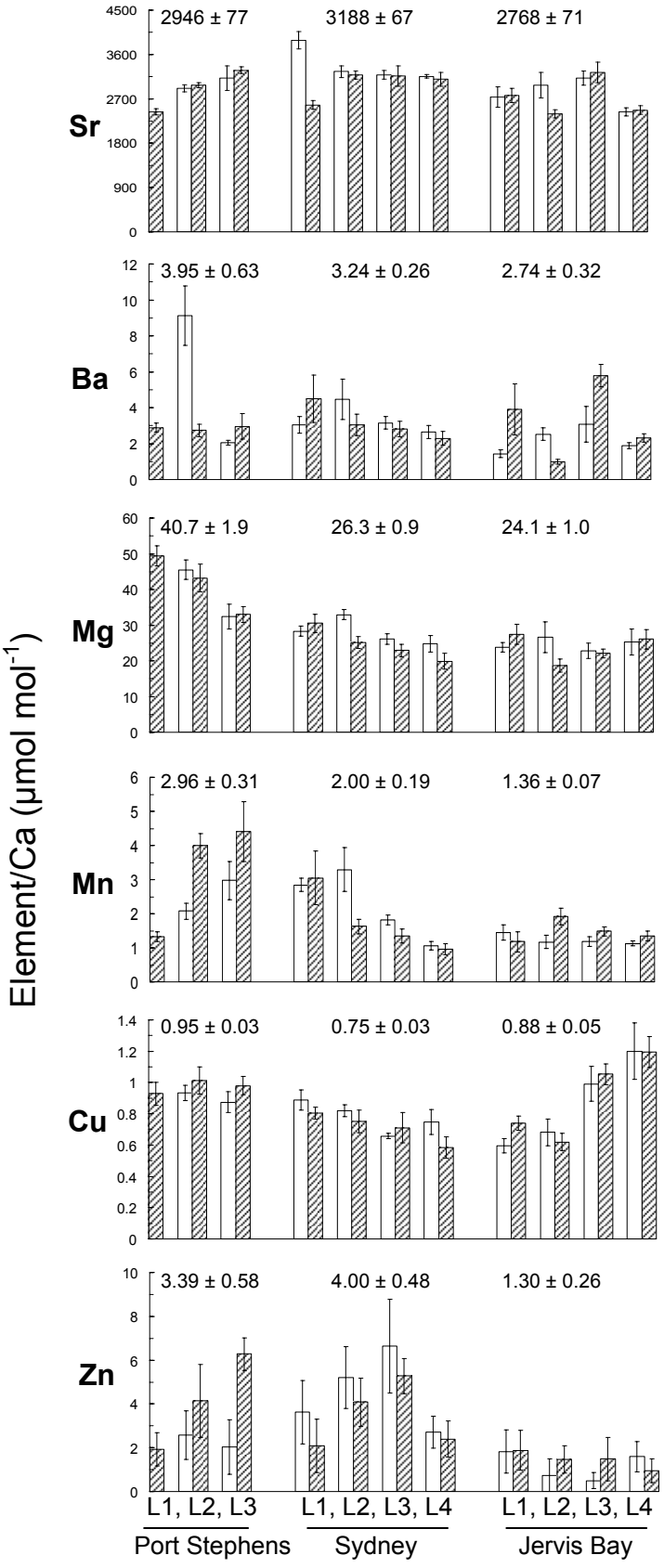

Fig. 3.4. Mean elemental concentrations (± SE) in *P. microlepis* collected from each of two sites (separated by 1-2 km) within locations (separated by 10-50 km) across three regions: Port Stephens, Sydney and Jervis Bay (separated by 70-80 m), n = 5 fish per site. Mean regional concentrations (± SE) are shown above each graph.

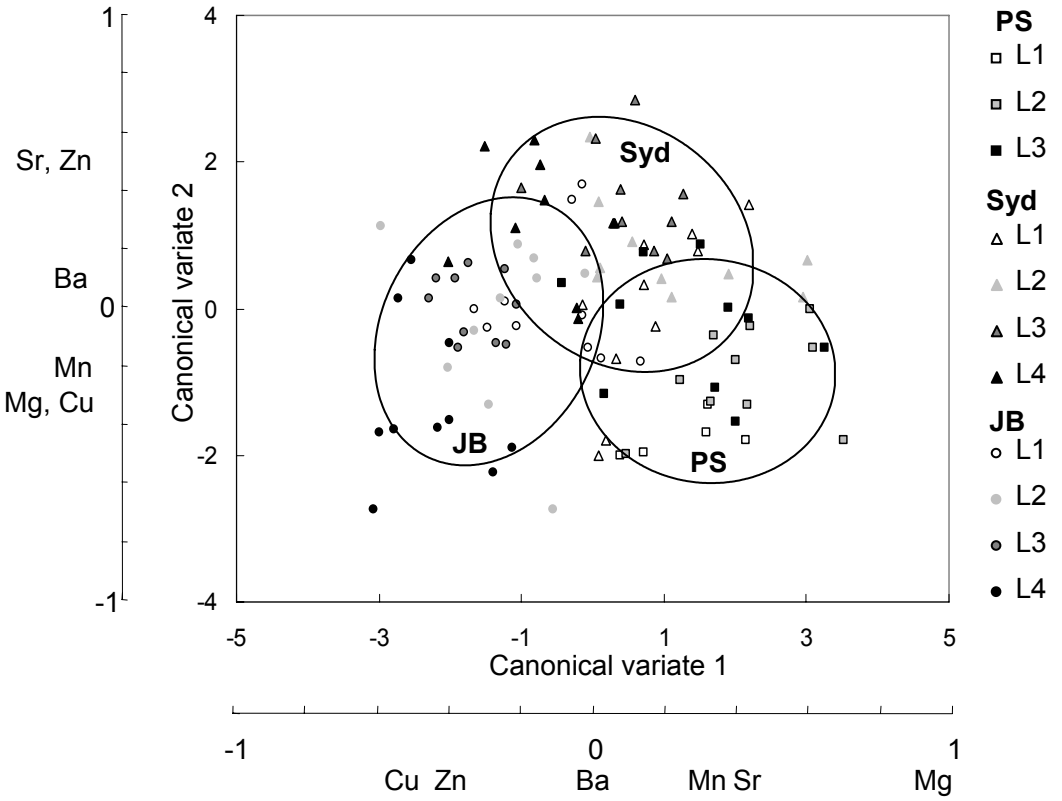

Fig. 3.5. Canonical variate plot summarising variation in otolith multi-element signatures for *P. microlepis* among three regions Port Stephens (PS), Sydney (Syd), Jervis Bay (JB) and locations (L) within regions. The plot is based on the first two canonical variates obtained through quadratic DFA. 95% confidence intervals are depicted around region centroids. Standardized discriminant function coefficients are shown on secondary axes. Coefficients are analogous to regression coefficients and range between -1.0 and 1.0. Values close to 1.0 or -1.0 indicates element/Ca that make the largest contribution to discrimination among regions. 76% of cumulative total dispersion was accounted for by function 1 (x-axis).

3.4. Discussion

This study demonstrated that otolith chemistry of the territorial fish *P. microlepis* varied at scales of kilometres to hundreds of kilometres in central NSW. Furthermore, multi-element signatures were good spatial discriminators, with 75-80% of fish correctly classified to the regions (separated by 70-80 km) in which they were collected. Previous studies have detected differences in the concentration of trace elements in otoliths, scales, spines and eye lenses of *P. microlepis* at scales of hundreds of metres to hundreds of kilometres within estuaries and along the open coast of NSW (Dove et al. 1996, Dove & Kingsford 1998, Kingsford & Gillanders 2000, Gillanders 2001). However, the objective of these studies was to compare the chemistry of different structures or among depth strata rather than examining spatial variation. My results indicate that the magnitude of environmental variability within open coastal environments was sufficient to produce measurable differences in otolith chemistry. The application of this technique to determine dispersal and levels of population connectivity in such environments is, therefore, highly viable.

Along-shore variation in otolith chemistry

Up to three-fold differences in mean concentrations of Sr/Ca, Mn/Ca and Zn/Ca were detected between Port Stephens, Sydney and Jervis Bay. Although significant differences in Mg/Ca concentrations were not detected in univariate analyses, Mg was found to be important for multi-element regional discrimination. Regional-scale variation in concentrations of elements (Mn, Hg) have been reported previously for *P. microlepis* collected from Sydney and Jervis Bay (Dove & Kingsford 1998). In contrast with the current study, Dove and Kingsford (1998) found no spatial variation in mean concentrations of Zn or Sr. The latter study, however, included less replication at fine spatial scales which most likely resulted in a less powerful test of regional differences.

The detection of large-scale variation in otolith chemistry in central NSW is not surprising. Sampling regions were located in distinct marine bioregions, as defined by differences in biophysical processes (e.g. climate, temperature, sediment, geology, bathymetry, riverine input, or water chemistry; Interim Marine and Coastal Regionalisation for Australia Technical Group 1998), and with varying degrees of

anthropogenic inputs. It was difficult, however, to establish simple causal relationships for regional variation in the concentration of individual elements. This is not uncommon, as physical and chemical properties of water masses, and thus otolith chemistry, are a product of the interaction of environmental variables across multiple spatial scales (Campana 1999, Elsdon & Gillanders 2003a, Dorval et al. 2007). Furthermore, relationships are often species-specific due to biological factors which affect rates and pathways of uptake (Elsdon & Gillanders 2003a). For example, although Sr/Ca changes in predictable ways with water chemistry, this relationship is not always linear due to effects of temperature, and is also subject to interspecific variation (Elsdon & Gillanders 2003a).

Despite this complexity, differential exposure to pollutants may partially explain the relatively high concentrations of Zn/Ca and Mn/Ca associated with Port Stephens and Sydney. These regions are highly populated, and have relatively high freshwater runoff which is subject to a wide range of urban, industrial and agricultural inputs. For example, sediments in Sydney have the highest recorded concentrations of heavy metals in marine sediment in Australia (e.g. Mn, Zn, Cu, Pb, Hg; Birch 2000). High concentrations of Hg in otoliths of *P. microlepis* have been attributed to high levels of pollutants in this region (Dove and Kingsford 1998). The relationship between ambient concentrations of heavy metals and their accumulation in otoliths is sometimes unclear. Ambient concentrations of Mn and Zn may not necessarily be reflected in otolith chemistry for reasons that are not fully understood (Thorrold et al. 1997, Hanson & Zdanowicz 1999, Elsdon & Gillanders 2003b). Other studies have found clear relationships between ambient concentrations and otoliths for Mn and other heavy metals (Geffen et al. 1998, Dorval et al. 2007). In any case, this study has shown that high concentrations of Zn/Ca and Mn/Ca were found in otoliths from regions where these elements are expected to be at high ambient concentrations. Consequently, otolith chemistry may reflect spatial variation in such pollutants, at least in some circumstances. This model and the mechanisms involved in uptake and incorporation of these marker elements into otoliths requires further investigation.

In contrast, regional-scale multi-element signatures were highly correlated with the behaviour of the East Australian Current (EAC). The EAC carries low-nutrient tropical water southward down the east Australian coast, and is associated with warm water

eddies, periods of upwelling and blooms of phytoplankton (Hallegraeff & Jeffrey 1993, Middleton et al. 1996). Although the influence of the EAC has the potential to reach 45^0S, it has a strong seasonal cycle below 32^0S (Port Stephens) (Middleton et al. 1996, Ridgway & Godfrey 1997). Consequently, Port Stephens is most influenced by subtropical waters, Jervis Bay by north-flowing temperate currents, while Sydney alternates between these two extremes (Breen et al. 2005). This spatial variation in water masses is a plausible explanation for why multi-element signatures of *P. microlepis* from Port Stephens and Jervis Bay are highly distinct but partially overlap with Sydney. Further spatial replication will be required to test this hypothesis.

Individual and multi-element signatures of *P. microlepis* also varied significantly within sampling regions, and often accounted for the largest percentage of variation in concentrations of individual elements. Concentrations of Cu/Ca and Zn/Ca varied among locations (10-50 km apart, within regions) and Sr/Ca, Ba/Ca and Mn/Ca varied among sites (1-2 km apart, within locations). Significant fine-scale variability in otolith chemistry has been found previously for *P. microlepis* in NSW (Dove & Kingsford 1998, Kingsford & Gillanders 2000) and is common for other species (e.g. Gillanders & Kingsford 2003, Dorval et al. 2005, Patterson et al. 2005). These findings emphasize the need for fine-scale replication in studies of otolith chemistry if potential confounding of larger-scale comparisons is to be prevented.

Localised variation in otolith chemistry is likely to be driven by a range of natural and anthropogenic processes. For example, fine-scale variation in Ba/Ca and Sr/Ca is potentially due to differential exposure to plumes of freshwater which can extend for kilometres from the mouth of rivers and estuaries in NSW (Kingsford 1999, Elsdon & Gillanders 2003a). Indeed, high levels of Ba have been found in past studies of *P. microlepis* and the labrid *Achoerodus viridis* collected from shallow estuarine habitats in NSW (Gillanders & Kingsford 1996, Dove & Kingsford 1998, Kingsford & Gillanders 2000). Plumes may also transport land-based sediment, nutrients and pollutants (Kingsford 1999) which may also explain high concentrations and fine-scale variability of Zn and Mn associated with Port Stephens and Sydney.

There was a trend for unique multi-element signatures at some sites and locations. For example, mean concentrations of Ba/Ca were three times higher at one site within Port

Stephens (location 2) relative to all other sites. This value was also several orders of magnitude higher than previous concentrations of Ba found in *P. microlepis* collected within estuaries and along the open coast of NSW (Dove & Kingsford 1998, Kingsford & Gillanders 2000). This site is located at the entrance to a drowned river valley, consequently, high Ba concentrations may be indicative of large amounts of freshwater input (Elsdon & Gillanders 2003b, 2005). Alternatively, high concentrations may be caused by current-induced upwelling, which occurs in this area (Hallegraeff & Jeffrey 1993); upwelled waters are enriched in Ba which can be incorporated into calcified structures (Lea et al. 1989).

Application for studies on population connectivity

Regardless of the mechanisms responsible, along-shore variation in otolith chemistry of *P. microlepis* demonstrates the viability of this technique for determining population connectivity across multiple spatial scales for reef fishes in central NSW. Several future applications warrant investigation. Firstly, otolith chemistry of 'resident' species such as *P. microlepis* may act as a baseline for comparison with other species of varying mobility. Although, interspecific variation in elemental signatures is poorly understood (Gillanders & Kingsford 2003, Swearer et al. 2003); comparative studies suggest that relative spatial differences can be consistent among species for some elements, particularly for closely related species with similar life histories, and/or for fishes which occupy the same ecological niche (Brown 2006, Hamer & Jenkins 2007). The use of *P. microlepis* as a 'proxy' should be examined by comparing otolith chemistry with sedentary species that occur in the same habitat, depth range, and from multiple family groups (e.g. *Parma unifasciata*, *Cheilodactylus fuscus*).

Secondly, the use of otolith chemistry for determining pre-settlement connectivity of reef fishes should be examined. Given differences in adult otolith chemistry, it is possible that the region of the larval otolith that forms prior to hatching (natal) or before significant dispersal has taken place (near-natal), will also show a site, location or region-specific signature. Although the environment may not be the primary determinant of the natal signature (Warner et al. 2005, Patterson 2004, Ashford et al. 2006), the natal region may exhibit similar broad geographic patterns to adults (Thorrold et al. 2001, Ashford et al. 2005, Patterson et al. 2005, Stransky et al. 2005, Ashford et al. 2006). Marine species with benthic eggs such as *P. microlepis* are ideal

candidates for this application as there is more time for natal signatures to develop (Warner et al. 2005). Finer-scale analyses of the otolith will be required to determine the utility of this application as the natal regions accounted for a very small percentage of the otolith matrix analysed in this study.

Additional requirements: 'atlas' of sources and temporal variability

Future applications of otolith chemistry for reef fishes in central NSW should be conducted across additional spatial scales, and must assess temporal variability in elemental signatures. When using elemental signatures to discriminate among fish from different places it is important that all possible groups contributing to the group mixture have been characterised (Campana 1999, Campana et al. 2000). Unlike species and life phases which have a limited number of potential sources (e.g. estuaries) it is more difficult to create a definitive 'atlas' or library of sources for coastal species (Warner et al. 2005). Future studies, therefore, should focus on targeting sites within each region that may exhibit great variation (e.g. entrances to estuaries, different depths). Outliers within each region may have to be grouped by factors other than geographic location (e.g. entrance to estuaries) and considered when testing hypotheses on population connectivity. The analyses of additional elements and stable isotopes in otoliths and other structures (e.g. spines, eye lenses, scales) may also improve the accuracy of discriminant functions. For example, Cu, Rb, Hg were found in higher concentrations in eye lenses of *P. microlepis* when compared to otoliths (Dove & Kingsford 1998, Kingsford & Gillanders 2000).

Temporal variability in elemental composition of otoliths within each geographic location should also be tested (Campana 1999, Gillanders & Kingsford 2000, Gillanders 2002a) as elemental signatures may fluctuate over time potentially confounding spatial comparisons (Swearer et al. 2003). The spatial patterns described in this study are based on whole-otolith analyses and thus are a measure of age-integrated elemental signatures. Most fishes used to build discriminant functions were ≤ 6 years old. However, *P. microlepis* can reach a maximum age of 37 years and press or pulse events over longer time periods could influence average elemental composition of otoliths (Tzioumis & Kingsford 1999, Kingsford & Gillanders 2000, Elsdon & Gillanders 2003a). Future studies should examine elemental concentrations at finer temporal scales using a probe-based approach (e.g. laser ablation ICP-MS). Comparisons based on the

same years (e.g. recruits by year) may also significantly improve the resolution of sites and broader spatial scales detected in this study. If temporal variability is found, a 'library' of signatures for each year class of fish will be required before applying this technique to studies on population connectivity (see discussion in Gillanders and Kingsford 2000, Gillanders and Kingsford 2003).

3.4.1. Conclusion

In summary, otolith chemistry of *P. microlepis* varied across multiple spatial scales and a convincing separation of three regions in central NSW was detected, suggesting that water masses are spatially distinct at these scales. Results indicated that the magnitude of environmental variability within open coastal environments such as central NSW facilitate the use of otolith chemistry for determining dispersal and population connectivity of pre- and post-settlement fishes at scales < 100's km. Knowledge of connectivity at these scales is pertinent to the logical design and effectiveness of individual and networks of MPAs relative to within-MPA, 'spillover' and 'recruitment effects' (Russ 2002). Future research on interspecific and temporal variation of otolith chemistry will enhance our understanding of the utility of this technique. Such studies will provide a powerful synergy with population genetics to provide a comprehensive understanding of connectivity and appropriate scales for management and conservation.

Chapter 4: Population connectivity in the temperate damselfish *Parma microlepis*: analyses of genetic structure across multiple spatial scales

4.1 Introduction

The majority of reef fishes have a bipartite lifecycle, which includes a pelagic pre-settlement phase and a relatively sedentary juvenile and adult phase (Sale 2004). Populations of reef fish have been traditionally viewed as 'open' and connected over large spatial scales due to the potential for passive transport of larvae via ocean currents (Leis 2002). This assumption has increasingly been challenged due to improved understanding of larval behaviour and ecology, more sophisticated modelling techniques and empirical data (Jones et al. 1999, Swearer et al. 1999, Atema et al. 2002, Kingsford et al. 2002, Leis 2002, Paris & Cowen 2004, Gerlach et al. 2007). It is now known that late-stage larvae are not passive but have strong sensory and swimming capabilities, which may be used in conjunction with oceanographic features to facilitate retention near natal reefs (e.g. Gerlach et al. 2007). Therefore, although reef fishes are capable of long-distance dispersal, it may be rare, and it's effect on downstream populations may be ecologically insignificant (Cowen et al. 2000, Strathmann et al. 2002). Evidence in support of self-recruitment has driven a need to readdress the assumptions of demographically-open population models and their applicability to the management of marine organisms (Swearer et al. 2002).

Estimating population connectivity is logistically challenging. The most appropriate methods vary according to the species, geographic region, and spatial and temporal scales of interest. Population genetic studies to assess gene flow among local populations have been widely used as an indirect method of estimating population connectivity in reef fishes (Van Herwerden et al. 2003, Carreras-Carbonell et al. 2006, Purcell et al. 2006). Unlike alternative methods (e.g. artificial tags, otolith chemistry), measures of connectivity using genetics are based only on those migrants (propagules, juveniles or adults), that successfully breed and contribute to the next generation (Hellberg et al. 2002). Furthermore, genetic markers provide longer-term estimates of population connectivity, as genetic structure among populations results from the

interaction between gene flow, genetic drift, selection and mutation averaged over multiple generations (Slatkin 1987, Palumbi 2003).

The use of genetic markers for estimating population connectivity in marine fishes was previously thought to be limited to large spatial scales, due to large population sizes which experience minimal genetic drift, and the potential for marine larvae to disperse and spread genes over large geographic distances (Ward et al. 1994). However an increasing number of studies have found genetic structure at scales of kilometres to hundreds of kilometres for species with a range of dispersal capabilities (Doherty et al. 1995, Bernal-Ramirez et al. 2003, Rhodes et al. 2003, Hoffman et al. 2005, Bay et al. 2006, Carreras-Carbonell et al. 2006, Purcell et al. 2006, Gerlach et al. 2007). This fine scale genetic differentiation has been attributed to smaller than expected effective population sizes, mechanisms that favour self-recruitment, and the use of more sensitive genetic markers (e.g. microsatellites) which can provide information on gene flow over ecological time scales (Hellberg et al. 2002). Consequently, studies which do not investigate fine-scale variation may confound comparisons made across larger spatial scales, leading to inaccurate conclusions regarding levels of connectivity (Doherty et al. 1995). Furthermore, genetic markers that are capable of detecting fine scale genetic differentiation, such as microsatellites, may offer a valid alternative for measuring population connectivity at spatial scales relevant to management techniques such as Marine Protected Areas (Palumbi 2003).

A diverse range of factors can interact to influence dispersal, and therefore the genetic structure of fish populations. These include geographic distance, discontinuities or fragmentation of habitats, oceanography, and the biology/ecology of species (Bohonak 1999, Kingsford et al. 2002, Taylor & Hellberg 2003, Hoffman et al. 2005, Rocha et al. 2005, Bay et al. 2006). It is often difficult to predict the scale at which genetic markers will be informative in demonstrating population subdivision for a particular species or geographical region (Bohonak 1999). For example, neither egg type (demersal or pelagic) or pelagic larval duration is an accurate and simple predictor of geographic structure in fish populations (Shulman & Bermingham 1995, Taylor & Hellberg 2003, Bay et al. 2006). More empirical studies are required for species that exhibit a range of dispersal capabilities and ecologies across a range of spatial scales and geographical provinces, to determine what factors promote genetic subdivision (Bohonak 1999).

Different analytical frameworks for interpreting genetic population structure may be used to describe connectivity among marine populations (reviewed in Palumbi 2003). Isolation-by-distance (IBD), based on a stepping-stone model of dispersal (Wright 1943, Kimura & Weiss 1964, Slatkin 1993) is typically the most suitable for describing connectivity among marine populations strung along a one, two, or three dimensional habitat lattice (e.g. coastal populations; Hellberg et al. 2002, Palumbi 2003). This model predicts that neighbouring populations will be linked by larger amounts of genetic exchange, such that genetic similarity decreases with increasing geographic distance. Significant patterns of IBD have been found for many marine fishes (Riginos & Nachman 2001, Buonaccorsi et al. 2004, Carreras-Carbonell et al. 2006, Purcell et al. 2006). Investigation of potential IBD increases the power to detect genetic structure and provide information on connectivity, particularly for species characterised by large population sizes, extensive gene flow and weak genetic differentiation (Palumbi 2003, Purcell et al. 2006).

The broad objective of this chapter was to determine whether microsatellite markers could elucidate population connectivity for a low dispersing reef fish, at scales ≤ 400 km in central NSW, Australia. The temperate damselfish *Parma microlepis* (Pomacentridae) is endemic to southern Australia and is relatively abundant on reefs in NSW (Curley et al. 2002). This species represents the lower end of dispersal capabilities for reef fishes in this region (Chapter 1, Table 1). *P. microlepis* is territorial, with limited post-settlement movement (< 100 m^2), and actively guards demersal eggs which hatch and enter a pelagic phase lasting 2-4 weeks (Moran & Sale 1977, Tzioumis & Kingsford 1995, Kingsford & Gillanders 2000). Local-scale differences in hydrographic processes may act to retain ichthyoplankton and minimize along-shore drift within some areas of central NSW (Smith et al. 1999, Booth et al. 2007). This process in combination with the active behaviour typically exhibited by pomacentrids during larval transport (Leis et al. 2003, Paris & Cowen 2004) may act to reduce long-distance dispersal and gene flow in *P. microlepis*. Given this, it was hypothesized that *P. microlepis* would exhibit population genetic structure and a significant pattern of IBD at scales ≤ 400 km.

4.2. Methods

4.2.1. Sampling design and genetic analyses

The population genetic structure of *P. microlepis* was examined at multiple spatial scales within a 400 km stretch of coastline in NSW, Australia, using a partially hierarchical design. Samples of *P. microlepis* were collected from three regions separated by 70-80 km (Port Stephens, Sydney and Jervis Bay) during 2003-04 (Fig. 4.1). Within each region, I sampled fish from two sites (separated by 1-2 km) at four locations separated by 10-50 kilometres; two locations where rocky reef was continuous for kilometres alongshore, and two locations where rocky reefs were separated by kilometres of sand. Sand and reef separated sites were included as this was representative of the NSW coastal environment, which consists of stretches of reef and intermittent beaches. *P. microlepis* could not be found on subtidal reefs separated by specified distances north of location 1 in Port Stephens, thus fish were collected from five sites in this region. Sixteen fish of a range of sizes were collected from each site, at depths < 5 m, using a handspear. This sample size was chosen as fish were rare at some sites and larger samples were not practical. Samples were kept on ice after collection and frozen until processed. Standard lengths (SL) of fish were measured and fin clips were preserved in 70% ethanol.

DNA was extracted from fins using a proteinase K/salting out method (Sunnucks & Hales 1996). Individual fish were genotyped at seven microsatellite loci: PM3K12, PM2N7, PM1E12, PM2D15, PM2G3, PM2G2, PM1M14 using methods described in Curley and Gillings, 2004 (Appendix C). The construction and screening of the microsatellite library is also described in this publication. Previous analyses of 100 *P. microlepis* samples found no significant linkage disequilibrium between these loci, therefore, they were considered statistically independent (Curley & Gillings 2004). Ten randomly selected individuals were re-amplified and scored to act as positive controls for genotyping methods. DNA from individuals used to generate the original microsatellite libraries was included as a positive control in each PCR.

4.2.2. Statistical analyses

Number of alleles, and observed (H_O) and expected heterozygosity (H_E) were calculated for each locus for individual locations (sites pooled) and across all samples. Departure from Hardy Weinberg Equilibrium (HWE) was used to test for potential biological processes such as inbreeding or population substructure, and methodological errors (e.g. genotyping errors). HWE was examined for each locus-location combination and across the entire data set using exact tests based on a Markov chain approach and 9999 dememorization steps in ARLEQUIN 3.0 (Guo & Thompson 1992, Excoffier et al. 2005).

Frequency-based tests were used to test the null hypotheses that allelic and genotypic frequency distributions were identical across sampling locations for each locus. Tests on allelic distributions were based on an unbiased estimate of the *P*-value of the probability test (Fisher exact test) (Raymond & Rousset 1995), and tests on genotypic distributions on a log-likelihood (G) based exact test (Goudet et al. 1996). Analyses were performed using 5000 markov chain iterations, 500 batches (5000 iterations per batch) in GENEPOP version 3.4 (Raymond & Rousset 1995). Paired comparisons among all locations were done when results of these tests were found to be significant. Results were adjusted for multiple tests, where appropriate, using sequential Bonferroni tests by the Dunn-Ŝidák method, $\alpha' = 1-(1-\alpha)^{1/\text{number of tests}}$, with $\alpha = 0.05$ (Sokal & Rohlf 1995).

A distance-based approach was used to examine genetic variation across different spatial scales, and to test for IBD. All tests were based on pairwise, individual-by-individual genetic distance matrices and were performed using GenAlEx 6.0 (Peakall & Smouse 2005). Two evolutionary models are typically used to interpret genetic variation at microsatellite loci; the infinite allele model (IAM) in which each allele may mutate to any other allele (Kimura & Crow 1964), and the stepwise mutation model (SMM) where mutations generally consist of a length change of one repeat unit (Kimura & Ohta 1978). Although microsatellites generally conform to the SMM, the mutation process is complex and poorly understood such that neither model is entirely adequate (Ellegren 2000, Whittaker et al. 2003). Given this, two types of distance matrices were used in analyses to account for different mutation models. Genotypic distances assuming the IAM, and allele-size-based distances assuming the SMM were calculated for each locus

and then summed across loci, under the assumption of independence as described in (Peakall et al. 1995, Smouse & Peakall 1999). There was missing data for some individuals at some loci due to failed amplifications or PCR products that could not be reliably scored (see Table 4.1). In these cases, missing individual-by-individual pair-wise distances were replaced with the average genetic distance for each region, location or site level pair-wise contrast.

A hierarchical analysis of molecular variance (AMOVA) was used to partition total genetic variation (genotypic or size-based) into three spatial scales; among regions, locations (within a region), and sites (within locations) (following methods of; Excoffier et al. 1992, Peakall et al. 1995). Variation was summarized as the proportion of the total variance and as analogues of *F*-statistics; Φ-statistics, based on genotypic distance matrices, and *R*-statistics based on size-based distance matrices. These statistics estimate the relative genetic variance among compared to within populations, or in this case sampling regions, locations and sites. Values may lie between zero (indicating no subdivision) and one (indicating complete genetic subdivision). Statistical significance was tested using non-parametric permutation procedures to calculate null distributions using 999 permutations. Two designs were analysed, with, and without regional partitioning. The first design compared 10 locations (P1 was excluded) and 2 sites within each location. The second compared 3 regions and 3 or 4 locations within each region. Sites were pooled for this analysis to increase statistical power, as no significant differences were detected between sites at $P \geq 0.25$ in the first design. This analysis was also performed on each locus separately to determine if the detection of genetic differentiation was independent of locus polymorphism. The relationship between genetic differentiation estimated using Φ_{PT} or R_{ST} values, the number of alleles, and the mean expected heterozygosity per location was tested using linear regression. Intra-individual variation was suppressed in all analyses. Pairwise comparisons were made when significant differences were detected by AMOVA.

