The genetic structure of New Zealand's coastal benthos: using the estuarine clam *Austrovenus stutchburyi*, to determine rates of gene flow and population connectivity

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Said the duck to the kangaroo,

‘Good gracious! How you hop!

Over the fields and the water too,

As if you never would stop!

My life is a bore in this nasty pond,

And I long to go out in the world beyond!

I wish I could hop like you!’

Said the duck to the kangaroo.

Edward Lear

Abstract

For many benthic marine taxa, dispersal over large distances is dependent on a pelagic larval phase. It is through the dispersal process that benthic taxa can found new populations, colonise disturbed or degraded habitats and achieve genetic exchange between geographically separated subpopulations. While it is widely accepted that the dispersal process is important in determining the dynamics of many marine communities, difficulties associated with the direct measurement of larval dispersal mean that detailed knowledge of the mechanisms controlling larval transport has been elusive. Consequently, indirect methods, such as population genetics, have been used to estimate dispersal pathways. This thesis examines the population genetic structure of New Zealand's coastal benthos with the aim of enhancing our understanding of population connectivity, as well as identifying physical and biological processes that might influence population genetic structure.

The population genetic structure of New Zealand's coastal benthos was examined in a quantitative literature review. Divergence between northern and southern populations was identified as the most frequently reported population genetic structure, with the divergence most often located in central New Zealand. Additional population structure although rare, was reported for a number of taxa, particularly those restricted to estuaries and with life history traits indicative of limited dispersal potential. A significant negative correlation between pelagic larval duration (PLD) and genetic divergence suggests that PLD may be a useful proxy for a species' dispersal ability. However, variation in

estimates of divergence for taxa with limited PLDs suggests that other factors may also influence dispersal potential.

To determine whether populations of an estuarine organism with life history traits indicative of widespread dispersal are genetically subdivided, I examined the population genetic structure of the clam *Austrovenus stutchburyi* using the mitochondrial gene cytochrome oxidase *c* subunit I (COI). Analyses indicated that dispersal was limited and that gene flow was mostly occurring among estuaries in close proximity. Genetic boundaries were detected in central New Zealand, about the East and North Capes and in the south of the South Island. Similar boundaries have been reported for estuarine taxa lacking a dispersive larval phase suggesting that distribution and habitat requirements may influence patterns of gene flow.

To further investigate gene flow among estuaries, I conducted a multi-scale genetic analysis of *A. stutchburyi* populations on New Zealand's west coast Analyses of COI and microsatellite loci showed populations to be well connected within estuaries and at the regional sampling scale (≤ 156 km) although not at a national scale (≤ 1226 km). Distance among populations explained much of the observed genetic variation as did distance between estuaries. Long stretches of open coast appear to act as barriers to dispersal among *A. stutchburyi* populations. However, as long stretches of open coast also coincide with putative hydrodynamic dispersal barriers there is some uncertainty about the roles of barriers versus inter-estuary distance in generating the observed divergences. Within-estuary genetic differences were evident from COI analyses, but may result from local adaptation to within-estuary environmental gradients.

I conclude that genetic connectivity among populations of New Zealand's estuarine taxa is generally low relative to coastal species. The data presented here also suggest that migration among patchily distributed habitats, such as estuaries, will be dependent on the spatial arrangement of habitats. Consequently, patchily distributed taxa may experience low rates of ecologically meaningful connectivity, requiring relatively cautious management at small spatial scales to ensure the persistence of intact biological communities in the face of persistent anthropogenic and natural disturbances.

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heterozygosity, Hardy-Weinberg equilibrium probability test *p*-values (*P*) and the null allele frequency for each locus (NULL). Heterozygosities and their significance were calculated in ARLEQUIN with exact tests using a Markov chain (100 000 steps; 1 000 000 chain length).

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Preface

The main body of this thesis comprises three chapters (Chapters 2-4). Chapters 2 and 3 have been published in peer reviewed international scientific journals. I have assumed responsibility for the field work, laboratory and data analysis, and for writing this thesis. The material in this thesis was produced from my own ideas except where referenced. This work was undertaken under the supervision of Conrad Pilditch, Ian Hogg, Carolyn Lundquist and Dick Wilkins.

Chapter 2 has been published by the *New Zealand Journal of Marine and Freshwater Research* (2009), Vol. 43: 1009-1028 under the title "Phylogeography of New Zealand's coastal benthos" by P.M. Ross, I.D. Hogg, C.A. Pilditch and C.J. Lundquist.

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Chapter 1

General Introduction

1.1 Introduction

Two of the most important and longstanding questions in the study of marine ecology are "*where do dispersing larvae go*", and "*where do settling larvae come from"* (Levin 2006). While it is widely accepted that pre-settlement processes such as larval dispersal are of considerable importance in controlling the dynamics of marine communities (Hastings and Harrison 1994; Botsford et al. 2001), we lack a good understanding of the mechanisms that control the transport of larvae and therefore drive variation in the supply of recruits (Levin 2006). As the adult life forms of many benthic marine taxa are sedentary or have limited mobility (Grantham et al. 2003), dispersal over large distances is often dependent on a pelagic larval phase. It is through this dispersal process that benthic taxa can found new populations, colonise disturbed or degraded habitats and achieve genetic exchange between geographically separated subpopulations thereby avoiding the genetic pitfalls associated with inbreeding, small population size and asexual reproduction (Conner & Hartl 2004).

Although interest in the fate of pelagic larvae dates back to the beginning of the twentieth century (Hjort 1926; Thorson 1950; Mayr 1954), recent research into larval sink-source dynamics is to a large extent motivated by the need to understand and manage anthropogenic impacts in the marine environment (Sale et al. 2005). As the exchange of larvae among geographically separated

subpopulations (i.e. population connectivity) will determine population and community dynamics, knowledge of dispersal pathways and migration rates would improve the certainty with which a population's response to disturbance could be predicted, and strategies developed for habitat restoration and the mitigation of anthropogenic impacts (Sale et al. 2005; Thrush et al. 2005). For example, it would be possible to identify populations that are vulnerable to overharvesting or disturbance due to low rates of external recruitment, or conversely, to identify populations that contribute significant numbers of recruits to neighbouring regions. Furthermore, this knowledge could be used to determine the optimal size, configuration and location of marine protected areas (MPAs) within MPA networks, to establish biologically meaningful population management units and to assist with understanding and controlling the spread of invasive species (Sale et al. 2005; Levin 2006).

Unfortunately, data on larval dispersal and connectivity has been elusive. This is primarily due to the difficulties associated with the direct measurement of larval dispersal (Thorrold et al. 2002; Levin 2006). Impediments to direct measurement include the large number and small size of propagules produced in a single spawning event, extremely high natural rates of larval mortality and the potentially large distances over which larvae might disperse. Consequently, direct tracking, telemetry and mark-recapture studies, the methods traditionally used to track dispersal on land have rarely been applied successfully to the measurement of larval dispersal in marine environments (Thorrold et al. 2002). A number of methods for the indirect measurement and estimation of larval dispersal, retention and connectivity have been developed for use in the marine

environment (Hellberg et al. 2002; Thorrold et al. 2002; Levin 2006). These methods include physical models, environmental markers (e.g. elemental fingerprinting) and population genetics (Hedgecock et al. 2007; Thorrold et al. 2007; Werner et al. 2007).

Geographical surveys of genetic variation are one of the methods used most frequently to estimate larval dispersal, providing evidence for the openness of populations (or lack of it) and rates of inter-population larval exchange (Hellberg et al. 2002). Benthic marine organisms often occur in dense aggregations as adults, are highly fecund, undergo external fertilisation, and experience extremely high rates of pre-settlement mortality (Stoner 1990, Grantham et al. 2003). Consequently, genetic drift as a result of non-random mating is expected to be minimal and population genetic structure to reflect patterns of gene flow and natural selection (Hellberg et al. 2002, Carr et al. 2003). For taxa that occur in smaller, less open populations and produce fewer offspring which experience lower levels of mortality, such as marine mammals or benthic invertebrates with direct developing larvae, estimation of connectivity from genetic data may be complicated by potentially high levels of genetic drift.

Although it was once assumed that species with long-lived pelagic larvae would have high levels of inter-population gene flow and consequently exhibit low levels of genetic subdivision (Mayr 1954; Scheltema 1986; Bohonak 1999), the genetic structure of many marine taxa does not conform to these generalisations (Weersing and Toonen 2009). These data suggest that larval retention and restricted dispersal may be more common than once thought. Unfortunately, the genetic makeup of an individual migrant cannot reveal its

origin in the same way as a physical tag. This is because genes are passed through multiple generations with a population's genetic structure reflecting both historical and contemporary gene flow (Avise 1992; Hedgecock 1994; Hellberg et al. 2002). The interpretation of genetic data is further complicated by natural selection which can generate environment specific genetic variants or range wide homogeneity, which may be incorrectly construed as an indication of rates of larval exchange (Schmidt et al. 2008). Population genetic data must therefore be interpreted with some caution and with consideration of processes occurring over multiple spatial and temporal scales.

In New Zealand, genetic surveys have shown for the most part that populations of coastal benthic invertebrates are not genetically homogenous (e.g. Apte and Gardner 2002; Stevens and Hogg 2004; Goldstien et al. 2006). Population structure and genetic subdivision have been identified in species with a variety of life history traits from a number of taxonomic groups. However, the spatial scale at which subdivision has generally been reported is much larger than the distances over which the larvae of these taxa would be expected to disperse (Shanks 2009). Accordingly, there is some uncertainty about the processes generating the observed genetic boundaries and the usefulness of these data in guiding fisheries or conservation resource management. Furthermore, in some cases where genetic differentiation has been detected, low sampling resolution has made it difficult to determine the nature of the genetic relationships (e.g. genetic clines vs. genetic boundaries) or the locations at which genetic changes occur. In this thesis, I aim to address these uncertainties by (1) reviewing existing research examining the geographic

distribution of genetic variation in New Zealand's coastal benthos and (2) performing my own genetic analyses with sampling conducted at a high spatial resolution across multiple sampling scales.

1.2 Organisation of thesis

The main body of this thesis comprises three chapters (2-4) examining the population genetic structure of New Zealand's coastal benthos. In **Chapter 2** I present a quantitative review of studies examining the genetic structure of New Zealand's coastal benthos. My specific objectives were to identify generalities or patterns of population genetic structure shared among taxa, identify the processes implicated in generating subdivision, examine the relationship between larval duration and population genetic structure, and to compare current knowledge of population subdivision with boundaries identified in New Zealand's biogeographic literature. From this review, I was able to identify deficiencies in the methodologies of previous research (e.g. low sampling resolution, few studies in which multiple markers are used, lack of *a priori* hypothesis testing) and gaps in our knowledge of population genetic structure for New Zealand's coastal benthos (e.g. research focused on rocky reef taxa, uncertainties regarding processes generating population structure). I have attempted to address these deficiencies and knowledge gaps in the subsequent chapters of this thesis.

Of particular interest was fact that studies to date have largely focused on open coast rocky reef taxa with life history traits indicative of widespread

dispersal. Relative to taxa lacking pelagic larvae or restricted to estuaries, open coast pelagic dispersers are expected to experience high degrees of connectivity and therefore exhibit low levels of population genetic structure (Bilton et al. 2002; Pelc et al. 2009). The prevalence of open coast taxa in New Zealand's population genetic literature may explain the predominantly large scales at which genetic subdivision has been reported. To examine this hypothesis, I have conducted geographical surveys of genetic variation in the estuary restricted clam *Austrovenus stutchburyi. A. stutchburyi* is a New Zealand endemic venerid clam often found at high densities (up to 1200 individuals m^{-2}) on intertidal sand and mud flats in estuaries throughout New Zealand (Hewitt et al. 1996). *A. stutchburyi* often account for a large proportion of total estuarine biomass and play a key role in nutrient dynamics and primary productivity (Jones et al. 2011; Sandwell et al. 2009). Spawning over several months during summer, *A. stutchburyi* are highly fecund and have a pelagic larval phase of 2-3 weeks during which dispersal over large distances and among geographically discrete populations is possible (Stephenson and Chanley 1979; Booth 1983). The results of my genetic surveys are reported in Chapters 3 and 4.

In **Chapter 3** I present an examination of genetic variation in *A. stutchburyi* populations at a New Zealand wide sampling scale using the mitochondrial cytochrome *c* oxidase subunit I (COI) gene. My specific objectives were to determine whether populations of *A. stutchburyi* are genetically subdivided, identify the locations of genetic boundaries and determine whether these boundaries coincide with previously identified biogeographic break points. A large number of populations were sampled relative to previous analyses to

ensure that the locations of genetic boundaries could be identified with some certainty.

In **Chapter 4,** my final research chapter, I present the results of multi-scale analyses of COI and nuclear microsatellite variation among populations of *A. stutchburyi* on New Zealand's west coast. My objective was to examine the role of inter-population distance in determining rates of connectivity. Specifically, I set out to determine the spatial scale at which *A. stutchburyi* populations are connected and to determine the extent to which geographic distance among populations and between estuaries explains patterns of population genetic structure.

My overall aim in this thesis was to provide information on the genetic connectivity of New Zealand's coastal benthos and the processes responsible for larval transport. The knowledge generated by this research will be applicable to a wide range of marine organisms, both coastal and estuarine as the principles and processes involved in larval transport are ubiquitous throughout the marine environment.

Chapter 2

The genetic structure of New Zealand's coastal benthos

2.1 Introduction

Determining the sources of new recruits to a population is fundamental to ecological research in marine benthic systems. However, determining recruitment pathways has been difficult for many taxa as adults are largely sedentary and dispersal is achieved during a pelagic larval stage (Grantham et al. 2003). Although it is widely acknowledged that pre-settlement pelagic processes play an important role in structuring benthic communities (e.g. Underwood 1981; Menge 1991; Gaines and Bertness 1993), the pelagic component of larval life history remains largely unknown. Numerous synergistic factors including ocean currents and larval duration, behaviour and mortality can determine patterns of larval dispersal (Roughgarden et al. 1985; 1988; Menge 1991; Cowen and Sponaugle 2009). These patterns govern larval exchange between populations, influencing local and metapopulation dynamics, community structure, genetic diversity and the resilience of populations to human exploitation (Hastings and Harrison 1994; Botsford et al. 2001; Cowen et al. 2007). Understanding population connectivity is therefore essential for the effective management of species and ecosystem resources (Botsford et al. 2001; Palumbi 2003; Levin 2006).

Previously, it was considered that few barriers to dispersal existed in the marine environment (Mayr 1954; Scheltema 1986). This paradigm was retained for many years, in part because of the difficulties associated with tracking larval dispersal (Levin 2006; Palumbi et al. 2003). With the advent and subsequent development of molecular techniques it is now possible to estimate rates of larval migration between populations, as a population's genetic structure is likely to reflect patterns of dispersal (Wright 1951; Neigel 1997). Molecular studies have produced conflicting evidence as to whether wide-ranging dispersal and high connectivity are pervasive among marine populations. For example, some studies have shown high levels of connectivity over thousands of kilometres (Takabayashi et al. 2003; Cassista and Hart 2007) whereas in others, boundaries have been identified between genetically distinct populations allowing the location of dispersal barriers to be inferred (e.g. Pelc et al. 2009; Kelly and Palumbi 2010). These results suggest that larval duration on its own may not be a suitable proxy for predicting dispersal potential (Bay et al. 2006). Species specific traits or behaviours and local hydrodynamic conditions are likely to exert considerable influence over rates of connectivity and dispersal pathways.

New Zealand is an archipelago of over 700 islands stretching 2700 km from the subtropical Kermadec islands (29°S) in the north to the subantarctic Auckland and Campbell islands (50°S and 52°S; respectively) in the south (Fig. 2.1; Laing and Chiswell 2003). The oceanography of this region is complex as the archipelago straddles the subtropical convergence, a meeting of subtropical and subantarctic waters (Heath 1982; de Lange et al. 2003; Laing and Chiswell 2003). Numerous major current systems divide as they reach New Zealand's continental

shelf creating many smaller coastal current systems and eddies. Coastal currents are further modified through interactions with topography (e.g. headlands, islands, and canyons) and temporally variable wind, waves, and tide. This spatial and temporal complexity makes it difficult to predict how oceanographic and physical features might influence larval transport and therefore connectivity among coastal populations of benthic marine organisms.

Genetic techniques have been used to examine New Zealand's coastal benthos in order to improve our understanding of meta-population dynamics, larval transport and the processes that determine connectivity among populations. In this chapter I review studies examining spatial variation in the distribution of genetic diversity for New Zealand's coastal flora and fauna. These studies are published as peer-reviewed journal articles, graduate theses, and government reports. I have focused specifically on benthic invertebrates and plants, excluding highly mobile taxa such as fish. Studies of recently introduced species (e.g. Smith et al. 1986) have also been excluded as their genetic structure is likely to be influenced by their method of introduction and therefore not indicative of natural patterns of population connectivity (Provan et al. 2005; Rius et al. 2008). I have identified generalities and/or patterns in population structure from the reviewed literature as well as the processes implicated in generating subdivision. I have also examined the relationship between larval duration and population differentiation and compared current knowledge of population subdivision with boundaries identified in biogeographic classification schemes.

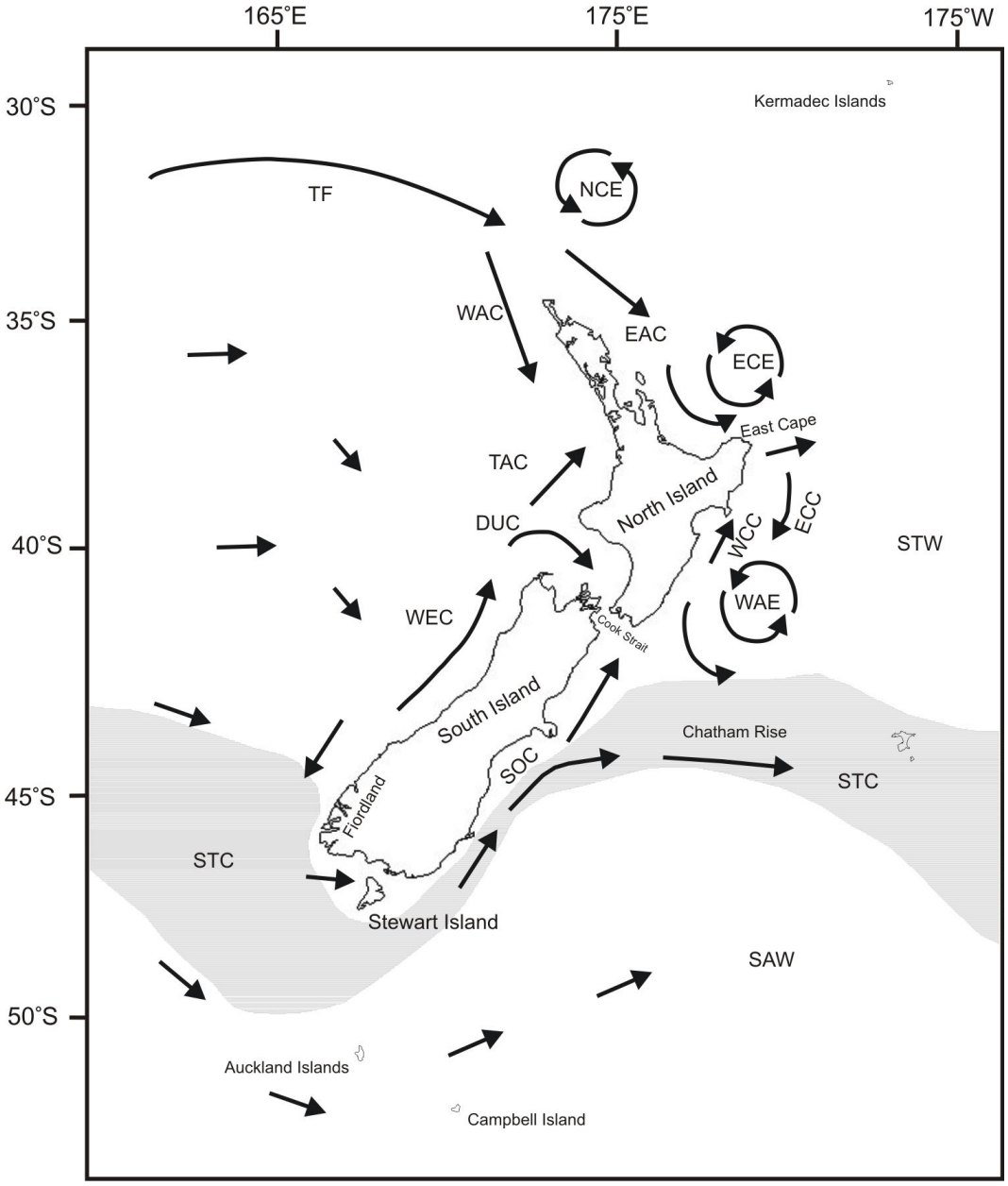

Figure 2.1. New Zealand's major coastal current systems and boundaries between water masses. D'Urville Current (DUC); East Auckland Current(EAC); East Cape Current (ECC); East Cape Eddy (ECE); North Cape Eddy (NCE); Southland Current (SOC); Subantarctic water (SAW); Subtropical Convergence (STC); Subtropical water (STW); Tasman Current (TAC); Tasman Front (TF); West Auckland Current (WAC); Wairarapa Eddy (WAE); Wairarapa Coastal Current (WCC); Westland Current (WEC). Figure redrawn after Heath (1982) and Carter et al. (1998).

2.2 New Zealand literature

Forty-two population genetic studies, published between 1980 and 2008 were included in this review and meta-analysis (Table 2.1). Where more than one species was included in a single publication, each was considered separately. During this period (1980-2008), there has been an increase in the number of studies using molecular techniques as well as in the types and number of molecular markers used (Fig. 2.2, Table 2.1). Early studies predominantly used allozyme electrophoresis, later increasing the use of mitochondrial sequence and fragment analyses and nuclear DNA analyses as these molecular techniques became increasingly available and affordable (for descriptions of markers and their uses in molecular ecology see Wan et al. 2004; DeYoung and Honeycutt 2005; Anne 2006). As molecular methods have developed, concerns about the comparability of genetic data have arisen where analyses of different markers in a single species have produced conflicting results (e.g. green lipped mussel *Perna canaliculus,* Smith 1988; Gardner et al. 1996; Apte and Gardner 2001, 2002). However, recent studies have produced results that are more consistent both within and across taxa (Apte and Gardner 2002; Apte et al. 2003; Star et al. 2003; Goldstien et al. 2006). It is possible that some of the variability in genetic patterns and lack of genetic structure reported among early studies are a reflection of the low numbers of populations and individuals typically sampled in early population genetic research (Table 2.1).

Table 2.1 Summary of population genetic studies of coastal benthic invertebrates and plants undertaken in New Zealand. Pelagic larval duration (PLD); number of populations sampled (*n*); New Zealand (NZ); mitochondrial DNA (mtDNA); restriction fragment length polymorphism (RFLP); amplified fragment length polymorphism (AFLP); nuclear DNA (nDNA); internal transcribed spacer (ITS); cytochrome c oxidase subunit I (COI); displacement loop (D–loop); NADH dehydrogenase subunit IV (NADH IV); Random Amplification of Polymorphic DNA (RAPD),. Unless specified, reproductive strategy and PLD were given in the cited study or references therein. *Number indicates sampling spread when North and South Island outgroups are included. (a) Stevens 1991, (b) Perrin et al. 2004, (c) Lamare 1997, (d) Tong et al. 1992, (e) Buchanan 1994.

Species	Subphylum or class	Reproductive strategy	PLD	Reference	Distribution of sampling	Sampling scale (°latitude)	*n*	Results	Genetic marker
Arthropoda									
Jasus edwardsii	Crustacea	Pelagic larvae	possibly >1yr	Smith & McKoy 1980	NZ wide	8	3	No structure	Allozymes
Jasus edwardsii	Crustacea	Pelagic larvae	possibly >1yr	Ovenden et al. 1992	NZ east coast	7	2	No structure	mtDNA RFLP
Jasus verreauxi	Crustacea	Pelagic larvae	> 8 months	Brasher et al. 1992	NZ north-east coast	3	2	No structure	mtDNA RFLP
Paracorophium excavatum	Crustacea	Brooded young	No pelagic larval phase	Schnabel 1998, Schabel et al. 2000	NZ east coast	8	4	North-south differentiation	Allozymes
Paracorophium excavatum	Crustacea	Brooded young	No pelagic larval phase	Stevens & Hogg 2004	NZ wide	9	21	North-south differentiation	Allozymes
Paracorophium lucasi	Crustacea	Brooded young	No pelagic larval phase	Schnabel 1998, Schabel et al. 2000	Central New Zealand	3	11	East-west & north-south differentiation	Allozymes
Paracorophium lucasi	Crustacea	Brooded young	No pelagic larval phase	Stevens & Hogg 2004	NZ wide	6	18	North-south & east-west differentiation	Allozymes
Pinnotheres novaezelandiae	Crustacea	Pelagic larvae	Unknown - Genera mean = 23 days (a)	Stevens 1990	NZ North Island	6	5	Differentiation within and among locations	Allozymes
Pinnotheres atrinicola	Crustacea	Pelagic larvae	Unknown - Genera mean = 23 days	Stevens 1991	NZ North Island	6	7	Latitudinal clinal pattern	Allozymes

Species	Subphylum or class	Reproductive strategy	PLD	Reference	Distribution of sampling	Sampling scale (°latitude)	*n*	Results	Genetic marker
Brachiopoda									
Liothyrella neozelanica	Articulata	Brooded young	No pelagic larval phase	Ostrow 2004	Fiordland	<1	6	Differentiation within and among fiords	AFLP
Terebratella sanguinea	Articulata	Pelagic Larvae	unknown	Ostrow 2004	Fiordland and Stewart Island	3	23	Some differentiation among fiords	Allozymes & AFLP
Cnidaria									
Actinia tenebrosa	Anthozoa	Brooded young	No pelagic larval phase	Veale 2007	NZ wide	11	27	Isolation by distance	Microsatellites
Antipathes fiordensis	Anthozoa	Marginally pelagic larvae	~10 days	Miller 1997	Fiordland & Stewart Island	3	28	Divergence within and among fiords	Allozymes
Antipathes fiordensis	Anthozoa	Marginally pelagic larvae	~10 days	Miller 1998	Doubtful Sound (Fiordland)	<1	3	Data suggests very limited dispersal within fiords	Allozymes
Errina novaezealandia	Anthozoa	Brooded young	No pelagic larval phase	Miller et al. 2004	Fiordland	<1	9	Differentiation within and among fiords	Allozymes
Echinodermata									
Amphipholis squamata	Ophiuroidea	Brooded young	No pelagic larval phase	Sponer 2002, Sponer & Roy 2002	NZ wide	12	16	North - south differentiation	mtDNA 16s (nDNA ITS)
Astrobrachion constrictum	Articulata	Pelagic Larvae	4-8 days	Steele 1999	Fiordland	1	7	No Structure	Allozymes & mtDNA COI
Coscinasterias muricata	Asteroidea	Pelagic larvae	~30 days (b)	Waters & Roy 2003	NZ wide	10	4	No Structure	mtDNA COI (& nDNA ITS2)
Coscinasterias muricata	Asteroidea	Pelagic larvae	~30 days	Perrin 2002, Perrin et al. 2004	Fiordland (& 2 South Island & 1 North Island sites)	2 (10)*	17	Isolation-by-distance amongst northern fiords. Restricted gene flow between southern fiords	mtDNA D-loop
Coscinasterias muricata	Asteroidea	Pelagic larvae	~30 days (b)	Skold et al 2003	Fiordland (& 2 South Island & 1 North Island sites)	2 (10)*	16	Differentiation among fiords	Allozymes

Species	Subphylum or class	Reproductive strategy	PLD	Reference	Distribution of sampling	Sampling scale (°latitude)	*n*	Results	Genetic marker
Evechinus chloroticus	Echinoidia	Pelagic larvae	3-6 weeks (b)	Mladenov et al. 1997	NZ wide	11	6	Doubtful Sound distinct from other NZ populations	Allozymes
Evechinus chloroticus	Echinoidia	Pelagic larvae	3-6 weeks (c)	Perrin 2002	Fiordland (& 2 South Island & 2 North Island sites)	2 (12)*	20	Differentiation within and among fiords	Microsatellites
Ophiomyxa brevirima	Ophiuroidea	Brooded young	No pelagic larval phase	Garrett 1995	South & Stewart Islands	5	4	Differentiation between populations	Allozymes
Patiriella regularis	Asteroidea	Pelagic larvae	9-10 weeks	Waters & Roy 2004	NZ wide	12	19	North-south differentiation.	mtDNA control region
Patiriella regularis	Asteroidea	Pelagic larvae	9-10 weeks	Ayers & Waters 2005	NZ wide	12	22	North-south differentiation.	mtDNA control region
Mollusca									
Austrovenus stutchburyi	Bivalvia	Pelagic larvae	2-3 weeks	Lidgard 2001	NZ wide			No structure	Allozymes
Cellana flava	Gastropoda	Pelagic larvae	3-11 days	Goldstien 2005, Goldstien et al. 2006	NZ wide	5	8	North-south differentiation	mtDNA cytochrome b
Cellana ornata	Gastropoda	Pelagic larvae	3-11 days	Goldstien 2005, Goldstien et al. 2006	NZ wide	12	31	North-south differentiation	mtDNA cytochrome b
Cellana radians	Gastropoda	Pelagic larvae	3-11 days	Goldstien 2005, Goldstien et al. 2006	NZ wide	12	31	North-south differentiation	mtDNA cytochrome b
Haliotis iris	Gastropoda	Pelagic larvae	5-9 days(d)	Smith and McVeagh 2006	NZ wide	11	4	Differentiation between locations	mtDNA COI & microsatellites
Nerita atramentosa	Gastropoda	Pelagic larvae	5-6 months	Waters et al. 2005	Northern NZ & Three Kings	4	10	No structure	mtDNA COI
Paphies subtriangulata	Bivalvia	Pelagic larvae	~3 weeks	Smith et al. 1989	NZ wide	11	13	Distinct north, central & Chatham Islands populations	Allozymes

Species	Subphylum or class	Reproductive strategy	PLD	Reference	Distribution of sampling	Sampling scale (°latitude)	*n*	Results	Genetic marker
Perna canaliculus	Bivalvia	Pelagic larvae	>4 weeks (e)	Smith 1988	NZ wide	11	6	North - south differentiation	Allozymes
Perna canaliculus	Bivalvia	Pelagic larvae	>4 weeks (e)	Gardner et al. 1996	NZ wide	11	10	Isolation by distance	Allozymes
Perna canaliculus	Bivalvia	Pelagic larvae	>4 weeks (e)	Apte & Gardner 2001	NZ wide	12	35	No structure	Allozymes
Perna canaliculus	Bivalvia	Pelagic larvae	>4 weeks (e)	Apte & Gardner 2002	NZ wide	12	22	North-south differentiation	mtDNA NADH IV
Perna canaliculus	Bivalvia	Pelagic larvae	>4 weeks (e)	Star et al. 2003	NZ wide	12	19	North-south differentiation	RAPD
Perna canaliculus	Bivalvia	Pelagic larvae	>4 weeks (e)	Apte et al. 2003	NZ wide	12	36	North-south differentiation	Allozymes, mtDNA & RAPD
Scutellastra kermadecensis	Gastropoda	Pelagic larvae	unknown - possibly 4-10 days	Wood & Gardner 2007	Kermadecs: northern and central Islands	1	11	Differentiation within and among regions: Isolation-by-distance at small scales	RAPD
Siphonaria raoulensis	Gastropoda	Pelagic larvae	unknown - possibly 9days - 10 weeks	Wood & Gardner 2007	Kermadecs: northern Islands	<1	6	Differentiation within and among regions	RAPD
Sypharochiton pelliserpentis	Gastropoda	Pelagic larvae	unknown - possibly <4 days	Veale 2007	NZ wide	12	28	North-south differentiation & east-west in northern NZ.	mtDNA COI
Plantae									
Zostera muelleri	Magnoliophyta	Mainly vegetative	No pelagic larval phase - dispersal as adult	Jones 2004, Jones et al. 2008	NZ wide	11	8	North-south & east-west differentiation	RAPD

The reviewed studies have covered twenty-nine species, the majority of which were crustaceans, echinoderms, or molluscs. These taxa possess a range of dispersal strategies including brooding species that lack a dispersive larval stage (e.g. the anemone *Actinia tenebrosa*), species with pelagic larval durations (PLDs) ranging from as little as a few days (e.g. the limpet *Cellana ornata*) to greater than 12 months (e.g. the rock lobster *Jasus edwardsii*), and one plant (the seagrass *Zostera muelleri*) able to undergo dispersal in its adult form (Table 2.1).

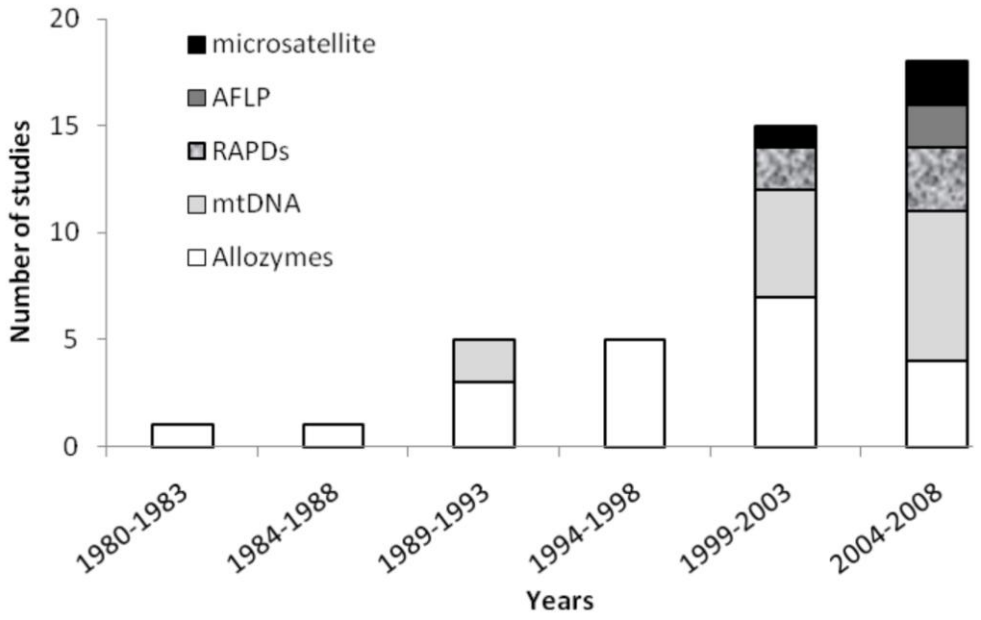

Figure 2.2 Number of population genetic studies of the New Zealand coastal benthos published between 1980 and 2008 and molecular techniques used. Amplified fragment length polymorphisms (AFLP); random amplification of polymorphic DNA (RAPD); mitochondrial DNA (mtDNA).