IBD by distance was tested using Mantel tests and spatial autocorrelation. Mantel tests were used to test for statistical relationship between pairwise Φ/*R*-statistics among locations and geographic distance. Relative geographic distance was calculated as the shortest distance between the two sampling locations. The hypothesis that genotypes of individuals separated by short geographical distances were more similar than those

further apart was tested using spatial autocorrelation (Smouse & Peakall 1999, Peakall et al. 2003). Pairwise genotypic and geographical distance matrices were compared and the spatial autocorrelation coefficient (*r*) was calculated across multiple distance classes within each region and across all regions. This coefficient is closely related to Moran's *I* and provides a measure of genetic similarity between pairs of individuals which are found within the specified distance class. The *r* statistic has a mean of '0' when there is a random non-linear relationship between genotypes and distance, and is bounded by [-1, +1] (Smouse & Peakall 1999). A significantly positive *r* is, therefore, expected over geographic scales at which gene flow is limited. Results were considered statistically significant when *r* exceeded the 95% confidence interval about the null hypothesis of zero (calculated using 999 permutations), and when the 95% error about r (calculated using 1000 bootstrap replicates) did not intercept the x-axis at $r = 0$ (Peakall et al. 2003). Principal coordinates analysis (PCA) was used to visualise patterns of genetic relatedness between individual fish among locations and regions based on genotype.

4.3. Results

A total of 336 fish were collected ranging in size from 50-150 mm SL, including juveniles (identified by colouration) and adults. Genotypes for the ten randomly chosen individuals used as positive controls were identical on repeat analyses indicating that genotyping methods were accurate. The genotypes of the individuals used to construct the microsatellite library were consistent in every run, confirming the accuracy of genotyping. Summary statistics for seven microsatellite loci in *P. microlepis* are given in Table 4.1. The number of alleles per microsatellite locus ranged from 2-49 across all samples, and 2-32 within sampling locations. Expected heterozygosities (H_E) ranged from 0.21-0.97 across all samples, and 0.12-0.97 within sampling locations. No significant deviations from HWE were detected at any locus within locations, or when all samples were pooled ($P < 0.05$). Overall allele frequency distributions were unimodal except for PM1E12 and PM2N7 which were multimodal (Appendix C, Fig. C.1). No significant differences in allelic and genotypic distributions were found across sampling locations for six of the seven loci (Table 4.2). Significant differences in allelic and genotypic distributions were, however, detected for locus PM1E12, $P < 0.01$. These differences were most likely due to substantial fluctuations in the frequency of allele sizes 121 (2-19%), 135 (3-19%), and 137 (3-28%) among locations (Appendix C, Fig. C.2; e.g. location P3 versus S3 allele, size 137). Although eight of 55 tests were

significant in pair-wise comparison of locations of both allelic and genotypic distributions for PM1E12, only one test (genotypic, location J1 versus J2) remained significant after sequential Bonferroni correction for multiple tests (Table 4.3).

No significant genotypic or size-based differentiation was detected by multi-locus AMOVA among regions (separated by 70-80 km), locations (separated by 10-50 km) or sites (separated by 1-2 km of sand or reef) regardless of the way in which data were partitioned (Table 4.4). The fraction of the total genetic variation that distinguished regions, locations or sites was effectively zero in all analyses (Φ/R-statistics ≤ 0.007, Φ_{PT} = 0.003 and R_{ST} = 0.004), with 99-100% of the total genetic variance being attributed to variation among individuals within sites or locations. Analyses using individual loci were also non-significant except for PM1E12 where significant differences between locations were detected (Φ_{PT} = 0.011, $P < 0.05$). These differences, however, only accounted for 1% of the total genetic variation. Although pair-wise comparisons among locations revealed significant differences between P3 or S3 and several other locations and between J1 and J2 with Φ_{PT} values ranging between 0.029 and 0.070, these differences were non-significant after applying the Bonferroni correction for multiple tests. No significant relationship was found between locus polymorphism and the level of genetic differentiation detected in analyses ($P > 0.05$). For example, R_{ST} for the most polymorphic locus, PM1M14, was higher than for four less polymorphic loci and Φ_{PT} values were greater or similar to values obtained using three less polymorphic loci (Fig. 4.2).

There was no evidence for IBD when analyzing allele-size-based or genotypic-based genetic distances. No relationship was found between pairwise Φ_{PT} or R_{ST} and geographic distance along the 360 km stretch of coastline using mantel tests (Fig. 4.3). Results for spatial autocorrelation were depicted by genetic correlograms which show genetic correlation as a function of distance between genotypes (Fig. 4.4). Spatial autocorrelation within each region and across the entire coastline sampled revealed a random non-linear relationship between genotypes and distance ($r \leq 0.003$) (Fig. 4.4). Therefore, the lack of IBD was consistent across the entire coastline. This was well illustrated in the PCA in which genotypes of individuals separated by small geographical distances (e.g. ≤ 2 km) were not necessarily more similar than those separated by distances of up to 360 km (Fig. 4.5).

Table 4.1. Summary statistics for seven microsatellite loci in *P. microlepis* collected at 11 locations in central NSW, Australia. Shown are sample sizes (*n*), the number of alleles (*A*), allele size range (bp) and the observed and expected heterozygosities (H_O and H_E) for individual locations and all samples combined. Locations and all samples were in Hardy-Weinberg equilibrium at $P > 0.05$ for all loci.

Locus	Location	P1	P2	P3	S1	S2	S3	S4	J1	J2	J3	J4	*All*
PM3K12	*n*	16	32	32	31	32	32	32	32	31	32	32	*334*
$(CA)_7(CT)_4$	*A*	2	2	2	2	2	2	2	2	2	2	2	*2*
	bp	327-329	327-329	327-329	327-329	327-329	327-329	327-329	327-329	327-329	327-329	327-329	*327-329*
	H_O	0.25	0.09	0.19	0.16	0.19	0.31	0.22	0.13	0.10	0.25	0.16	*0.18*
	H_E	0.37	0.12	0.32	0.20	0.30	0.29	0.23	0.15	0.12	0.22	0.23	*0.21*
PM2N7	*n*	16	32	32	32	32	32	32	32	31	32	32	*335*
$(GA)_{16}$	*A*	9	11	9	8	12	9	9	8	9	13	10	*18*
	bp	236-270	236-260	240-260	236-259	236-266	236-256	236-260	246-260	236-258	236-268	236-266	*236-270*
	H_O	0.88	0.81	0.88	1.00	0.88	0.91	0.78	0.88	0.84	0.88	0.81	*0.87*
	H_E	0.86	0.82	0.85	0.85	0.81	0.84	0.83	0.84	0.81	0.86	0.84	*0.83*
PM1E12	*n*	16	32	32	32	32	32	32	32	31	32	32	*335*
$(GT)_{13}$	*A*	9	14	14	13	12	16	16	11	14	14	13	*22*
	bp	121-145	121-159	121-151	121-151	121-159	121-157	121-159	121-163	121-153	121-165	121-159	*121-165*
	H_O	0.88	0.91	0.94	0.88	0.81	0.91	0.91	0.91	1.00	0.84	0.91	*0.90*
	H_E	0.87	0.91	0.89	0.87	0.88	0.88	0.91	0.88	0.89	0.89	0.87	*0.89*
PM2D15	*n*	16	32	32	32	32	32	32	32	31	32	32	*335*
$(AC)_{19}$	*A*	12	15	16	16	14	15	15	14	15	14	15	*22*
	bp	117-145	115-143	117-149	117-153	117-151	111-149	111-153	117-147	117-149	119-145	117-157	*111-157*
	H_O	0.81	0.91	0.78	0.75	0.94	0.81	0.84	1.00	0.90	0.81	0.84	*0.86*
	H_E	0.86	0.88	0.88	0.78	0.88	0.80	0.83	0.89	0.87	0.84	0.85	*0.85*

Table 4.1. cont.

Locus	Location	P1	P2	P3	S1	S2	S3	S4	J1	J2	J3	J4	*All*
PM2G3	*n*	16	32	32	32	32	32	32	32	31	32	32	*335*
$(TG)_{19}$	*A*	14	16	17	16	18	16	19	18	19	15	16	*23*
	bp	129-163	127-163	127-167	133-163	133-167	127-165	127-167	129-179	127-171	135-165	129-163	*127-179*
	H_O	0.88	0.94	0.84	0.97	0.91	0.94	0.97	0.97	0.90	0.94	0.84	*0.92*
	H_E	0.93	0.92	0.94	0.92	0.94	0.92	0.95	0.94	0.95	0.92	0.92	*0.93*
PM2G2	*n*	16	32	32	32	32	32	32	32	31	32	32	*335*
$(ACAG)_9(ATAG)_{21}$	*A*	14	15	13	16	17	16	15	15	18	17	14	*23*
	bp	135-227	159-219	155-211	159-219	135-235	135-211	159-219	155-211	135-215	155-223	135-219	*135-235*
	H_O	0.94	0.94	0.81	0.88	0.94	0.94	0.91	0.91	1.00	0.91	0.84	*0.91*
	H_E	0.93	0.91	0.90	0.92	0.93	0.91	0.93	0.92	0.91	0.92	0.91	*0.92*
PM1M14	*n*	16	32	32	32	32	32	32	32	30	32	32	*334*
$(CA)_5(CR)_8(CA)_{24}$	*A*	21	26	32	30	31	31	27	31	28	25	28	*49*
	bp	332-406	330-444	338-436	336-406	334-428	336-498	338-406	334-498	334-430	336-430	336-406	*330-498*
	H_O	1.00	0.94	0.94	0.97	0.97	0.91	0.94	0.94	0.97	0.94	1.00	*0.95*
	H_E	0.97	0.96	0.97	0.97	0.97	0.97	0.96	0.97	0.96	0.96	0.97	*0.97*

Table 4.2. Allelic and genotypic differentiation at seven microsatellite loci in *P. microlepis* collected from 11 locations. *P*-values are shown ± S.E.

Locus	Differentiation *Allelic*	*Genotypic*
PM3K12	0.128 ± 0.002	0.197 ± 0.002
PM2N7	0.861 ± 0.006	0.820 ± 0.007
PM1E12	**0.008 ± 0.001**	**0.007 ± 0.002**
PM2D15	0.235 ± 0.007	0.350 ± 0.012
PM2G3	0.317 ± 0.008	0.403 ± 0.013
PM2G2	0.359 ± 0.009	0.476 ± 0.013
PM1M14	0.366 ± 0.011	0.546 ± 0.017
Average over all loci	0.055 chi2: 23.314 df: 14	0.121 chi2: 20.300 df: 14

Table 4.3. Pairwise comparisons of allelic (above diagonal) and genotypic (below diagonal) differentiation for locus PM1E12 in *P. microlepis* collected from 11 locations. *P*-values < 0.05 are shown, and bold values were significant after sequential Bonferroni correction.

	P1	**P2**	**P3**	**S1**	**S2**	**S3**	**S3**	**J1**	**J2**	**J3**	**J4**
P1											
P2								0.005			0.033
P3						0.006			0.006		0.047
S1								allelic			
S2						0.020					
S3			0.020		0.041						
S3											
J1		0.002					0.029		0.001		
J2			0.006		genotypic			**0.000**			0.049
J3											
J4		0.029							0.035		

Table 4.4. AMOVA showing partitioning of genotypic (Φ-statistics) and size-based (R-statistics) variation across regions (separated by 70-80 km), locations (separated by 10-50 km) and sites (separated by 1-2 km). (a) Without regional partitioning of data, n = 16; (b) data partitioned into three regions (Port Stephens, Sydney and Jervis Bay), sites pooled n = 32. ns $P \geq 0.05$.

Source of variation	df	Sums of squares	% Total variation	Φ-statistics
(a) No regional partition				
Among Locations	9	55.975	0%	$\Phi_{RT} = 0.003$ ns
Among Sites (Locations)	10	56.252	0%	$\Phi_{PR} = 0.001$ ns
Within Sites	300	1668.839	100%	$\Phi_{PT} = 0.004$ ns
Total	319	1781.066		
(b) Data partitioned by region (sites pooled)				
Among Regions	2	12.833	0%	$\Phi_{RT} = 0.001$ ns
Among Locations (Regions)	8	48.035	0%	$\Phi_{PR} = 0.003$ ns
Within Locations	325	1812.470	100%	$\Phi_{PT} = 0.003$ ns
Total	335	1873.338		

Source of variation	df	Sums of squares	% Total variation	R-statistics
(a) No regional partition				
Among Locations	9	9463.111	1%	$R_{RT} = 0.007$ ns
Among Sites (Locations)	10	7215.230	0%	$R_{SR} = 0.000$ ns
Within Sites	620	480098.032	99%	$R_{ST} = 0.005$ ns
Total	639	496776.373		
(b) Data partitioned by region (sites pooled)				
Among Regions	2	1808.235	0%	$R_{RT} = 0.000$ ns
Among Locations (Regions)	8	7962.106	0%	$R_{SR} = 0.005$ ns
Within Locations	661	512975.895	100%	$R_{ST} = 0.004$ ns
Total	671	522746.236		

* For regional analyses Φ/R_{RT} = the fraction of the total variance that distinguishes regions; Φ_{PR}/R_{SR} = the fraction of the variation within a region that distinguishes locations; Φ_{PT}/R_{ST} = the fraction of all the variation that distinguishes locations, without regard to regional partitioning. Similar interpretation for analyses (a).

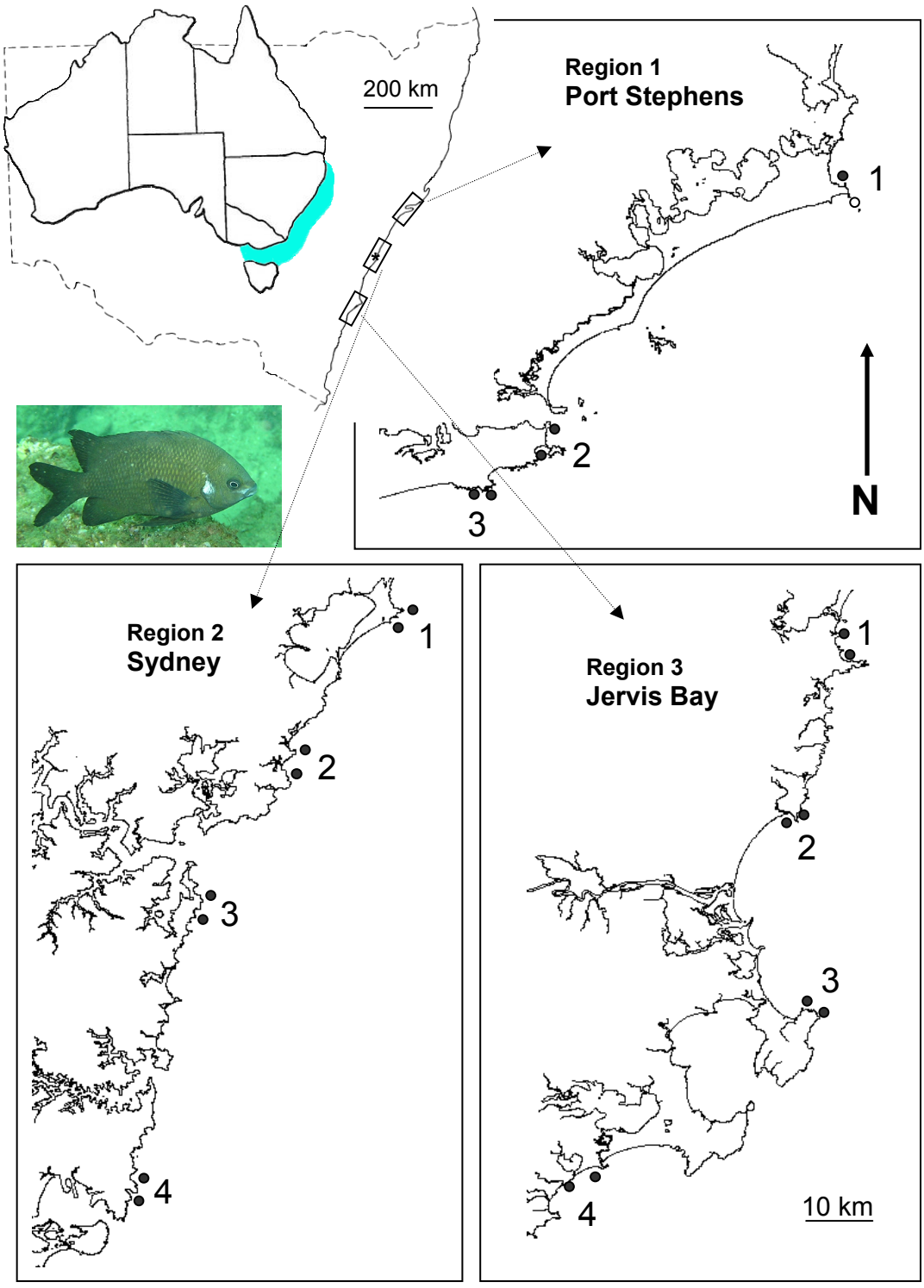

Fig. 4.1. Geographic distribution of *P. microlepis* in Australia including three sampling regions in central NSW: Port Stephens, Sydney and Jervis Bay. Within each region, the locations (1-4) and sites (within locations) at which fish were collected (●) are shown. Fish were not collected at this site (○). GPS co-ordinates for collection sites are given in Appendix B, Table B.1.

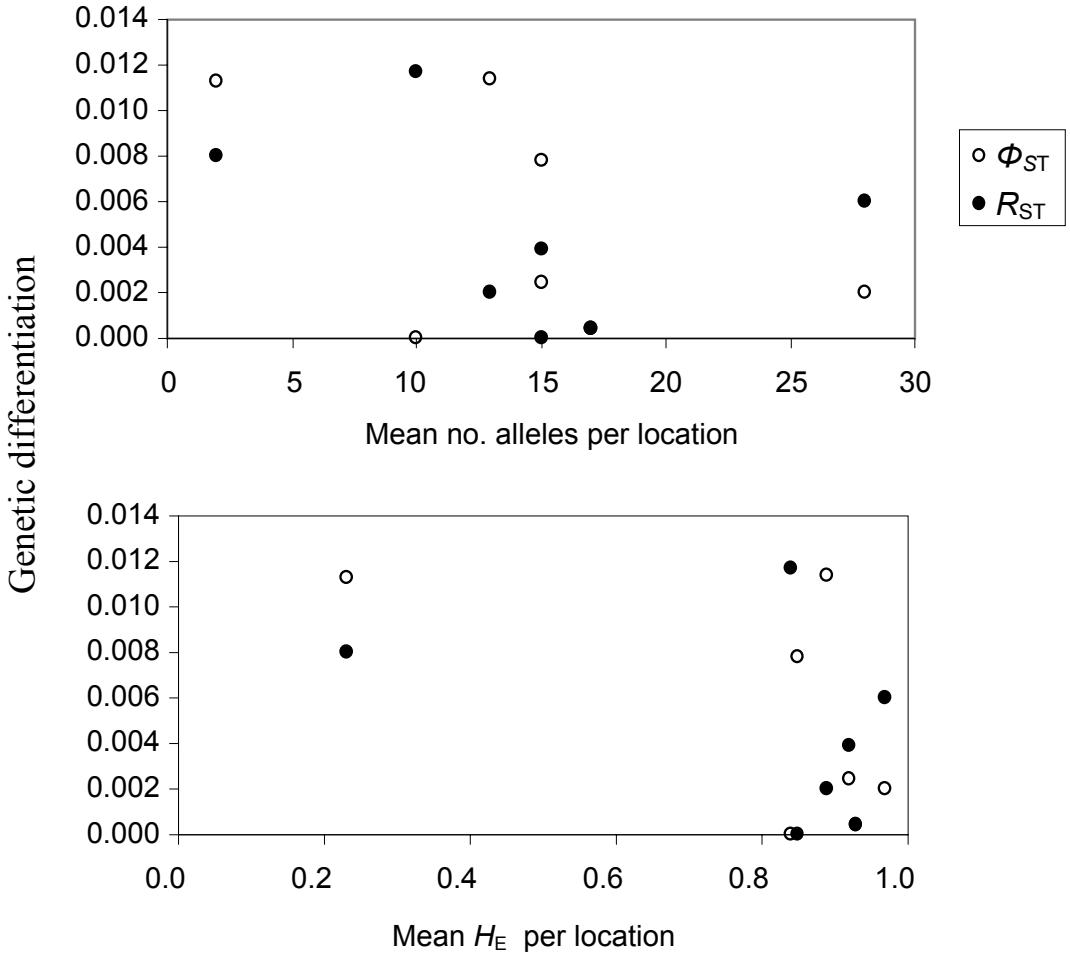

Fig. 4.2. The relationship between locus polymorphism and the magnitude of genetic differentiation detected in *P. microlepis*.

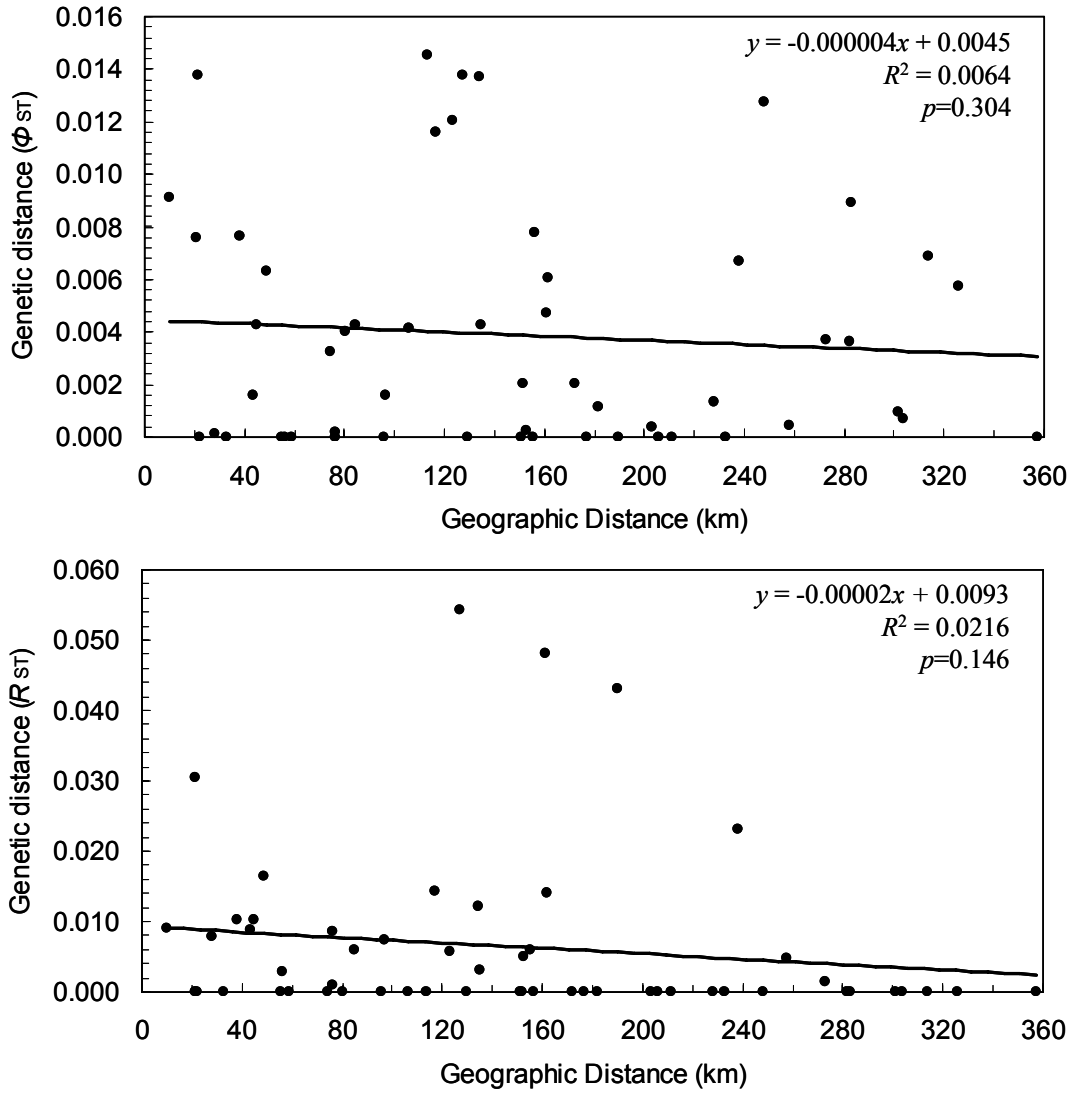

Fig. 4.3. The relationship between geographic distance (km) and genetic distance (Pairwise R_{ST} /Φ_{ST}) for *P. microlepis* collected from 11 locations.

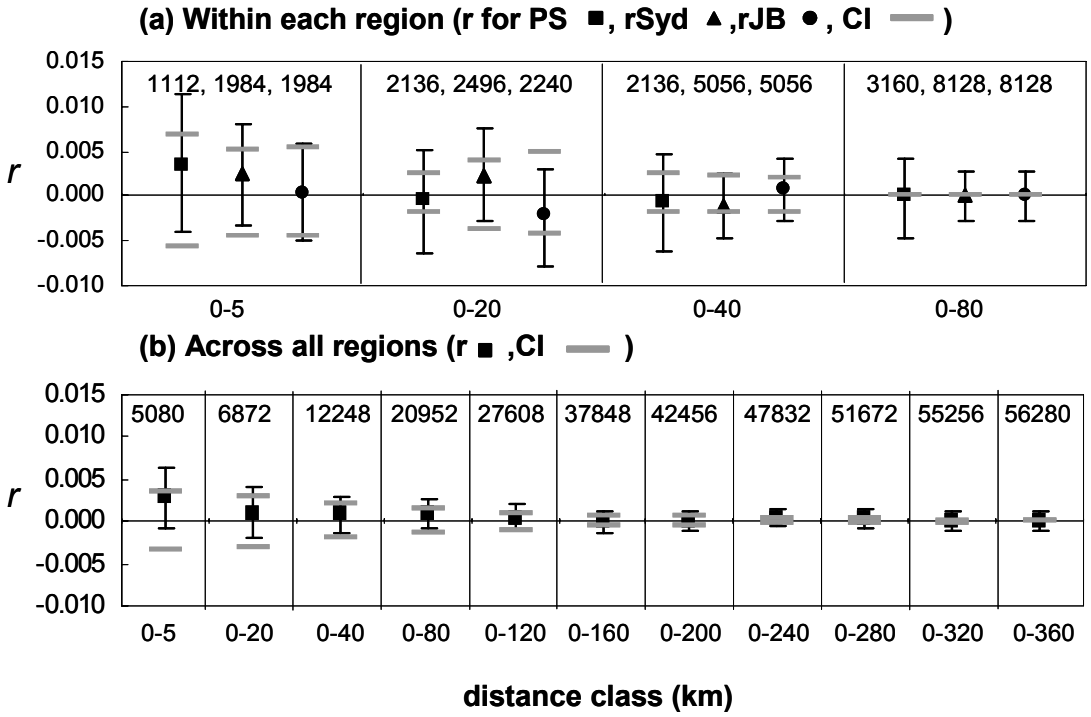

Fig. 4.4. Spatial autocorrelation analyses (a) within each region: Port Stephens (PS), Sydney (Syd) and Jervis Bay (JB), and (b) across all regions. Shown are a range of geographical distance classes, with 95% confidence error bars. The 95% confidence intervals (CI) around the null hypothesis of $r = 0$ are given. Number of comparisons made in each class are shown above each r value. Genotypic distances were based on seven microsatellite loci, n = 336.

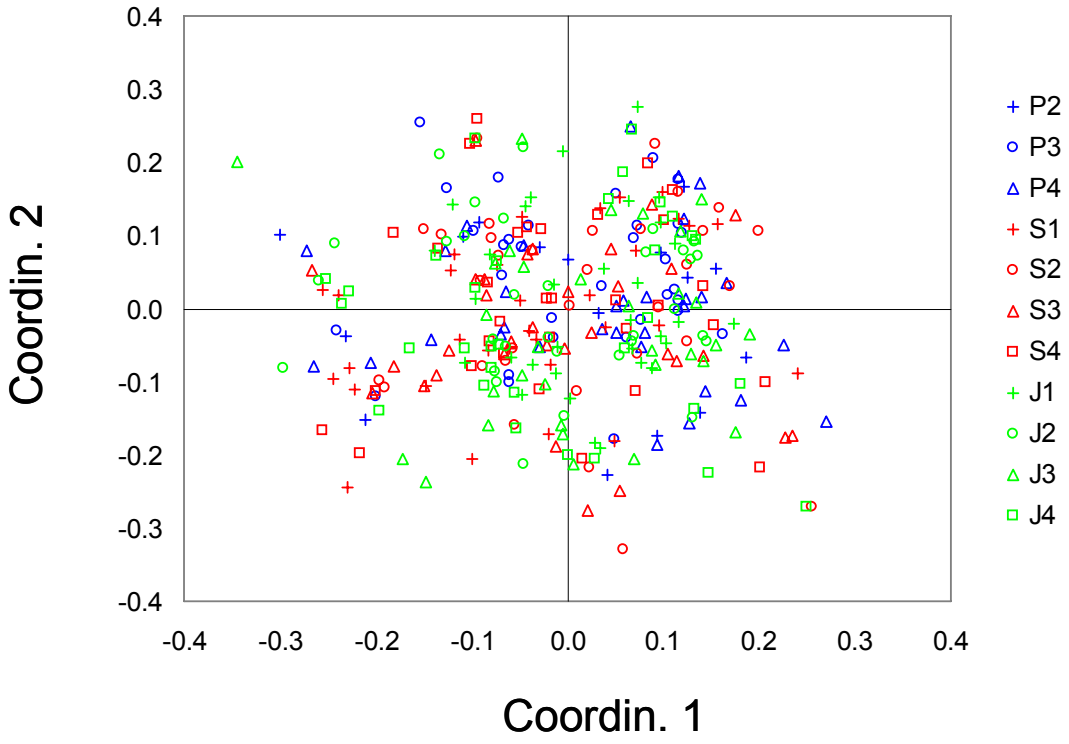

Fig. 4.5. Principal coordinates analyses of multi-locus genotypes of *P. microlepis* collected from three regions in NSW. Port Stephens (blue) n = 80, Sydney (red) n = 128, Jervis Bay (green) n = 128. Fish collected from the same location within a region have the same symbol. Principal coordinates 1 and 2 accounted for 20.2 and 19.2% of the total variation.