2.2.1 Panmictic taxa

An absence of genetic subdivision was reported for eight of the reviewed studies covering seven taxa (Table 2.1). This suggests that for these species, gene flow is high, barriers to dispersal are absent, and that populations can be considered as part of a single interbreeding unit. For the rock lobsters *J. edwardsii* and *J. verreauxi* with PLDs of 8–15 months and the gastropod *Nerita atramentosa* with a PLD of 5–6 months this lack of genetic structure was understandable (Smith and McKoy 1980; Ovenden et al. 1992; Brasher et al. 1992; Waters et al. 2005). Connectivity across great distances is expected for taxa with extended PLDs (Mayr 1954; Scheltema 1986) regardless of temporal variability in coastal oceanography or the occurrence of short-lived hydrological dispersal barriers (e.g. Roberts and Paul 1978). Conversely, for the bivalve *Austrovenus stutchburyi* and the echinoderm *Coscinasterias muricata*, both with shorter PLDs (2–5 weeks), the lack of population subdivision was unexpected (Lidgard 2001; Waters and Roy 2003).

2.2.2 North-south population differentiation

The population genetic pattern most frequently reported in the reviewed studies was that of genetically distinct northern and southern populations. This pattern was detected in 16 of the 26 studies in which populations were sampled at a New Zealand-wide scale (5-12° latitude; Table 2.1). On the west coast of New Zealand, the location of this divergence has been consistently reported at the top of the South Island or in the vicinity of Cook Strait (e.g. Apte et al. 2003; Stevens and Hogg 2004; Ayers and Waters 2005; Goldstien et al. 2006; Veale 2007; Jones et al. 2008), whereas on the east coast the location of divergence has been more

variable. A division was detected in the East Cape region (Fig. 2.1) for two species of estuarine amphipod (*Paracorophium lucasi* and *P. excavatum*; Stevens and Hogg 2004), whereas for other taxa the demarcation between northern and southern populations has been located in the vicinity of Cape Campbell (Fig. 2.3) on the east coast of the South Island (e.g. limpet; (*C. ornata*, Goldstien et al. 2006), the green lipped mussel (*P. canaliculus*, Apte and Gardner 2002; Apte et al. 2003), the cushion star (*Patiriella regularis*, Waters and Roy 2004; Ayers and Waters 2005), the brittle star (*Amphipholis squamata*, Sponer and Roy 2002) and the snakeskin chiton (*Sypharochiton pelliserpentis*, Veale 2007)). Divergence between northern and southern populations was also detected in some Fiordland-focused studies that included North Island outgroups (e.g. Perrin 2002; Skold et al. 2003; Perrin et al. 2004). However, owing to the small number of northern populations included in these studies, no conclusions were able to be made about the location of genetic breaks occurring between northern and southern populations.

Where a genetic division between northern and southern populations occurs in the upper South Island, upwelling has often been implicated as a barrier to larval dispersal (Apte and Gardner 2002; Star et al. 2003; Waters and Roy 2004; Ayers and Waters 2005; Veale 2007). It has been hypothesised that upwellings on the northern east and west coasts of the South Island could cause larvae passing into these regions to be advected offshore and lost to coastal systems. Upwelling as a barrier to population connectivity and larval dispersal has been studied in a number of systems, particularly in areas with well characterised oceanography such as Chile (Poulin et al. 2002; Narvaez et al.

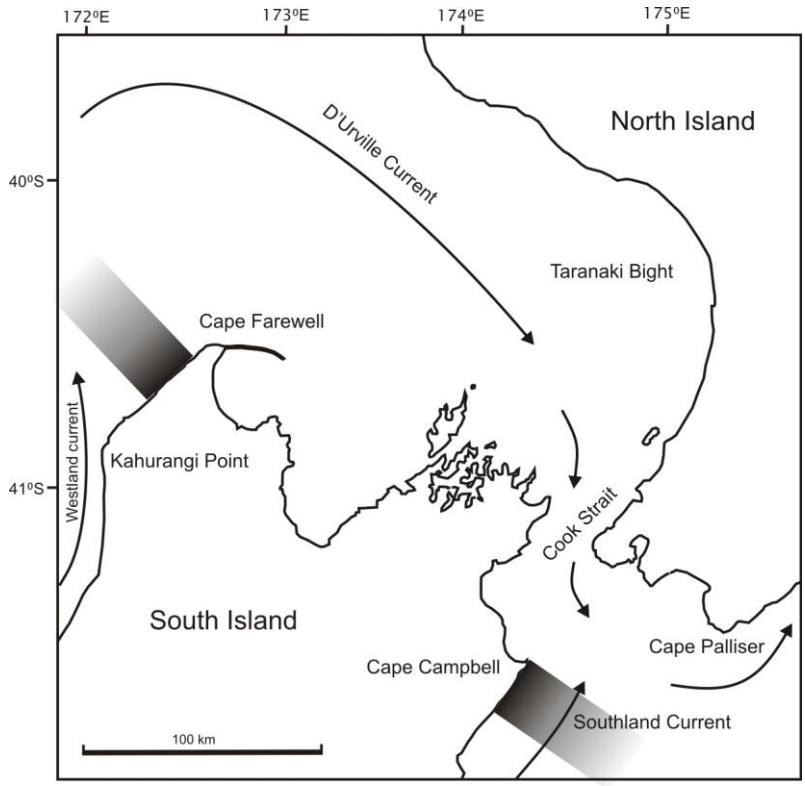

Fig. 2.3 The Cook Strait region (New Zealand) with major currents and locations of interest indicated (redrawn after Heath (1982) and Carter et al. (1998)). Dotted lines south of Cook Strait indicate the location of upwelling zones implicated in preventing larval transport between northern and southern populations (Apte and Gardner 2002).

2006), the west coast of the United States (Roughgarden et al. 1988; Hohenlohe 2004), the Iberian Peninsula, Portugal (Santos et al. 2007), and the west coast of Africa (Lett et al. 2007). In these areas, which are dominated by major current systems (i.e. Humbolt, California, Canary, and Benguela currents), upwelling occurs seasonally and once established may persist for months (Largier et al. 1993). Even in these systems, there is limited evidence to suggest that upwelling isolates coastal populations (Hohenlohe 2004; Shanks and Brink 2005; Narvaez et al. 2006; Lett et al. 2007; Johansson et al. 2008). There is, however, evidence to

suggest that the effects of upwelling on larval transport are strongly modified by larval behaviour (Poulin et al. 2002, Shanks and Brink 2005). Shanks and Brink (2005) demonstrated that vertical positioning could determine the cross-shelf transport of larvae under upwelling conditions. They observed that the larvae of some species were transported offshore during upwelling as predicted, whereas others were moved shoreward or maintained position relative to the coast despite experiencing the same cross-shelf currents. Consequently, in the absence of detailed sampling, upwelling should not be invoked as a cause of variation in larval settlement patterns.

New Zealand, weather patterns are highly variable and wind-driven oceanographic features such as upwellings will vary at equivalent time scales (Heath 1972; de Lange et al. 2003; Laing and Chiswell 2003). Throughout any given breeding season, particularly where spawning duration is protracted, it is likely that larvae will experience both upwelling and downwelling conditions. Accordingly, upwelling alone is unlikely to be responsible for the genetic divergence observed across this region. Detailed small spatial scale studies of oceanography and inter-population genetic variation and a more complete understanding of the consequences of larval behaviour are required if this hypothesis is to be thoroughly tested.

An alternative to upwelling as the cause of north-south subdivision on the east coast is the East Cape Current (ECC) and Wairarapa Eddy. The ECC flows from East Cape down the east coast of the North Island to approximately 42°S– the same latitude as the upwelling at the top of the South Island (cf. Fig. 2.1 and 2.3). At the southern limit of its flow, the ECC splits with some of its flow

proceeding to the north-east while the remainder flows into and along the subtropical convergence (de Lange 2003; Laing and Chiswell 2003; Chiswell 2005). The transport of larvae from northern to southern populations on the east coast could be prevented if larvae carried south by the ECC are advected either back to the north or out across the Chatham Rise. Chiswell and Roemmich (1998) simulated larval trajectories around the East Cape region and suggested that if larvae were passively drifting they could potentially be retained in the Wairarapa and East Cape eddies for up to 2–3 years. For taxa with short pelagic larval durations (e.g. *Cellana* spp., PLD 3-11 days; Goldstien et al. 2006), retention within an eddy even for a short time would probably be terminal as larvae attaining settlement maturity would not reach the required habitat within their larval life stage. Conversely, for *J. edwardsii* larvae which have a pelagic duration of over 12 months (Booth 1994) and for other species with long-lived pelagic larvae, retention in eddies or gyres could act to promote local recruitment.

On the west coast of the South Island there is little data to support the hypothesis that upwelling is a barrier to gene flow and a cause of divergence between northern and southern populations. The Westland Current generally flows in a northerly direction along the west coast of the South Island before merging with the D'Urville Current and feeding into the South Taranaki Bight (Fig. 2.3; Heath 1982; de Lange et al. 2003; Laing and Chiswell 2003). The D'Urville Current sweeps into Cook Strait from the northwest, mixing with water from the Southland and East Cape currents before moving eastwards across Cook Strait and around Cape Palliser (Heath 1982). Upwelling does occur on the northwest coast between Kahurangi Point and Cape Farewell (Fig. 2.3), but is temporally

variable in intensity and occurrence (Shirtcliffe et al. 1990). During upwelling, water is advected offshore with much of it moving northeast and into the D'Urville Current then Cook Strait (Bradford-Grieve et al. 1993). Rather than preventing the transport of larvae between populations north and south of Cape Farewell, this hydrology may promote the mixing of larvae from the greater Cook Strait region, although the degree of larval transport will vary with larval behaviour and PLD (Bradford-Grieve et al. 1993; Shanks and Brinks 2005). Possible indications of this mixing may be found in the genetic makeup of some Cook Strait populations where both northern and southern haplotypes are present (e.g. Veale 2007) or where differentiation among central New Zealand populations is small (e.g. Waters and Roy 2004).

2.2.3 East-west population differentiation

Of the 15 studies in which multiple populations were sampled on both east and west coasts of the North and South islands, divergent east and west coast populations were detected in four studies of three species. These include the amphipod *P. lucasi* (Schnabel et al. 2000; Stevens and Hogg 2004) and the snakeskin chiton *S. pelliserpentis* (Veale 2007) in which North Island populations were distinct between coasts, and the seagrass *Z. muelleri* (Jones et al. 2008) in which distinct east and west coast populations were detected in both the North and South Islands.

Upwelling at the top of the North Island (Roberts and Paul 1978) was suggested as a possible impediment for gene flow between coasts (Veale 2007). However, without detailed knowledge of local oceanography and larval life history, this hypothesis should be treated with caution (Shanks and Brink 2005).

It has also been hypothesised that east-west differentiation in the North Island is a consequence of the significant geological changes that New Zealand has undergone over the last 65 million years (Stevens and Hogg 2004). Marine intrusions during the upper Miocene and Pliocene may have turned what is now the North Island into an archipelago of smaller ephemeral islands (Fleming 1979; Stevens et al. 1995). East-west migration among populations would have been possible until sea level dropped, creating a barrier that physically separated populations. The few examples of east-west differentiation may relate to the timing with which species arrived in New Zealand and whether their population structure has been influenced by geological processes (Lamb and Avise 1992; Knowlton et al. 1993; Stevens and Hogg 2004). Alternatively, differences in population subdivision may relate to species-specific differences in ability to disperse across hydrodynamic features such as the north coast upwelling as suggested by Veale (2007) and/or the requirement for specific settlement habitats (e.g. estuaries; Stevens and Hogg 2004; Jones et al. 2008).

2.2.4 Small-scale population genetic structure

Few studies have detected population structure either within a region or along stretches of continuous coast. Where reported, it has largely been in studies designed specifically to test hypotheses at small spatial scales, with the majority being conducted in Fiordland. An exception is Veale's (2007) New Zealand-wide study in which microsatellite loci were used to examine the population genetic structure of the Waratah anemone *A. tenebrosa*. Seven regional *A. tenebrosa* subpopulations were described and an isolation-by-distance relationship among populations was attributed to the reproductive characteristics of the species

(Veale 2007). As most comparable studies have used mtDNA, it is not possible to determine whether the finer-scale resolution attained in this study resulted from differences in the molecular marker used (mtDNA versus microsatellite) or life history characteristics of *A. tenebrosa*. Smith and McVeagh (2006) also used microsatellite markers and found significant geographic differentiation among populations of the abalone *Haliotis iris*. However, as only four locations were sampled it was not possible to determine the spatial scale at which differentiation occurred.

Fiordland in the southwestern corner of the South Island is an ideal system for examining population connectivity as each fiord constitutes a discrete habitat isolated by geography and hydrology (Stanton and Pickard 1981; Lamare 1998; Gibbs 2001). As a consequence of extremely high rainfall in the region (up to 7 m per year), the fiords have a low salinity surface layer and two layer estuarine circulation (Stanton and Pickard 1981; Gibbs 2001). This circulation will determine the degree to which larvae are physically transported within and among fiords (Lamare 1998; Metaxas 2001; Bilton et al. 2002; Wing et al. 2003). Depending on the life history characteristics of larvae, such as vertical positioning in the water column and tolerance to the low salinity outflowing surface waters, it is possible that larvae will be retained within the fiords limiting connectivity among fiord populations (Lamare 1998).

Population genetic structure has been examined in nine studies of Fiordland taxa (Table 2.1). Genetic differentiation has been reported at very small spatial scales (<50 m) for some species (e.g. the coral *Antipathes fiordensis*; Miller 1998), whereas other species such as the snake star (*Astrobrachion*

constrictum; Steele 1999) with similar dispersal potential (i.e. PLD) showed little evidence of restricted larval exchange. Although all but one of these studies detected population subdivision either within or among the fiords, interpretation of genetic patterns has been difficult. For example, Skold et al. (2003) detected significant variation among fiord populations of *Coscinasterias muricata* using allozyme electrophoresis, but found no correlation between genetic structure and geographic distribution. In contrast, Perrin et al. (2004) analysed mtDNA of the same species at the same sites and detected an isolation-by-distance relationship among populations in the northern fiords and restricted gene flow between southern fiords particularly Long Sound.

In another study, populations of the urchin, *Evechinus chloroticus* clustered into two groups corresponding to inner and outer fiord environments (Perrin 2002). Inner fiord populations among fiords were more similar to each other than were inner and outer populations within the same fiord. This was also the case for outer fiord populations among fiords. Although often thought to be neutral (Schlotterer 2000), microsatellite loci may be under selection themselves or linked to DNA that is under selection (*sensu* Wright and Andolfatto 2008). If the loci used in the Perrin (2002) study were under selection, the observed genetic structure could reflect adaptation to and/or differential settlement/survival in sub-habitats within the fiords rather than patterns of connectivity among populations.

In the only study conducted on offshore islands, Wood and Gardner (2007) examined the population genetic structure of two limpets (*Siphonaria raoulensis* and *Scutellastra kermadecensis*) endemic to the isolated Kermadec

Islands (Fig 2.1). The assumption of self-recruitment was used to test hypotheses on connectivity among islands and to examine the scale over which self-recruitment occurs. Limited connectivity was observed among populations separated by less than 1 km suggesting that larvae did not disperse or alternatively settle far from their population of origin (Wood and Gardner 2007).

2.3 Larval duration and population genetic structure

Although some common patterns of geographical subdivision have emerged from New Zealand population genetic studies, patterns have not been consistent across taxa, suggesting that species-specific traits and behaviours are likely to influence genetic structure and connectivity. While it is widely accepted that larval behaviour interacts with hydrology to determine physical transport (Shanks and Brink 2005), the complexities of coastal oceanography have made it difficult to predict dispersal pathways and explain the observed differences in genetic structure. In many instances estimates of PLD are available (e.g. Sponaugle et al. 2006), but, there is no consensus as to how variation in the length of larval duration might determine the distances over which propagules might disperse or the genetic structure of populations (Mayr 1954; Ehrlich and Raven 1969; Burton 1983; Scheltema 1986; Bohonak 1999; Bay et al. 2006; Bradbury et al. 2008; Miller and Ayre 2008).

For species with limited dispersal ability, it is expected that migration among subpopulations will be rare and subpopulations may diverge owing to genetic drift (Wright 1951). Conversely, for taxa with extended dispersal stages, gene flow among populations is expected to be high and populations genetically

homogeneous (Wright 1951, Mayr 1954; Scheltema 1986). However, the relationship between early life history characteristics and genetic structure varies significantly among taxa (Bohonak 1999; Bay et al. 2006; Bradbury et al. 2006). Bohonak (1999) and Bradbury et al. (2008) compared estimates of genetic differentiation among species and reported inverse relationships between larval duration and genetic differentiation, suggesting that increased larval duration is associated with decreases in genetic structure. Moreover, Bradbury et al. (2008) in their analysis of 246 species, including echinoderms, molluscs, crustaceans and sea grasses, reported that genetic differentiation was more variable for species with limited larval duration.

To investigate the effect of larval duration on the population structure of New Zealand's coastal benthos, I have examined the relationship between estimates of larval duration and measures of population differentiation as reported in the published literature. The two most commonly reported measures of differentiation are F_{ST} (and PHI_{ST}) and Nei's genetic distance (D) (Nei 1972). F_{ST} and PHI_{ST} are measures of population differentiation with a maximum of value of one indicating complete fixation of different alleles in each population (i.e. no gene flow), and a value of zero indicating an absence of subdivision (i.e. high gene flow; Wright 1951). Despite controversy over the use of *F*-statistics as a measure of relative differentiation (Hedrick 1999, 2005; Neigel 2002; Palumbi 2003; Bradbury and Betzen 2007; Jost 2008), F_{ST} and its analogues are the measures of genetic differentiation most frequently used in population genetics and therefore the most readily incorporated into a multi-study analysis (e.g. Bohonak 1999; Bradbury et al. 2008).

Estimates of population differentiation (F_{ST} or PHI_{ST}) were available for 29 of the 42 New Zealand studies, with several studies presenting multiple values where a number of markers were analysed. Estimates based on mitochondrial DNA were corrected following the methods of Kinlan and Gaines (2003) to allow comparison with markers possessing biparental inheritance and diploid gene flow. Research has been conducted at a variety of spatial scales. Twenty three studies included populations from across the entire length of New Zealand, whereas in the other 19 studies sampling was conducted regionally or restricted to specific locations. To test whether the geographical scale of sampling influenced the detection of population genetic differentiation, F_{ST} was regressed against the latitudinal range of sampling in each study. The relationship was not significant indicating no effect of sampling scale on the detection of significant differentiation ($r^2 = 0.0042$, $p = 0.72$, $n = 29$).

In most studies, PLDs were provided (Table 2.1), although it should be noted that PLD estimates are generally based on laboratory rather than field studies and are in some instances based on congeners or similar invertebrate families (e.g. Wood and Gardner 2007). Where PLD was presented as a range (e.g. 20-30 days), the longest estimate (i.e. 30 days) was used in my analyses. A significant linear relationship between F_{ST} and PLD was observed ($r^2 = 0.27$, $p = 0.002$, n = 29; Fig. 2.4a) and a natural log transformation of F_{ST} values improved the fit ($R^2 = 0.39$ $p = 0.0001$, $n = 29$; Fig. 2.4a).

Nei's measure of genetic distance was only available in 12 of the 42 reviewed studies. Although frequently used to construct phylogenetic trees, raw values are published infrequently. As with F_{ST}, Nei's D was more variable for taxa

with reduced PLDs (Fig. 2.4b). Nei's D was always low for taxa with extended PLDs while the highest values of D were reported for populations with limited larval durations. The relationship between Nei's D and PLD was not significant (r^2 = 0.19, p =0.179, n = 12; Fig. 2.4b).

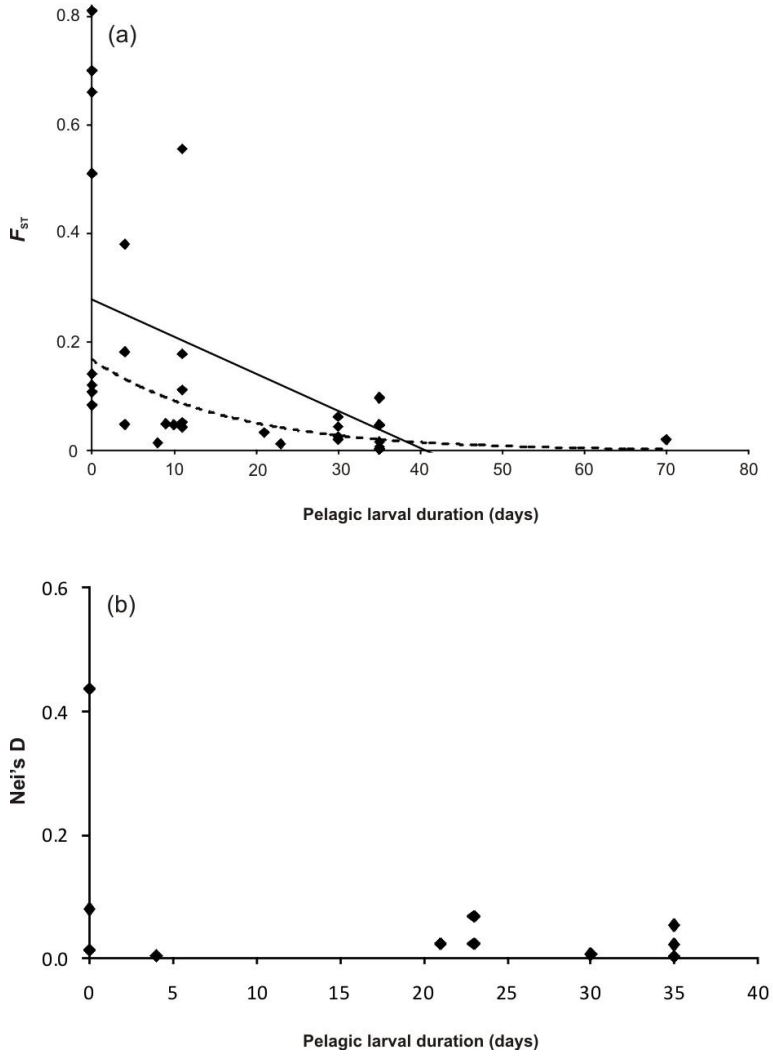

Fig. 2.4 Scatter plots showing the relationship between pelagic larval duration (PLD) and: (a) genetic differentiation (F_{ST} or PHI_{ST}; continuous line slope, F_{ST} = -0.0068*PLD+0.028, R^2 = 0.27, p =0.002, n = 29) and natural log transformed genetic differentiation (dashed line slope, LN(F_{ST}) = -0.0595*PLD-1.785, R^2 = 0.39, p =0.0001, n = 29); (b) Nei's measure of genetic distance (D). Data taken from population genetic studies of the New Zealand coastal benthos.

The low levels of differentiation reported for taxa with extended PLDs suggests that larvae of these taxa disperse over large distances. In contrast, for taxa with shorter PLDs, differentiation among populations is generally greater but is highly variable. This result suggests that when PLD is short, other factors either biological (e.g. larval behaviour and post-settlement dispersal) or physical (e.g. hydrodynamic) become important in determining the scale of dispersal and population genetic structure. As similar relationships have been reported in more extensive meta-analyses of the global literature (Bohonak 1999; Bradbury et al. 2008) it is likely that the patterns observed for New Zealand taxa are indicative of this global trend and are not biased by the small number of genetic studies conducted in New Zealand.

2.4 Genetic versus biogeographic classification

The notion of genetic and biogeographic boundaries coinciding is attractive as the processes limiting the distribution of species (i.e. barriers to dispersal and environmental variables) would be expected to influence the genetic structure of populations through the regulation of gene flow or natural selection (Avise 1992, 1994). This hypothesis has been tested across a number of biogeographic boundaries providing conflicting evidence for a close association between biogeographic boundaries (divisions based on on the geographical distribution of biodiversity) and boundaries based on geographical distribution of genetic variation (e.g. Burton 1998; Lamb and Avise 1992; Knowlton et al. 1993). Consequently, it has been suggested that the different processes responsible for

causing biogeographic breaks will have varying impacts on the genetic structure of wide-ranging species and that the occurrance of genetic boundaries may be governed by the geological history of a region (Burton 1998).

Biogeographic classification schemes based on the distribution of macroalgae, benthic invertebrates and reef fish have divided the New Zealand coast into between 2 and 11 biogeographic regions (Moore 1949; Knox 1975; Walls 1995; Nelson 1994; Francis 1996; Shears et al. 2008) with the most recent scheme describing eleven bioregions falling within two bioprovinces (Shears et al. 2008). Comparison with boundaries identified in genetic studies provides mixed evidence for congruence between genetic and biogeographic boundaries. Biogeographic boundaries have been consistently detected either at finer spatial scales or at different locations from the genetic breaks observed in populations of wide-ranging species (e.g. *P. canaliculus*). Seven of 28 New Zealand-wide populution genetic studies found no population subdivision, 12 studies detected two genetically distinct subpopulations, 3 studies detected three subpopulations and a single study detected four distinct subpopulations.

Despite the absence of a high degree of congruence between genetic and biogeographic boundaries, where genetic boundaries were reported they were often located in the vicinity of biogeographic divisions. For example, the divergence between northern and southern populations (located in the vicinity of Cook Strait) reported for a number of taxa (e.g. Ayers and Waters 2005; Goldstien et al. 2006; Veale 2007) is closely aligned with the major biogeographic boundary decribed by Shears et al. (2008). Further congruence between the biogeographic boundaries of Shears et al. (2008) and genetic boundaries is

apparent in the population structures of the anemone *A. tenebrosa* and seagrass *Z. muelleri.* Veale's (2007) examination of *A. tenebrosa* revealed seven regional subpopulations using microsatellite data. When the biogeographic divisions proposed by Shears et al. (2008) are overlayed on Veale's (2007) genetic groupings, parallels between the two schemes are apparent with Veale's subpopulations falling within single or adjacent bioregions (Fig. 2.5). Jones et al. (2008) using random amplification of polymorphic DNA (RAPD) analysis of the seagrass *Z. muelleri* also found a similar agreement between genetic and biogeographic boundaries albeit with fewer sampling locations.

The lack of consistent conguence between genetic boundaries and biogeographic classification schemes may relate to the molecular markers used, their differing rates of evolution, and suitability for detecting fine scale genetic structure (Wan et al. 2004; DeYoung and Honeycutt 2005; Anne 2006). Alternatively, the discrepancy may relate to differences in the processes responsible for structuring the species and population boundaries measured in genetic and biogeographic studies.

2.5 Conclusions

Genetic divergences have been identified across large spatial scales for several marine benthic taxa in New Zealand and may indicate regions across which dispersal is limited. Two areas of particular interest are the East Cape and the Cook Strait regions. Upwellings south of Cook Strait have been widely proposed as a barrier to larval transport. However, there are limited oceanographic data to support this hypothesis and few populution genetic studies have had sufficient

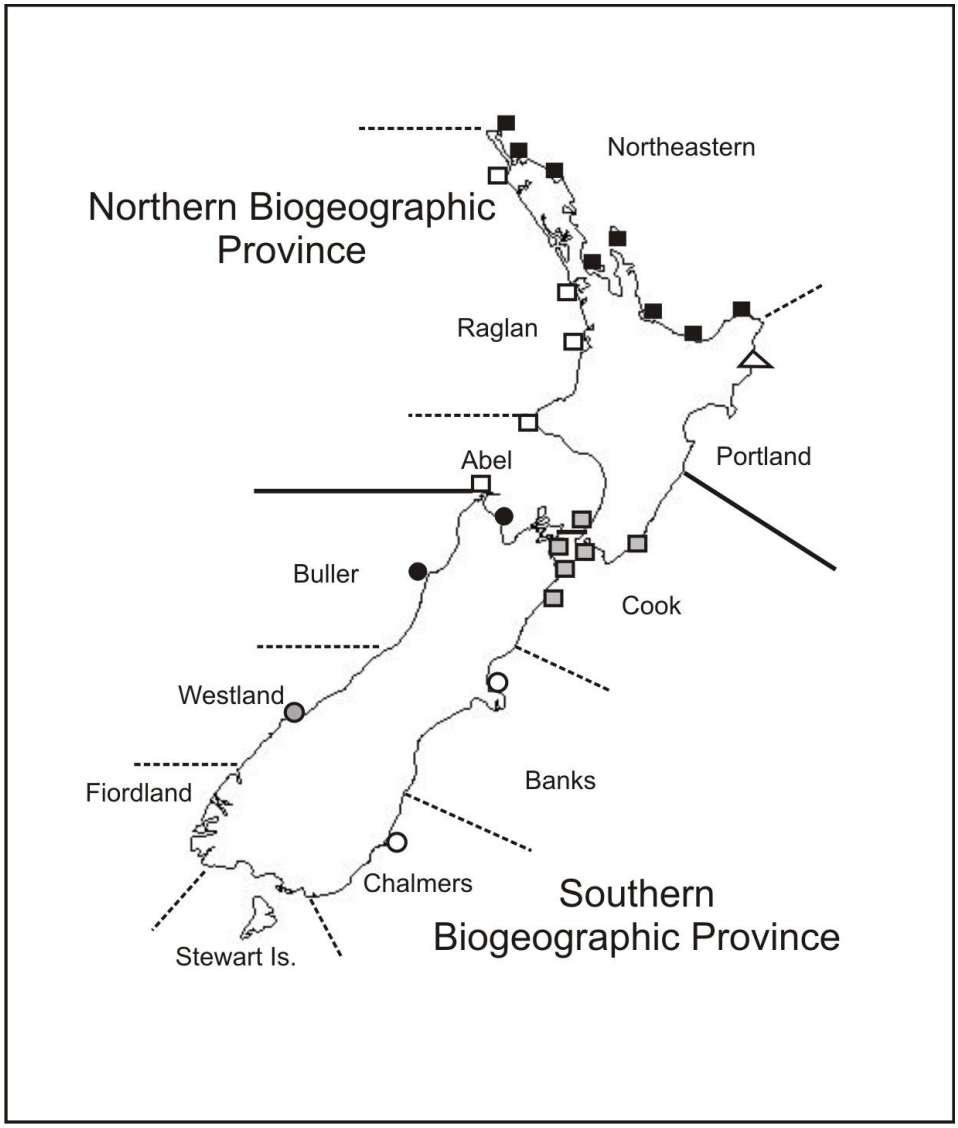

Fig. 2.5 *Actinia tenebrosa* population clusters (as defined by Veale (2007) based on data at four microsatellite loci) and biogeographic regions (as defined by Shears et al. (2008)). Dashed lines denote boundaries between bioregions; solid lines denote boundaries between bioprovinces (redrawn after Veale (2007) and Shears et al. (2008)).

sampling resolution to adequately test it. Genetic divergences have also been detected between the east and west coasts on both the North and South islands and among offshore islands. The mechanisms responsible for generating and maintaining these divergences remain largely unknown. In contrast, the hydrology of Fiordland is well characterised which has aided in the interpretation of genetic patterns observed there.

The New Zealand taxa studied to date have predominantly been rocky reef dwellers. In many parts of New Zealand inter- and sub-tidal reefs occur either continuously along stretches of coast or in close proximity separated by stretches of sandy beach. Because of the small distances between suitable habitats, larvae may be able to move incrementally around the coast over successive generations and genetic divergences that could infer dispersal barriers may be masked by successive dispersal events. For organisms with specific habitat requirements (e.g. estuarine or island restricted species) occurring as discrete populations separated by greater distances, gene flow among populations is likely to be lower. These species are also more likely to exhibit the genetic effects of dispersal barriers and may be a useful target for future studies (Pelc et al. 2009).

Different molecular markers can provide information at different temporal and spatial scales (Anne 2006). For example, fast evolving neutral markers are likely to provide information on breeding systems and/or gene flow among populations, whereas slower evolving markers may provide information about evolutionary relationships with other taxa. The selection of suitable molecular markers is therefore crucial for testing hypotheses at specific spatial or

temporal scales and is likely to have influenced the genetic patterns observed in New Zealand taxa to date. The use of different markers has also hampered comparisons among studies, especially where there is uncertainty over the spatial and temporal scale of information provided by the specific markers (Anne 2006).

Studies of dispersal and/or population connectivity have generally used selectively neutral markers rarther than genes that might reflect environmental gradients or transitions that drive shifts in community structure (e.g. Gardner and Kathiravetpillai 1997). However, the study of ecologically significant genes may further improve our understanding of population connectivity. Specifically, the capacity of individuals to disperse great distances is irrelevant if new recruits lack the ability to survive and reproduce in a new environment. Dispersal potential is therefore likely to be a function of both physical transport and biological suitability with the relative importance of each varying between locations. The identification and inclusion of ecologically significant genes (*sensu* Schmidt et al. 2008) in population genetic studies would help determine the role of genetic variation in adaptation to environmental heterogeneity and is also likely to provide insight into the relationship between biogeographic and genetic boundaries.

Populution genetic research can contribute to the management of the marine environment (Hauser and Carvalho 2008). Many marine ecosystems are threatened by anthropogenic disturbances such as fishing, coastal development and pollution, and resource managers are increasingly required to identify areas or habitats requiring protection (Himes 2007; Wood and Dragicevic 2007).

Understanding population connectivity and subdivision would better allow for characterisation of population units for exploitation and/or conservation purposes. With few exceptions (e.g. *A. tenebrosa*), current knowledge of population connectivity and subdivision is inadequate to aid in management level decision making.

New Zealand with its variable and extensive coastal landscape is ideal for studying the processes that drive larval dispersal patterns and population genetic structure. However, much of the research to date has been characterised by haphazard sampling and *post-hoc* speculation rather than driven by *a priori* hypotheses. Consequently, patterns of genetic subdivision have been identified while the processes responsible for generating them remain elusive. Future studies would benefit greatly from a more structured sampling regime and a multidisciplinary approach designed to test hypotheses driven by physical and biological oceanography. This approach would result in a better understanding of the processes generating population subdivision as well as factors responsible for recruitment, dispersal and population connectivity.