4.4. Discussion

Patterns of microsatellite differentiation across a hierarchy of spatial scales did not support predictions that *P. microlepis* would exhibit spatial structuring of genes or a pattern of IBD at scales ≤ 400 km in central NSW, Australia. Broad-scale genetic homogeneity was well supported, regardless of the evolutionary model (IAM or SMM) used to interpret genetic variation. The proportion of the total genetic variation attributable to differences among sampling regions (separated by 70-80 km), locations (separated by 10-50 km) or sites (separated by 1-2 km) was effectively zero (e.g. Φ_{PT} = 0.003 and R_{ST} = 0.004), with 99-100% of the variation occurring within sites and locations. A lack of genetic structure was further supported by the random non-linear relationship between genetic and geographic distance over the 400 km sampling area. Although subtle genetic structure was detected among locations at one locus, PM1E12 (Φ_{PT} = 0.011, $P < 0.05$) these differences were very weak, accounting for 1% of the total genetic variation, and could not be attributed consistently to any locations. Given that this locus is not homologous to any known gene (and therefore potentially subject to selection), it is likely that results were due to stochastic processes rather than biologically important differentiation.

The geographic distribution of genetic homogeneity and the high polymorphism found for *P. microlepis* is common in marine fishes, and is indicative of high mutation rates, large effective population sizes, and high rates of gene flow (Gyllensten 1985, DeWoody & Avise 2000, O'Reilly et al. 2004). Such patterns have been reported for many reef fishes, including tropical damselfishes in which genetic structure is either absent or only detectable at scales of 1000's km (Bernardi et al. 2001, Van Herwerden et al. 2003, Bay et al. 2006, Gilbert-Horvath et al. 2006). Although there are no published data on other reef fishes in central NSW, patterns of genetic variation in *P. microlepis* are consistent with invertebrates which inhabit near-shore coastal environments in NSW, and like *P. microlepis*, have limited post-settlement movement and a pelagic larval phase. A lack of genetic structure at non-neutral loci has been described for several invertebrate species including the: starfish *Patiriella calcar* F_{ST} = 0.000 over 230 km (Hunt 1993), bivalve *Donax deltoids* F_{ST} = 0.009 over 1200 km (Murray-Jones & Ayre 1997), gastropod *Morula marginalba* F_{ST} = 0.017 over 180 km (Hoskin 1997), and the anemone *Oulactis muscosa* F_{ST} = 0.03 over 735 km (Hunt &

Ayre 1989). The lack of genetic structure at scales < 100's km in many reef fishes, and for invertebrates with pelagic dispersal in central NSW suggests that observed patterns in *P. microlepis* are due to biological and environmental factors which oppose the process of genetic differentiation.

Effective population size and genetic drift

Genetic drift due to finite population size, and natural selection, results in the accumulation of unique mutations within local populations which have been separated for a sufficient period of time (Slatkin 1987, Bernardi et al. 2001). The rate at which isolated populations lose genetic variability due to genetic drift, is inversely related to the total number of individuals that contribute genetically to the next generation (effective population size (N_e)) (Hellberg et al. 2002). The high genetic polymorphism in *P. microlepis* and relatively large census sizes in NSW indicate that N_e is large. This species is one of the most abundant reef fishes in central NSW with temporally stable densities ranging from 2-20 fish per 125 m^2 in urchin-grazed barrens habitat (Holbrook et al. 1994, Curley et al. 2002). Given that total barrens habitat within the sampling area was conservatively estimated at 20 km^2 (calculated using data from Andrew & O'Neill 2000), total census numbers are likely to be in the order of millions.

Although N_e may be much lower than the census population due to variation in spawning success and pelagic survival (Hellberg et al. 2002, Turner et al. 2002), *P. microlepis* also displays several biological and ecological traits which are predicted to increase N_e (see Turner et al. 2002 and references therein). *P. microlepis* become reproductively active between 2-5 years of age and may live in excess of 37 years, thus potentially contributing to the reproductive output of local populations for more than 30 years (Tzioumis & Kingsford 1999). This long reproductive life span together with overlapping generations can act to limit the variance in lifetime reproductive success (Turner et al. 2002). *P. microlepis* also display a non-biased sex ratio and a mating system which is unlikely to produce high variance in male and/or female reproductive success (Turner et al. 2002). The species forms spawning pairs in which adults of both sexes defend territories (Moran & Sale 1977, Tzioumis & Kingsford 1999). Individuals may spawn asynchronously many times throughout a 3.5-4 month breeding season, with males guarding demersal eggs until hatching (Tzioumis & Kingsford 1995, Tzioumis & Kingsford 1999). Such high parental investment is predicted to enhance larval survival,

as highly developed larvae are more likely to exhibit active behaviour and can settle after a shorter pelagic duration (Sponaugle et al. 2002). These characteristics may also ensure that representative individuals have an opportunity to disperse novel alleles through their offspring over ecological time scales.

Gene flow

The East Australian Current

Given that *P. microlepis* exhibits minimal post-settlement movement, a lack of genetic structure suggests that gene flow via dispersal of pelagic larvae is sufficient to oppose processes which lead to genetic differentiation. Dispersal of pelagic larvae in central NSW is likely to be facilitated by the East Australian Current (EAC), which carries tropical water, including vagrant tropical fish larvae, southwards down the east Australian coast. The EAC flows strongly and consistently parallel to the shore between 26 and 32^0S (Port Stephens region) before separating from the coast and flowing south east. Although the influence of the EAC has the potential to reach 45^0S, it is has a strong seasonal cycle below 32^0S and is generally present as southward moving filaments and eddies of warm water (Middleton et al. 1996, Ridgway & Godfrey 1997). Although the heterogeneous nature of the EAC has been correlated with the degree of genetic subdivision in the direct developing snail *Bedeva hanleyi* (Hoskin 2000), there is no indication that this behaviour affects gene flow in *P. microlepis* or in other invertebrates with pelagic dispersal in this region (e.g. Hunt & Ayre 1989, Hunt 1993, Murray-Jones & Ayre 1997). This could be partly due to opposing northward current pulses caused by coastal trapped waves (Middleton et al. 1996), which would facilitate south-north dispersal, thus counteracting the effects of the EAC.

The lack of correlation between the behaviour of the EAC and genetic population structure of *P. microlepis* may also be partially explained by the timing of reproduction in this species. In the Sydney region, spawning is restricted to three months during the late austral spring and early summer (Tzioumis & Kingsford 1999). This period corresponds with the strongest southward flow of the EAC during which associated currents and eddies may reach speeds of 1.5 m s^{-1} (Middleton et al. 1996, Ridgway & Godfrey 1997, Kingsford 1999). Under these conditions *P. microlepis* may theoretically be transported thousands of kilometres during their 14-28 day pelagic larval phase. Despite potentially high levels of diffusion and/or larval mortality it is likely that the

small number of migrants required to maintain genetic homogeneity could be supplied by this process. Such large-scale transport of pre-settlement fishes by the EAC during the summer months is well supported by the presence of tropical recruits as far south as 37^{o}S (Booth et al. 2007). Therefore, although local-scale differences in hydrographic processes may act to retain pelagic larvae and minimize alongshore drift within some parts of central NSW, the EAC has the potential to facilitate long-distance dispersal at rates that prevent genetic differentiation.

Habitat continuity

The distribution of habitat in central NSW may also promote gene flow in *P. microlepis*. Evidence suggests that continuous habitat promotes contact between populations, thereby reducing genetic differentiation, while large discontinuities of sand or deep-water channels often restrict gene flow (Bernardi 2000, Bernardi et al. 2001, Riginos & Nachman 2001, Taylor & Hellberg 2003, Hoffman et al. 2005, Carreras-Carbonell et al. 2006, Gilbert-Horvath et al. 2006). Although subtidal rocky reefs in central NSW are regularly intercepted by beaches and the mouths of rivers and estuaries, they are probably not of sufficient magnitude to significantly limit dispersal and hence gene flow of pre-settlement *P. microlepis*. All deep water channels in the study area are < 4 km and most beaches are < 10 km in length with only one stretching for approximately 30 km. In addition, urchin-grazed barrens, the preferred reef habitat of *P. microlepis*, are one of the most frequently occurring habitat types in central NSW (Underwood et al. 1991, Curley et al. 2002). Thus availability of suitable habitat would not significantly restrict along-shore settlement.

Distribution of habitat could also influence the role of larval behaviour on dispersal of *P. microlepis* in NSW. Settling fish larvae may use olfactory discrimination of adjacent reefs separated by as little as 3-23 km to aid retention in natal areas (Gerlach et al. 2007). It has been hypothesized however, that the strength of homing behaviour will vary according to the possibility of unsuccessful settlement. For example, species with stronger swimming larvae (e.g. pomacentrids) may be more capable of returning to a reef once they have been advected away, than weaker swimmers (Gerlach et al. 2007). In these cases responding to generic rather than natal reef odor may be adequate to facilitate their return to reef habitat (Gerlach et al. 2007). Likewise, it has been proposed that species like *P. microlepis* which are found along continuous habitats may avoid

offshore dispersal due to the greater opportunity for encountering settlement habitat and the continuous reception of sensory information regarding its location (Swearer et al. 2002). Exhibition of this behaviour by *P. microlepis* larvae would not act to prevent along-shore dispersal and gene flow in central NSW.

Sampling effects

Finally, it is possible that *P. microlepis* does exhibit spatial genetic structure but it could not be detected in this study (Type II error). The power to detect genetic structure is influenced by several factors including: the magnitude of genetic differentiation, intrinsic variability of the population, sample size, spatial replication, and the number and characteristics of the microsatellite loci used (e.g. polymorphism, homoplasy, mutation rates) (Underwood 1997, Ruzzante 1998, O'Reilly et al. 2004, Olsen et al. 2004, Ryman et al. 2006). Although biases in estimates were limited by the use of equal sample sizes and intensive replication across multiple spatial scales (Ruzzante 1998, Leberg 2002), genotypic diversity at fine spatial scales was characterised by high variance, low precision, and hence low statistical power. Although there was no indication that the level of genetic variation detected was due to locus polymorphism, six of seven loci tested were highly polymorphic (H_E 0.86-0.95, Number of alleles 18-49). It is possible that the use of less polymorphic loci and larger sample sizes would improve accuracy of estimates and therefore the ability to detect structure (O'Reilly et al. 2004). However, given the evidence for large effective population sizes and high gene flow, detectable structure is likely to be weak, making ecological implications difficult to interpret (Palumbi 2003). Regardless, the null hypothesis that *P. microlepis* forms a single, largely panmictic population within central NSW could not be rejected in this study.

Population connectivity

In theory only a small amount of gene flow ($\geq$ 5 effective migrants per generation, (Shulman 1998) would be required for ecologically distinct populations of *P. microlepis* to be genetically homogeneous (Mora & Sale 2002). Quantification of gene flow is difficult due to the unrealistic assumptions of models (e.g. symmetrical gene flow between populations) and large errors associated with estimates of very small values of F_{ST} (Palumbi 2003). Therefore, genetic similarity of *P. microlepis* at scales $\leq$ 400 km may suggest populations are largely 'open' with high exchange of individuals (> 10 000)

or largely 'closed' with sporadic but demographically inconsequential inter-population migration (Hellberg et al. 2002, Mora & Sale 2002). Although the scale of genetic homogeneity may not reflect ecologically relevant dispersal distances, it does imply that populations of *P. microlepis* are well connected from an evolutionary perspective.

4.4.1. Conclusion

A high level of genetic population connectivity was found for the low dispersing fish, *P. microlepis* at spatial scales ≤ 400 km in central NSW, Australia. Detectable genetic structure, however, may be found for this species across broader spatial scales using alternative markers, or in different geographic regions, particularly those unaffected by the EAC. Repeated geographic sampling and genetic analyses of *P. microlepis* recruits may also give insight into spatio-temporal patterns of recruitment, even in areas like central NSW where adult populations are genetically homogeneous (Hellberg et al. 2002, Thorrold et al. 2002). It is likely, however, that alternative methods (e.g. otolith tagging/chemistry, modelling) will be required to determine levels of connectivity for *P. microlepis* and similar species, particularly at fine spatial scales. In conclusion, the use of population genetics for determining population connectivity of reef fishes in central NSW should be tested on additional species with varying life history traits. The primary focus, however, should include species with extremely limited dispersal capabilities (e.g. no or short pelagic phases), small population sizes, short life spans, and whose habitats are rare or patchily distributed along-shore.

Chapter 5: Population connectivity in the highly dispersive temperate fish *Girella tricuspidata*: analyses of population genetic structure

5.1. Introduction

Knowledge of connectivity among local populations of reef fishes is fundamental to conservation and fisheries management. Levels of genetic differentiation among local populations can be used to estimate population connectivity, even across relatively fine spatial scales of kilometres to hundreds of kilometres (Hoffman et al. 2005, Carreras-Carbonell et al. 2006, Purcell et al. 2006, Froukh & Kochzius 2007, Gerlach et al. 2007). The recent detection of population genetic structure at fine spatial scales has been attributed to small effective population sizes relative to total population numbers, mechanisms that favour self-recruitment, and the use of sensitive genetic markers (e.g. microsatellites) which provide information on gene flow over ecological time scales (Hellberg et al. 2002). Consequently, genetic markers such as microsatellites, could be used to measure population connectivity at spatial scales relevant to management techniques such as Marine Protected Areas (MPAs) (Palumbi 2003). Furthermore, the investigation of fine-scale variation is now a prerequisite if comparisons made across larger spatial scales are to be considered valid (Doherty et al. 1995).

Dispersal capabilities have been shown to affect genetic differentiation in many organisms (Bohonak 1999). Despite this, it is difficult to make generalizations about the dispersal potential of marine organisms and observed levels of genetic structure in populations (Avise 1998). For example, neither egg type (demersal or pelagic) nor pelagic larval duration are accurate, simple predictors of genetic structure in fish populations, with relationships varying among studies and geographic regions (Waples 1987, Doherty et al. 1995, Shulman & Bermingham 1995, Taylor & Hellberg 2003, Bay et al. 2006). Dispersal potential may not translate directly to gene flow or genetic structure for several reasons (Bohonak 1999). Realized dispersal may be significantly lower than potential dispersal due to physical, chemical, or biological processes which act to limit dispersal continuity (Hedgecock 1986, Bohonak 1999, Taylor & Hellberg 2003, Hoffman et al. 2005, Rocha et al. 2005, Bay et al. 2006). In addition, levels of genetic differentiation result from interactions between gene flow, genetic drift,

selection and mutation rather than gene flow alone (Slatkin 1987). It is, therefore, difficult to predict the utility of genetic markers for providing information on population connectivity for a particular species or geographical region (Bohonak 1999).

Research on the influence of dispersal on levels of genetic differentiation in reef fishes has generally focused on the pre-settlement phase of their life histories (e.g. Waples 1987, Shulman & Bermingham 1995, Bay et al. 2006). The influence of post-settlement dispersal has received less attention, perhaps due to a focus on coral reef fishes, which often exhibit limited post-settlement movement among reefs (Sale 2004), and the assumption that highly mobile species will be genetically homogeneous over large spatial scales. Although site attachment is also common in temperate reef fishes, others may travel tens to hundreds of kilometres alongshore, or between estuarine and reef habitats (Gillanders 1997, Griffiths & Wilke 2002, Edgar et al. 2004a). In addition, genetic differentiation has been detected for migratory marine fishes and invertebrates (Shaw et al. 1999, Gold & Turner 2002, Bernal-Ramirez et al. 2003, Knutsen et al. 2003), suggesting that while species may be capable of large-scale movements, these may be rare or impeded by oceanographic features, or may not be the primary factor influencing genetic structure (Shaw et al. 1999, Bernal-Ramirez et al. 2003, Knutsen et al. 2003). Furthermore, movements and spatial patterns of spawning in marine organisms could influence the dynamics and mechanisms of pre-settlement dispersal (Sponaugle et al. 2002) and population genetic structure. Investigations on the role of dispersal should, therefore, include species with a range of pre and post-settlement dispersal capabilities across a range of spatial scales within the same geographical provinces and using the same protocols (Waples 1987, Bohonak 1999).

The broad objective of this thesis was to examine the influence of post-settlement movement on the degree of population connectivity among local populations of temperate reef fishes across a hierarchy of spatial scales (kilometres, tens of kilometres, and > 60 km). It was predicted that population connectivity would be greater for fishes that were capable of large-scale post-settlement movements than for relatively sedentary species. Hypotheses were tested using two species that were representative of the lowest and highest dispersal capabilities of reef fishes in central New South Wales (NSW), Australia (Table 1.1). Population genetic structure of the low dispersing damselfish *Parma microlepis*, which exhibits limited post-settlement movement (< 100 m^2), was

investigated in Chapter 4 using microsatellite markers. This chapter uses microsatellites to examine the population genetic structure of the highly dispersive fish *Girella tricuspidata* within the same geographic region.

Girella tricuspidata (common names: Luderick, Blackfish, Parore) is a primarily herbivorous fish which is common in shallow estuarine and coastal waters along the eastern and southern seaboard of Australia, and in north-eastern New Zealand (Russell 1977, Kailola et al. 1993). *G. tricuspidata* are harvested as part of the estuarine and ocean haul fisheries in NSW, and are an important recreational species (Kingsford et al. 1991, Gray et al. 2000). They are considered to be highly mobile, generally recruiting to estuarine habitats, and migrating to, from, and along the open coast as adults (Morrison 1990, McNeill et al. 1992, Kingsford 2002). *G. tricuspidata* undertake pre-spawning migrations along the coast of NSW, traversing open coastal beaches. Tagged estuarine fish have been recorded as travelling distances of > 150 km between estuaries (Thomson 1959, Morrison 1990, West 1993, Gray et al. 2000). They are broadcast spawners with pre-settlement fish spending approximately four weeks in the pelagic environment (based on related species, Sparidae, T. Trnski Pers. Comm.), before settling primarily into estuarine habitat. Given the high potential for dispersal throughout its life history, it was hypothesized, that *G. tricuspidata* would exhibit genetic homogeneity at scales ≤ 300 km in central NSW. The study focused on coastal rather than estuarine populations of *G. tricuspidata*, consistent with the major objectives of the thesis.

5.2. Methods

5.2.1. Sampling design and genetic analyses

Population genetics of *G. tricuspidata* was examined within a 300 km stretch of coastline in central NSW, Australia. This corresponded to the area sampled for *P. microlepis* (Chapter 4). Samples of *G. tricuspidata* were collected from each of three regions separated by 70-100 km (Port Stephens, Sydney and Jervis Bay) during 2002-05 (Fig. 5.1). Within each region, I sampled fish at two locations separated by 50-60 km in which *P. microlepis* samples had been collected. Adequate sample sizes could not be obtained from a second location in Port Stephens; therefore, only one location was used for analyses in this region. Fish were collected at each location via spearing or were

obtained from recreational line fishers. Sample size varied from 18 – 32 among locations (Table 5.1). Samples were kept on ice after collection and frozen until processed. Fork lengths (FL) of fish were measured and fin clips were preserved in 70% ethanol.

DNA was extracted from fins using a proteinase K/salting out method (Sunnucks & Hales 1996). Individual fish were genotyped at seven microsatellite loci: GT2M11, GT1N8, GT2J21, GT2C1, GT1E22, GT2A10, GT1A9 using methods described in Curley and Gillings (2006) (Appendix D). The construction and screening of the microsatellite library is also described in this publication. Previous analyses of sixty four *G. tricuspidata* samples found no significant linkage disequilibrium between these loci, and therefore they were considered statistically independent (Curley & Gillings 2006). DNA from the individuals that were used to generate the original microsatellite library was included as a positive control in every reaction set.

5.2.2. Statistical analyses

Number of alleles, observed (H_O) and expected heterozygosity (H_E) were calculated for each locus for individual locations and across all samples. Departure from Hardy Weinberg Equilibrium (HWE) was used to test for potential biological processes such as inbreeding or population substructure, and methodological errors (e.g. genotyping errors). HWE was examined for each locus-location combination and across the entire data set using exact tests based on a Markov chain approach and 9999 dememorization steps in ARLEQUIN 3.0 (Guo & Thompson 1992, Excoffier et al. 2005). The software MICRO-CHECKER (Van Oosterhout et al. 2004) was used to infer potential technical causes of departure from HWE.

Frequency-based tests were used to test the null hypotheses that allelic and genotypic distributions were identical across sampling locations for each locus. Tests on allelic distributions were based on an unbiased estimate of the *P*-value of the probability test (Fisher exact test; Raymond & Rousset 1995), and tests on genotypic distributions on a log-likelihood (G) based exact test (Goudet et al. 1996). Analyses were performed using 5000 markov chain iterations, 500 batches (5000 iterations per batch) in GENEPOP version 3.4 (Raymond & Rousset 1995). Paired comparisons among all locations were done when results of these tests were found to be significant. Results were adjusted for

multiple tests, where appropriate, using sequential Bonferroni tests by the Dunn-Ŝidák method, $\alpha' = 1-(1-\alpha)^{1/\text{number of tests}}$, with $\alpha = 0.05$ (Sokal & Rohlf 1995).

A distance-based approach was used to examine genetic variation across different spatial scales and to test for isolation-by-distance (IBD). All tests were based on pairwise, individual-by-individual genetic distances matrices and were performed using GenAlEx 6.0 (Peakall & Smouse 2005). Alternative evolutionary models have been used to interpret genetic variation at microsatellite loci. These include the infinite allele model (IAM) in which each allele may mutate to any other allele (Kimura & Crow 1964), and the stepwise mutation model (SMM) where mutations generally consist of a length change of one repeat unit (Kimura & Ohta 1978). Although microsatellites generally conform to the SMM, the mutation process is complex and poorly understood such that neither model is entirely adequate (Ellegren 2000, Whittaker et al. 2003). Given this, two types of distance matrices were used in analyses to account for different mutation models. Genotypic distances assuming the IAM, and allele-size-based distances assuming the SMM were calculated for each locus and then summed across loci, under the assumption of independence as described in (Peakall et al. 1995, Smouse & Peakall 1999). There were missing data for some individuals at some loci due to failed amplifications or PCR products that could not be reliably scored (see Table 5.1). In these cases, missing individual-by-individual pair-wise distances were replaced with the average genetic distance for each region, location or site level pair-wise contrast.

Analysis of molecular variance (AMOVA) was used to partition total genetic variation (genotypic or size-based) (following methods of Excoffier et al. 1992, Peakall et al. 1995). Variation was summarized as the proportion of the total variance and as analogues of *F*-statistics; Φ-statistics, based on genotypic distance matrices, and *R*-statistics based on size-based distance matrices. These statistics estimate the relative genetic variance among, compared to within populations, or in this case sampling regions and locations. Values may lie between zero (indicating no subdivision) and one (indicating complete genetic subdivision). Estimated values can sometimes be negative, particularly in small samples, if the true value is close to zero. Statistical significance was tested using non-parametric permutation procedures to calculate null distributions using 999 permutations. Two designs were analysed, with, and without regional partitioning. The first design compared 5 locations. The second compared 3 regions;

locations were pooled for this analysis to increase statistical power, as no significant differences were detected between locations with $P \geq 0.25$ in the first design. The first design was also performed on each locus separately to determine if the detection of genetic differentiation was independent of locus polymorphism. The relationship between genetic differentiation estimated using Φ_{PT} or R_{ST} values, the number of alleles, and the mean expected heterozygosity per location was tested using linear regression. Intra-individual variation was suppressed in all analyses. Pairwise comparisons were made when significant differences were detected by AMOVA.

Isolation-by-distance (IBD) was tested using Mantel tests and spatial autocorrelation. Mantel tests were used to test for statistical relationship between pairwise Φ/*R*-statistics among locations and geographic distance. The hypothesis that genotypes of individuals separated by short geographical distances were more similar than those further apart was tested using spatial autocorrelation (Smouse & Peakall 1999, Peakall et al. 2003). Pairwise genotypic and geographical distance matrices were compared and the spatial autocorrelation coefficient (*r*) was calculated across multiple distance classes within each region and across all regions. This coefficient is closely related to Moran's *I* and provides a measure of genetic similarity between pairs of individuals which are found within the specified distance class. The *r* statistic has a mean of '0' when there is a random non-linear relationship between genotypes and distance, and is bounded by [-1, +1] (Smouse & Peakall 1999). A significantly positive *r* is, therefore, expected over geographic scales at which gene flow is limited. Results were considered statistically significant when *r* exceeded the 95% confidence interval about the null hypothesis of zero (calculated using 999 permutations), and when the 95% error about r (calculated using 1000 bootstrap replicates) did not intercept the x-axis at $r = 0$ (Peakall et al. 2003). Principal coordinates analysis (PCA) was used to visualise patterns of genetic relatedness between individual fish among locations and regions based on genotype.

5.3. Results

A total of 137 fish were collected ranging in size from 210-430 mm FL. All fish were genotyped at seven microsatellite loci. The genotypes of individuals used as positive controls were identical on all repeat analyses indicating that genotyping methods were accurate. Significant departure from HWE was detected for Locus GT1N8 within all sampling locations. MICRO-CHECKER indicated that null alleles may be present at

this locus, due to the general excess of homozygotes for most allele size classes. In addition, stuttering may have resulted in scoring errors at one location as indicated by the highly significant shortage of heterozygote genotypes with alleles of one repeat unit difference. Although it is possible to adjust genotypes using MICRO-CHECKER this process would not allow multi-locus genotypic analysis. Locus GT1N8 was therefore removed from subsequent analyses.

Summary statistics for the remaining six microsatellite loci in *G. tricuspidata* are given in Table 5.1. The number of alleles per microsatellite locus ranged from 7-50 across all samples, and from 4-37 within sampling locations. Expected heterozygosities (H_E) ranged from 0.65-0.97 across all samples and from 0.46 to 0.98 within sampling locations. No significant deviations from HWE were detected at any locus within locations, or when all samples were pooled ($P < 0.05$). Overall allele frequency distributions were unimodal except for GT1E22 and GT1A9 which were multimodal (Appendix D, Fig. D.1). No significant differences in allelic and genotypic distributions were found across sampling locations for five of the six loci (Table 5.2). Significant differences in genotypic distributions were, however, detected for locus GT2A10, $P < 0.05$. Although three of ten tests were significant in pair-wise comparison of locations of genotypic distributions for GT2A10, these differences were not geographically logical. For example differences in genotype frequencies were detected between location J2 (the southern-most sampling location) and S2 (located in the middle of the sampling region) rather than locations separated by the largest geographic distances. Furthermore, all tests were non-significant after corrections for multiple tests (Table 5.3).

No significant genotypic or size-based differentiation was detected by multi-locus AMOVA among locations or regions separated by 50-100 km (Table 5.4). The fraction of the total genetic variation that distinguished regions or locations was not significantly different from zero in all analyses ($\Phi_{PT} \leq 0.002$ and $R_{ST} \leq -0.008$), with 100% of the total genetic variance being attributed to variation among individuals within locations or regions. Analyses using individual loci were also non-significant except for GT2J21 where significant difference between locations was detected ($R_{ST} = 0.050$, $P < 0.05$). These differences, accounted for 5% of the total genetic variation. Although pair-wise comparisons among locations revealed significant differences between locations S2 and

S1, and J2 versus P1, S1, and J1 with R_{ST} values ranging between 0.072 and 0.214, only one test J2 versus S2 remained significant after applying the Bonferroni correction for multiple tests. Furthermore differences among locations were not consistent with differences in genotype frequencies detected for locus GT2A10. No significant relationship was found between locus polymorphism and the level of genetic differentiation detected in analyses ($P > 0.1$).

There was no evidence for IBD when analyzing size-based or genotypic-based genetic distances. No relationship was found between pairwise Φ_{PT} or R_{ST} and geographic distance along the 300 km stretch of coastline using mantel tests (Fig. 5.2). Results for spatial autocorrelation were depicted by genetic correlograms which show genetic correlation as a function of distance between genotypes (Fig. 5.3). Spatial autocorrelation across the entire coastline sampled revealed a random non-linear relationship between genotypes and distance ($r \leq 0.002$) (Figure 5.3). This was well illustrated in the PCA in which genotypes of individuals separated by small geographical distances (e.g. ≤ 50 km) were not necessarily more similar than those separated by distances of up to 300 km (Figure 5.4).

Table 5.1. Summary statistics for six microsatellite loci in *G. tricuspidata* collected at five locations in central NSW, Australia. Shown are sample sizes (*n*), the number of alleles (*A*), allele size range (bp) and the observed and expected heterozygosities (H_O and H_E) for individual locations and all samples combined. Locations and all samples were in Hardy-Weinberg equilibrium at $P > 0.05$ for all loci

Locus	Locations	P1	S1	S2	J1	J2	*All*
GT2M11	*n*	30	32	31	25	18	136
$(GT)_{14}$	*A*	6	7	7	6	4	7
	bp	156-166	148-166	148-166	156-166	158-164	148-166
	H_O	0.70	0.69	0.61	0.68	0.44	0.64
	H_E	0.71	0.67	0.65	0.67	0.46	0.65
GT2J21	*n*	30	32	32	25	18	137
$(CA)_{17}$	*A*	12	15	8	11	12	18
	bp	261-291	261-301	259-277	261-285	261-301	259-301
	H_O	0.87	0.88	0.81	0.92	0.89	0.87
	H_E	0.85	0.88	0.80	0.87	0.88	0.85
GT2C1	*n*	30	32	32	25	18	137
$(AC)_{25}$	*A*	18	13	16	17	12	24
	A_E	9.52	7.45	11.01	10.59	8.20	10.56
	bp	147-195	153-189	153-185	149-189	161-191	147-195
	H_O	0.90	0.84	0.91	0.88	0.89	0.88
	H_E	0.91	0.88	0.92	0.92	0.90	0.91
GT1E22	*n*	30	32	32	25	18	137
$(GT)_{34}$	*A*	17	18	20	16	15	24
	bp	152-194	154-204	148-196	152-194	154-192	148-204
	H_O	1.00	0.84	0.94	0.96	1.00	0.94
	H_E	0.92	0.91	0.94	0.93	0.92	0.92
GT2A10	*n*	30	32	32	25	18	137
$(TG)_{32}$	*A*	26	32	29	28	20	43
	bp	213-279	205-291	205-291	217-295	219-293	205-295
	H_O	1.00	1.00	1.00	1.00	0.94	0.99
	H_E	0.96	0.97	0.96	0.97	0.96	0.97
GT1A9	*n*	30	32	32	21	18	133
$(GT)_{27}$	*A*	33	33	37	25	24	50
	bp	294-418	296-376	296-394	300-382	298-390	294-418
	H_O	1.00	0.97	0.91	1.00	0.94	0.96
	H_E	0.98	0.97	0.98	0.97	0.98	0.97

Table 5.2. Allelic and genotypic differentiation at six microsatellite loci in *G. tricuspidata* collected from five locations. *P* values ± S.E. are shown.