Chapter 3

Population genetic structure of the New Zealand estuarine clam *Austrovenus stutchburyi* reveals population subdivision and partial congruence with biogeographic boundaries

3.1 Introduction

Many marine benthic invertebrates can only disperse over large distances during a pelagic larval phase (Thorson 1950; Pechenik 1999; Grantham et al. 2003). For inshore species, larvae are exported to coastal or shelf waters and develop during a period of weeks to months before returning to suitable habitat in late larval or early post-larval form (Pechenik 1999). For estuarine taxa, the process of inter-estuary dispersal is more complex. Larvae must be exported from their natal estuary, develop in coastal waters and be transported to suitable settlement habitat within another estuary when developmentally capable of settling (Bilton et al. 2002). Inter-estuary larval dispersal may be further limited by the often large distances between estuaries, the physical properties of estuarine waters and their interface with the coastal ocean (Mann 1988; Largier 1993), as well as the physiological challenges associated with a life history alternating between estuarine and coastal waters (Cognetti and Maltagliati

2000). Consequently, it has been suggested that connectivity among populations of estuarine taxa will be reduced compared to taxa occurring on the open coast and that different spatial management strategies may be needed for estuarine versus coastal taxa (Bilton et al. 2002; Watts and Johnson 2004; Pelc et al. 2009). However, determining rates of connectivity has proven challenging owing to the difficulties associated with physically tracking or predicting the dispersal of larvae (Levin 2006; Gawarkiewicz et al. 2007).

A number of indirect methods of estimating connectivity have been developed (Levin 2006) with population genetics one of the most widely utilized. If rates of inter-estuary gene flow are low as hypothesized, then estuarine taxa would be expected to exhibit a greater degree of genetic subdivision relative to taxa occurring on the open coast (Bilton et al. 2002; Pelc et al. 2009). However, as natural selection (Schmidt et al. 2008; Richards et al. 2010 and references therein) and patterns of historical gene flow (Avise et al. 1987; Kelly et al. 2006) can also influence population genetic structure, the interpretation of genetic patterns and estimation of gene flow has been difficult (Sotka et al. 2004).

Because larval dispersal and natural selection will also determine species' distribution and abundance, it has been suggested that boundaries between groups of genetically distinct populations will be geographically congruent with biogeographic break points (the boundaries between taxonomically distinct communities; Avise et al. 1987). Although tested across a number of biogeographic boundaries (e.g. Avise 1992; Hare and Avise 1996; Dawson 2001, 2005; Cárdenas et al. 2009), conflicting results suggest that agreement between

genetic and biogeographic boundaries may be location and taxon specific (Burton 1998; Pelc et al. 2009).

The New Zealand archipelago with its complex oceanography (Heath 1982; Laing and Chiswell 2003), dynamic geological history (Fleming 1979) and well documented biogeography (Shears et al. 2008), provides an opportunity to examine the processes that generate population subdivision and the relationship between genetic and biogeographic boundaries. Straddling the subtropical convergence, New Zealand's marine climate follows a steep gradient from the subtropical north to subantarctic south (Laing and Chiswell 2003; Hadfield et al. 2007). The resulting temperature gradient coupled with spatial variation in swell regime, geological processes and other environmental factors (Laing and Chiswell 2003), has created regional variation in the distribution and abundance of flora and fauna. This biogeographical variation has been described in a number of classification schemes that have divided New Zealand's coastline into distinct biogeographic regions (reviewed by Shears et al. 2008). In contrast, the influence of physical, environmental and geological processes on a population's genetic structure is less understood.

Population genetic structure has been examined in at least 29 New Zealand coastal benthic species with the majority of studies focused on taxa that occur on the open coast (see Chapter 2; Ross et al. 2009 for review). Where populations have been sampled across a wide latitudinal range there has usually been an absence of genetic structure (e.g. rock lobster*;* Smith and McKoy 1980; Ovenden et al. 1992, seastar; Waters & Roy 2003), or the detection of genetically divergent northern and southern populations divided through central New

Zealand. Differentiation about a central boundary has now been recorded in at least ten species including open coast taxa with pelagic larvae (seastar; Waters and Roy 2004; Ayers and Waters 2005, limpets; Goldstien et al. 2006, mussel; Apte and Gardner 2002; Apte et al. 2003, chiton; Veale 2007), open coast taxa without pelagic larvae (brittle star; Sponer and Roy 2002) and estuarine taxa without pelagic larvae (Corophiid amphipods; Stevens and Hogg 2004; Knox et al. 2011, seagrass; Jones et al. 2008). While it is possible that taxa sharing this pattern of population subdivision have experienced a common set of contemporary or historical processes (Kuo and Avise 2005; Pelc et al. 2009), the identification of these processes has thus far proved difficult (see Apte and Gardner 2002; Goldstien et al. 2006).

Genetic structure in addition to the central New Zealand divergence has only been reported for a few species, all of which exhibit characteristics of limited dispersal capacity such as non-pelagic larvae or restriction to estuarine habitats (e.g. Corophiid amphipods; Knox et al. 2011; Stevens and Hogg 2004, seagrass; Jones et al. 2008). As most of the estuarine taxa examined to date lack a pelagic larval phase, it is uncertain which characteristic (estuarine distribution versus larval mode) is driving patterns of additional population subdivision.

The clam *Austrovenus stutchburyi* (Wood 1828), is the only New Zealand taxon that both disperses via pelagic larvae and is restricted to estuaries that has previously been assessed for population genetic structure (Lidgard 2001). In contrast to other estuarine taxa with limited potential for long distance dispersal, *A. stutchburyi* spawn over a period of several months and have a pelagic larval

phase of 2-3 weeks during which dispersal among geographically discrete populations is possible (Stephenson and Chanley 1979; Booth 1983). A previous analysis of allozyme polymorphism in *A. stutchburyi* did not detect any spatial genetic subdivision (although temporal patterns may have been detected; Lidgard 2001). This supports the notion that for previously examined taxa, the lack of pelagic larval phase rather than an estuary-restricted distribution *per se*, might be responsible for the additional genetic structure observed (e.g. Stevens and Hogg 2004; Jones et al, 2008). However, as most recent genetic studies of New Zealand taxa have used mitochondrial DNA sequences to assess population structure (Chapter 2; Ross et al. 2009), it is uncertain whether the lack of subdivision reported for *A. stutchburyi* can be attributed to their pelagic larval phase or to differences in methodology (allozymes versus mtDNA).

To determine whether populations of an estuarine species with pelagic larvae will be genetically subdivided, population genetic analyses of *A. stutchburyi* were conducted using the mitochondrial cytochrome *c* oxidase subunit I (COI) gene. A Mantel test and spatial analysis of molecular variance (SAMOVA) were performed to test for patterns of genetic isolation-by-distance (IBD) and to identify divergent subpopulations. An analysis of molecular variance (AMOVA) was also used to determine whether genetic subdivisions are congruent with known biogeographic break points. A recent biogeographic classification of New Zealand (Shears et al. 2008; Fig. 3.1a) was chosen for comparison because it incorporated results from previous biogeographic classifications, and included taxa similar to those considered here and in previous population genetic studies. Shears et al. (2008) described a major biogeographic

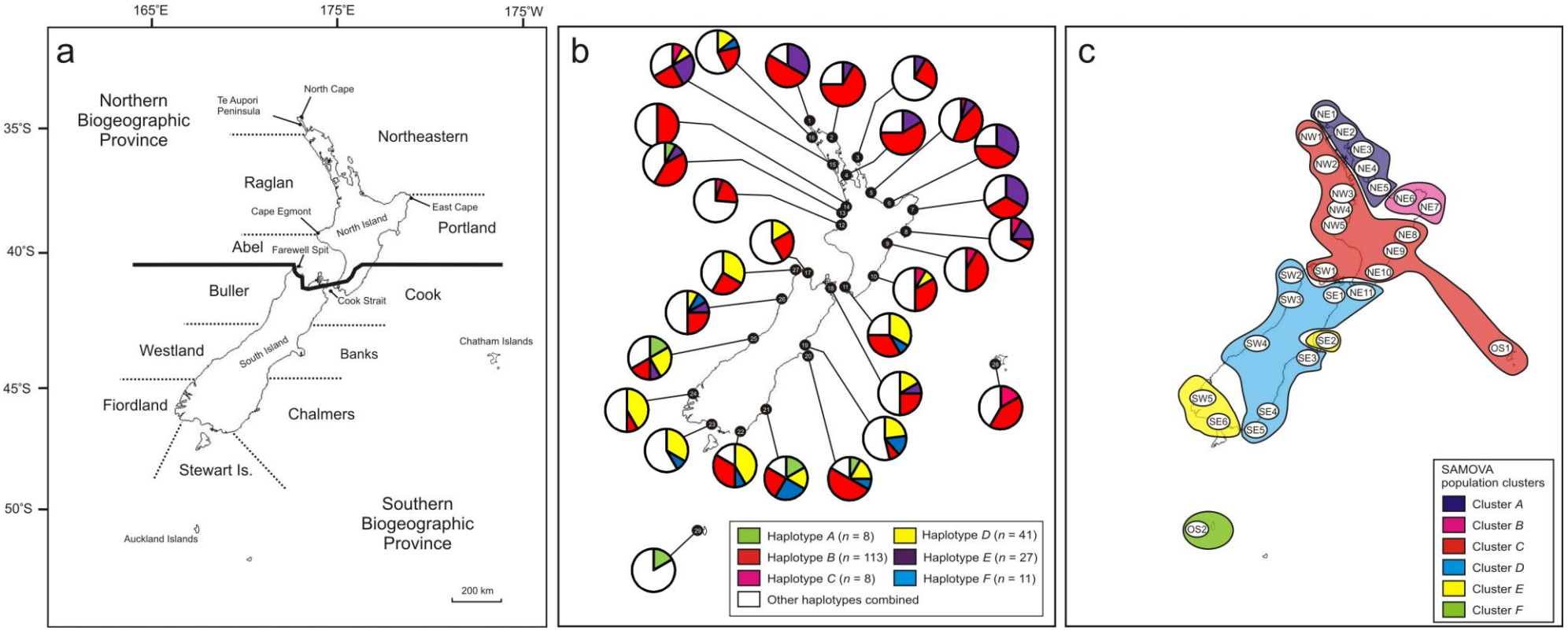

Figure 3.1 (a) New Zealand biogeographic provinces and regions described by Shears et al. (2008). Solid black line indicates boundary between biogeographic provinces while dashed lines indicate boundaries between biogeographic regions. Specific locations referred to in the text are named and indicated by arrows. (b) Sampling locations of *Austrovenus stutchburyi* populations and the frequencies of common cytochrome *c* oxidase I (COI) haplotypes at each site. Labels refer to estuary locations detailed in Table 3.1. Pie segment size indicates relative frequencies of COI haplotypes in each population. Haplotypes detected in fewer than eight specimens are grouped (white segments) for clarity of presentation. (c) Population clusters designated by SAMOVA analysis.

division through central New Zealand and another nine minor biogeographic boundaries located throughout New Zealand (Fig. 3.1a).

3.2 Methods

3.2.1 Study sites and sample collection

A. stutchburyi from 29 New Zealand estuaries were collected between January 2007 and January 2010 (Table 3.1; Fig. 3.1b). Fourteen of the sampled estuaries were located in the North Island and 12 in the South Island. Specimens were also collected from estuaries on one inshore island and the only two offshore islands where *A. stutchburyi* is present; Great Barrier Island (NE3) less than 20 km off the northeast coast of the North Island, the Chatham Islands (OS1) 660 km to the east of central New Zealand and the Auckland Islands (OS2) 460 km to the south of the South Island (Fig. 3.1b). Between 12 and 25 *A. stutchburyi* were collected from each estuary and stored at -80°C prior to DNA extraction.

3.2.2 DNA extraction and sequencing

A 0.25 - 0.50 cm^2 piece of adductor mussel was dissected from each specimen and genomic DNA extracted using the Zymo Research Genomic DNA II Kit (Zymo Research Corporation, Orange, CA, USA). A 710 base pair (bp) fragment of the mitochondrial COI gene was then amplified using the universal primers LCO1490 and HCO2198 (Folmer et al. 1994). PCR amplifications were conducted in 10 µl reactions containing 4.8 µl Intron i-Taq 2x PCR master mix, 5 pmol of each primer and 1 µl of unquantified template DNA. PCR reactions consisted of an initial

Table 3.1 Summary of *Austrovenus stutchburyi* populations sampled. Labels correspond to population markers in Figures 3.1b and 3.1c. Approximate locations of populations are provided as well as the number of COI sequences obtained (*N*), number of haplotypes detected (N_{hap}), number of polymorphic sites (*S*), haplotype diversity (*H*), mean number of pairwise differences (π_1) and nucleotide diversity (π_2) at each location.

Sampling location	Label	Position (Lat.-Long.)	*N*	N_{hap}	*S*	*H*	π_1	π_2
Parengarenga	NE1	34°31' S-172°55' E	12	4	6	0.682	1.79	0.0027
Paihia	NE2	35°18' S-174°06' E	12	5	10	0.576	2.21	0.0034
Waitemata	NE3	36°52' S-174°42' E	12	5	5	0.667	1.32	0.0020
Whangapoua	NE4	36°08' S-175°26' E	12	9	12	0.939	2.55	0.0039
Tauranga	NE5	37°29' S-175°57' E	25	12	17	0.803	1.86	0.0028
Ohiwa	NE6	38°23' S-178°18' E	12	5	6	0.758	1.88	0.0029
Uawa	NE7	37°59' S-177°06' E	12	6	7	0.818	1.86	0.0028
Mahia	NE8	39°04' S-177°54' E	12	10	18	0.970	4.98	0.0076
Ahuriri	NE9	39°29' S-176°53' E	12	7	7	0.773	2.23	0.0034
Riversdale	NE10	41°05' S-176°05' E	12	9	18	0.909	4.05	0.0062
Hutt River	NE11	41°14' S-174°54' E	12	7	7	0.864	2.26	0.0034
Herekino	NW1	35°18' S-173°10' E	14	11	17	0.956	3.98	0.0061
Kaipara	NW2	36°15' S-174°15' E	12	8	14	0.909	3.64	0.0055
Raglan	NW3	37°48' S-174°52' E	12	6	9	0.758	2.05	0.0031
Kawhia	NW4	38°03' S-174°50' E	12	8	10	0.848	2.44	0.0037
Tongaporutu	NW5	38°49' S-174°36' E	19	16	25	0.965	4.51	0.0069
Hakahaka	SE1	41°18' S-174°07' E	12	9	16	0.939	3.88	0.0059
Avon-Heathcote	SE2	43°32' S-172°44' E	13	9	12	0.936	3.44	0.0052
Akaroa	SE3	43°45' S-172°56' E	12	6	6	0.758	1.74	0.0027
Otago	SE4	45°50' S-170°40' E	12	6	6	0.879	1.91	0.0029
Waikawa	SE5	46°29' S-169°42' E	12	5	8	0.758	2.44	0.0037
Riverton	SE6	46°21' S-168°01' E	13	9	15	0.910	3.79	0.0058
Pakawau	SW1	40°37' S-172°41' E	12	9	13	0.939	3.21	0.0049
Whanganui Inlet	SW2	40°34' S-172°35' E	12	7	11	0.864	3.08	0.0047
Orowaiti	SW3	41°45' S-171°38' E	12	10	14	0.955	3.21	0.0049
Okarito	SW4	43°13' S-170°10' E	12	8	8	0.924	2.38	0.0036
Doubtful Sound	SW5	45°26' S-167°08' E	12	8	11	0.848	2.59	0.0039
Chatham Island	OS1	43°55' S-176°27' W	12	7	12	0.833	3.17	0.0048
Auckland Island	OS2	50°33' S-166°07' E	12	7	12	0.879	3.21	0.0049
New Zealand		**-**	**372**	**125**	**99**	**0.889**	**3.06**	**0.0047**

denaturing phase of 94°C (4 min.), followed by 35 cycles consisting of 94°C (60 s), 52°C (90 s) and 72°C (90 s) and a final extension period at 72°C (5 min.). Unincorporated nucleotides and primers were removed by adding 2 units of Exonuclease I, 0.1 unit of Shrimp Alkaline Phosphatase and 2.7 µl H_2O and incubating at 37°C (30 min) then 80°C (15 min). Sequencing reactions used Big Dye terminator sequencing chemistry (Applied Biosystems) on an Applied Biosystems 3130 Genetic Analyzer. DNA strands were edited in Geneious (ver. 4.8.4) to produce an alignment of 658 bp. Sequences have been deposited in the Barcode of Life Datasystems (BOLD) database under project NZCOC (New Zealand Marine Bivalves).

3.2.3 Population genetic analyses

Indices of genetic diversity were quantified using DnaSP ver. 5 (Librado and Rozas 2009). The number of COI haplotypes (N_{hap}), number of segregating sites (S), haplotype diversity (H_e), mean number of pairwise differences (π_1) and nucleotide diversity (π_2) were calculated for each estuary and for the entire data set. The GTR model of sequence evolution was selected in jMODELTEST 0.1.1 (Posada 2008) and a GTR corrected distance matrix was generated in PAUP* 4.0 (Swofford 2000) for use in subsequent analyses. Estimates of population pairwise F_{ST} were then calculated in ARLEQUIN ver. 3.11 (Excoffier et al. 2005) to determine if any two populations differed significantly in their genetic composition. A Mantel test was implemented in ARLEQUIN to assess the relationship between genetic and geographic distance. Geographic distance between populations, measured in Google Earth as the shortest distance over

water between two estuaries was regressed against estimates of population pairwise F_{ST} to determine whether *A. stutchburyi* dispersal conformed to a pattern of IBD.

Population structure was further investigated using SAMOVA 1.0 (Dupanloup et al. 2002). This method is based on a simulated annealing procedure that maximizes the proportion of total genetic variance due to differences among groups of populations. SAMOVA can be used to define population clusters that are geographically homogeneous and maximally differentiated from each other without the prior assumption of subpopulation composition. Genetic variance (F_{ST}) is partitioned into two components, F_{SC} and F_{CT}, indicating respectively the differentiation among populations within and among groups (note that I have adopted the ARLEQUIN and SAMOVA subscript definitions to define differentiation within and among groups of populations). SAMOVA analyses ran for 10 000 iterations from each of 100 random initial conditions for a predetermined number of subpopulations (k) ranging from 2 to 14.

Tajima's (1989) D, Fu and Li's (1993) F* and D* and Fu's (1997) F_S were calculated in DnaSP to test for deviation from the Wright-Fisher model of neutral evolution consistent with either non-neutral evolution or population expansion under neutral evolution. The mismatch distributions of pairwise differences between all individual haplotypes (calculated in DnaSP) were then used to further test for population stability or growth.

3.2.4 Comparison of genetic and biogeographic boundaries

To test for congruence between genetic and biogeographic boundaries (sensu Avise et al. 1987), the locations of all boundaries between *A. stutchburyi* subpopulations identified in SAMOVA were compared with biogeographic break points described by Shears et al. (2008; Fig 3.1a). Populations were grouped according to the zonation of the biogeographic classification and AMOVA performed at both bioprovince and bioregion spatial scales (as defined by Shears et al. 2008), to assess how well this biogeographic classification represented the spatial distribution of genetic variation in *A. stutchburyi.*

3.3 Results

3.3.1 Population genetic analyses

Three hundred and seventy two *A. stutchburyi* were sequenced for the mitochondrial COI gene. Of the 658 positions analysed, 99 were variable leading to the delineation of 125 haplotypes (Table 3.1). The most abundant haplotype (H2; Fig. 3.1b), occurred in 27 of 29 sampled populations, accounting for 30% of the total data set. Another 25 haplotypes were recorded in at least two populations (43% of data set), while the remaining 99 haplotypes were recorded only in single populations. Haplotype diversity was high throughout all populations with at least four haplotypes recorded at each location ($\overline{H}_e$ = 0.85 ± 0.10 (± SD); Table 3.1). In contrast to haplotype diversity, nucleotide diversity was low with most COI sequences differing only by a small number of base changes ($\overline{\pi}_1$ = 2.8 ± 0.93, $\overline{\pi}_2$ = 0.004 ± 0.001; Table 3.1).

A plot of haplotype frequency and distribution suggested regional differences in the genetic composition of populations (Fig. 3.1b). In contrast to the most abundant haplotype (H2), which was detected throughout sampled populations (with the exception of sites SE6 and OS2; Fig. 3.1b), other haplotypes were either restricted to, or were detected more frequently, in specific regions. For example, haplotypes H1, H4 and H6 were most abundant in southern populations, H3 in lower North Island populations and OS1 to the east of the South Island and H5 in northern populations (Fig. 3.1b). The most dramatic shift in genetic composition appeared to be between northern and southern populations to the north of NE11 in the lower North Island.

Population pairwise F_{ST}-values (Table 3.2) indicated that, for the most part, North and South Island populations were significantly differentiated from each other. The exceptions were NE11 which was significantly different to most North Island populations but not South Island populations and SW1 (Fig. 3.1b) located in the north of the South Island which was not significantly different from either lower North Island or upper South Island populations. While OS1 was significantly different from most South Island and northeast coast North Island populations, it did not differ significantly from lower North Island populations. OS2, located to the south of the South Island, was differentiated from most northern populations and the southernmost South Island populations. The two East Cape populations (NE6 and NE7; Fig. 3.1b), were significantly different from adjacent east coast populations.

A Mantel test revealed a highly significant positive correlation between geographic and genetic distances (F_{ST}) among all sampled populations ($P < 0.001$; Fig. 3.2). Distance between estuaries accounted for 28% of inter-population COI variability, a result indicative of genetic IBD, implying that for *A. stutchburyi* dispersal over large distances may be limited.

SAMOVA obtained its best partitioning of genetic variance when populations were assigned to six groups (Table 3.3). All grouping options (k = 2 to 14) produced populations clusters that were significantly differentiated. However, it was only when SAMOVA generated six or more clusters that differentiation within groups (F_{SC}) became non-significant, indicating that populations within each cluster were similar to each other. At k = 6, populations were assigned to clusters that were spatially coherent, with the exception of cluster *E* in which SE6 and SW5 in the southwest were grouped with SE2 located *c.* 600km to the northeast (Fig. 3.1c). Once k exceeded six, populations were removed one at a time from the established clusters, providing no further groupings that were informative of population structure. When k = 6 the SAMOVA assigned populations into five mainland clusters and the Auckland Islands as a sixth (Fig. 3.1c). Mainland populations were grouped on the northeast coast (Fig. 3.1c; cluster *A*), around East Cape (cluster *B*), across the lower North Island (including both SW1 in the upper South Island and OS1 to the east of the South Island; cluster *C*), across the upper and central South Island (included NE11 in the lower North Island; cluster *D*) and in the south-western Fiordland region also incorporating SE2 (cluster *E*). Tests of neutrality were all

Table 3.2 F_{ST} values among all *Austrovenus stutchburyi* populations (below diagonal), and significance (above diagonal). **P*<0.05; ***P*<0.01; ****P*<0.001. Population site labels detailed in Table 3.1 and locations displayed on Figure 3.1b.

	NE1	NE2	NE3	NE4	NE5	NE6	NE7	NE8	NE9	NE10	NE11	NW1	NW2	NW3	NW4	NW5	SW1	SW2	SW3	SW4	SW5	SE1	SE2	SE3	SE4	SE5	SE6	OS1	OS2
NE1		.	.	.	.	.	.	.	*	*	***	*	.	.	.	*	*	*	*	*	***	.	***	*	***	*	***	**	***
NE2	0.01		.	.	.	.	.	.	.	.	*	.	.	.	.	.	.	*	*	*	***	.	***	.	***	*	***	.	**
NE3	0.01	-0.04		.	.	.	.	.	.	.	***	.	.	.	.	.	.	**	**	**	***	.	***	.	***	***	***	.	*
NE4	-0.03	-0.02	-0.02		.	.	.	.	.	*	***	*	.	.	.	*	*	**	***	***	***	*	***	*	***	***	***	*	***
NE5	0.07	-0.01	-0.01	0.01		**	**	**	.	*	***	**	*	.	.	***	*	***	***	***	***	**	***	***	***	***	***	*	***
NE6	-0.04	0.03	0.04	0.02	**0.10**		.	.	**	**	***	*	.	.	.	*	*	*	.	**	***	.	***	**	***	***	***	*	***
NE7	-0.04	0.02	0.01	0.00	**0.09**	-0.04		.	*	*	***	*	.	.	*	***	**	***	***	***	***	*	***	***	***	***	***	***	***
NE8	0.07	0.02	0.03	0.08	**0.09**	0.07	0.07		.	.	*	.	.	.	.	.	.	.	.	*	***	.	*	.	*	*	***	.	*
NE9	**0.13**	0.01	0.02	0.10	0.05	**0.14**	**0.14**	-0.01		.	***	.	.	.	.	.	.	**	*	***	***	.	**	*	*	***	***	.	***
NE10	**0.08**	0.00	0.02	**0.07**	**0.05**	**0.09**	**0.10**	-0.02	-0.02		.	.	.	.	.	.	.	.	.	*	***	.	*	.	.	*	***	.	.
NE11	**0.19**	**0.17**	**0.17**	**0.24**	**0.21**	**0.21**	**0.24**	**0.10**	**0.18**	0.07		.	*	*	***	**	.	.	.	.	.	.	.	.	.	.	.	*	.
NW1	**0.06**	0.02	0.02	**0.07**	**0.06**	**0.07**	**0.08**	-0.01	0.00	-0.04	0.04		.	.	.	.	.	.	.	.	***	.	.	.	.	.	***	.	.
NW2	0.00	-0.03	-0.01	0.02	**0.05**	0.00	0.01	-0.03	-0.01	-0.02	**0.10**	-0.02		.	.	.	.	.	.	.	***	.	**	.	*	*	**	.	*
NW3	0.00	-0.02	-0.03	-0.05	0.00	0.03	0.02	0.06	0.07	0.05	0.18	0.05	0.02		.	.	*	**	*	**	***	*	***	.	***	***	***	.	*
NW4	0.05	-0.02	-0.02	0.03	0.00	0.07	**0.07**	0.00	-0.04	-0.03	**0.12**	-0.02	-0.03	0.01		.	.	.	.	*	***	.	**	.	.	**	***	.	.
NW5	**0.09**	0.02	0.04	**0.09**	**0.09**	**0.08**	**0.10**	-0.02	-0.02	-0.02	**0.12**	0.00	-0.02	0.07	-0.01		.	*	.	*	***	.	**	.	*	***	***	.	*
SW1	**0.09**	0.02	0.02	**0.09**	**0.06**	**0.11**	**0.11**	0.00	-0.01	-0.04	0.03	-0.03	-0.01	0.05	-0.04	0.00		.	.	.	***	.	*	.	.	.	*	.	.
SW2	**0.15**	**0.13**	**0.13**	**0.19**	**0.16**	**0.18**	**0.19**	0.08	**0.14**	0.03	-0.05	0.02	0.07	0.14	0.08	**0.10**	0.01		.	.	.	.	.	.	.	.	.	*	.
SW3	**0.09**	**0.08**	**0.08**	**0.13**	**0.12**	0.09	**0.12**	0.05	**0.08**	0.00	0.00	-0.01	0.03	0.09	0.04	0.05	0.01	-0.01		.	*	.	.	.	.	.	.	.	.
SW4	**0.15**	**0.16**	**0.15**	**0.21**	**0.20**	**0.17**	**0.20**	**0.09**	**0.18**	**0.07**	-0.04	0.03	0.07	0.17	**0.10**	**0.10**	0.03	-0.03	-0.02		.	.	.	.	.	.	.	**	.
SW5	**0.31**	**0.32**	**0.31**	**0.39**	**0.36**	**0.32**	**0.36**	**0.18**	**0.31**	**0.16**	0.01	**0.13**	**0.20**	**0.32**	**0.26**	**0.19**	**0.14**	0.02	**0.08**	0.03		*	.	*	.	.	.	***	**
SE1	0.04	0.04	0.04	**0.08**	**0.09**	0.06	**0.09**	-0.01	0.03	-0.02	-0.01	-0.04	-0.02	0.06	0.00	0.01	-0.03	-0.01	-0.03	-0.02	**0.07**		.	.	.	.	.	.	.
SE2	**0.23**	**0.22**	**0.21**	**0.28**	**0.25**	**0.22**	**0.26**	**0.12**	**0.18**	**0.07**	0.04	0.05	**0.12**	0.23	**0.14**	**0.11**	**0.07**	0.01	-0.01	0.03	0.03	0.02		*	.	.	.	***	.
SE3	**0.10**	0.06	0.06	**0.12**	**0.07**	**0.14**	**0.15**	0.06	**0.09**	0.01	0.00	0.00	0.03	0.07	0.02	0.06	-0.03	0.00	-0.01	0.00	**0.15**	-0.02	**0.09**		.	.	*	.	.
SE4	**0.18**	**0.16**	**0.15**	**0.23**	**0.17**	**0.18**	**0.22**	**0.09**	**0.14**	0.03	-0.01	0.01	**0.08**	**0.16**	0.08	**0.08**	0.02	-0.02	-0.05	-0.03	0.07	-0.02	-0.01	-0.01		.	.	*	.
SE5	**0.20**	**0.18**	**0.18**	**0.25**	**0.23**	**0.23**	**0.25**	**0.11**	**0.20**	**0.08**	-0.06	0.05	**0.11**	**0.20**	**0.14**	**0.13**	0.04	-0.04	0.02	-0.02	0.00	0.00	0.04	0.02	0.01		.	*	*
SE6	**0.24**	**0.25**	**0.24**	**0.30**	**0.29**	**0.24**	**0.28**	**0.15**	**0.23**	**0.12**	0.02	**0.10**	**0.16**	**0.25**	**0.19**	**0.15**	**0.10**	0.02	0.02	0.03	-0.02	0.04	-0.03	**0.09**	0.020	0.03		***	*
OS1	**0.13**	0.03	0.05	**0.12**	**0.08**	**0.11**	**0.13**	-0.01	-0.01	-0.03	**0.11**	-0.01	0.00	0.07	-0.01	-0.02	-0.01	**0.09**	0.03	**0.11**	**0.20**	0.01	**0.11**	0.04	**0.07**	**0.13**	**0.14**		.
OS2	**0.13**	**0.09**	**0.07**	**0.13**	**0.11**	**0.13**	**0.15**	**0.08**	**0.10**	0.03	0.08	0.03	**0.07**	**0.09**	0.05	**0.07**	0.02	0.05	0.00	0.06	**0.16**	0.02	0.06	0.03	0.010	**0.10**	**0.09**	0.06	

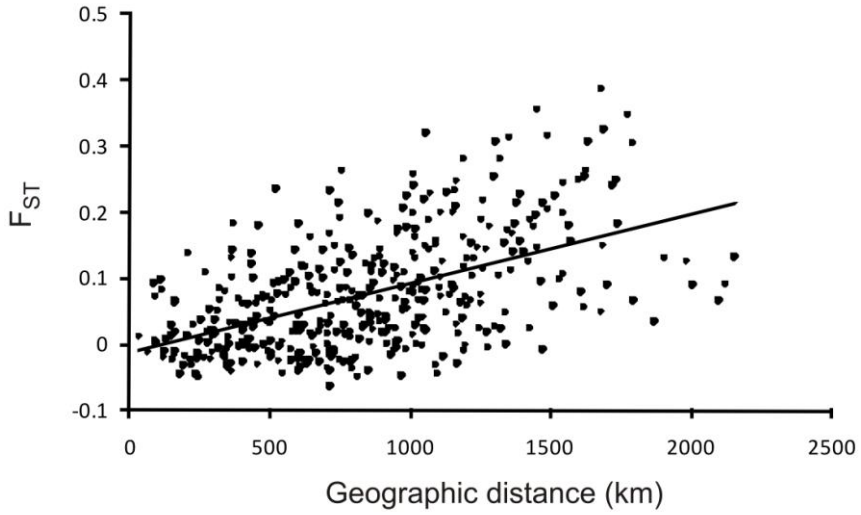

Figure 3.2 Relationship between genetic disimilarity (estimated as Fst) and geographical distance among *Austrovenus stutchburyi* populations. The Mantel relationship was significant (R^2 = 0.28, p = <0.001).

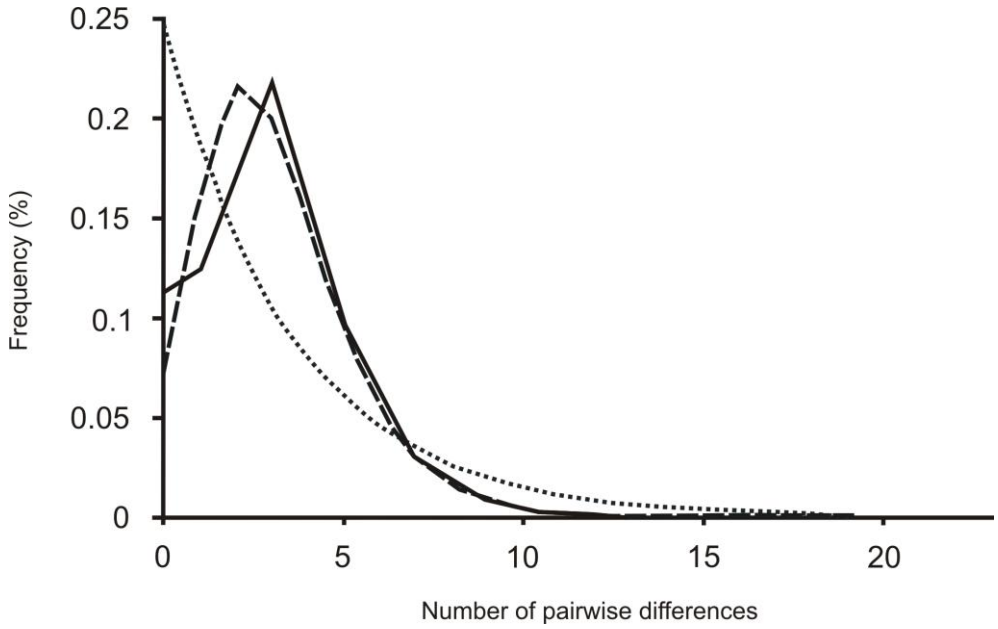

Figure 3.3 Frequency distribution of pairwise differences among cytochrome oxidase subunit I (COI) haplotypes in *Austrovenus stutchburyi*. Solid line indicates observed frequencies while dashed and dotted lines indicate respectively the expected frequencies under models of population expansion and constant population size.