Locus	Differentiation	
	Allelic	Genotypic
GT2M11	0.302 ± 0.003	0.284 ± 0.003
GT2J21	0.343 ± 0.005	0.243 ± 0.005
GT2C1	0.211 ± 0.005	0.177 ± 0.005
GT1E22	0.520 ± 0.005	0.452 ± 0.007
GT2A10	0.052 ± 0.003	0.039 ± 0.003
GT1A9	0.985 ± 0.001	0.986 ± 0.001
Average over all loci	0.248 chi2: 14.882 df: 12	0.153 chi2: 16.900 df: 12

Table 5.3. Pairwise comparisons of genotypic differentiation for locus GT2A10 in *G. tricuspidata* collected from five locations. All tests were non-significant after sequential Bonferroni correction. 'No information' indicates tables for which all rows or all columns marginal sums are 1 (i.e. no common genotypes).

Locations	***P*-values**	**S.E.**
P1 vs. S1	0.03751	0.00063
P1 vs. S2	0.12754	0.00133
P1 vs. J1	0.18959	0.00152
P1 vs. J2	0.68756	0.00182
S1 vs. S2	0.66728	0.00204
S1 vs. J1	0.19297	0.00154
S1 vs. J2	No information	
S2 vs. J1	0.02115	0.00044
S2 vs. J2	0.04471	0.00067
J1 vs. J2	0.19083	0.00136

Table 5.4. AMOVA showing partitioning of genotypic (Φ-statistics) and size-based (R-statistics) variation across different spatial scales: (a) among locations (separated by 50-60 km); (b) among regions (separated by 70-100 km, locations pooled). Φ_{PT}/R_{ST} = the fraction of all the variation that distinguishes locations or regions. ns $P \geq 0.05$. Sample sizes are given in Table 5.1.

Source of variation	df	Sums of squares	% Total variation	Φ-statistics
(a) No regional partitioning				
Among Locations	4	21.982	0%	Φ_{PT} = 0.002 ns
Within Locations (residual)	132	694.005	100%	
Total	136	715.987		
(b) Data pooled by region				
Among Regions	2	10.864	0%	Φ_{PT} = 0.001 ns
Within Regions (residual)	134	705.209	100%	
Total	136	716.073		

Source of variation	df	Sums of squares	% Total variation	R-statistics
(a) No regional partitioning				
Among Locations	4	2400.659	0%	R_{ST} = -0.008 ns
Within Locations (residual)	269	294776.434	100%	
Total	273	297177.093		
(b) Data pooled by region				
Among Regions	2	513.882	0%	R_{ST} = -0.009 ns
Within Regions (residual)	271	297022.872	100%	
Total	273	297536.754		

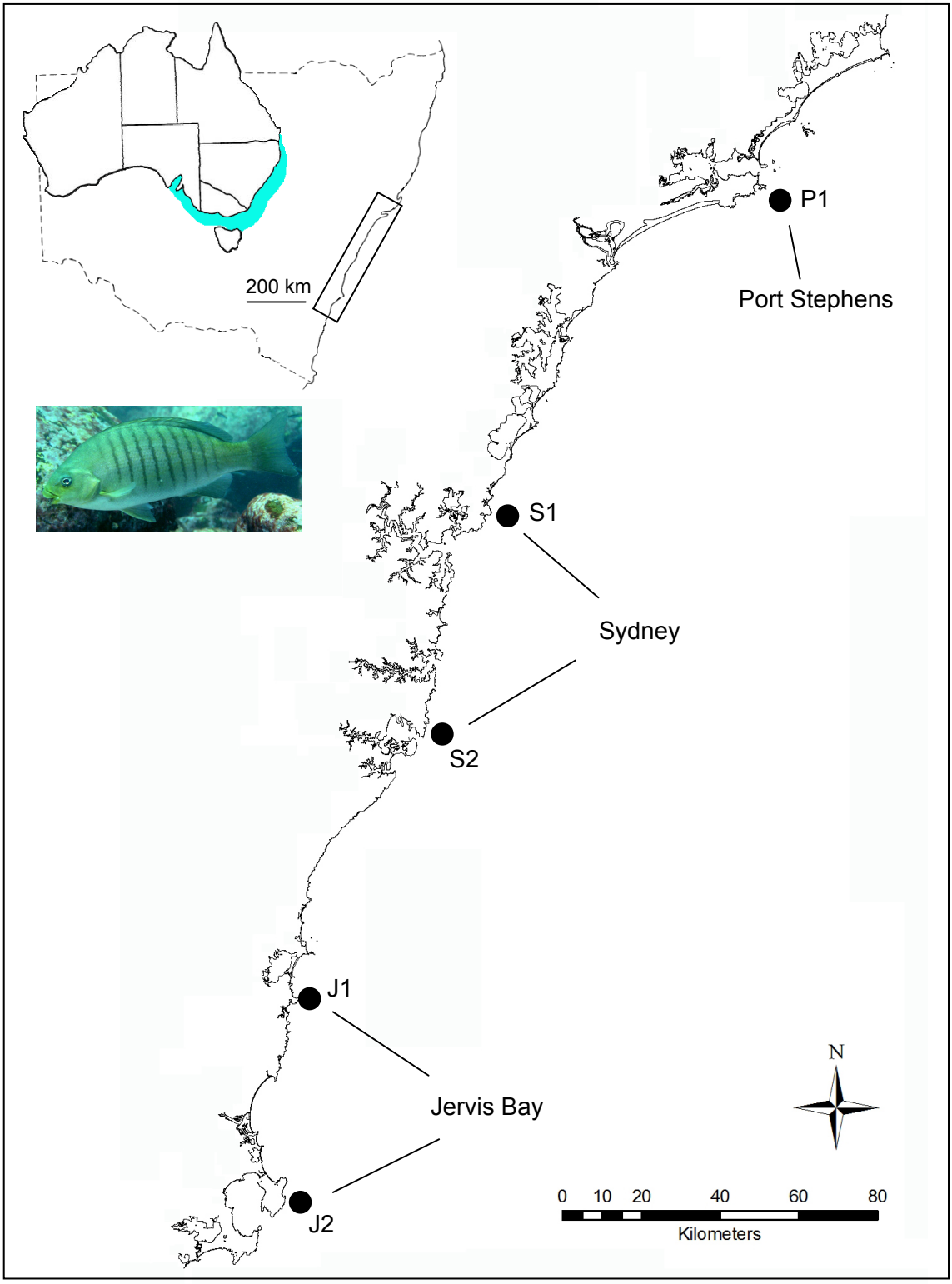

Fig. 5.1. Geographic distribution of *G. tricuspidata* in Australia, and three sampling regions in central NSW (Port Stephens, Sydney and Jervis Bay). Sampling locations within each region are shown.

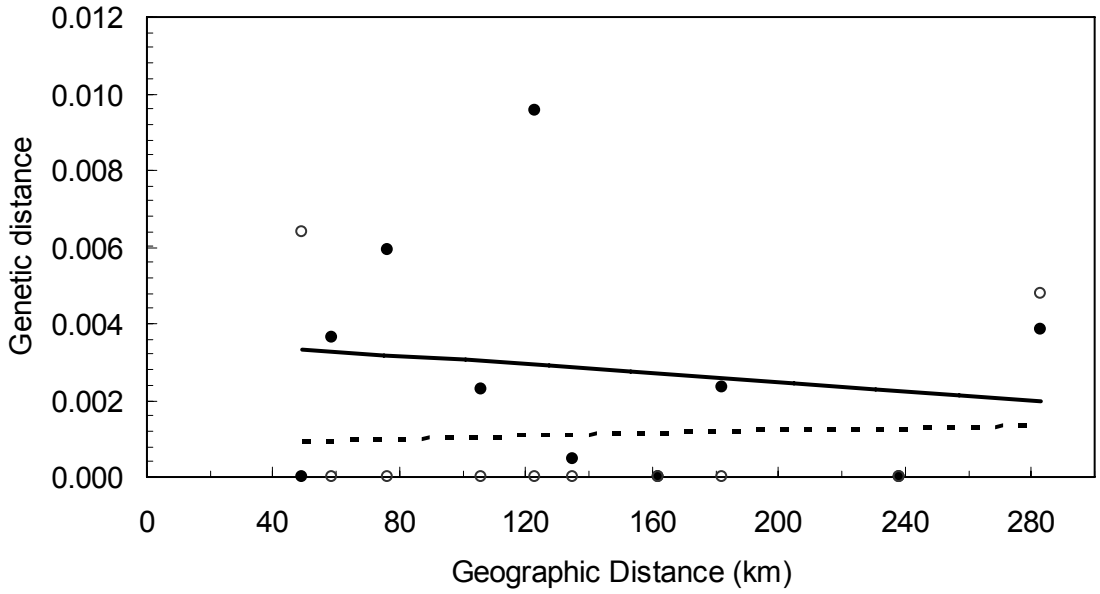

Fig. 5.2. The relationship between geographic distance (km) and genetic distance (pairwise Φ_{ST} or R_{ST}) for *G. tricuspidata* collected from five locations. Solid circles/line is Φ_{ST} (y = - 0.000006x + 0.0036, R^2 = 0.0206, P = 0.426). Open circles/dotted line = R_{ST} (y = 0.000002x + 0.0009, R^2 = 0.003, P = 0.376).

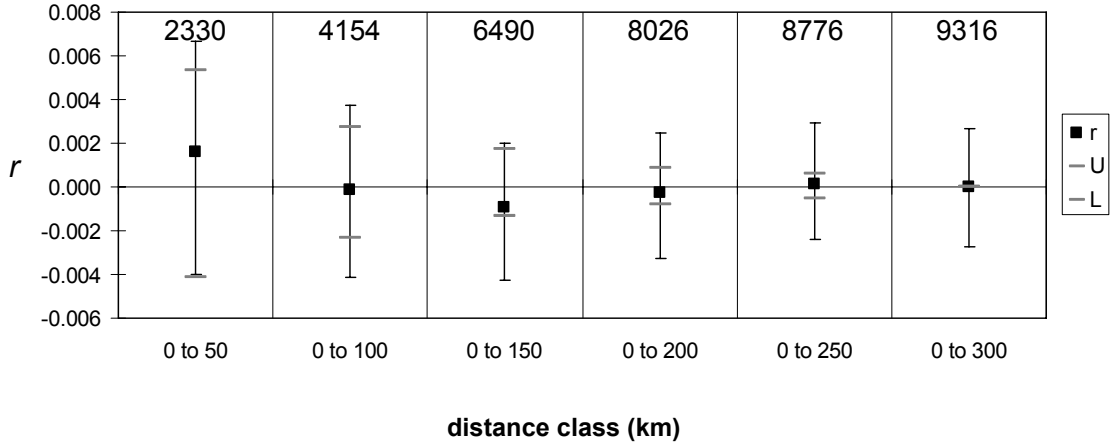

Fig. 5.3. Spatial autocorrelation analyses of genotypic distances in *G. tricuspidata* across a range of geographical distance classes. Shown are the 95% confidence error bars, upper (U) and lower (L) 95% confidence intervals (CI) around the null hypothesis of r = 0. Number of comparisons made in each class are shown above each r value. Genotypic distance based on six microsatellite loci, n = 137.

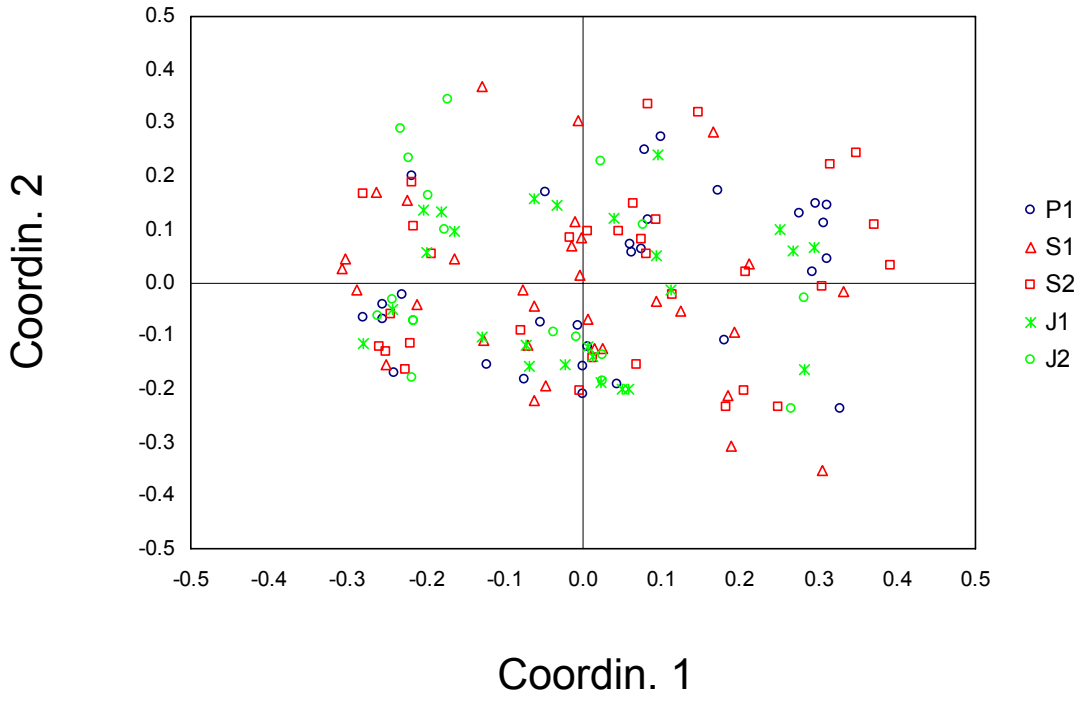

Fig. 5.4. Principal coordinates analyses of multi-locus genotypes of G. *tricuspidata* collected from five locations within three regions in central NSW. Port Stephens (blue) n = 30, Sydney (red) n = 64, Jervis Bay (green) n = 43. Principal coordinates 1 and 2 accounted for 28.0 and 19.2% of the total variation. Six microsatellite loci used in analyses, n = 137. Fish collected from the same location within a region have the same symbol.

5.4. Discussion

Patterns of microsatellite differentiation supported predictions that coastal populations of *G. tricuspidata* would exhibit genetic homogeneity at scales ≤ 300 km. This conclusion was consistent, regardless of the evolutionary model (IAM or SMM) used to interpret genetic variation. The proportion of the total genetic variation attributable to differences among locations (separated by 50-60 km) or sampling regions (separated by 70-100 km) was effectively zero (e.g. $\Phi_{PT} \leq 0.002$ and $R_{ST} = 0.000$), with 100% of the genetic variation occurring within locations or regions. Broad-scale homogeneity was further supported by a lack of IBD. Although subtle genetic structure was detected among locations using two loci, GT2A10 and GT2J21, these differences were relatively weak (e.g. GT2J21 accounted for 5% of the total genetic variation) and could not be attributed consistently to specific locations. Given that these loci are not homologous to any known genes (and therefore potentially subject to selection), and the high sampling variance associated with high polymorphism, it is likely that results are due to stochastic processes rather than biologically important differentiation

The power to detect genetic structure is influenced by several factors including: the magnitude of genetic differentiation, intrinsic variability of the population, sample size, spatial replication, and the number and characteristics of the microsatellite loci used (e.g. polymorphism, homoplasy, mutation rates; Underwood 1997, Ruzzante 1998, O'Reilly et al. 2004, Olsen et al. 2004, Ryman et al. 2006). Genotypic diversity at fine spatial scales was characterised by high variance, low precision and hence low statistical power. Although there was no indication that the level of genetic variation detected was associated with locus polymorphism and associated sampling variance, five of six loci used in analyses were highly polymorphic (H_E 0.85-0.97, number of alleles 18-50). It is possible that the use of less polymorphic loci and larger sample sizes would improve accuracy of estimates and therefore the ability to detect structure (O'Reilly et al. 2004). However, the geographic distribution of genetic homogeneity and the high polymorphism found for *G. tricuspidata* is common in other marine fishes, and is indicative of high mutation rates, large effective population sizes, and high rates of gene flow (Gyllensten 1985, DeWoody & Avise 2000, O'Reilly et al. 2004). Given this, if detectable structure exists, it is likely to be weak, making ecological implications difficult to interpret (Palumbi 2003, Waples & Gaggiotti 2006).

Gene flow via pre- and post-settlement dispersal

The observed lack of population structure at scales ≤ 300 km in coastal *G. tricuspidata* is most likely driven by high rates of gene flow. Findings suggest a strong link between the high dispersal potential during pre- and post-settlement and realized dispersal; with little evidence that physical, chemical or biological processes (e.g. oceanographic features, behaviour) significantly influenced dispersal continuity important for gene flow. For example, it was possible that habitat discontinuities (e.g. beaches, deep-water channels at mouths of rivers and estuaries) would impede dispersal and restrict gene flow (Riginos & Nachman 2001, Bernal-Ramirez et al. 2003, Carreras-Carbonell et al. 2006). Potential barriers in central NSW are, however, small (< 30 km) relative to the dispersal capabilities of *G. tricuspidata*, with fishes known to transverse such barriers (e.g. beaches) during spawning migrations (Gray et al. 2000). In fact the distribution of estuaries in central NSW is likely to promote mixing of *G. tricuspidata* recruits from different estuaries when they move to the open coast, as there are 35 major estuaries within the sampling area (NSW Department of Natural Resources) which are generally only separated by tens of kilometres of coastline.

It has been proposed that *G. tricuspidata* exhibits intraspecific variation in movement, with some fish displaying high site fidelity and others being migratory (Morrison 1990). This model is supported by tagging studies of estuarine and coastal populations of *G. tricuspidata* in which some tagged individuals were re-captured several months after tagging within their release estuary or coastal reef, while others travelled distances up to 150 km between estuaries (Thomson 1959, West 1993, Gray et al. 2000; Curley unpublished data). In addition, although local densities of *G. tricuspidata* fluctuate dramatically over time, this species has been shown to respond to protection within a small coastal Marine Protected Area (MPA) ≤ 0.2 km^2 indicating that some individuals may be relatively sedentary (Chapter 2). Although population genetic structure has been detected in other species which exhibit intraspecific variation in movements (e.g. Snapper, Pagrus auratus; Bernal-Ramirez et al. 2003), there was no evidence that this characteristic is an important determinant of population genetic structure for *G. tricuspidata* in this study.

Large-scale post-settlement movement has the potential to increase gene flow in fishes such as *G. tricuspidata*. However, existing genetic studies suggest it is not the primary

mechanism for maintaining genetic homogeneity in central NSW. Lack of population genetic structure among coastal *G. tricuspidata* is concordant with genetic homogeneity found for the territorial damselfish *P. microlepis* (Φ_{PT} = 0.003 and R_{ST} = 0.004) across the same sampling geographic region using the same protocols (Chapter 4), and for several near-shore coastal invertebrates in central NSW which exhibit limited post-settlement movement and a pelagic larval phase. For example some species of starfish, bivalves, gastropods, and anemones were found to be genetically homogeneous at scales of 180-1200 km using non-neutral loci (Hunt & Ayre 1989, Hunt 1993, Hoskin 1997, Murray-Jones & Ayre 1997). This suggests that genetic homogeneity in coastal populations of *G. tricuspidata* and other nearshore marine organisms is primarily due to biological and environmental factors operating during pre-settlement dispersal which oppose the process of genetic differentiation.

The East Australian Current (EAC) is likely to have an important influence on pre-settlement dispersal in *G. tricuspidata* and similar organisms. This current is a major oceanographic feature of NSW, carrying tropical water, including pre-settlement tropical fish, south down the east Australian coast (Booth et al. 2007). Associated currents and eddies of the EAC can reach speeds of 1.5 m s^{-1} (Middleton et al. 1996, Ridgway & Godfrey 1997, Kingsford 1999) potentially transporting pre-settlement fishes, including *G. tricuspidata*, thousands of kilometres southward during their 2-4 week pre-settlement phase. The EAC has a strong seasonal cycle below 32^0S (Port Stephens) generally present as southward moving filaments and eddies of warm water (Middleton et al. 1996, Ridgway & Godfrey 1997). Although it has been proposed that the heterogeneous nature of the EAC and local-scale differences in hydrographic processes, particularly in the Sydney region, may impede along-shore transport of pre-settlement marine organisms (Smith et al. 1999, Booth et al. 2007) there is little evidence that these processes influence gene flow in fishes (e.g. *G. tricuspidata*, *P. microlepis*) or near-shore invertebrates in this region (Hunt & Ayre 1989, Hunt 1993, Murray-Jones & Ayre 1997, Ward & Elliot 2001; Chapter 4; but see Hoskin 2000). This could be partly due to strong northward current pulses caused by coastal trapped waves (Middleton et al. 1996) which would compensate for the southward displacement of larvae by the EAC. Northward spawning migrations of *G. tricuspidata* and other fishes in NSW (e.g. bream) could also operate to compensate for the EAC in a similar fashion. Thus, despite potentially high levels of diffusion and/or larval mortality, large-scale

oceanographic processes, in conjunction with post-settlement movement of *G. tricuspidata* appear to assist long-distance pre-settlement dispersal at rates that prevent genetic differentiation at scales of ≤ 300 km in central NSW.

Effective population size and genetic drift

Large effective population sizes may also contribute to genetic homogeneity in coastal populations of *G. tricuspidata*. The rate at which isolated populations lose genetic variability due to genetic drift, and become genetically differentiated, is inversely related to the total number of individuals that contribute genetically to the next generation (the effective population size N_e) (Hellberg et al. 2002). The high genetic polymorphism in *G. tricuspidata* and relatively large census sizes in NSW indicate that N_e is relatively large. The average annual commercial ocean and estuarine landings, and recreational catch for *G. tricuspidata* in NSW are 80 000, 400 000, and 280 000 kg respectively (Gray et al. 2000, Henry & Lyle 2003). These catches equate to approximately 1.5 million fish (based on 0.5 kg per fish). Actual population sizes should be many orders of magnitude higher as *G. tricuspidata* stocks are not considered to be heavily exploited (West 1993, Gray et al. 2000).

N_e may be much lower than the census population due to variation in spawning success and pelagic survival, particularly in broadcast spawners like *G. tricuspidata* (Hellberg et al. 2002, Turner et al. 2002). It has been suggested that even when populations consist of millions of individuals, only hundreds to thousands may successfully contribute to the next generation (Hauser et al. 2002). *G. tricuspidata*, however, displays some biological and ecological traits which are predicted to increase N_e (see Turner et al. 2002 and references therein). *G. tricuspidata* become reproductively active at ≥ 2 years of age and may live for more than 24 years (West 1993, Gray et al. 2000). Thus individuals potentially contribute to the reproductive output of local populations for more than 20 years. This long reproductive life span together with overlapping generations and an extended spawning period in south-eastern Australia (Smith & Sinerchia 2004), is likely to limit the variance in life time reproductive success for *G. tricuspidata* (Turner et al. 2002). In addition sex ratios of the fishery are only slightly biased towards females (Gray et al. 2000) indicating that variance in male and/or female reproductive success is likely to be low (Turner et al. 2002).

Overall population genetic structure in G. tricuspidata

The current study focused on coastal populations of *G. tricuspidata*, however, this species forms spawning aggregations, recruits to estuarine habitats and may remain in estuaries as adults. It is unclear if genetic homogeneity applies to all life history stages or only applies to coastal populations during non-spawning periods. It is possible that fish aggregate and return to, or near natal estuaries to spawn (e.g. Sciaenidae, Thorrold et al. 2001), with spawning aggregations and resulting offspring consisting of individuals that are genetically more similar. Settling fish larvae can use olfactory discrimination of reefs to aid retention in natal areas (Gerlach et al. 2007). Estuarine and riverine plumes which extend for kilometres from shore-lines in NSW, could act as navigational cues for pre-settlement estuarine dependent species such as *G. tricuspidata* (Kingsford et al. 2002). This is a likely scenario for *G. tricuspidata* as settlement-stage fish are strong swimmers, demonstrate complex behaviour such as directed swimming orientation (Trnski 2002), and accumulate in frontal regions of estuarine and sewage plumes (Gray 1996, Kingsford & Suthers 1996).

Estuarine-dependence may also enhance genetic subdivision of marine fishes (Watts & Johnson 2004). For example, gene flow in *Sciaenops ocellatus* (Red Drum) was inversely related to geographic distance from a natal bay or estuary (Gold & Turner 2002). Often there is more genetic subdivision among estuarine populations of fishes than among marine populations of the same species, and differentiation between estuarine and coastal populations have been detected even for highly dispersive species (e.g. *P. auratus*) (Johnson et al. 1986, Bastow et al. 2002, Watts & Johnson 2004). The challenge for future studies will be to test these potential scenarios through sampling of spawning aggregations, and juveniles and adults within and among estuaries relative to coastal populations.

Population connectivity

In theory only a small amount of gene flow (≥ 5 effective migrants per generation, (Shulman 1998) would be required for ecologically distinct populations of *G. tricuspidata* to be genetically homogeneous (Mora & Sale 2002). Estimates of gene flow are difficult due to the unrealistic assumptions of models (e.g. symmetrical gene flow between populations) and large errors associated with estimates of very small values of F_{ST} (Palumbi 2003, Waples & Gaggiotti 2006). Therefore, genetic similarity

of *G. tricuspidata* at scales ≤ 300 km may suggest populations are largely 'open' with high exchange of individuals (> 10 000) or largely 'closed' with sporadic but demographically inconsequential inter-population migration (Hellberg et al. 2002, Mora & Sale 2002). For example, *P. auratus* recruit to estuarine habitats or bare substrata and then move to the open coast. Studies of connectivity using otolith chemistry between juvenile and adult habitats in NSW have shown that adults on the open coast in the Sydney region have come from local estuaries with 11% from other estuaries in NSW (Gillanders 2002a). Although there are no genetic studies for comparison, it is likely that there is enough mixing to prevent genetic differentiation of this demographically closed population. This scenario may also apply to *G. tricuspidata* within this region as it has similar life history traits which interact with similar environmental processes.

Although the scale of genetic homogeneity may not reflect ecologically relevant dispersal distances, it does imply that populations of *G. tricuspidata* are well connected from an evolutionary perspective and have large effective population sizes, thus reducing the genetic risks associated with exploitation (e.g. reduced adaptability and localised extinction) (Hauser et al. 2002, Hutchings & Reynolds 2004). Detectable population genetic structure for *G. tricuspidata* may be found across broader spatial scales, with alternative markers, or in different geographic regions where the effect of the EAC is minimal, outside migrations routes, or where estuarine habitats are rare or separated by large geographic distances. Repeated geographic sampling and genetic analyses of *G. tricuspidata* recruits may also give insight into spatial and temporal patterns of recruitment, even in areas such as central NSW where adult populations are genetically homogeneous (Hellberg et al. 2002, Thorrold et al. 2002). However, it is likely that alternative methods (e.g. otolith chemistry; Chapter 3) will be required to elucidate the relationship between genetic and demographically important connectivity for coastal populations of *G. tricuspidata*, and similar species, particularly at fine spatial scales in central NSW.

5.4.1. Conclusion

A high level of genetic connectivity was found among coastal populations of the high dispersing fish, *G. tricuspidata*, at scales ≤ 300 km in central NSW. Although large-scale post-settlement movement may increase gene flow in *G. tricuspidata*, existing data suggest that pre-settlement processes are the primary mechanism for maintaining

genetic homogeneity for this and other near-shore species in central NSW. Further comparative studies of fishes, using similar sampling protocols, will be required to test the generality of this model and to investigate the predictive value of other biological characteristics (e.g. pelagic larval duration, effective population sizes, and ecological niches) which could be important for determining population genetic structure in this region.

Chapter 6: Key findings, implications and future research

Currently, we have few data on the magnitude of dispersal and levels of population connectivity in marine organisms. This impedes the application of ecologically relevant management strategies and our ability to predict the consequences of such management. This thesis is the first program of study to focus on dispersal and population connectivity for temperate reef fishes in central NSW, at scales relevant to the implementation of Marine Protected Areas (hundreds of metres to hundreds of kilometres). The study provides: (1) empirical data on the localised benefits of small MPAs relative to the mobility of fishes; (2) baseline data on the utility of different methods (microsatellite markers and otolith chemistry) for determining levels of population connectivity and the potential scales of benefits of MPAs to unprotected areas. The work on microsatellite markers compared population genetic structure in two species which span the post-settlement dispersal potentials of reef fishes in this region (*Parma microlepis* and *Girella tricuspidata*), and provides a benchmark for understanding general mechanisms which govern gene flow and population connectivity in central NSW. Overall results contribute to global literature to further our understanding of responses of reef fishes to MPAs, and general patterns and processes governing dispersal and connectivity in reef fishes. The thesis also provides clear implications for management of reef fishes in central NSW, and testable hypotheses and priorities for future research.

6.1. Marine Protected Areas

6.1.1. Is there a correlation between mobility and response to protection?

This study provided clear evidence that small MPAs ≤ 0.2 km^2 do not encompass the average movements of most commonly exploited fishes in central NSW (Table 1.1) (Chapter 2). With the exception of *C. fuscus* and *A. viridis*, all species exhibited high temporal variation in abundances suggesting that movements were at scales > km's. Despite this, small MPAs were shown to have significant localised benefits even for highly mobile species. MPA effects were detected for two 'mobile' species, *Acanthopagrus australis* (Yellow-fin Bream) and *G. tricuspidata* (Luderick) within the 2.5 year old MPA (Cabbage Tree Bay; CTB), and for one 'sedentary' species, *Cheilodactylus fuscus* (Red Morwong), within the 12.5 year old MPA (Gordon's Bay;

GB). The response of highly mobile fishes to protection was contrary to initial predictions and adds to a growing body of literature which fails to establish simple relationships between estimated mobility, duration of protection and the response of fishes to protection (Micheli et al. 2004, Palumbi 2004).

What can temporal patterns of abundance combined with MPA effects tell us about the mobility of fishes?

Results support existing studies which have found that highly mobile fishes can benefit from relatively small MPAs (Roberts et al. 2001, Apostolaki et al. 2002, Willis et al. 2003). Densities of *A. australis* and *G. tricuspidata* were up to 2.6-times higher within CTB relative to unprotected locations, with similar trends observed within GB (for *A. australis* only). Several models may explain the response of highly mobile species such as *G. tricuspidata* and *A. australis* to MPA protection and require further investigation. Importantly, effects may indicate the exhibition of intraspecific variation in mobility, with some fishes displaying high site fidelity and being effectively protected within MPA boundaries, and others being migratory. The unanticipated response of a related species *Pagrus auratus* (Sparidae) to small MPAs has been attributed to bi-modal patterns of movement (Parsons et al. 2003, Egli & Babcock 2004). Such studies illustrate that mobility of reef fishes can be complex, and emphasises the utility of MPAs, and the approach used in this study, to elucidate mobility. While some support for 'sedentary' behaviour in *G. tricuspidata* can be gleaned from previous studies of spatial and temporal variation in abundances and tagging (Morrison 1990, Gray et al. 2000; Curley unpublished data), dedicated tagging studies will be required to test this hypotheses for *G. tricuspidata* and *A. australis*.

6.1.2. Other important factors for determining responses to protection

Protection of habitats and aggregation sites

Interaction between mobility and other factors critical in determining the recovery of populations (e.g. MPA attributes, previous local fishing pressure) can also prevent simple relationships between mobility and the response of organisms to MPAs (Palumbi 2004). For example, the placement of MPAs relative to the type and amount of habitat within MPA boundaries may dictate the efficacy of MPAs, regardless of mobility (Gell & Roberts 2003, Jones et al. 2004, Barrett et al. 2007). Furthermore, even though the home range of fish may be large, movements may be restricted to a small number of

preferred sites (Eristhee & Oxenford 2001, Popple & Hunte 2005). In such cases, MPAs may provide short-term refugia for highly mobile species at times or locations when they are highly vulnerable to fishing (e.g. feeding or spawning aggregations). This scenario was well supported for *G. tricuspidata* which exhibited large-site specific aggregations and responded to protection within CTB but not in GB. Inappropriate habitat protection is also a likely cause for the lack of response observed for *Girella elevata* within both MPAs. Although this species is thought to move at scales < km's adjacent schools may be separated by hundreds of metres to kilometres and site-associated aggregations occur in wave exposed areas and deep holes adjacent to rock platforms. Such habitats were generally found on the edge of GB and CTB resulting in large temporal variation in abundances in visual counts, and poor response to protection.

Previous studies have shown that small, randomly placed MPAs such as CTB and GB are unlikely to include a representative range of habitats and depths (Curley et al. 2002). If the size of MPAs is limited by geography or social restrictions then careful placement of MPAs relative to known aggregations and habitats becomes highly critical. Obtaining precise data on the magnitude of average movements and habitat use by fishes is, therefore, imperative to maximise the benefits of small MPAs. Depth and habitat-related patterns of abundance are well documented for most commonly exploited fishes in central NSW (Gillanders 1997, Curley et al. 2002, Kingsford 2002; Chapter 2). These data coupled with further studies using real-time tagging techniques (e.g. acoustic telemetry; Eristhee & Oxenford 2001, Popple & Hunte 2005) should be used to provide ecologically relevant placement of future MPAs. The location of fish aggregation sites may also be aided by surveys of the distribution of recreational fishers, who target locations where catch-rates (and presumably fish densities) are high. Protecting such areas will also result in significantly reduced fishing effort and greater MPA effects, as opposed to the protection of unproductive, rarely fished areas (Lynch 2006).

Previous fishing pressure

Strong responses by highly mobile and other species may also be attributed to high levels of previous fishing pressure (Palumbi 2004). This is a likely factor contributing to responses of *G. tricuspidata* and *A. australis* to protection, as both species are heavily targeted by recreational fishers in central NSW (Kingsford et al. 1991). It was difficult, however, to determine the importance of fishing pressure for responses of these species

due to the confounding factors discussed above. In contrast, MPA effects for the 'sedentary' *C. fuscus* provided conclusive evidence that spear fishing significantly influences the abundance, size structure and depth distribution of local populations in central NSW. Legal-sized fishes were 2.8-times more abundant and larger inside GB (protected from spear fishing only), with higher densities of legal fish found in shallow areas of reef when compared to unprotected areas. These results were not surprising given that *C. fuscus* move at scales of 1865 ± 268 m^2 during the day, form aggregations and have a docile behaviour conducive to heavy exploitation, even by novice spear fishers (Lincoln Smith et al. 1989, Lockett & Suthers 1998, Lowry & Suthers 2004). Importantly, these are the first empirical data to support claims that recreational fishing negatively impacts reef fishes in central NSW, and demonstrates the strength of MPAs to act as human-exclusion 'experiments' (Micheli et al. 2004). *C. fuscus* is clearly an ideal candidate for management using MPAs. In contrast to existing studies, the response of *C. fuscus* indicates that partial protection, as opposed to 'no-take' MPAs, can benefit selected species (Denny & Babcock 2004, Denny et al. 2004, Shears et al. 2006).

***Partial protection versus 'no-take'* status**

Although partial protection may benefit select species, the cost-benefit of this approach is poor. Different responses of fishes within CTB and GB were expected given the different levels of protection offered. While CTB is a 'no-take' MPA, GB protects fish (with the exception of *A. viridis*) from spear fishing only. Most of the species examined in this study are highly targeted by recreational line fishers (e.g. *A. australis*, *G. tricuspidata*, *G. elevata*) as well as spear fishers (Kingsford et al. 1991) and did not respond to the level of protection offered within GB. There was no evidence that lack of response in this species was due to increased line fishing pressure inside GB relative to unprotected areas as suggested in previous studies (Denny & Babcock 2004). Rather, results imply that while reduction in fishing pressure via removal of spear fishing may be adequate for some species (*C. fuscus*), it is insufficient to allow recovery of species targeted by additional methods. I would strongly recommend that GB be converted to a complete 'no-take' MPA. This would significantly improve the potential of GB to benefit a wider range of species, and would provide clearer guidelines for MPA use. Data collected in this study could then act as 'before-data' to assess the impact of removing line fishing, allowing for more direct comparisons with CTB.

Duration of protection

The duration of protection for CTB (2.5 yrs) was probably inadequate to cause detectable changes in many species, regardless of mobility. Although increases in the density of target species can be rapid (< 3 yrs; Halpern & Warner 2002), full recovery of a fished stock to a 'natural state' may take considerably longer (10-40 yrs; Russ & Alcala 2004, Barrett et al. 2007). Theoretical recovery rates depend on several factors including; initial population size, intrinsic rates of population increase, life-history characteristics, recruitment variation, reduction in fishing mortality, immigration rates and local habitat quality (Jennings 2001, Denny et al. 2004). For example, duration of protection was predicted to be the major factor accounting for the lack of response observed for *C. fuscus* within CTB when compared with GB (12.5 yr old MPA). Evidence of over fishing for *C. fuscus* (discussed above) suggests that recovery of local populations will be slow, particularly when recovery is reliant on recruitment rather than immigration from adjacent areas (Polunin & Roberts 1993, Denny et al. 2004), as seems to be the case within CTB. Recovery from heavy exploitation will also be slow, particularly for the accumulation of large individuals of long-lived species (Roberts et al. 2001, Russ et al. 2003) characteristic of temperate regions (e.g. Tzioumis & Kingsford 1999, Lowry 2003). These factors necessitate long-term monitoring of MPAs to accurately assess benefits and determine mechanisms of recovery.

The location of MPAs will significantly influence the mechanism and rate at which recovery takes place. Recovery can be rapid when it is achieved via the immigration of fishes from adjacent habitat. A previous study which involved the experimental removal of > 70% of large *C. fuscus* from a reef demonstrated that densities could return to pre-speared levels within 2-4 months when re-colonised by fish from adjacent areas (Lowry & Suthers 2004). Immigration may also be the only mechanism of recovery for some species. For example, rapid recovery of local populations of *A. australis* and *G. tricuspidata* within CTB was attributed to immigration of fishes, as both species recruit to estuarine habitats (Pollock et al. 1983, Hannan & Williams 1998). Immigration-based recovery will be site-dependent and may be facilitated by careful placement of MPAs relative to estuarine environments and/or continuity of adjacent habitats. For example, although MPAs flanked by sandy habitats may prevent the movement of species beyond MPA boundaries, it may also prevent immigration from

contributing to recovery of populations (Curley et al. 2002). Specific locations also appeared more favourable for recruitment in this and previous studies (Curley et al. 2002). Protection of such 'recruitment hot spots' is an important selection criterion in future design of MPAs, as higher levels of local recruitment may lead to faster recovery of populations following protection. Furthermore, even 'sedentary' species may show little MPA effect if recovery is reliant on recruitment and levels of recruitment are low (Palumbi 2004).

6.1.3. Conclusions on the benefits of small MPAs for protecting reef fishes

This study is the first to clearly demonstrate the ecological value of small MPAs in central NSW for protecting reef fishes. This provides a strong scientific and social basis to justify existing and future MPAs in this region. Knowledge of the utility of small MPAs (≤ 1 km^2) is relatively poor (see review by Halpern 2003). However, results support existing evidence that small MPAs can be effective even for highly mobile species. Furthermore, the magnitude of effects are consistent with meta-analyses which indicate that protection generally leads to doubling of densities and a 20-30% increase in the mean size (e.g. *C. fuscus*) of exploited organisms, relative to unprotected areas, irrespective of the size of MPAs (Halpern 2003).

MPAs should ideally be larger so as to encompass the average movements of commonly exploited reef fishes. However, small MPAs may be the only option in some parts of central NSW where reefs are regularly intercepted by beaches (60% of reefs in central NSW are < 2 km in length; Curley et al. 2002), necessitating the use of several small rather than single large MPAs. Importantly, as MPAs become smaller the choice of location relative to habitat, known aggregations of species, and recruitment 'hotspots' becomes highly critical in determining the outcomes of protection and rates of recovery. Overall results support the consensus that response to protection is species-specific, slow, complex, and varies with MPA design and local geography (Russ & Alcala 2004, Barrett et al. 2007). This necessitates the collection of long-term empirical data on individual MPAs rather than reliance on broad-generalities obtained through the study of MPAs in other geographic areas.

Although small MPAs were shown to have significant localised benefits, the spatial scales over which spillover of eggs, larvae and adults may improve sustainability in

unprotected areas remains unknown. For example, does an increase in abundance and size of *C. fuscus* inside GB equate to increased reproductive capacity and export to unprotected areas? Empirical data on 'spillover' from MPAs are limited (Roberts et al. 2001, Russ et al. 2003) due to the considerable design, scale and logistic difficulties of measuring net export (particularly of eggs and larvae) from MPAs (Russ 2002, Palumbi 2004). Determining levels of population connectivity of reef fishes in this region is an essential step towards an understanding of such benefits. Such studies should ideally focus on species which demonstrated 'within MPA' effects in this study (i.e. *C. fuscus*, *G. tricuspidata*, *A. australis* and possibly *G. elevata*).

6.2. Population Genetics

6.2.1. Population genetic structure for fishes with varying dispersal potential at scales ≤ 400 km

This is the first study to evaluate the use of microsatellite markers for elucidating population connectivity for reef fishes in central NSW. It is also one of the few studies to have used a comparative approach to analyse population genetic structure in species with varying post-settlement dispersal capabilities within the same geographic region, using the same protocols. Results showed that the territorial damselfish *P. microlepis* and the highly mobile species *G. tricuspidata* exhibited high polymorphism (*P. microlepis*: H_E 0.86-0.95; *G. tricuspidata*: H_E 0.65-0.97) broad-scale genetic homogeneity and a lack of isolation-by distance at spatial scales ≤ 400 km. For example, the proportion of the total genetic variation attributable to differences among sampling regions (separated by 70-100 km), locations (separated by 10-60 km) or sites (separated by 1-2 km) was effectively zero (e.g. $\Phi_{PT} \leq 0.003$ and $R_{ST} \leq 0.004$). Such patterns have been reported for many reef fishes in which genetic structure is either absent or only detectable at scales of 1000's km (Bernardi et al. 2001, Van Herwerden et al. 2003, Bay et al. 2006, Gilbert-Horvath et al. 2006); and is indicative of high mutation rates, large effective population sizes, and high rates of gene flow (Gyllensten 1985, DeWoody & Avise 2000, O'Reilly et al. 2004).

Although genetic homogeneity may be extrapolated to all local *P. microlepis* populations in central NSW, interpretation of results for *G. tricuspidata* are complex. Sampling of *P. microlepis* in this study was spatially and temporally representative of

populations found in central NSW, as this species resides exclusively within reef habitat and has limited post-settlement movement. In contrast, *G. tricuspidata* forms migratory spawning aggregations, recruits to estuarine habitats and may remain within estuaries as adults. The focus of this study was limited to coastal populations during non-spawning periods. Consequently, the conclusion that populations are genetically homogenous only applies to the sampled fish. It is possible that *G. tricuspidata* aggregate and return to (or near) natal estuaries to spawn (e.g. Thorrold et al. 2001) such that individuals in spawning aggregations and resulting offspring are genetically more similar. If this is the case, genetic subdivision could be found among estuarine populations of juvenile and/or adult *G. tricuspidata*. Genetic structure may also occur between estuarine and coastal populations, even for highly mobile fishes (e.g. Snapper, *Pagrus auratus*) (Johnson et al. 1986, Watts & Johnson 2004). Investigation of these scenarios is imperative, as genetic subdivision during specific life history stages, will have significant consequences for management of this recreationally and commercially exploited species.

Is post-settlement mobility important for determining levels of genetic connectivity in central NSW?

The comparative approach taken in this thesis allowed insight into the general mechanisms which are important for determining gene flow and population genetic structure at scales ≤ 400 km in central NSW. Genetic homogeneity for *P. microlepis* and *G. tricuspidata* is concordant with lack of detectable structure for many near-shore invertebrates in this region, which exhibit limited post-settlement movement and pelagic larval phases (Hunt & Ayre 1989, Hunt 1993, Hoskin 1997, Murray-Jones & Ayre 1997). This suggests that gene flow important to genetic structure is driven by processes operating during the pre-settlement phase. Thus, although large-scale post-settlement movement may increase gene flow in species such as *G. tricuspidata*, it does not appear to be important for the maintenance of gene flow at rates that prevent genetic differentiation among populations. Thus post-settlement capabilities appear to be of little predictive value for predicting genetic structure in this region. Having said this, post-settlement mobility may still be important in cases where movements act to limit pre-settlement dispersal (e.g. choice of spawning location, time and depth; Largier 2003). Factors which influence pre-settlement dispersal may provide some predictive benefits and should be the focus of future studies (e.g. demersal versus pelagic eggs,

pelagic larval duration; Waples 1987, Doherty et al. 1995, Shulman & Bermingham 1995, Taylor & Hellberg 2003, Bay et al. 2006).

6.2.2. Major factors contributing to genetic homogeneity in central NSW

Genetic homogeneity among populations of reef fishes and nearshore invertebrates indicate that there are no major oceanographic barriers to gene flow in central NSW. Long-distance pre-settlement dispersal is probably driven by the East Australian Current (EAC) and north-flowing counter-current. Currents and eddies associated with the EAC may reach speeds of 1.5 m s^{-1} (Middleton et al. 1996, Ridgway & Godfrey 1997, Kingsford 1999) theoretically transporting fishes thousands of kilometres during their 2-4 week pre-settlement phase. Long-distance intermittent transport of pre-settlement fishes by the EAC is well supported by the presence of tropical fish recruits as far south as 37^{o}S (Booth et al. 2007). Subsequently, the small number of migrants required to maintain genetic homogeneity are likely to be supplied by this process, despite high levels of diffusion and/or larval mortality, and the potential for meso-scale oceanographic features to retain ichthyoplankton within some regions of central NSW (Smith et al. 1999, Booth et al. 2007).

The distribution of reef habitat in central NSW may also facilitate long-distance dispersal and gene flow. Evidence suggests that continuous habitat promotes contact between populations, thereby reducing genetic differentiation, while large discontinuities of sand or deep-water channels often restrict gene flow (Bernardi 2000, Bernardi et al. 2001, Riginos & Nachman 2001, Taylor & Hellberg 2003, Hoffman et al. 2005, Carreras-Carbonell et al. 2006, Gilbert-Horvath et al. 2006). Although subtidal rocky reefs in central NSW are regularly intercepted by beaches and the mouths of rivers and estuaries, they do not appear to be of sufficient magnitude relative to pre-settlement dispersal potential of reef fishes, to significantly limit dispersal at rates that influence population genetic structure. Habitat continuity may also reduce the exhibition of larval behaviours that promote retention in natal area. Recruitment habitats for *P. microlepis* and *G. tricuspidata* are common along central NSW. Therefore, pre-settlement fish only need to avoid offshore dispersal as there is greater potential for encountering settlement habitat, and the reception of sensory information regarding its location is continuous (Swearer et al. 2002). This model is in direct contrast to coral reef

environments where recruitment habitats may be extremely isolated, necessitating the exhibition of traits that favour retention in natal areas and subsequent population genetic structure (Sponaugle et al. 2002).

Lastly it was proposed that *P. microlepis* and *G. tricuspidata* probably have large effective population sizes (N_e) in central NSW thus reducing the rate at which populations become genetically differentiated via genetic drift. Both occur naturally in relatively large abundances and display several biological characteristics which are predicted to increase N_e including: long reproductive life spans, overlapping generations, reproductive behaviour that promotes larval survival and representative sampling of gametes, and extended spawning periods (Turner et al. 2002). These characteristics may also ensure that representative individuals have an opportunity to disperse novel alleles through their offspring over ecological time scales.

6.2.3. What can genetics tell us about population connectivity for *P. microlepis* and *G. tricuspidata*?

The ecological implications of the genetic homogeneity observed for *P. microlepis* and *G. tricuspidata* are difficult to interpret. Scales of genetic homogeneity may not reflect demographically relevant dispersal distances, as even a 10% level of exchange of individuals is many orders of magnitude higher than required for retaining genetic homogeneity (Cowen et al. 2000). Genetic similarity may, therefore, indicate that populations are largely 'open' with high exchange of individuals or 'closed' with sporadic but demographically inconsequential inter-population exchange (Cowen et al. 2002, Hellberg et al. 2002, Mora & Sale 2002). For example, long-distance dispersal via the EAC may be rare and the effect on downstream populations demographically insignificant. Therefore, although meso-scale oceanographic features which operate at scales < 100's km (e.g. tidally driven flow, eddies in lee of headlands) do not restrict gene flow in central NSW, they may still limit demographically important dispersal (Cowen et al. 2000, Sponaugle et al. 2002). Repeated geographic sampling and genetic analyses of recruits could show inter-class genetic differentiation and provide insight into spatial and temporal patterns of recruitment, even in areas of central NSW where adult populations are genetically homogeneous (Hellberg et al. 2002, Thorrold et al. 2002). It is likely, however that alternative methods (e.g. otolith chemistry, modelling)

will be required to distinguish between these different scenarios and to determine scales over which MPAs may benefit unprotected areas.

Local populations of many commonly exploited fishes (Table 1.1) are likely to be genetically homogeneous within central NSW. Most species are thought to have similar pre-settlement durations as *P. microlepis* and *G. tricuspidata* (≥ 2 weeks) and will be exposed to similar environmental factors that promote gene flow within the region (e.g. EAC). While the current study suggests that microsatellite markers will provide limited information on demographically relevant dispersal distances for many reef fishes in central NSW, it does not negate the potential of this technique for select species. Rather, it suggests that future studies using microsatellite markers should focus on species which exhibit biological and ecological traits not represented in *P. microlepis* or *G. tricuspidata* and which may favour genetic differentiation (e.g. shorter or no pelagic larval phase, small effective population sizes, short-life spans, and whose habitats are rare or patchily distributed). For example, species which occur in low numbers either naturally (e.g. Black Reef Leatherjacket, *Eubalichthys bucephalus*) or due to heavy exploitation (e.g. *C. fuscus*; Chapter 2) may have low effective population sizes leading to higher rates of genetic drift and lower rates of gene flow.

6.2.4. Implications of genetic homogeneity for management and MPAs

Although the absence of population genetic structure provides little information on demographically relevant population connectivity, it has several clear consequences for management and design of MPAs in this region. Genetic homogeneity and patterns of genetic diversity indicate that *P. microlepis*, *G. tricuspidata* and possibly other commonly exploited species are well connected or 'open' from an evolutionary perspective and have large effective population sizes, thereby reducing genetic risks associated with exploitation and local extinction (Hauser et al. 2002). Ongoing monitoring of genetic diversity over time may also indicate changes in effective population size and risk of extinction for exploited species (e.g. *G. tricuspidata*, *C. fuscus*) as a loss in genetic variability can lead to reduced adaptability, population persistence, and productivity (Hauser et al. 2002, Hutchings & Reynolds 2004). Secondly, microsatellite diversity could act as a surrogate for functional diversity that may be adaptive significance (Hauser et al. 2002). If this is the case, genetic diversity

across spatial scales ≤ 400 km could be conserved within relatively small MPAs such as those examined in Chapter 2, as 99-100% of the total genetic variation for *P. microlepis* and *G. tricuspidata* was represented within 1-2 km of reef. Larger or multiple areas, however, may be required for less abundant and heavily exploited species. For example, exploitation of *C. fuscus* via spear fishing can result in lower abundances and sizes of local populations (Chapter 2), potentially leading to localised losses of genetic diversity. The implementation of MPAs may not only restore natural abundances and size structure of target species but may result in significantly higher allelic richness (e.g. *Diplodus sargus*; Perez-Ruzafa et al. 2006).

6.3. Otolith chemistry

6.3.1. Is there enough environmental variability at spatial scales < 100's km to facilitate use of otolith chemistry in central NSW?

Otolith chemistry is one of few available techniques which may distinguish levels of population connectivity for genetically homogeneous populations such as those described for central NSW. Chapter three demonstrated that the magnitude of environmental variability within open coastal environments such as central NSW facilitates the use of otolith chemistry for determining population connectivity of reef fishes at scales < 100's km. Otolith chemistry of the territorial damselfish *P. microlepis* varied at scales of kilometres to hundreds of kilometres with trends for unique individual and multi-element signatures at some sites and locations (within regions). Furthermore, multi-element signatures were good spatial discriminators with 75-80% of fish correctly classified to the regions (separated by 70-80 km) in which they were collected. These results are exceptional, as most studies of otolith chemistry for measuring population connectivity utilise environmental variability found within and among estuaries, between estuarine and coastal environments, and/or across large spatial scales (Thorrold et al. 1998, Gillanders & Kingsford 2000, Forrester & Swearer 2002, Swearer et al. 2003, Dorval et al. 2005, Fowler et al. 2005).

Environmental factors determining patterns of variation in otolith chemistry

It was difficult to establish simple causal relationships for the variation in otolith chemistry observed for *P. microlepis*. This is not uncommon, as variation is a consequence of the interaction between three major factors: water chemistry, salinity

and temperature across multiple spatial scales; and relationships are often species specific (Campana 1999, Elsdon & Gillanders 2003a, Dorval et al. 2007). For example, fine-scale differences in otolith chemistry for *P. microlepis* were attributed to the interaction between large-scale biophysical processes which define bio-geographic regions in NSW (climate, sediment, riverine input) and fine-scale natural and anthropogenic processes (e.g. input of freshwater, localised upwelling, and pollutants associated with urbanisation, agriculture and industry). In contrast, several models for regional-scale patterns were proposed. There was a clear correlation between differences in regional-scale elemental signatures and the behaviour of the EAC. High levels of pollutants (e.g. Zn) were associated with the heavily populated Sydney region. Mensurative and manipulative studies designed to test specific hypotheses are required to investigate the processes determining patterns described in this study, and will broaden our understanding of the application of otolith chemistry for studies on population connectivity in this region. For example, depth-related patterns in elemental composition may be caused by differential exposure to estuarine plumes and could confound spatial comparisons (Kingsford & Gillanders 2000).

6.3.2. Applications of otolith chemistry to determine levels of dispersal and population connectivity

Otolith chemistry of 'resident' species such as *P. microlepis* could act as a baseline for comparison with other species of unknown mobility. For example, sites which had unique elemental signatures would be ideal for comparative studies and could elucidate small scale movement of fishes. Fish that were also relatively sedentary would be expected to exhibit similar relative differences among adjacent sites whereas fish moving at greater spatial scales would not. This is a potential approach for investigating the intraspecific variation in mobility hypothesised for *A. australis* and *G. tricuspidata* (Chapter 2). This type of application is complex, as interspecific variation in elemental signatures is poorly understood (Gillanders & Kingsford 2003, Swearer et al. 2003). Comparative studies suggest that this application will be most useful for closely related species with similar life histories and/or for fishes which occupy the same ecological niche (Brown 2006, Hamer & Jenkins 2007). Subsequently, the use of *P. microlepis* as a 'proxy' requires further work, and should initially focus on comparisons with other sedentary species from a range of family groups across the same sites, habitats, and depths (e.g. *Parma unifasciata*, *C. fuscus*).

Variability in otolith chemistry may also be used to determine pre-settlement connectivity for *P. microlepis* and other reef fishes. Although the environment may not be the primary determinant of natal signatures (Warner et al. 2005, Patterson 2004, Ashford et al. 2006), this region of the otolith may still exhibit similar broad-scale geographic patterns as adults (Thorrold et al. 2001, Ashford et al. 2005, Patterson et al. 2005, Stransky et al. 2005, Ashford et al. 2006). The region of the larval otolith that forms prior to hatching ('natal' or core region) or before significant dispersal has taken place (near-natal or edge of core) could incorporate a site, location or region-specific signature reflective of those found for post-settlement *P. microlepis*. *P. microlepis* is an ideal candidate to test these hypotheses as it lays benthic eggs and there is more time for natal signatures to develop. Modelling techniques which integrate hydrodynamic models, biological traits and settlement habitats should be used to produce spatio-temporal predictions of larval pathways (James et al. 2002; Paris et al. 2005) and formulate hypotheses for these applications. This approach could be complemented with spatially discrete batch-tagging of eggs with antibiotics or manipulated isotope ratios (e.g. Ba 138/137; Jones et al. 1999, Thorrold et al. 2002, Almany et al. 2007).

What other pre-requisites must be examined?

While demonstration of variability in otolith chemistry is an important pre-requisite, the applications described above require further studies which include intensive spatial replication and assessment of temporal variability in signatures. When using elemental signatures to discriminate among fish from different places it is important that all possible groups contributing to the group mixture are characterised (Campana 1999, Campana et al. 2000). This process may be challenging for coastal species due to high number of potential sources. The hierarchical design used in chapter three provides a baseline for the range of elemental concentrations found across central NSW. Future studies, could focus on targeting sites within each region that are likely to exhibit great variation (e.g. entrance to estuaries, different depths). Outliers within each region may have to be grouped by factors other than geographic location (e.g. entrance to estuaries) and considered when testing hypotheses on population connectivity.

Temporal variability in elemental composition should also be tested as elemental signatures may fluctuate over time, potentially confounding spatial comparisons

(Campana 1999, Gillanders 2002b). The spatial patterns described in this study were based on whole-otolith analyses and thus were a measure of age-integrated elemental signatures. Further studies should examine elemental chemistry at finer temporal scales using a probe-based approach (e.g. laser ablation ICP-MS). Spatial comparisons based on this data would significantly improve the resolution of differences among sites, locations or regions found in this study. The establishment of a 'library' of signatures for each class of fish may also be required prior to the application of this technique, if significant temporal variability is detected (see discussion in; Gillanders & Kingsford 2000, Gillanders & Kingsford 2003).

6.4. Concluding remarks

The multi-disciplinary approach of this thesis provided empirical data relevant to the design of MPAs, and a strong baseline for further investigations of dispersal and population connectivity for reef fishes in central NSW. Results demonstrated that small MPAs can be effective for protecting 'sedentary' and highly 'mobile' fishes, and the genetic diversity of population's representative of broader-spatial scales. Importantly, as MPAs become smaller, their location relative to habitat and depth, local aggregations, recruitment 'hotspots', adjacent habitats, and existing fishing pressure is critical in determining responses and recovery rates of fish populations. The determination of scales of 'spillover' from MPAs remains the greatest challenge for future studies. Realistically, the answer is unlikely to be derived from empirical experiments alone, but will probably require a modelling approach done with the best possible empirical data on population connectivity, and from large temporal and spatial-scale management experiments (Russ 2002). This study suggests that microsatellite markers are unlikely to provide useful information on demographically relevant population connectivity for many reef fishes in central NSW. A combination of otolith chemistry, artificial tags (e.g. batch-tagging of eggs with antibiotics or manipulated isotope ratios, acoustic telemetry), and modelling appear to be the most promising techniques for future investigations.

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Appendix A: Marine Protected Areas

Table A.1. Asymmetrical ANOVA comparing the abundance of *A. viridis* at two MPAs (CTB, Cabbage Tree Bay; GB, Gordon's Bay) and three control locations. Cochran's *C* -test (*C*) and ANOVA were done on ln(x+1) transformed data. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. *F* vs., first number shown is the row number for term used as denominator in *F* test, second number is row for alternative test whose use is indicated in superscript next to the MS estimate. R., redundant for hypotheses, NT., no valid test due to significance of terms requiring elimination at $P \leq 0.25$.