Table 3.3 SAMOVA fixation indices (F_{CT}, F_{SC} and F_{ST}) as a function of predefined numbers of groups (*k*). Significant values are in bold. *p<0.01: **p<0.001.

k	F_{CT}	F_{SC}	F_{ST}
2	**0.143****	**0.060****	**0.194****
3	**0.133****	**0.052****	**0.179****
4	**0.121****	**0.029****	**0.147****
5	**0.117****	**0.021***	**0.135****
6	**0.114****	-0.014	**0.101****
7	**0.112****	-0.010	**0.103****
8	**0.113****	-0.020	**0.010****
9	**0.114****	-0.020	**0.095****
10	**0.115****	-0.023	**0.095****
11	**0.116****	-0.025	**0.093****
12	**0.116****	-0.027	**0.093****
13	**0.116****	-0.027	**0.092****
14	**0.116****	-0.029	**0.090****

Table 3.4 *Austrovenus stutchburyi* population statistics for the mtDNA COI gene (*n*=372) and interpretation with respect to selection and population expansion. Significant values are in bold. **P*<0.02.

Population statistics		Expectation under	
		Selection	Expansion
Number of Haplotypes	125	–	–
Number of polymorphic sites	99	–	–
Nucleotide diversity	0.0047	Low	Low
Tajima's D	**-2.38***	Significant	Significant
Fu & Li's (1993) F*	**-6.51***	Significant	NS
Fu & Li's (1993) D*	**-5.34***	Significant	NS
Fu's (1997) F_s	**-217.4***	NS	Significant

significant, indicating either natural selection or population expansion (Table 3.4). Further support for a recent population expansion was provided by a mismatch analysis in which the distribution of pairwise differences was a close fit under the expectations of expansion, but departed from expectations under a model of population stability (Fig. 3.3; but see Wares 2010).

3.3.2 Comparison of phylogeographic and biogeographic boundaries

The boundaries between all SAMOVA clusters were located in close proximity to the biogeographic break points of Shears et al. (2008). The boundary between clusters *C* and *D* (Fig. 3.1c), which pairwise F_{ST}-values indicated to be the most significant genetic boundary (Table 3.2), was a close match to the major break point between biogeographic provinces (Fig. 3.1a). Other boundaries between SAMOVA clusters were located in proximity to more minor inter-bioregional break points. However, genetic divisions were not detected at all biogeographic break points. Shears et al. (2008) described 11 biogeographic boundaries delineating 11 bioregions within two bioprovinces. In comparison, seven *A. stutchburyi* genetic boundaries (excluding SE2 boundaries in cluster *E;* Fig. 3.1c) were identified, delineating five mainland subpopulations. Boundaries identified by both biogeographic and genetic methods were located around the North and East Capes, to the north of Fiordland, in the southeast of the South Island and across Cook Strait from Farewell Spit to the southeast coast of the North Island.

When *A. stutchburyi* populations were grouped according to the biogeographic classification (Fig. 3.1a), AMOVA indicated significant genetic differentiation between biogeographical provinces (F_{CT} = 0.109, P < 0.001) and among bioregions (F_{CT} = 0.072, P < 0.001). While these levels of differentiation

were less than those detected among SAMOVA assigned population clusters (F_{CT} = 0.114), the result suggests that this biogeographic classification provides a reasonable representation of the spatial distribution of genetic variation, especially at the larger bioprovince spatial scale. Differentiation among populations within bioregions was marginally significant (F_{SC} = 0.018, P = 0.045 compared to F_{SC} = -0.014^{NS} in SAMOVA), indicating that populations within bioregions were not all genetically homogenous. When the Northeastern-Portland and Portland-Cook boundaries (Fig. 3.1a) were relocated southward (*c*. 150 km) to match the boundaries suggested by SAMOVA (thereby shifting NE7 and NE10 populations between bioregions; Fig. 3.1c), differentiation among populations within bioregions became non-significant (F_{SC} = 0.006, P = 0.245).

3.4 Discussion

A greater number of genetic subdivisions among populations of *A. stutchburyi* were detected relative to most reported examples of New Zealand's open coast benthos. SAMOVA identified six genetically distinct subpopulations, with the most significant change in the genetic composition of populations occurring through central New Zealand in the vicinity of Cook Strait. The transition across this genetic boundary was not an abrupt or complete shift as has been reported for other taxa (e.g. Goldstien et al. 2006). Instead, some COI haplotypes were found throughout New Zealand but at different frequencies in northern and southern populations, while others were restricted to either side of this genetic boundary. The boundary between clusters *C* and *D* roughly coincides with Cook

Strait which separates the North and South Islands. However, as there are similarities between some upper South Island and lower North Island populations, this genetic transition does not appear to solely be a function of limited dispersal across the strait.

A similar genetic boundary has previously been reported for several species with varied developmental characteristics (planktonic larvae vs. direct developers) and habitat requirements (coastal vs. estuarine; Ross et al. 2009). Despite some inter-taxa variation in the location of this central genetic division (usually within 100 km of Cook Strait), the frequency with which the division has been detected, and the taxonomic diversity of species exhibiting this population structure, suggest that a similar genetic division might be found for much of New Zealand's coastal benthos.

For most open coast taxa, this central division has been the only genetic boundary reported (e.g. Apte and Gardner 2002; Apte et al. 2003; Waters and Roy 2004; Ayers and Waters 2005; Goldstien et al. 2006). For other coastal taxa, an additional boundary has been identified about North Cape (e.g. *S. pelliserpentis*; Veale 2007). In contrast, for *A. stutchburyi* SAMOVA was able to identify seven genetic boundaries, of which five (North Cape, north and south of East Cape, Farewell Spit and southeast North Island), are in the vicinity of genetic divisions reported previously for other estuarine taxa (Stevens and Hogg 2004; Jones et al. 2008; Knox et al. 2011). Surprisingly, SAMOVA grouped SE2 within the *E* cluster despite it being located in the centre of the *D* cluster of populations. It is possible that this grouping is an artefact of a sample size. Alternatively, it

may result from human mediated translocation of *A. stutchburyi* among estuaries.

The detection of a pattern of genetic IBD indicates that for *A. stutchburyi,* long distance dispersal among estuaries may be limited (Wright 1943). IBD has for the most part only been reported for New Zealand marine taxa lacking a planktonic larval stage (Sponer and Roy 2002; Stevens and Hogg 2004; Veale, 2007), those dependent on specific host organisms (Stevens 1991), or those sampled from estuaries (Perrin et al. 2004; Stevens and Hogg 2004). The population structure detected for *A. stutchburyi* is more similar to that of taxa with putatively limited dispersal than to open coast taxa with pelagic larvae (e.g Stevens and Hogg 2004; Veale 2007; Jones et al. 2008). This suggests that distribution and habitat requirements, as well as larval characteristics, may determine patterns of gene flow among populations.

3.4.1 Possible causes of population structure

Several mechanisms could explain the additional genetic structure (IBD) and greater number of genetic subdivisions detected in *A. stutchburyi* and other estuarine taxa relative to open coast taxa. For example, differences between estuarine and coastal taxa may result from limited present-day connectivity among estuarine populations (e.g. Watts and Johnson 2004). Connectivity will be restricted if larvae are retained within their natal estuary. However, while retention has been observed in species that occur in the upper reaches of an estuary (e.g. Little and Epifanio 1991; Cartaxana 1994; Paula 1998), retention is less likely for taxa such as *A. stutchburyi* which inhabit a broader range of estuarine habitats (Lundquist et al. 2009). A more plausible explanation is that

larvae are exported from their natal estuary but fail to reach or recruit to distant populations. For some taxa, mean dispersal distances may be as little as 2-50 km (e.g. DeBoer et al. 2008; Piggott et al. 2008; Puebla et al. 2009). Where this is the case, distance between suitable habitats will be critical in determining dispersal success. Where both dispersal capacity and the distance between settlement habitats are small, gene flow over large distances can occur in small increments over many generations. However, as the distance separating suitable habitats increases, widespread gene flow becomes less likely (Alberto et al. 2010).

In some parts of New Zealand, estuaries are separated by 200-300 km of open coast (e.g. west coast of both North and South Islands and northeast coast of South Island). Three of the genetic boundaries detected in *A. stutchburyi* occur along such sections of coast possibly reflecting the lack of estuarine habitat in these regions. Although rocky reef is also a disjunct habitat, in New Zealand there are fewer large stretches of coastline along which no hard strata can be found. Even if patches of reef are not ideal, open coast taxa can utilize sub-optimal habitats as stepping stones between more ideal habitats (Ayre et al. 2009). Similar flexibility is unlikely for estuarine taxa, limiting their potential for dispersal where estuaries are scarce.

Conversely, the genetic composition of offshore island populations (OS1 and OS2; Figures 3.1b and 3.1c), may imply that *A. stutchburyi* can disperse over larger distances. Genetic similarities were evident between offshore and mainland populations, particularly for the OS1 Chatham Islands population. Although *c.* 660 km to the southeast of the North Island, the Chatham Islands are located in the easterly flowing subtropical convergence (Heath 1982; Hadfield et

al. 2007), which may facilitate the transport of larvae from mainland to Chatham Island populations. In contrast, the Auckland Islands (OS2), while geographically closer (*c.* 460 km), lie outside of the predominantly easterly track of subantarctic water which flows past the lower South Island (Heath 1982) and appear to experience a lesser degree of connectivity.

Coastal circulation might also impede the transport of larvae among populations (e.g. Lamare 1998). It has been demonstrated that large and persistent eddies located to the north and south of East Cape (Chiswell and Booth 1999), and the semi-closed estuarine circulation typical of the fiords in southwest New Zealand (Lamare 1998), can entrain pelagic larvae. Simulations suggest that the time of entrainment could often exceed the larval duration of many benthic invertebrates (Chiswell and Roemmich 1998). As such, these hydrodynamic features may act as physical barriers to the dispersal of larvae and explain the genetic boundaries detected about East Cape and differences between Fiordland and more northerly populations.

A second possible explanation for the observed genetic subdivision is that present-day genetic boundaries are a consequence of historic dispersal barriers that no longer exist (Avise et al. 1987). Additional genetic subdivision would be expected in estuarine taxa if historic events (e.g. glaciation or topographical alteration with sea-level fluctuation) generated dispersal barriers for estuarine taxa that were easily traversed by coastal taxa on account of their greater dispersal potential. Alternatively, historic processes may have subdivided both estuarine and coastal taxa. High rates of dispersal in coastal taxa once dispersal barriers lapsed could quickly erase the genetic signatures of this subdivision,

while the introgression of allopatric populations would be slower for taxa with lesser dispersal capabilities.

A third possibility is that regional environmental differences rather than patterns of gene flow are determining the genetic structure of *A. stutchburyi*. There is evidence for natural selection on mtDNA (Fontanillas et al. 2005; Ballard et al. 2007; Oliviera et al. 2008, Diaz-Ferguson et al. 2010) and other genetic markers (Bernardi et al. 1993; Eanes 1999) with temperature suggested as a likely selective force (Schmidt et al. 2008; Balloux et al. 2009). New Zealand encompasses a large latitudinal range and steep environmental gradients exist between northern and southern locations for variables such as air and sea surface temperature (SST). It is currently unknown whether environmental variation will generate regional differences in the genetic composition of New Zealand's coastal and estuarine benthos. However, the detection of a similar population composition on east and west coasts, particularly in the South Island where east and west coast *D* cluster populations are disjunct, suggests that certain haplotypes may be favoured at specific latitudes. If survivorship or fecundity co-vary with haplotype and environmental variables such as temperature, regional variation in haplotype frequency and a major genetic divergence through central New Zealand (as detected in *A. stutchburyi* and other taxa), could be explained by a latitudinal gradient in SST and the relatively abrupt transition between subtropical and subantarctic waters off the coast of central New Zealand (Hadfield et al. 2007). Tests of neutrality provide support for the hypothesis that *A. stutchburyi* are experiencing selection, possibly in conjunction with a population or range expansion. Rapid population expansion following a

period of restricted abundance and distribution could further increase regional genetic differentiation, particularly if regionally restricted haplotypes evolved in response to environmental variation during periods of relative isolation. Given the ephemeral nature of estuaries when sea level fluctuates (Fleming 1979), repeated episodes of population and range expansion and contraction are a plausible scenario for estuarine taxa.

3.4.2 Comparison of genetic and biogeographic boundaries

Congruence between biogeographic and genetic boundaries is expected and could be explained by a combination of historical and contemporary processes (Avise et al. 1987). While the degree of congruence appears to be greater for *A. stutchburyi* and other estuarine taxa relative to coastal species, genetic differentiation was not detected at five of the eleven biogeographic boundaries described by Shears et al. (2008). This lack of complete congruence could result from the use of molecular markers that are inappropriate for detecting genetic variation across biogeographic break points, or where sampling resolution was inadequate to detect subtle genetic differences. Alternatively, the applicability of the hypothesis of congruence (Avise et al. 1987) may be location and taxon specific (Burton 1998).

While it is difficult to assess the suitability of molecular markers and the adequacy of sampling designs without further analyses, the idea that congruence will vary among species and biogeographic break points has already been the subject of considerable debate (e.g. Burton 1998; Dawson 2001; Pelc et al. 2009). In a review of population genetic studies across the southeast and southwest coasts of the United States, Pelc *et al*. (2009) found for taxa with potentially

limited dispersal ability (estuarine taxa and direct developers), that genetic boundaries were congruent with biogeographic break points, while for open coast taxa with planktonic larvae genetic boundaries were not. The available data suggest that a similar pattern may exist for New Zealand's marine benthos. However, as available research has largely focussed on open coast taxa, additional studies of estuarine and direct developing species will be required to further test this hypothesis.

3.4.3 Conclusions and Management Implications

The results of this study indicate that the estuarine clam, *A. stutchburyi* is genetically subdivided and that genetic boundaries are partially congruent with biogeographic break points. The genetic structure detected in *A. stutchburyi* was similar to that reported previously for estuarine taxa, and generally greater than the structure reported for open coast taxa. Accordingly, long-distance inter-population gene flow may be more frequent in coastal compared with estuarine taxa. Historical events and environmental processes can also cause geographical variation in genetic composition and may act either individually or together with present-day dispersal to generate the genetic structure observed in this study. Congruence between genetic and biogeographic boundaries suggests that some of the genetic subdivisions detected may be attributed to environmental variation or historical events. However, with a single non-recombining molecular marker (mtDNA), it will be difficult to fully determine which mechanisms are generating this subdivision (Balloux 2010).

Estuaries are one of the most highly impacted marine environments (Kennish 2002), with anthropogenic and natural disturbances often resulting in

the alteration, degradation or loss of estuarine habitats and communities. Recovery will depend on the spatial and temporal scales of disturbance and the rate of recruitment from intact populations (Thrush et al. 1996, 2005). Where disturbances are estuary-wide, recovery may rely on recruitment from other estuaries. These results results indicate that dispersal among estuaries may in some cases be limited. If true, estuarine communities may be slower to recover relative to coastal taxa and more vulnerable to localised population failures. Estuarine taxa may therefore need to be managed more conservatively and at smaller spatial scales than coastal species.

While the analyses conducted here identified six genetically distinct subpopulations it has been suggested that analyses of mtDNA may underestimate subdivision and overestimate connectivity (Goudet et al. 1996; Buonaccorsi et al. 1999). Further analyses incorporating multiple autosomal markers will provide more reliable estimates of connectivity and subdivision (Balloux 2010). Until such data is available environmental managers must use other tools to define population units. The partial congruence detected with biogeographic boundaries suggests that classifications based on taxonomic diversity may provide a suitable proxy for population subdivision in estuarine taxa until genetic data becomes available.

Chapter 4

Multi-scale analyses of mitochondrial and nuclear DNA provide both congruent and contrasting estimates of inter-estuary connectivity

4.1 Introduction

The importance of larval supply in determining the dynamics of adult populations of benthic marine organisms is well recognised (Underwood 1981; Menge 1991; Gaines and Bertness 1993). Since the advent of supply side ecology in the 1980's (Gaines and Roughgarden 1985), the far reaching consequences of variation in larval supply and recruitment have been demonstrated repeatedly, providing strong motivation for research into the mechanisms that underpin variation in larval delivery. While it was once believed that marine populations were largely 'open' and coastal waters an almost homogenous pool of passively drifting potential recruits (Mayr 1954; Scheltema 1986), it is now widely accepted that populations of many taxa are less open than once thought and that larval transport is spatially and temporally variable and influenced by a range of behavioural and life history traits and physical processes (Taylor and Hellberg 2003; Cowen et al. 2007).

In recent times, understanding larval sink-source dynamics and the mechanisms which govern inter-population connectivity has become a research priority as attempts are made to comprehend and manage anthropogenic

impacts on the oceans biological resources (Sale et al. 2005; Jones et al. 2007; Fogarty and Botsford 2007). Where estimates of connectivity are available they can be used to predict how populations might respond to disturbance or degradation, guiding resource and conservation management decision making. Unfortunately, our understanding of the processes that influence dispersal is far from complete, largely due to difficulties associated with physically tracking dispersing larvae (Levin 2006). However, a number of methods such as hydrodynamic modelling, elemental fingerprinting and population genetics, have been used to indirectly measure dispersal and connectivity, greatly improving our ability to estimate migration and species specific dispersal capacity (Werner et al. 2007; Hedgecock et al. 2007; Thorrold et al. 2007). Accordingly, there is now evidence for larval retention and local recruitment in taxa with long-lived pelagic larvae (Swearer et al. 2002; Taylor and Hellberg 2003), debunking the paradigm that a pelagic larval phase guarantees wide dispersal and high inter-population connectivity.