				Large (≥ 200 mm SL)		Small (≤ 150 mm SL)	
				CTB	GB	CTB	GB
Row	**Source**	**df**	***F* vs.**	**MS**	**MS**	**MS**	**MS**
1	Time	5	9	0.548	0.813	0.100	0.809
2	Location	3		2.921^{R}	2.932^{R}	1.064^{R}	2.295^{R}
3	MPA vs. Controls	1	4,5	0.082	0.115	0.382	4.073^{NT}
4	Among C's	2	5	4.340*	4.340^{NT}	1.405^{NT}	1.405^{NT}
5	Site(L)	4	12	0.483	0.807*	0.299*	0.620*
6	Si(MPA)	1	12	0.035	1.330*	0.152	1.439**
7	Si(C's)	3	12	0.632	0.632*	0.347**	0.347
8	Depth	1	16	2.852^{NT}	0.181^{NT}	0.775	3.944^{NT}
9	T x L	15	12	0.397	0.345	0.164*	0.490*
10	T x (MPA vs. C's)	5	11,12	0.341^{12}	0.187	0.109	1.087^{**12}
11	T x Controls	10	12	0.424	0.424	0.192*	0.192
12	T x Si(L)	20	28	0.355	0.199	0.067	0.171*
13	T x Si(MPA)	5	28	0.776**	0.152	0.058	0.475**
14	T x Si(C's)	15	28	0.215	0.215	0.070	0.070
15	T x D	5	22	0.599	0.815*	0.187*	0.654*
16	L x D	3	19	0.233	1.316	0.112	1.524*
17	(MPA vs. C's) x D	1	18,22	0.124^{NT}	3.372	0.022	4.259^{NT}
18	C's x D	2	19	0.288	0.288	0.157	0.157
19	Si(L) x D	4	25	0.846*	0.828*	0.201	0.200
20	Si(MPA) x D	1	25	0.082	0.007	0.004	0.002
21	Si(C's) x D	3	25	1.101*	1.101*	0.266	0.266
22	T x L x D	15	25	0.272	0.222	0.048	0.188
23	T x (MPA vs. C's) x D	5	24,25	0.459^{25}	0.310^{25}	0.084^{25}	0.506^{*25}
24	T x (C's) x D	10	25	0.178	0.178	0.029	0.029
25	T x Si(L) x D	20	28	0.269	0.256	0.100	0.149
26	T x Si(MPA) x D	5	28	0.327	0.277	0.087	0.283*
27	T x Si(C's) x D	15	28	0.250	0.250	0.104	0.104
28	Residual	384		0.226	0.234	0.091	0.106
C	MPA and C's			0.038	0.036	0.110**	0.095**
C	Controls only			0.050		0.129**	

Table A.2. Asymmetrical ANOVA comparing the abundance of Monacanthidae at two MPAs (CTB, Cabbage Tree Bay; GB, Gordon's Bay) and three control locations. Cochran's *C*-test (*C*) and ANOVA were done on ln(x+1) transformed data. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. *F* vs., first number shown is the row number for term used as denominator in *F* test, second number is row for alternative test whose use is indicated in superscript next to the MS estimate. R., redundant for hypotheses, NT., no valid test due to significance of terms requiring elimination at $P \leq 0.25$.

				Large (≥ 200 mm SL)		Small (≤ 150 mm SL)	
				CTB	GB	CTB	GB
Row	**Source**	**df**	***F* vs.**	**MS**	**MS**	**MS**	**MS**
1	Time	5	9	0.027	0.154	0.305	0.551*
2	Location	3		0.393^{R}	0.170^{R}	0.586^{R}	0.551^{R}
3	MPA vs. Controls	1	4,5	0.726^{NT}	0.058^{NT}	0.201	0.095
4	Among C's	2	5	0.226^{NT}	0.226^{NT}	0.779^{NT}	0.779^{NT}
5	Site(L)	4	12	0.304**	0.183	0.234*	0.231
6	Si(MPA)	1	12	0.692**	0.206	0.022	0.009
7	Si(C's)	3	12	0.175*	0.175	0.305**	0.305
8	Depth	1	16	1.761**	1.045^{NT}	0.002	0.006^{NT}
9	T x L	15	12	0.146*	0.144	0.236**	0.189
10	T x (MPA vs. C's)	5	11,12	0.275*	0.271^{12}	0.205	0.065
11	T x Controls	10	12	0.081	0.081	0.251**	0.251*
12	T x Si(L)	20	28	0.048	0.071	0.053	0.104
13	T x Si(MPA)	5	28	0.037	0.132	0.054	0.257*
14	T x Si(C's)	15	28	0.051	0.051	0.053	0.053
15	T x D	5	22	0.044	0.144	0.029	0.129
16	L x D	3	19	0.023	0.086	0.055	0.057^{NT}
17	(MPA vs. C's) x D	1	18,22	0.007^{22}	0.197^{22}	0.000	0.004^{NT}
18	C's x D	2	19	0.030	0.030	0.083	0.083^{NT}
19	Si(L) x D	4	25	0.056	0.077	0.017	0.212**
20	Si(MPA) x D	1	25	0.022	0.107	0.022	0.799***
21	Si(C's) x D	3	25	0.067	0.067	0.016	0.016
22	T x L x D	15	25	0.092	0.079	0.030	0.066
23	T x (MPA vs. C's) x D	5	24,25	0.132^{25}	0.094^{25}	0.014	0.122^{*25}
24	T x (C's) x D	10	25	0.072	0.072	0.038	0.038
25	T x Si(L) x D	20	28	0.130	0.099	0.023	0.036
26	T x Si(MPA) x D	5	28	0.198	0.075	0.009	0.064
27	T x Si(C's) x D	15	28	0.108	0.108	0.027	0.027
28	Residual	384		0.106	0.095	0.091	0.110
C	MPA and C's			0.063	0.096**	0.131**	0.109**
C	Controls only			0.097**		0.143**	

Table A.3. Asymmetrical ANOVA comparing the abundance of *G. elevata* at two MPAs (CTB, Cabbage Tree Bay; GB, Gordon's Bay) and three control locations. Cochran's *C*-test (*C*) and ANOVA were done on ln(x+1) transformed data. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. *F* vs., first number shown is the row number for term used as denominator in *F* test, second number is row for alternative test whose use is indicated in superscript next to the MS estimate. R., redundant for hypotheses, NT., no valid test due to significance of terms requiring elimination at $P \leq 0.25$.

				Legal (≥ 250 mm SL)		Small (≤ 200 mm SL)	
				CTB	GB	CTB	GB
Row	**Source**	**df**	***F* vs.**	**MS**	**MS**	**MS**	**MS**
1	Time	5	9	0.388	0.411*	0.713***	1.102***
2	Location	3		0.473^{R}	0.355^{R}	0.189^{R}	0.200^{R}
3	MPA vs. Controls	1	4,5	0.367^{NT}	0.015^{5}	0.437^{5}	0.472^{5}
4	Among C's	2	5	0.526	0.526	0.065	0.065
5	Site(L)	4	12	1.484**	1.272**	0.992*	0.953*
6	Si(MPA)	1	12	0.955*	0.108	0.682	0.524
7	Si(C's)	3	12	1.660**	1.660***	1.096*	1.096*
8	Depth	1	16	12.552^{NT}	10.144^{NT}	10.062^{NT}	11.476^{NT}
9	T x L	15	12	0.225	0.114	0.075	0.087
10	T x (MPA vs. C's)	5	11,12	0.371^{12}	0.040^{12}	0.064^{12}	0.098^{12}
11	T x Controls	10	12	0.152	0.152	0.081	0.081
12	T x Si(L)	20	28	0.192	0.179	0.287	0.231
13	T x Si(MPA)	5	28	0.218	0.164	0.474	0.251
14	T x Si(C's)	15	28	0.184	0.184	0.224	0.224
15	T x D	5	22	0.389	0.340	0.622*	1.102***
16	L x D	3	19	0.590	0.482	0.076	0.200
17	(MPA vs. C's) x D	1	18,22	0.324^{NT}	0.002	0.098	0.472
18	C's x D	2	19	0.722	0.722	0.065	0.065
19	Si(L) x D	4	25	1.387**	1.233**	0.867*	0.953*
20	Si(MPA) x D	1	25	0.724	0.108	0.180	0.524
21	Si(C's) x D	3	25	1.608**	1.608**	1.096*	1.096*
22	T x L x D	15	25	0.246	0.120	0.168	0.087
23	T x (MPA vs. C's) x D	5	24,25	0.415^{25}	0.038^{25}	0.344^{25}	0.098^{25}
24	T x (C's) x D	10	25	0.161	0.161	0.081	0.081
25	T x Si(L) x D	20	28	0.219	0.194	0.276	0.231
26	T x Si(MPA) x D	5	28	0.264	0.164	0.433	0.251
27	T x Si(C's) x D	15	28	0.204	0.204	0.224	0.224
28	Residual	384		0.207	0.194	0.263	0.270
C	MPA and C's			0.073	0.087*	0.103**	0.106**
C	Controls only			0.110**		0.108**	

Table A.4. Asymmetrical ANOVA comparing the abundance of *G. tricuspidata* and *A. australis* at two MPAs (CTB, Cabbage Tree Bay; GB, Gordon's Bay) and three control locations. Cochran's *C*-test (*C*) and ANOVA were done on ln(*x*+1) transformed data. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. *F* vs., first number shown is the row number for term used as denominator in *F* test, second number is row for alternative test whose use is indicated in superscript next to the MS estimate. R., redundant for hypotheses, NT., no valid test due to significance of terms requiring elimination at $P \leq 0.25$.

				G. tricuspidata		***A. australis***	
				Legal (≥ 200 mm SL)		**Legal (≥ 200 mm SL)**	
				CTB	**GB**	**CTB**	**GB**
Row	**Source**	**df**	***F* vs.**	**MS**	**MS**	**MS**	**MS**
1	Time	5	9	1.382	0.524	0.035	0.221
2	Location	3		20.143^{R}	1.289^{R}	0.786^{R}	0.650^{R}
3	MPA vs. Controls	1	4,5	56.634^{NT}	0.072^{5}	2.118**^{5}	1.711
4	Among C's	2	5	1.897	1.897	0.120	0.120^{NT}
5	Site(L)	4	12	13.416**	5.888**	0.080	0.833**
6	Si(MPA)	1	12	30.253**	0.141	0.033	3.043***
7	Si(C's)	3	12	7.803*	7.803**	0.096	0.096
8	Depth	1	16	24.096*	25.026*	0.086	0.638
9	T x L	15	12	2.365	1.091	0.210	0.166
10	T x (MPA vs. C's)	5	11,12	5.057^{12}	1.236^{12}	0.237^{12}	0.106
11	T x Controls	10	12	1.019	1.019	0.196	0.196
12	T x Si(L)	20	28	1.993***	1.222*	0.224	0.118
13	T x Si(MPA)	5	28	3.407***	0.321	0.618**	0.191
14	T x Si(C's)	15	28	1.522*	1.522**	0.093	0.093
15	T x D	5	22	1.537	0.416	0.082	0.099
16	L x D	3	19	1.298	1.488	0.027	0.395
17	(MPA vs. C's) x D	1	18,22	2.808^{NT}	3.379^{22}	0.037	1.140*
18	C's x D	2	19	0.542	0.542	0.022	0.022
19	Si(L) x D	4	25	0.950	0.729	0.450	0.757**
20	Si(MPA) x D	1	25	0.910	0.028	0.207	1.434**
21	Si(C's) x D	3	25	0.963	0.963	0.531*	0.531*
22	T x L x D	15	25	1.142	0.735	0.119	0.089
23	T x (MPA vs. C's) x D	5	24,25	1.934^{25}	0.711^{25}	0.144^{25}	0.053^{25}
24	T x (C's) x D	10	25	0.746	0.746	0.106	0.106
25	T x Si(L) x D	20	28	1.264*	1.235*	0.167	0.150
26	T x Si(MPA) x D	5	28	0.812	0.697	0.314	0.247
27	T x Si(C's) x D	15	28	1.415*	1.415*	0.118	0.118
28	Residual	384		0.749	0.675	0.146	0.113
C	MPA and C's			0.056	0.062	0.158**	0.204**
C	Controls only			0.076		0.031**	

Table A.5. Asymmetrical ANOVA comparing the abundance of *K. sydneyanus* at two MPAs (CTB, Cabbage Tree Bay; GB, Gordon's Bay) and three control locations. Cochran's *C*-test (*C*) and ANOVA were done on ln(x+1) transformed data. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. *F* vs., first number shown is the row number for term used as denominator in *F* test, second number is row for alternative test whose use is indicated in superscript next to the MS estimate. R., redundant for hypotheses, NT., no valid test due to significance of terms requiring elimination at $P \leq 0.25$.

				Legal (≥ 200 mm SL)		Small (≤ 150 mm SL)	
				CTB	GB	CTB	GB
Row	**Source**	**df**	***F* vs.**	**MS**	**MS**	**MS**	**MS**
1	Time	5	9	0.094	0.109	0.104	0.090
2	Location	3		0.362^{R}	0.086^{R}	0.053^{R}	0.071^{R}
3	MPA vs. Controls	1	4,5	0.871^{NT}	0.042^{5}	0.024^{5}	0.076^{5}
4	Among C's	2	5	0.107	0.107	0.068	0.068
5	Site(L)	4	12	0.153	0.137	0.097	0.108
6	Si(MPA)	1	12	0.341	0.276	0.086	0.130
7	Si(C's)	3	12	0.090	0.090	0.100	0.100
8	Depth	1	16	0.370^{NT}	0.152^{NT}	0.376^{NT}	0.433^{NT}
9	T x L	15	12	0.095	0.053	0.048	0.047
10	T x (MPA vs. C's)	5	11,12	0.173^{12}	0.047^{12}	0.035^{12}	0.032^{12}
11	T x Controls	10	12	0.055	0.055	0.054	0.054
12	T x Si(L)	20	28	0.096	0.075	0.060	0.081
13	T x Si(MPA)	5	28	0.199*	0.115	0.041	0.123
14	T x Si(C's)	15	28	0.061	0.061	0.067	0.067
15	T x D	5	22	0.172	0.151*	0.160	0.120
16	L x D	3	19	0.276	0.095	0.036	0.048
17	(MPA vs. C's) x D	1	18,22	0.824^{NT}	0.279^{*22}	0.036	0.072
18	C's x D	2	19	0.003	0.003	0.036	0.036
19	Si(L) x D	4	25	0.096	0.079	0.137	0.229
20	Si(MPA) x D	1	25	0.341	0.276*	0.009	0.377
21	Si(C's) x D	3	25	0.014	0.014	0.180	0.180
22	T x L x D	15	25	0.074	0.033	0.055	0.058
23	T x (MPA vs. C's) x D	5	24,25	0.144^{25}	0.021^{25}	0.053^{25}	0.061^{25}
24	T x (C's) x D	10	25	0.039	0.039	0.056	0.056
25	T x Si(L) x D	20	28	0.087	0.062	0.074	0.088
26	T x Si(MPA) x D	5	28	0.216**	0.115	0.056	0.112
27	T x Si(C's) x D	15	28	0.044	0.044	0.080	0.080
28	Residual	384		0.068	0.064	0.070	0.076
C	MPA and C's			0.1636**	0.225**	0.174**	0.182**
C	Controls only			0.292**		0.243**	

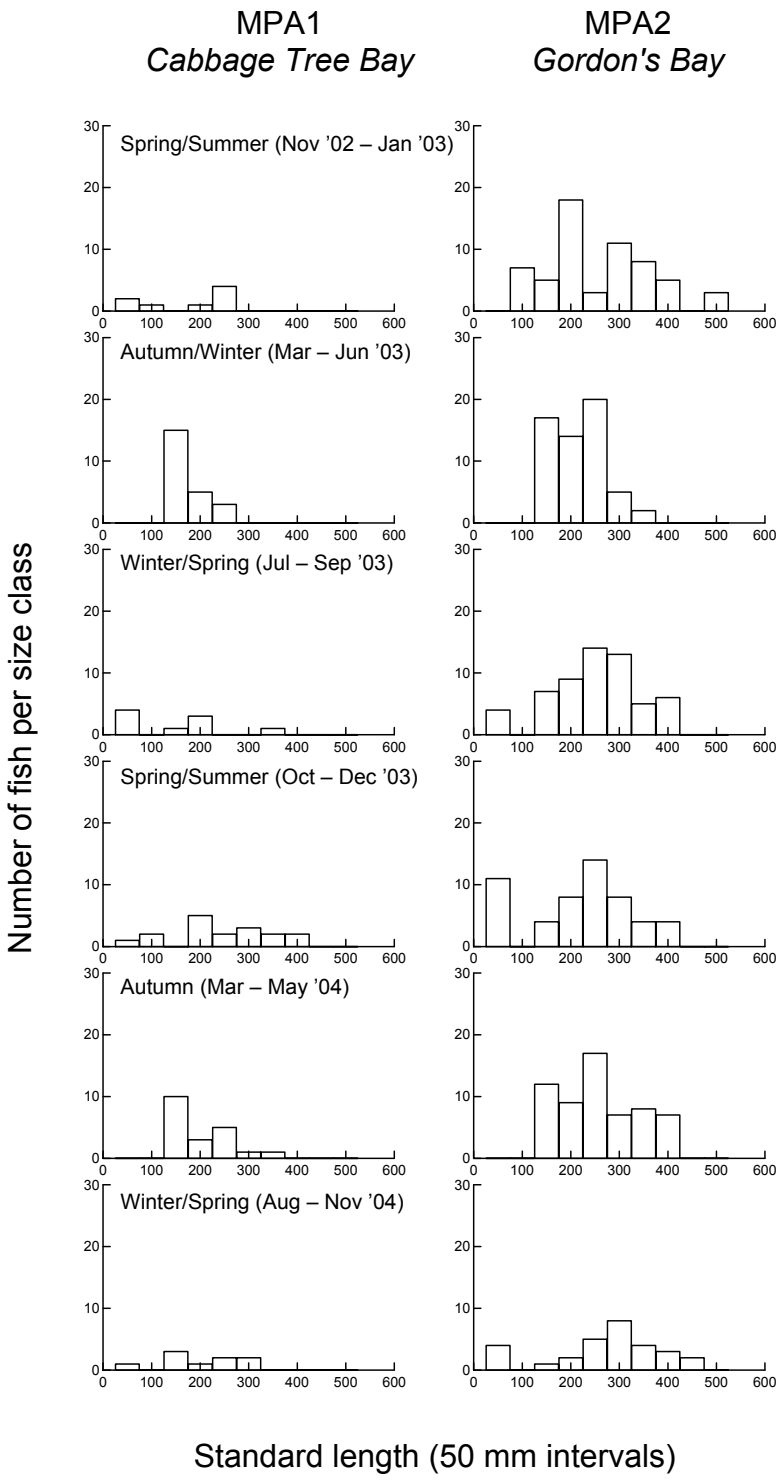

Fig. A.1. Size frequency of *C. fuscus* at MPA and control locations for six sampling times.

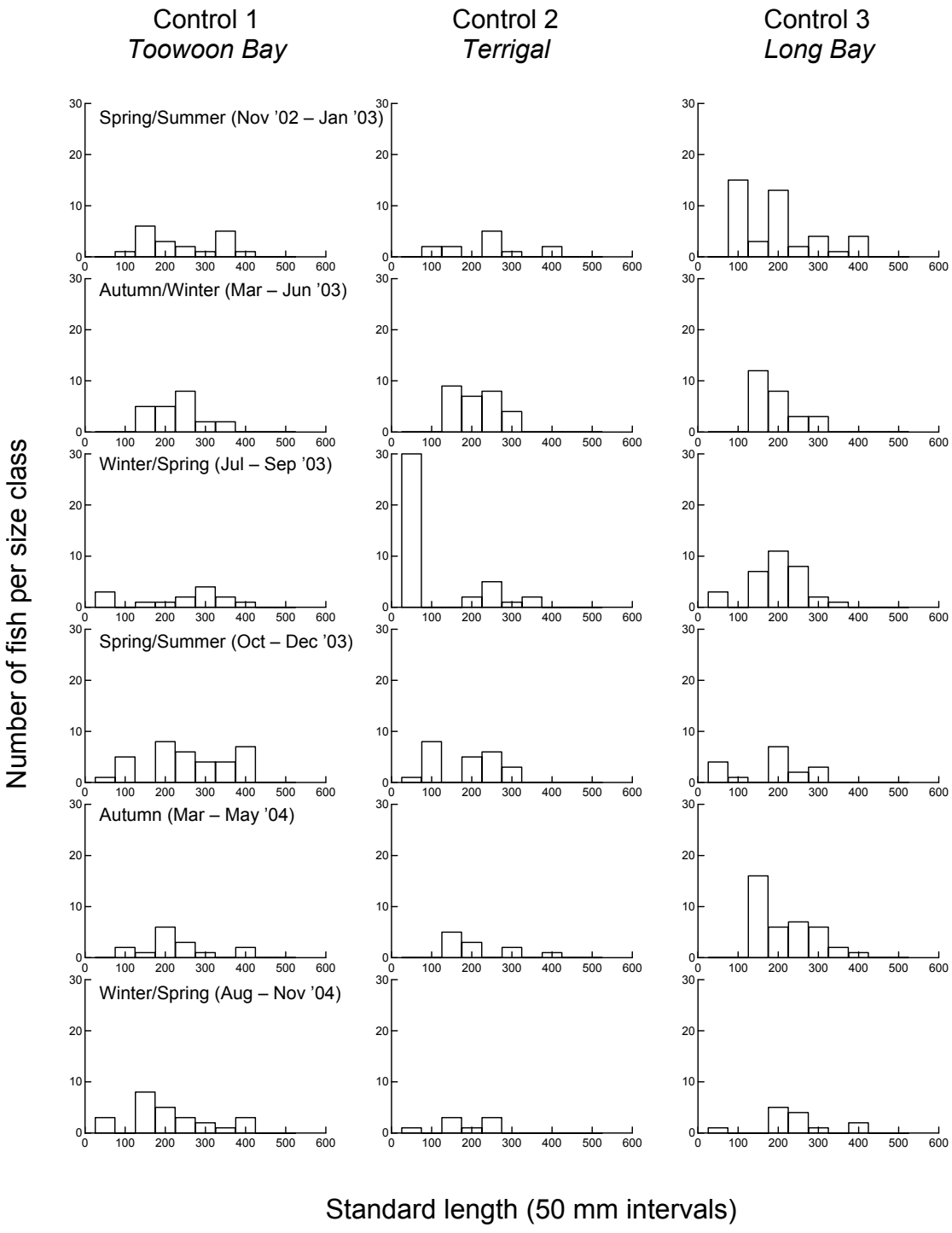

Fig. A.1. *C. fuscus* cont.

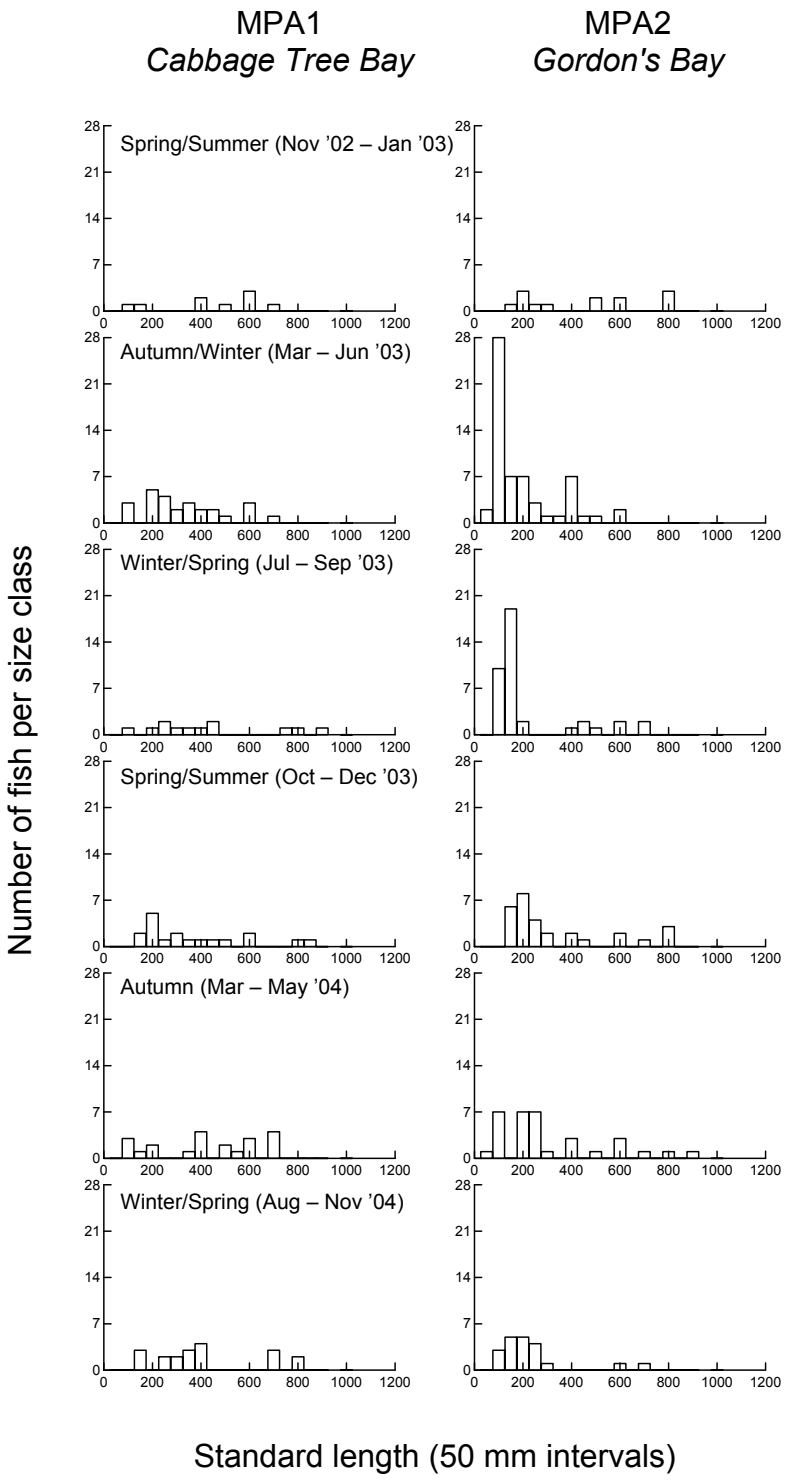

Fig. A.2. Size frequency of *A. viridis* at MPA and control locations for six sampling times.

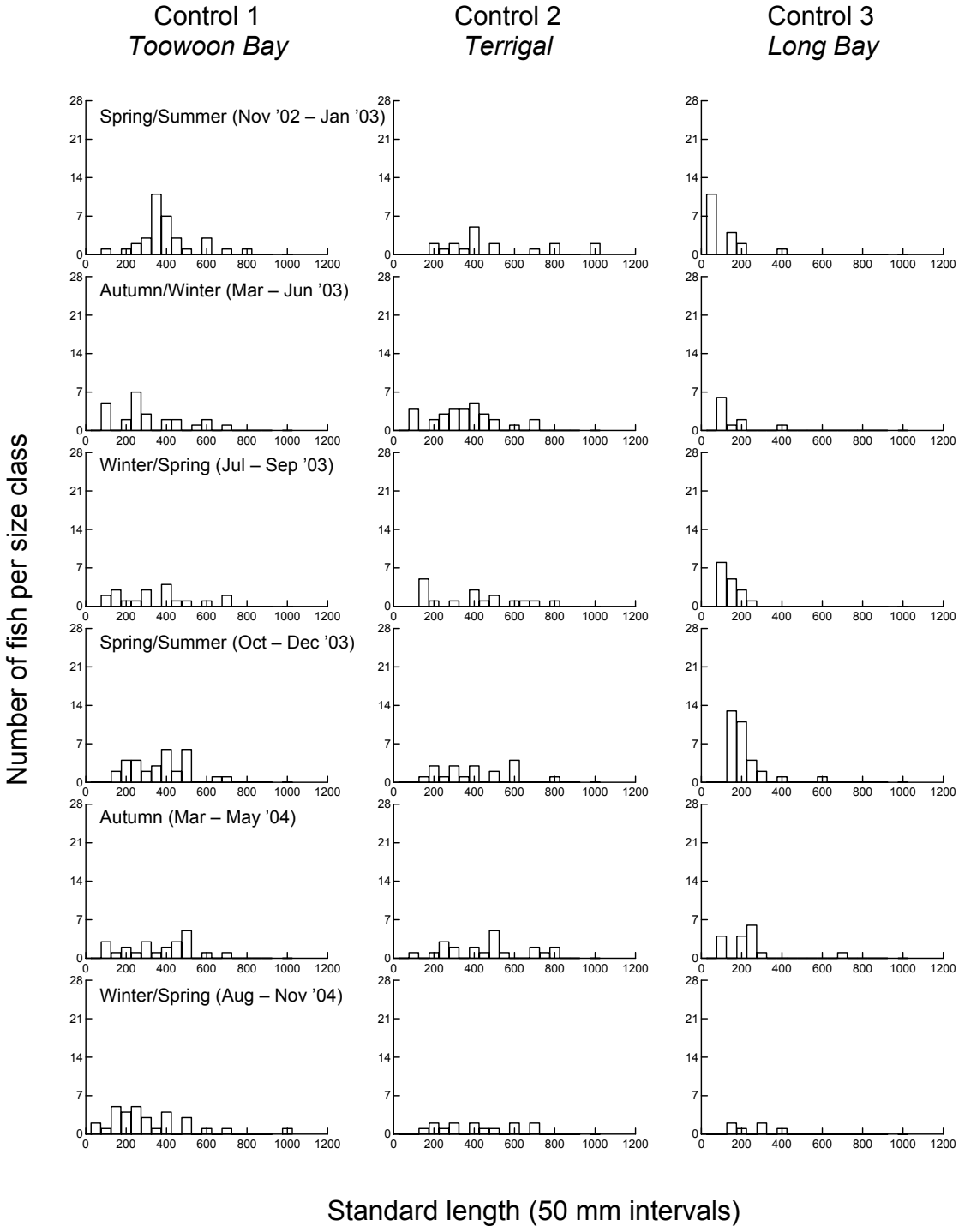

Fig. A.2. *A. viridis* cont.

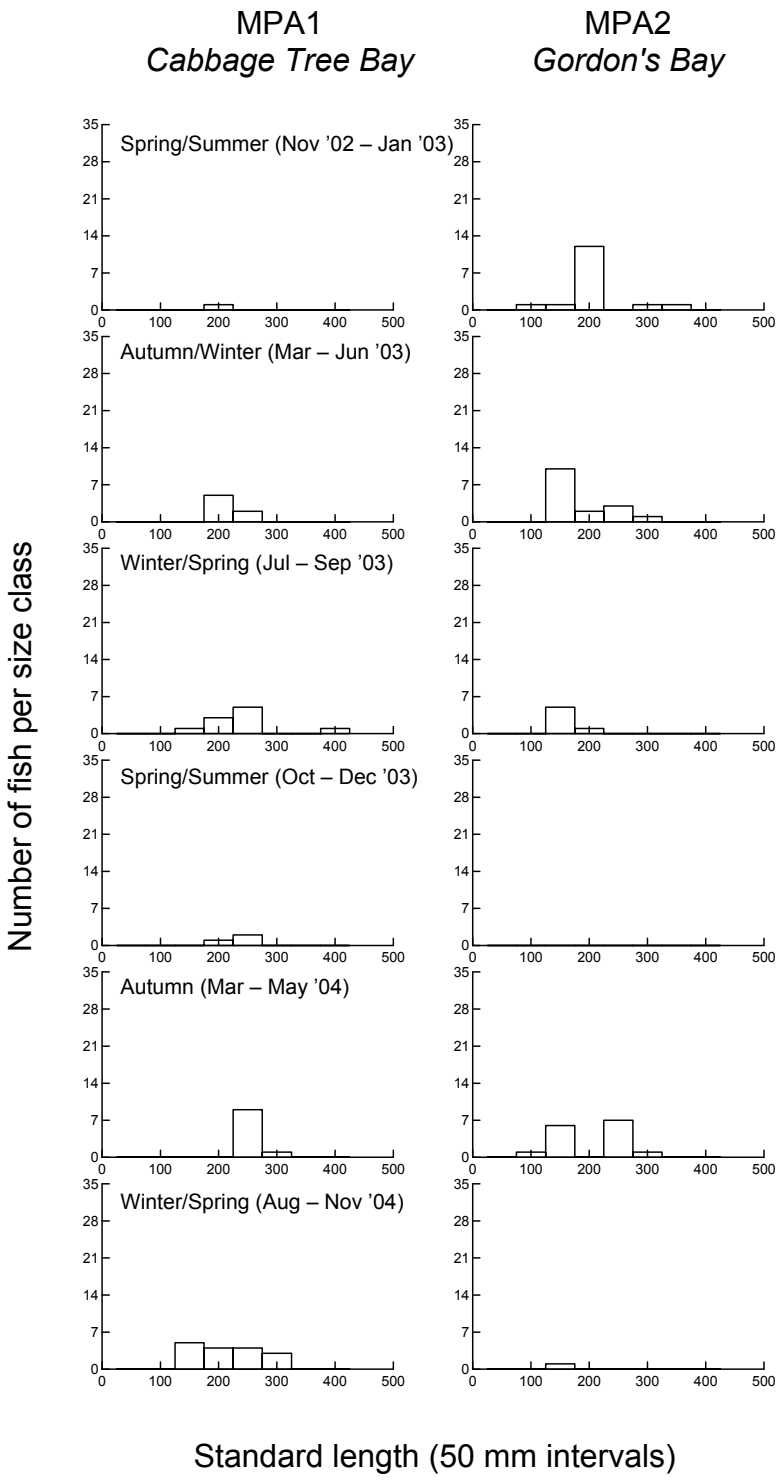

Fig. A.3. Size frequency of Monacanthidae at MPA and control locations for six sampling times.

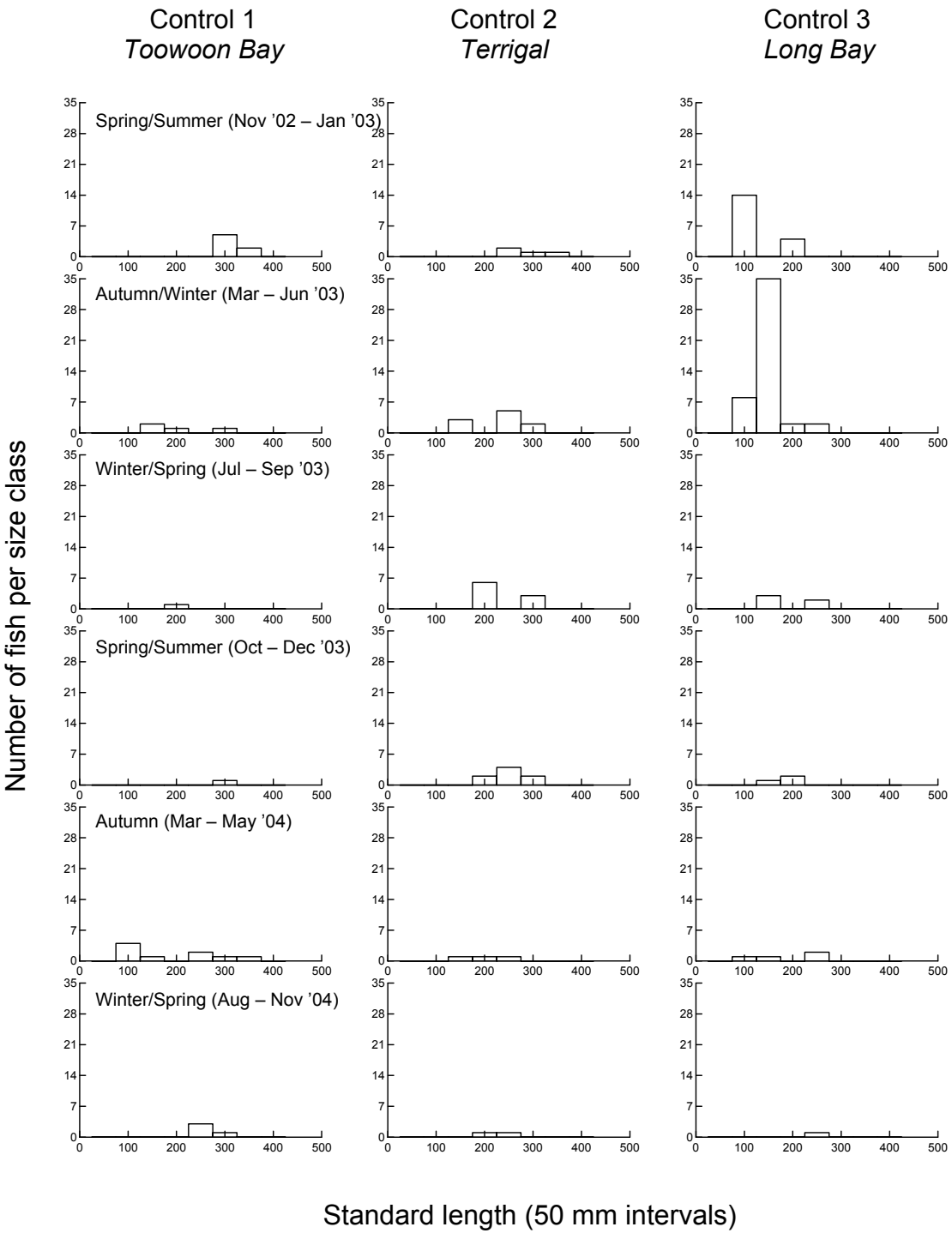

Fig. A.3. Monacanthidae cont.

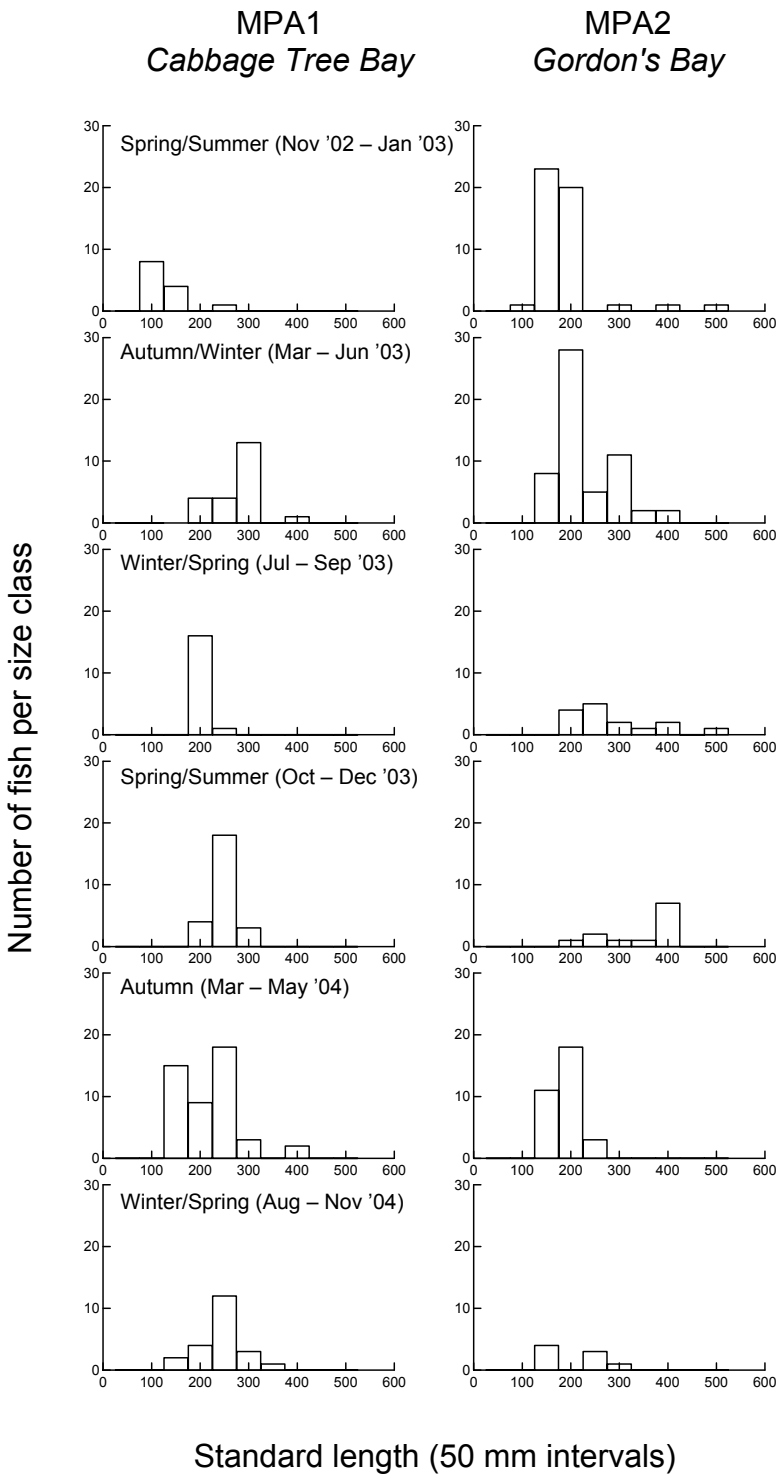

Fig. A.4. Size frequency of *G. elevata* at MPA and control locations for six sampling times.

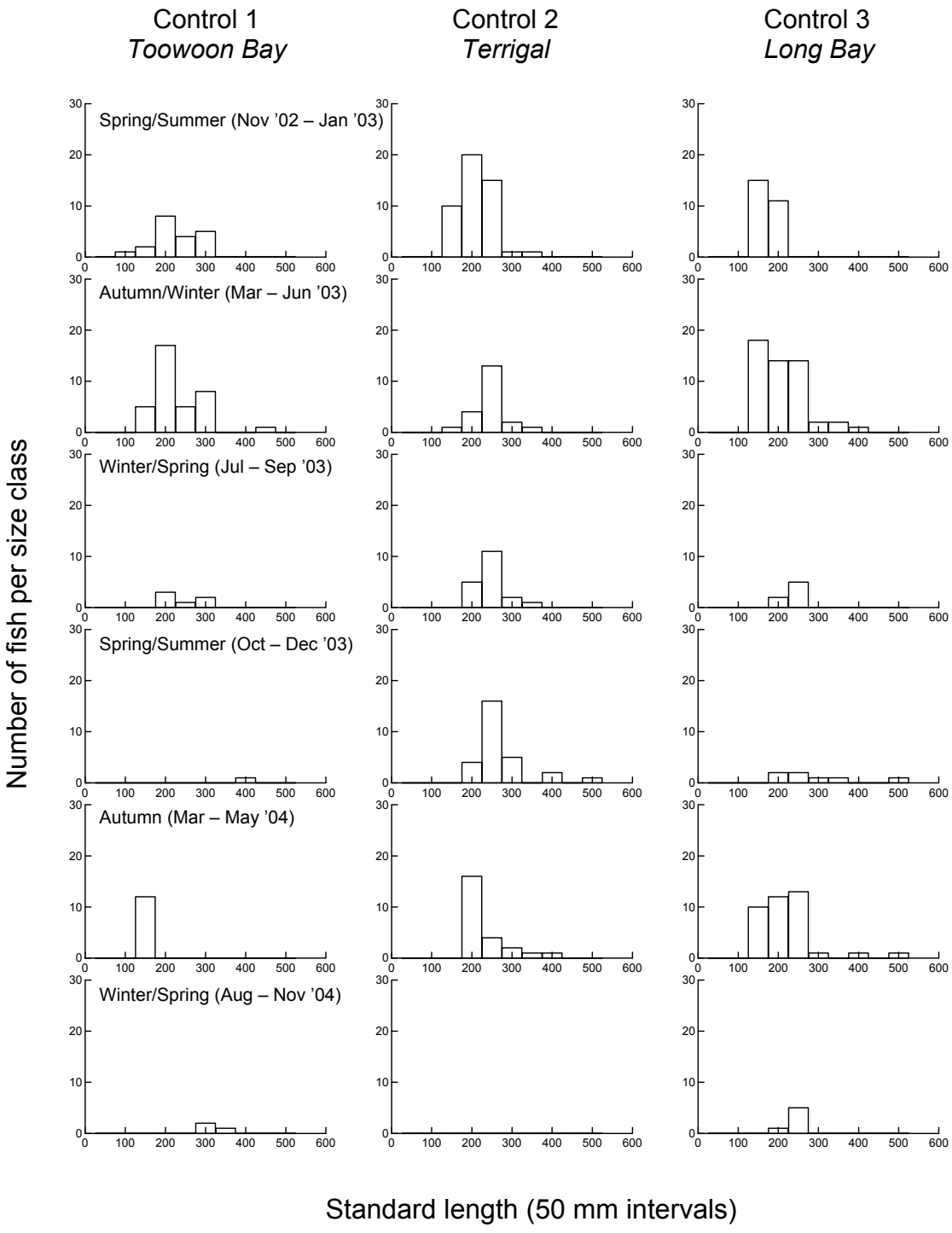

Fig. A.4. *G. elevata* cont.

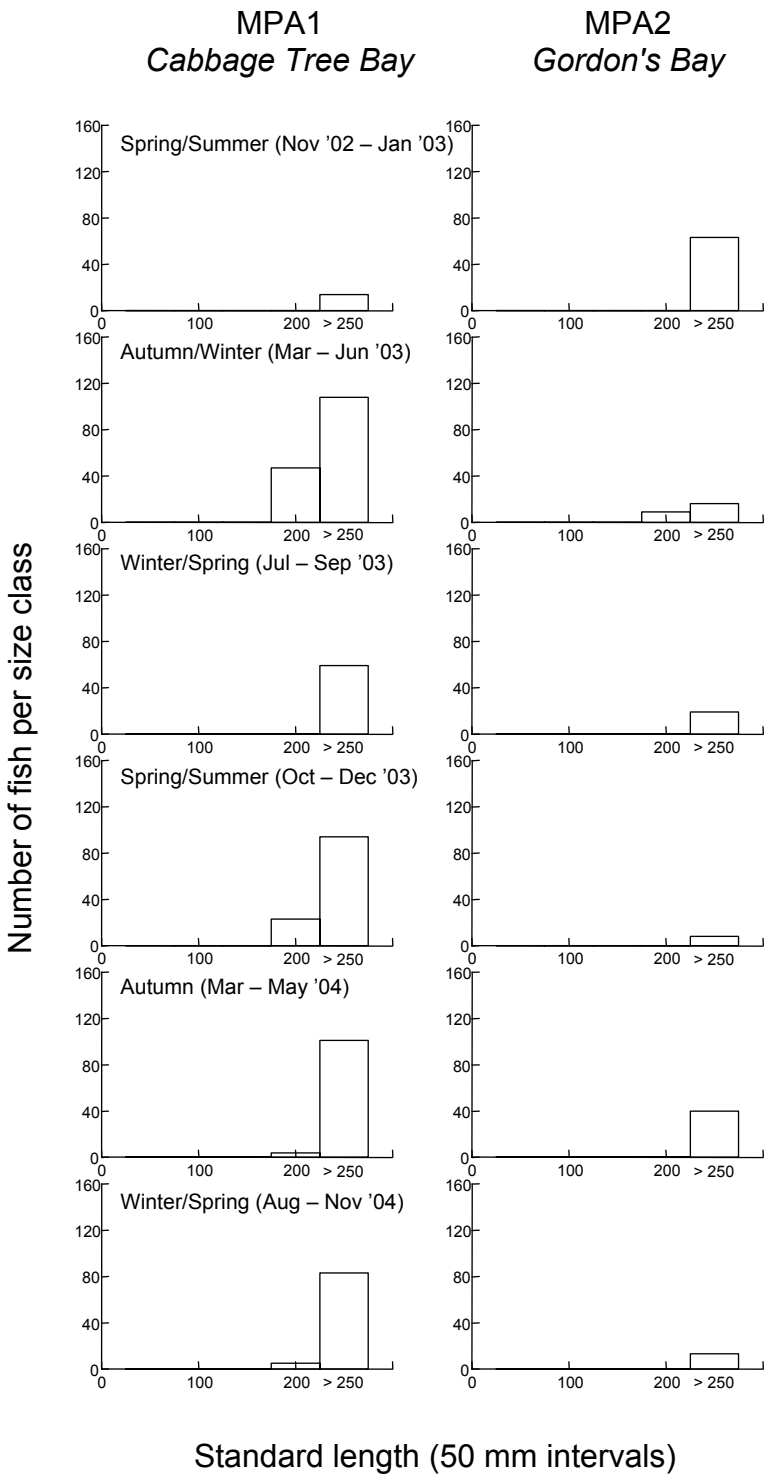

Fig. A.5. Size frequency of *G. tricuspidata* at MPA and control locations for six sampling times. Fish between 250 – 400 mm SL were grouped together in a single size class (> 250 mm).

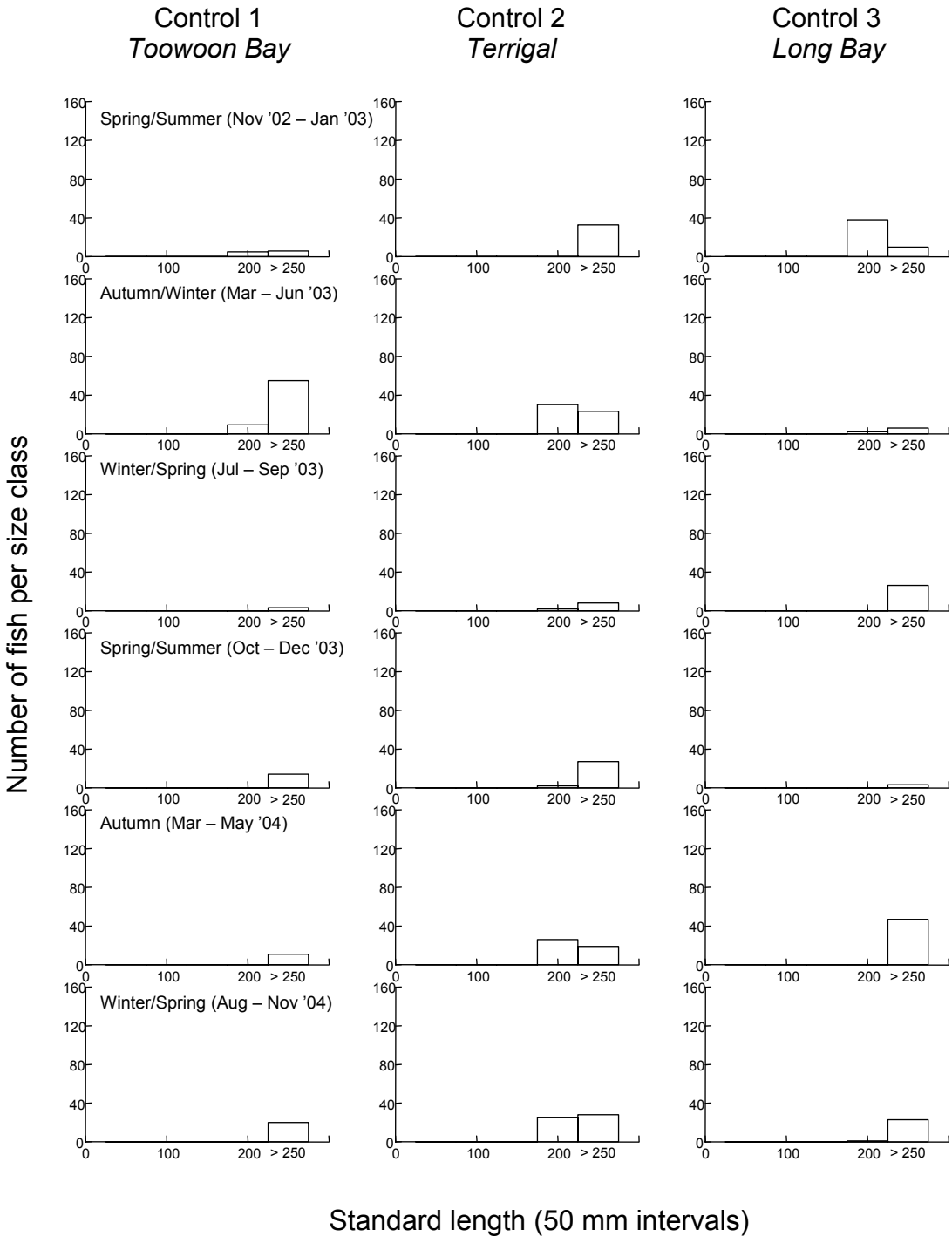

Fig. A.5. *G. tricuspidata* cont.

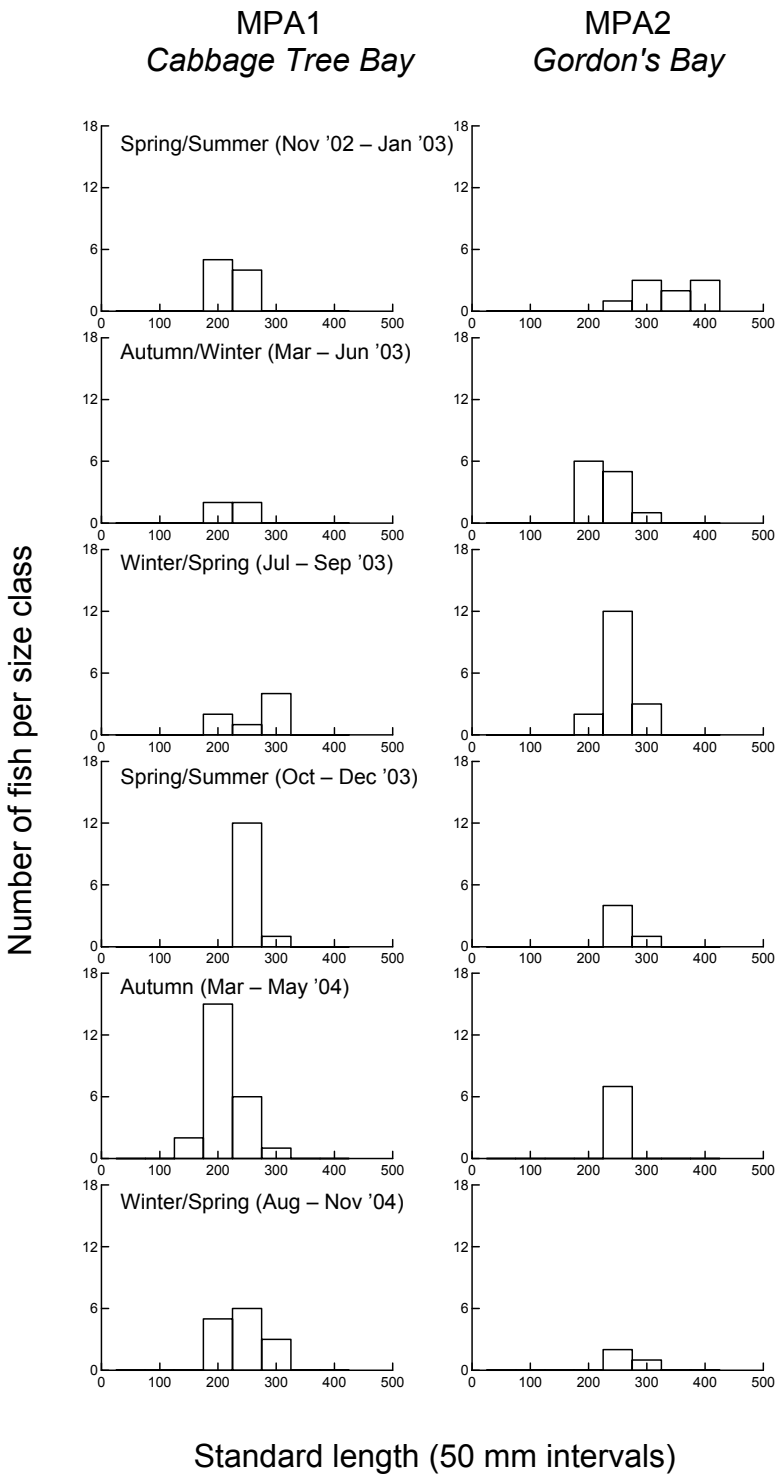

Fig. A.6. Size frequency of *A. australis* at MPA and control locations for six sampling times.

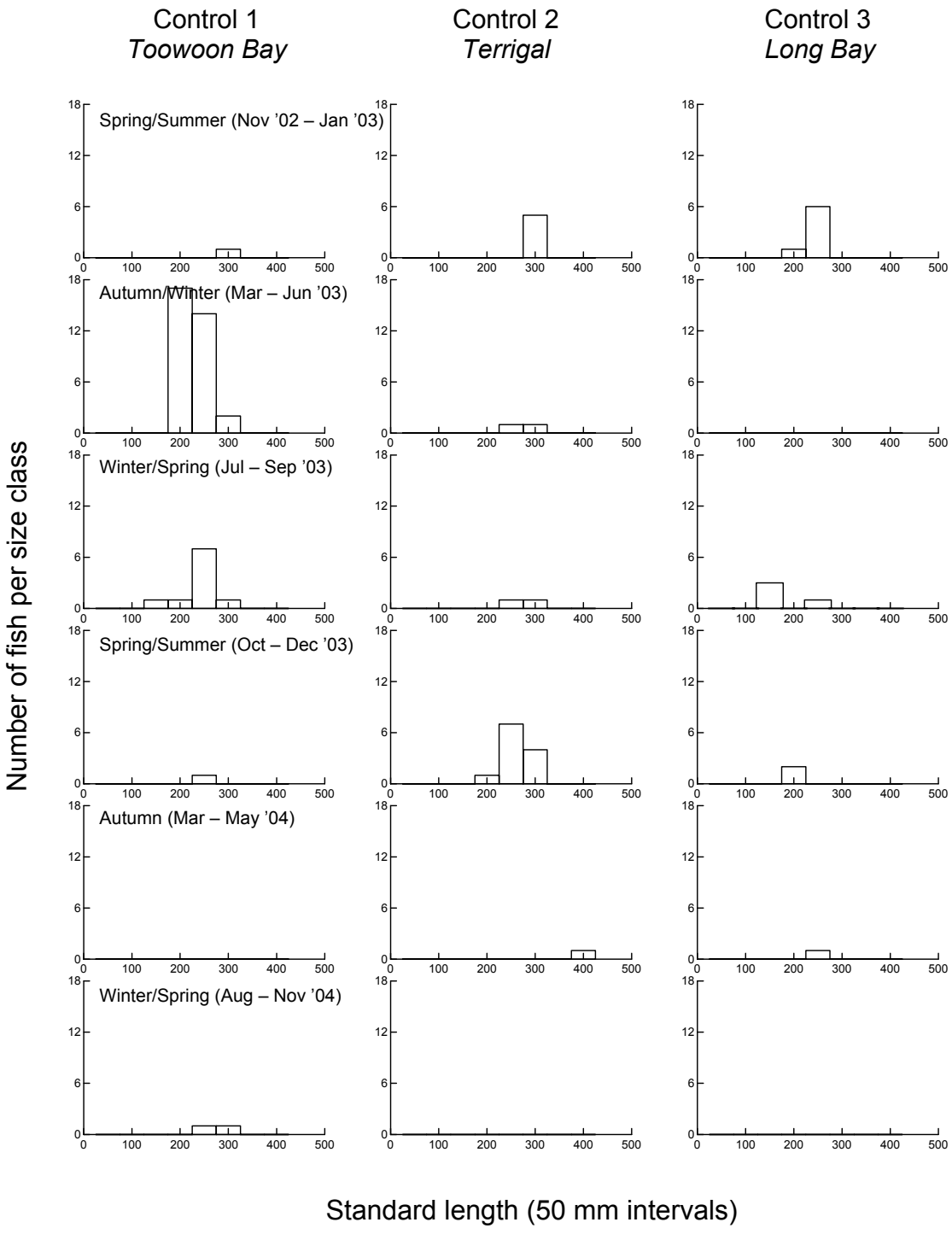

Fig. A.6. *A. australis* cont.

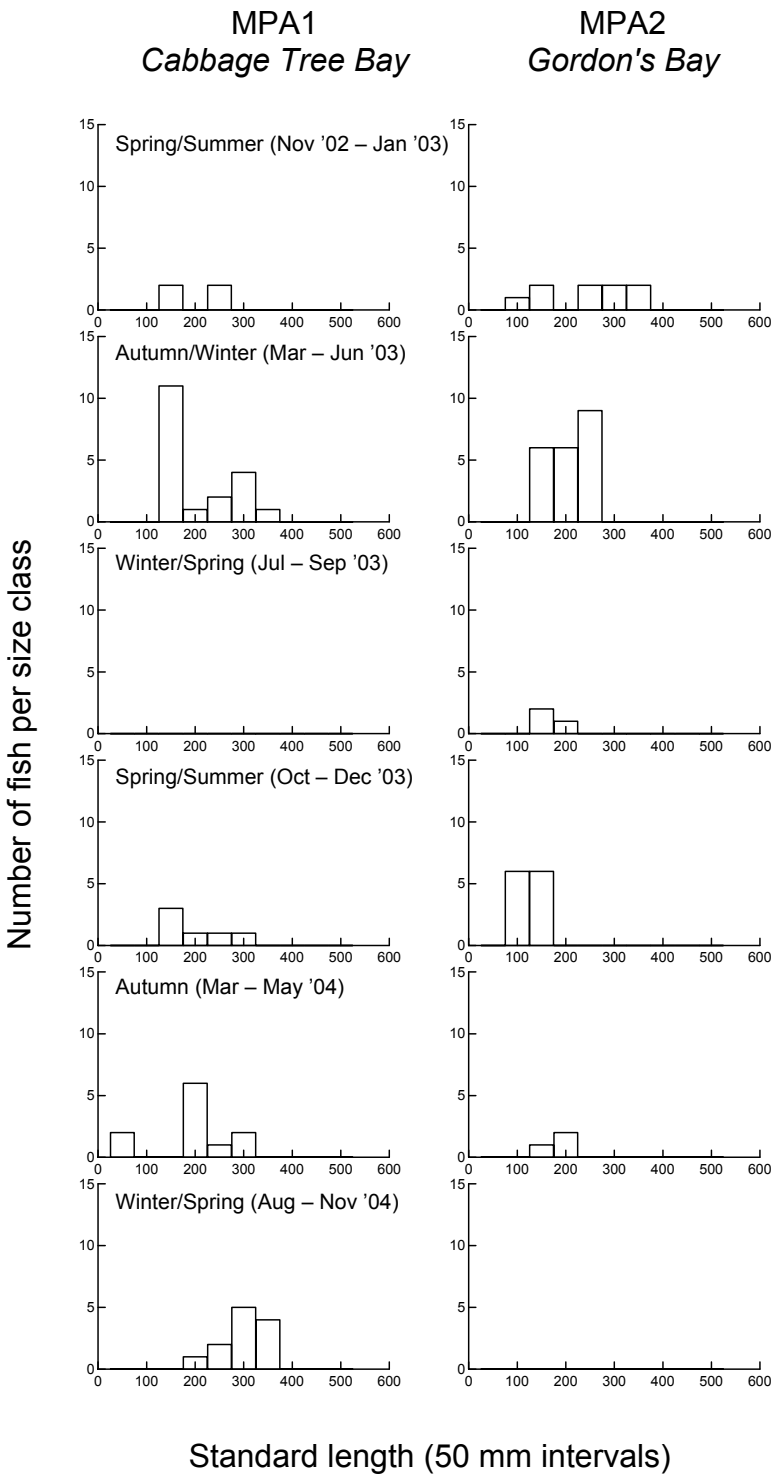

Fig. A.7. Size frequency of *K. sydneyanus* at MPA and control locations for six sampling times.

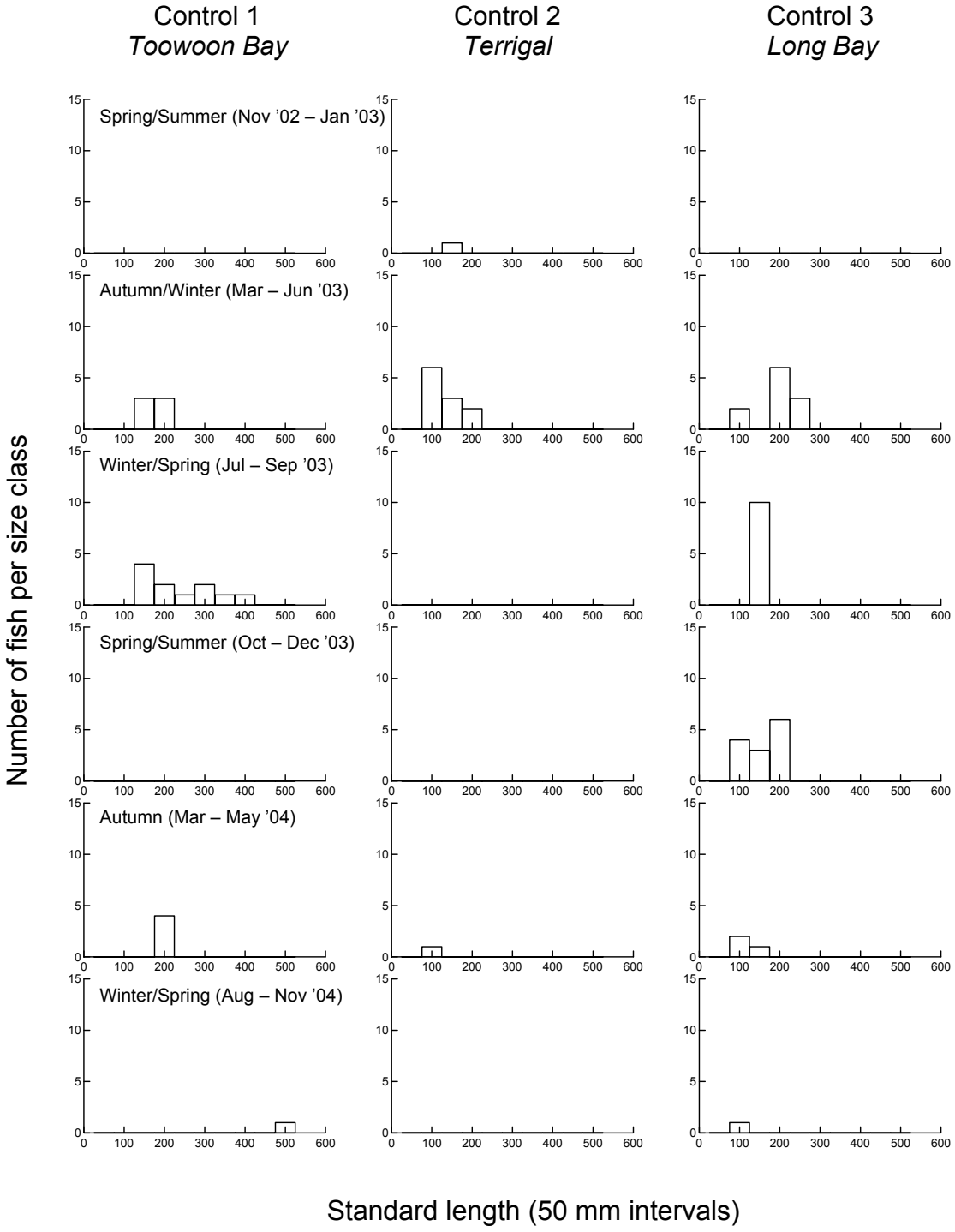

Fig. A.7. *K. sydneyanus* cont.

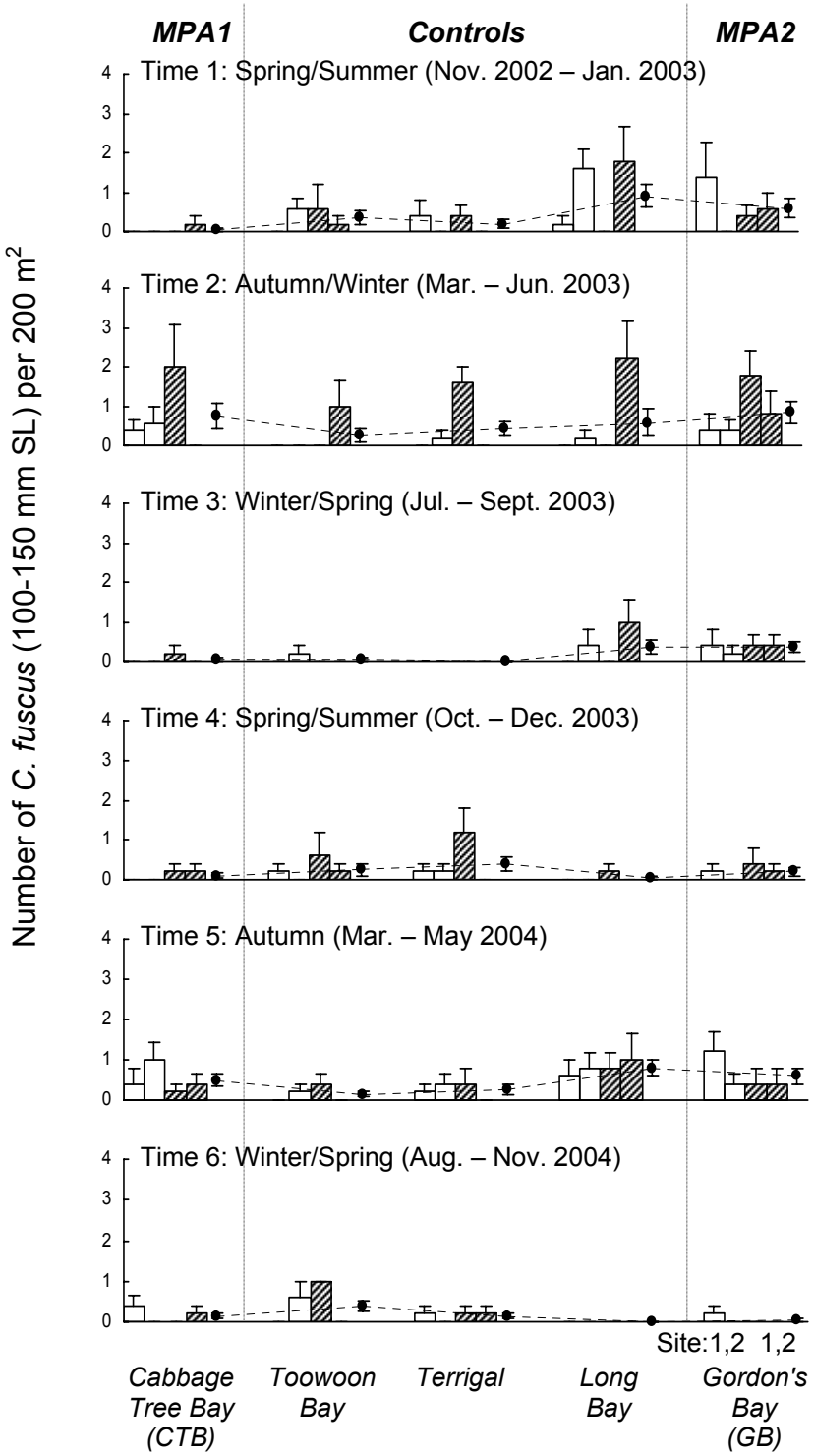

Fig. A.8. Mean abundance (+ SE) of sub-legal *C. fuscus* (100-150 mm standard length) in shallow (□) and deep (▨) areas of reef in MPA and control locations at six sampling times. Abundances are shown for each of two sites (separated by 100's m) within each location. n = 5 replicates at each depth. ● = Mean abundance for location (± SE).

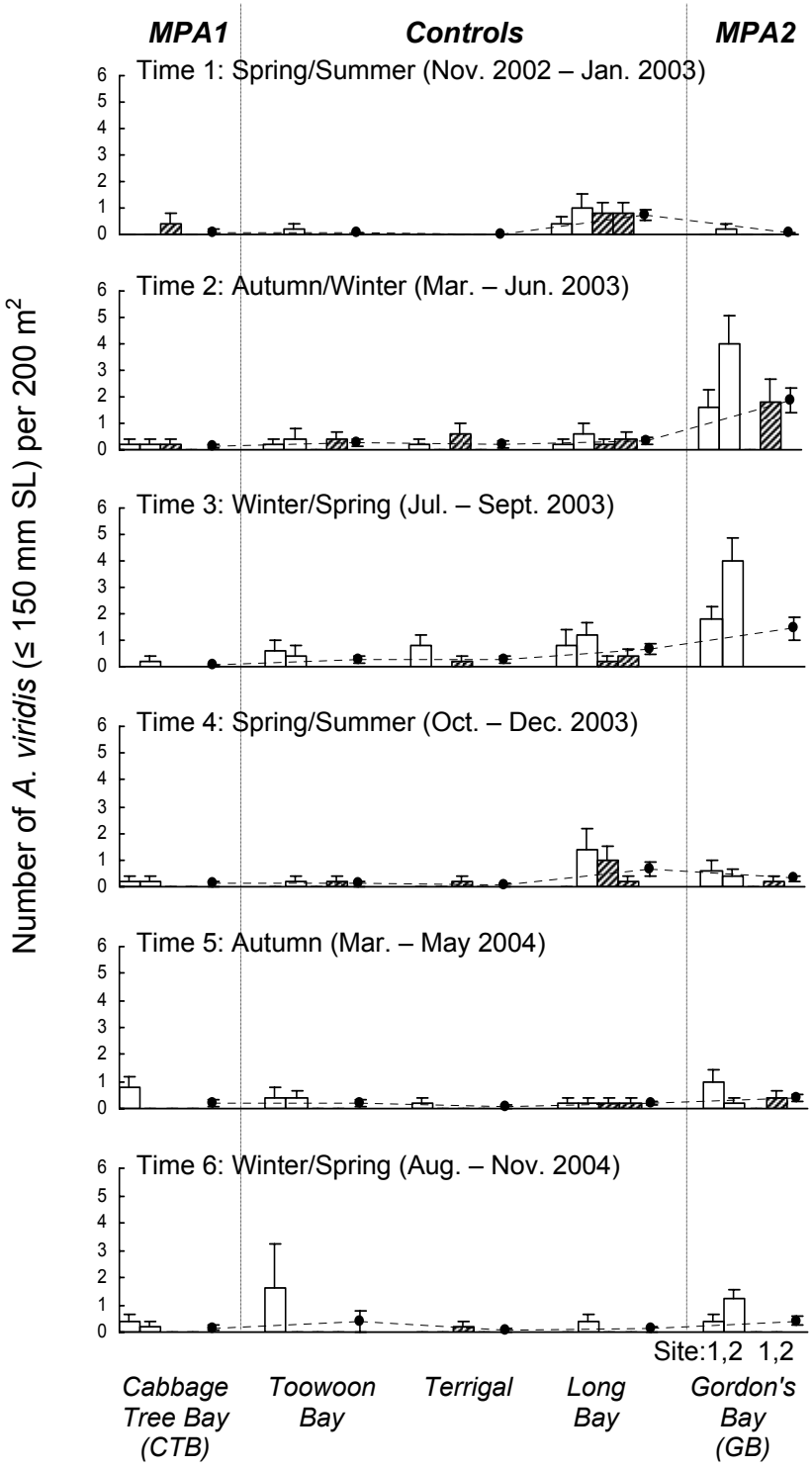

Fig. A.9. Mean abundance (+ SE) of small *A. viridis* (≤ 150 mm standard length) in shallow (☐) and deep (▨) areas of reef in MPA and control locations at six sampling times. Abundances are shown for each of two sites (separated by 100's m) within each location. n = 5 replicates at each depth. ● = Mean abundance for location (± SE).

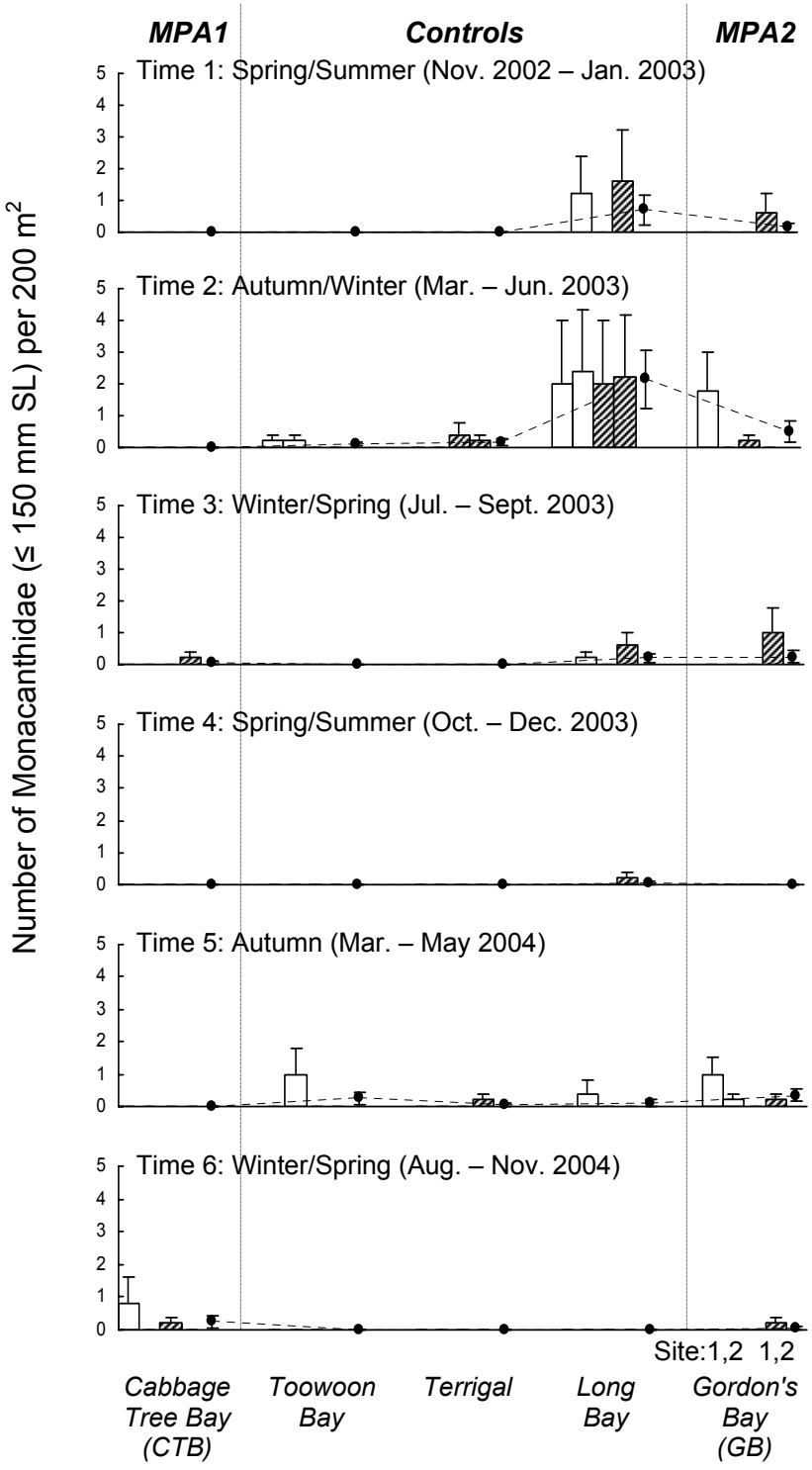

Fig. A.10. Mean abundance (+ SE) of small Monacanthidae (≤ 150 mm standard length) in shallow (□) and deep (▨) areas of reef in MPA and control locations at six sampling times. Abundances are shown for each of two sites (separated by 100's m) within each location. n = 5 replicates at each depth. ● = Mean abundance for location (± SE).

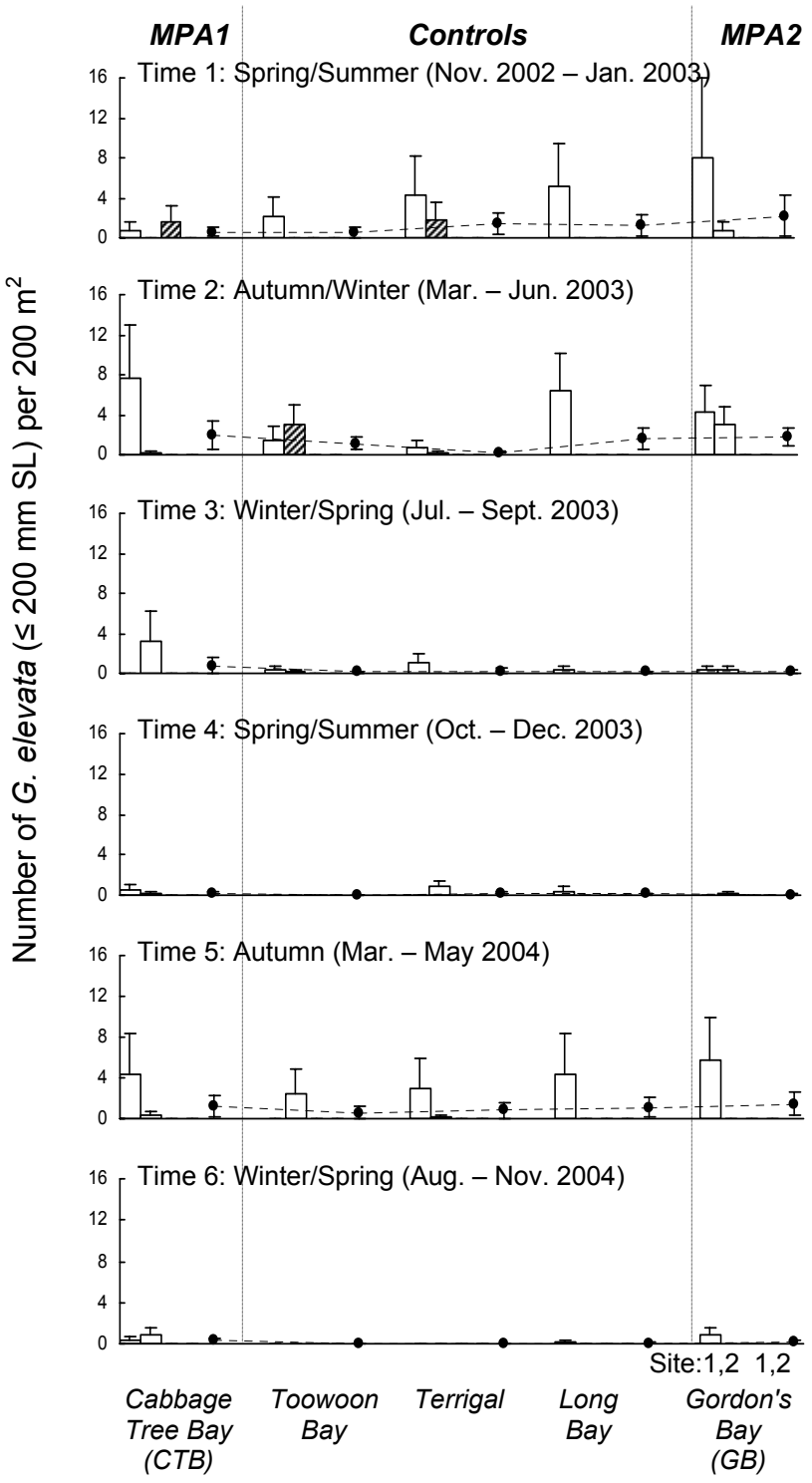

Fig. A.11. Mean abundance (+ SE) of sub-legal *G. elevata* (≤ 200 mm standard length) in shallow (□) and deep (▨) areas of reef in MPA and control locations at six sampling times. Abundances are shown for each of two sites (separated by 100's m) within each location. n = 5 replicates at each depth. ● = Mean abundance for location (± SE).

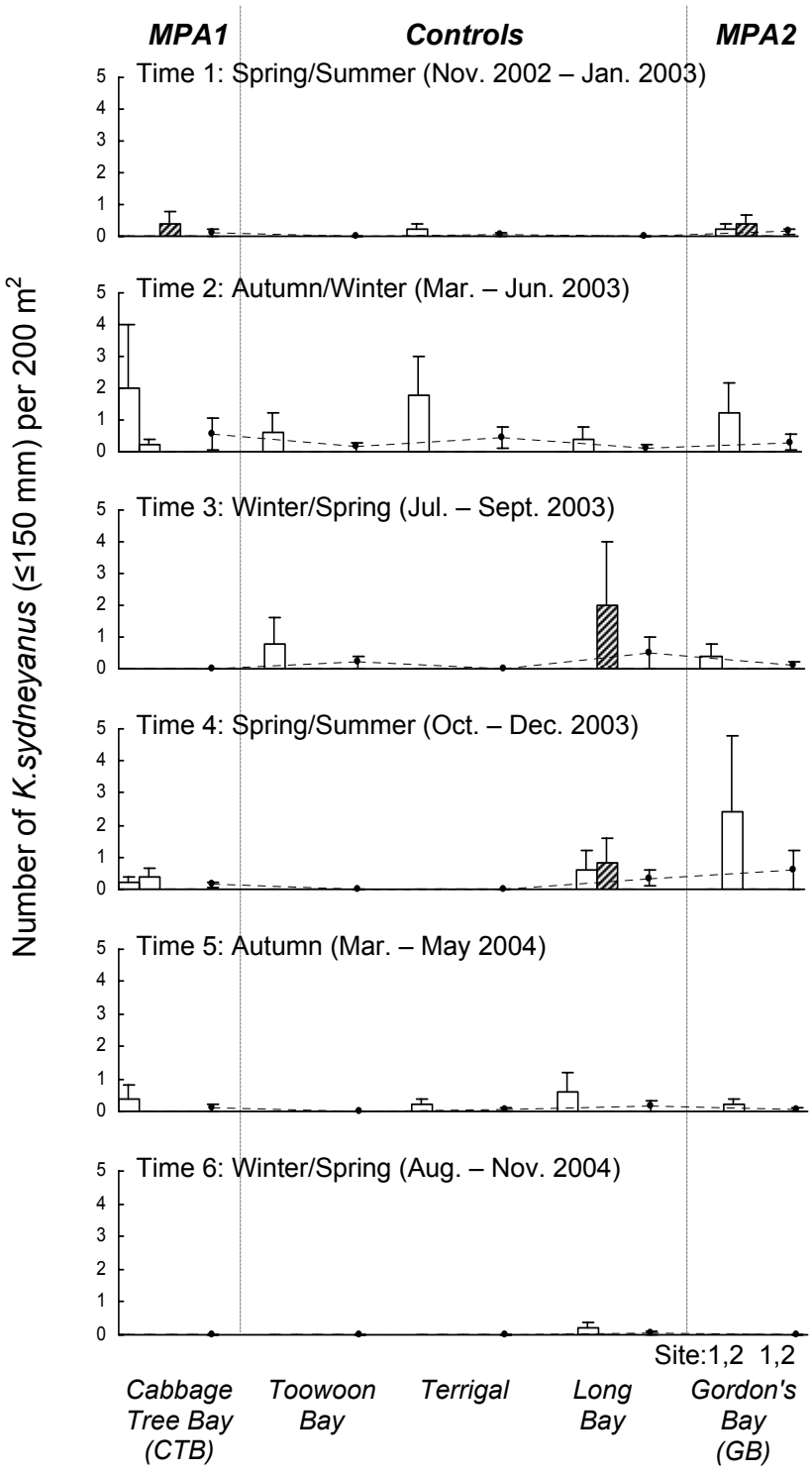

Fig. A.12. Mean abundance (+ SE) of small *K. sydneyanus* (≤ 150 mm standard length) in shallow (☐) and deep (▨) areas of reef in MPA and control locations at six sampling times. Abundances are shown for each of two sites (separated by 100's m) within each location. n = 5 replicates at each depth. ● = Mean abundance for location (± SE).

Appendix B: GPS Co-ordinates

Table B.1. GPS co-ordinates for collection sites given in Fig. 3.1 and Fig. 4.1

Region/location	Site	Latitude/longitude
Port Stephens		
1	1	32°43'S, 152°52'E
2	1	32°72'S, 152°19'E
	2	32°75'S, 152°17'E
	1	32°79'S, 152°11'E
	2	32°79'S, 152°09'E
Sydney		
1	1	33°28'S, 151°57'E
	2	33°30'S, 151°56'E
2	1	33°45'S, 151°45'E
	2	33°47'S, 151°44'E
3	1	33°62'S, 151°33'E
	2	33°64'S, 151°34'E
4	1	33°95'S, 151°26'E
	2	33°97'S, 151°25'E
Jervis Bay		
1	1	34°56'S, 150°87'E
	2	34°59'S, 150°88'E
2	1	34°75'S, 150°83'E
	2	34°78'S, 150°82'E
3	1	35°01'S, 150°83'E
	2	35°01'S, 150°84'E
4	1	35°19'S, 150°59'E
	2	35°21'S, 150°56'E

Appendix C: Population Genetics *P. microlepis*

Molecular Ecology Notes (2004) 4, 551-553

Isolation of highly polymorphic microsatellite loci from the temperate damselfish *Parma microlepis*

Belinda G. Curley and Michael R. Gillings

Abstract
Microsatellites were isolated from the damselfish *Parma microlepis* (Gunther 1862) (*Pomacentridae*) and screened for 100 individuals. Seven of the eight loci tested were highly polymorphic, having 14-43 alleles with average heterozygosities between 0.86 and 0.97. These loci should be informative for studies on population genetics of this species.

Keywords: connectivity, genetic diversity, microsatellites, *Parma microlepis, Pomacentridae,* temperate reefs

Appendix D: Population Genetics G. tricuspidata

Molecular Ecology Notes (2006) 6, 428-430

Isolation of microsatellites from *Girella tricuspidata*

Belinda G. Curley and Michael R. Gillings

Abstract
Microsatellites were isolated from *Girella tricuspidata (Girellidae*) and screened for 64 individuals collected from coastal reefs in New South Wales, Australia. All seven loci tested were highly polymorphic, having seven to 42 alleles with average heterozygositites between 0.44 and 1.0. One locus (GT1N8) had a significant excess of homozygotes, probably due to the presence of null alleles. These microsatellite loci should be informative for examining population genetics of this species.

Keywords: temperate reefs, connectivity, microsatellites, genetic diversity, *Girellidae*