Despite recent demonstrations of limited connectivity and population subdivision (e.g. Pelc et al. 2009; Kelly and Palumbi 2010), the genetic structure of many marine taxa implies long-distance dispersal and high connectivity over 1000s of km. This includes taxa for which alternate methodologies (e.g. elemental finger printing), life history characteristics or our knowledge of local physical processes suggest long-distance dispersal is likely to be rare (Jones et al. 1999; Jones et al. 2010). One explanation for this apparent incongruence between genetic data and other information sources (e.g. elemental finger printing, physical models, life history traits) is that stepping-stone dispersal

(Kimura and Weiss 1964) can mask genetic signals that would otherwise accompany limited dispersal. Where suitable settlement habitats occur frequently along coastlines or among islands, genetic material can be transported large distances in small increments over multiple generations (e.g. Ayre et al. 2009). This will result in low genetic divergences at large scales for taxa with limited dispersal ability, potentially leading to the incorrect conclusion that long-distance dispersal is possible or frequent when, in fact, it is not.

One possible strategy for separating the genetic signals associated with single versus multi-generational gene flow is to examine taxa that are restricted to patchily distributed or rare habitats. The spatial arrangement of habitat patches and the genetic structure of populations that occur within such patches could be used to make inferences about the distances over which larvae can disperse. Where two adjacent (nearest neighbour) habitat patches (i.e. populations) are separated by a distance easily traversed by dispersing larvae, gene flow will be high and populations genetically homogenous. Alternatively, if the separation between adjacent habitats is greater than the distances that larvae can traverse (given the local hydrodynamic conditions and duration of larval period) or if physical barriers to larval exchange are present, genetic divergence would be expected due to isolation and genetic drift. For example, estuaries are patchily distributed habitats, occupied by numerous estuary obligate taxa for which population genetic structure has been analysed. A high degree of genetic structure has been reported for estuarine taxa relative to those occurring on the open coast (Watts and Johnson 2004; Pelc et al. 2009; Chapter 3). It has been suggested that much of this additional genetic structure is

due to the scarcity of estuaries in some regions and accordingly, a lack of opportunities for long distance multi-generational gene flow.

To the best of our knowledge, no studies have used the spatial arrangement of estuaries to specifically test hypotheses about geographic separation of habitat patches and gene flow among populations of benthic invertebrates. This is unfortunate as such studies would improve our understanding of the determinants of inter-population connectivity. To address this gap, I examined the population genetic structure of the New Zealand endemic estuarine clam *Austrovenus stutchburyi* using a hierarchical sampling protocol to estimate connectivity at multiple spatial scales. *Austrovenus stutchburyi* occurs in estuaries spread over 16° of latitude (>1800 km including offshore islands) and has a pelagic larval period of 2-3 weeks (Stephenson and Chanley 1979), both characteristics indicative of considerable dispersal ability. However, a recent analysis of *A. stutchburyi* mitochondrial DNA (mtDNA) reported a high degree of population subdivision at large spatial scales suggesting the dispersal ability of this taxon may be less than its life history traits would suggest (Chapter 3; Ross et al. 2011). In the present study, the genetic composition of *A. stutchburyi* populations was analysed at three spatial scales using the mitochondrial gene cytochrome *c* oxidase subunit I (COI) and three nuclear microsatellite loci. The aims of this study were to determine the spatial scale at which *A. stutchburyi* populations are connected and determine the extent to which geographic distance among populations and between habitat patches explains patterns of population genetic structure. Specifically, I test the hypotheses that genetic divergence among populations will be less at small

spatial scales compared with large scales, and that levels of inter-population divergence will be correlated with geographic separation.

4.2 Methods

4.2.1 Sampling protocol

A. stutchburyi were collected from seven estuaries located on New Zealand's west coast (Table 4.1; Fig. 4.1). The selected estuaries were spread over more than 10° of latitude (>1400 km of coastline) encompassing much of the species' latitudinal range. Analyses grouped sampled locations at three spatial scales: local scale (within estuary), regional scale (across a 160 km section of coastline) and national scale (across > 1400 km of coastline; Fig 4.1). Local scale analyses compared specimens collected at two locations within a single estuary (N3 and N4; Fig 4.1a). Within-estuary sampling locations were separated by *c.* 15 km. Regional-scale analyses compared specimens collected at five locations (N1-N5; Fig 4.1b) within the four northernmost west coast estuaries. The four estuaries were separated by 12, 22 and 125 km of open coast (Fig. 4.1b). National-scale analyses compared all eight populations (from seven estuaries) from which specimens were collected. This included populations examined at local and regional scales (N1-N5) as well as populations from three additional estuaries (N6, S1 and S2) separated from regional-scale sampling locations by 270, 620 and 1150 km (Fig 4.1c). While regional-scale sampling incorporated all estuaries on New Zealand's north-west coast, at the national scale, the sampled estuaries were a subset of those in which *A. stutchburyi* occur. Specimens were collected between January 2007 and May 2011 and stored at -80°C prior to DNA extraction.

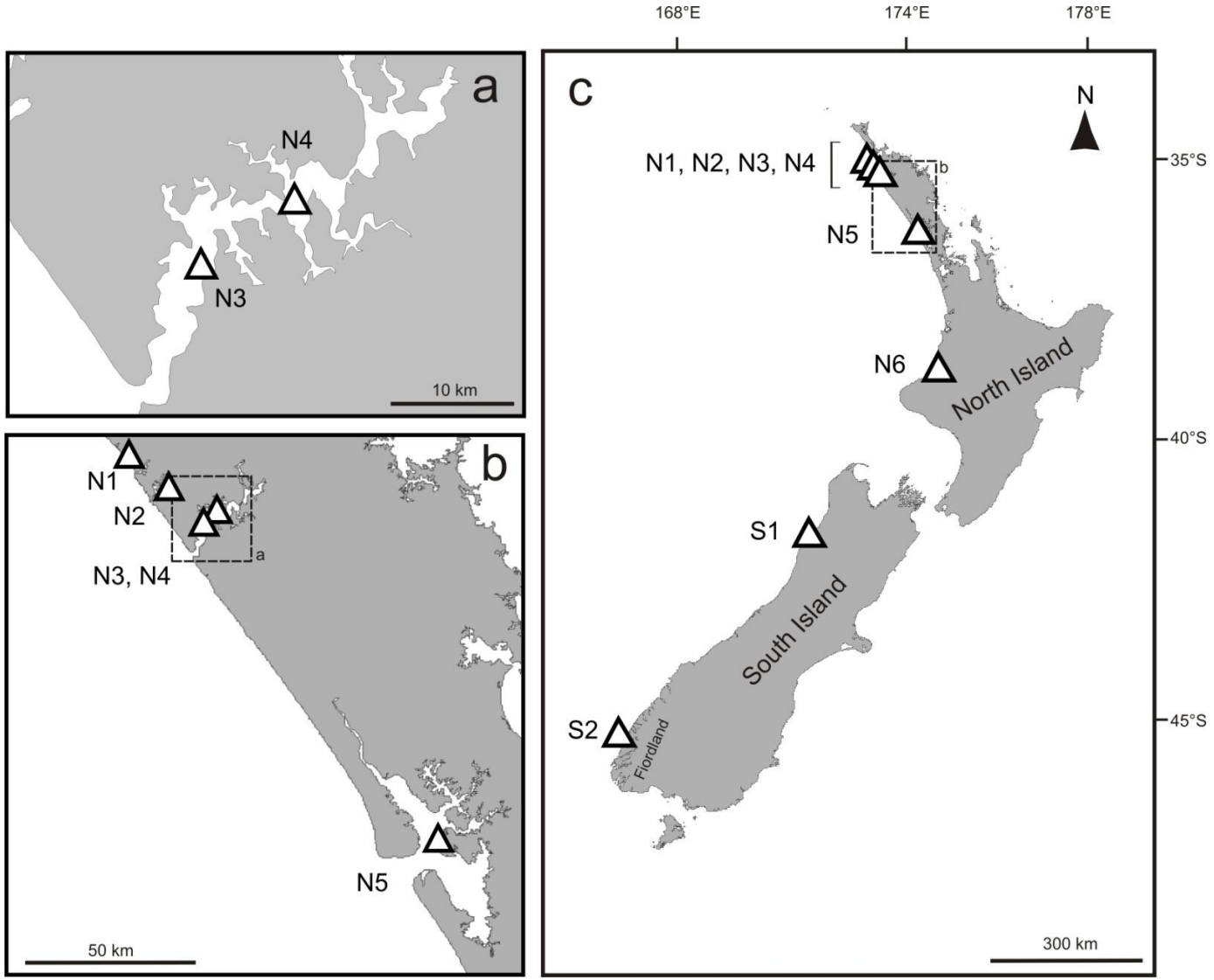

Figure 4.1 Maps of New Zealand showing locations of A*ustrovenus stutchburyi* populations sampled at (a) local, (b) regional and (c) national sampling scales. Dashed boxes (---) within map panels b and c indicate local and regional scale sampling areas. Site labels refer to estuary locations detailed in table 1.

Table 4.1 Summary of *Austrovenus stutchburyi* populations sampled. Labels correspond to population markers in Figure 1. Approximate locations of populations are provided as well as the number of cytochrome *c* oxidase subunit I (COI) sequences obtained (N_{COI}), number of haplotypes detected (N_{hap}), number of polymorphic sites (S), haplotype diversity (H), mean number of pairwise differences (π_1), nucleotide diversity (π_2), number of individuals genotyped for microsatellite loci (N_{MSAT}), number of alleles recorded across all loci (N_{ALL}) and allelic richness (A_r) at each location.

Location	Label	Position (Lat.-Long.)	N_{COI}	N_{hap}	S	H	π_1	π_2	N_{MSAT}	N_{ALL}	A_r
Herekino	N1	35°18' S - 173°10' E	14	11	17	0.96	4.0	0.006	42	72	23.2
Whangape	N2	35°21' S - 173°15' E	13	8	11	0.91	2.5	0.004	42	64	20.7
Opononi	N3	35°29' S - 173°24' E	13	12	21	0.99	4.5	0.007	48	76	23.4
Rawene	N4	35°24' S - 173°30' E	13	7	11	0.80	2.6	0.004	45	66	20.7
Kaipara	N5	36°15' S - 174°15' E	12	8	14	0.91	3.6	0.006	40	71	23.1
Tongaporutu	N6	38°49' S - 174°36' E	19	16	25	0.97	4.5	0.007	44	74	23.4
Orowaiti	S1	41°45' S - 171°38' E	12	10	14	0.96	3.2	0.005	39	69	22.6
Doubtful Sound	S2	45°26' S - 167°08' E	12	8	11	0.85	2.6	0.004	37	62	20.7
Total	**-**	**-**	**108**	**58**	**63**	**0.93**	**3.7**	**0.006**	**337**	**114**	**22.2**

4.2.2 DNA extraction and PCR amplification

A 0.25-0.50 cm^2 piece of adductor mussel was dissected from each specimen and genomic DNA extracted using the Zymo Research Genomic DNA II Kit (Zymo Research Corporation, Orange, CA, USA). DNA extracts were then stored at 4°C until needed for polymerase chain reaction (PCR) amplification.

A 710 base pair (bp) fragment of the mitochondrial COI gene was amplified using the universal primers LCO1490 and HCO2198 (Folmer et al. 1994). PCR amplifications were conducted in 10 µl reactions containing 4.8 µl Intron i-Taq 2x PCR master mix, 5 pmol of each primer and 1 µl of unquantified template DNA. PCR reactions consisted of an initial denaturing phase (94°C, 4 min), followed by 35 thermocycles of denaturation (94°C, 60 s), annealing (52°C, 90 s) and extension (72°C, 90 s), followed by a final extension period (72°C, 5 min). Unincorporated nucleotides and primers were removed by adding 2 units of Exonuclease I, 0.1 unit of Shrimp Alkaline Phosphatase and 2.7 µl H_2O and incubating at 37°C (30 min) then 80°C (15 min). Sequencing reactions used Big Dye terminator sequencing chemistry (Applied Biosystems) on an Applied Biosystems 3130 Genetic Analyzer. DNA strands were edited in Geneious (version 4.8.4) to produce an alignment of 658 bp. Sequences have been deposited in the Barcode of Life Datasystems (BOLD) database under project NZCOC (New Zealand Marine Bivalves).

Seventeen microsatellite primer pairs developed specifically for *A. stutchburyi* were tested on a subset of specimens from all sampled locations. Of the 17 primers sets, only three (Cocdi 5, Cocdi 13, Cocdi 15; Table 4.2) amplified consistently in all populations and produced eletropherogram peaks consistent

with those expected for microsatellites (Peakall and Smouse, 2009). These three loci were then amplified for all specimens from all sampled populations (Table 4.1).

PCR amplifications were conducted in 20 µl reactions containing 0.2 µl Bioline MyTaq HS DNA polymerase, 4 µl 5x MyTaq reaction buffer, 4 pmol of each primer and 0.4 µl of unquantified template DNA. Forward primers were 5' end-labelled with the fluorescent dye 6-FAM to allow amplified DNA fragments to be visualised and their length quantified. PCR reactions consisted of an initial denaturing step (94°C, 3 min), followed by 30 thermocycles of denaturation (94°C, 30 s), annealing (52°, 57° or 61°C, 30 s; Table 4.2) and extension (72°C, 90 s), followed by a final extension period (72°C, 30 min). Amplified products were processed on an Applied Biosystems 3130 Genetic Analyser with LIZ600 size standard. Fragment size was calculated using Peak Scanner v1.0 (Applied Biosystems).

4.2.3 Population genetic analyses

Indices of COI genetic diversity were quantified using DnaSP version 5 (Librado and Rozas 2009). For each population the number of COI haplotypes (N_{hap}), number of segregating sites (S), haplotype diversity (H_e), mean number of pairwise differences (π_1) and nucleotide diversity (π_2) were calculated. A haplotype network was then constructed in TCS v1.21 (Clement et al. 2000) and groups of closely related haplotypes identified and assigned to haplogroups. The abundance of each haplogroup was then plotted at sampled locations to visualise spatial variation in the genetic composition of populations.

Table 4.2 Characteristics and diversity indices of three microsatellite loci for all *Austrovenus stutchburyi* specimens (*N* = 337). Primer sequences are provided as well as the repeat motif as first identified (Repeat), annealing temperature (T_a), number of alleles recorded across all populations (NULL), observed (H_O) and expected (H_E) heterozygosity, Hardy-Weinberg equilibrium probability test *p*-values (*P*) and the null allele frequency for each locus (NULL). Heterozygosities and their significance were calculated in ARLEQUIN with exact tests using a Markov chain (100 000 steps; 1 000 000 chain length).

Locus	Primer sequences (5'-3')	Repeat	T_a(°C)	N_{ALL}	Allele size range (bp)	A_r	H_o	H_e	*P*	NULL
COCDI 5	F: CCCTAACCATGTTAAATAGGCA R: CCACTGTCAGCTTGAAACATTC	$(CT)_6(CT)_9$	61°C	49	148-274	29.3	0.75	0.96	<0.001	0.13
COCDI 13	F: GTTTGTGGAAACCATAGATGCA R: CCACTGTCAGCTTGAAACATTC	$(AG)_{15}(GA)_{10}$	57°C	28	198-282	18.2	0.47	0.92	<0.001	0.27
COCDI 15	F: CCAAGCATTTTAGAGAGAGA R: AAGTTAATTGTCCTCAATGTGA	$(AG)_{15}(GA)_{10}$	52°C	37	129-221	23.1	0.59	0.94	<0.001	0.21

The GTR model of sequence evolution was selected in jMODELTEST 0.1.1 (Posada 2008) and a GTR corrected distance matrix was generated in PAUP* 4.0 (Swofford 2000) for use in subsequent analyses. Estimates of population pairwise Φ_{ST} were calculated in ARLEQUIN version 3.11 (Excoffier et al. 2005) to determine if any two populations differed significantly in their genetic composition. Mantel tests, also implemented in ARLEQUIN were then conducted at regional and national sampling scales, to assess the relationship between genetic (Φ_{ST}) and geographic distance. Geographic distance was regressed against estimates of population pairwise Φ_{ST} to determine whether *A. stutchburyi* population genetic structure conformed to a pattern of IBD. Geographic distances among populations were measured in Google Earth as the shortest distance over water between any two estuaries.

At the national sampling scale, Spatial Analysis of Molecular Variance (SAMOVA version 1.0; Dupanloup et al. 2002) was used to further investigate spatial variation in the genetic composition of populations. This method is based on a simulated annealing procedure that maximizes the proportion of total genetic variance due to differences among groups of populations. SAMOVA can be used to define population clusters that are geographically homogeneous and maximally differentiated from each other without the prior assumption of subpopulation composition. Genetic variance (F_{ST}) is partitioned into two components, F_{SC} and F_{CT}, indicating respectively the differentiation among populations within and among groups. SAMOVA analyses ran for 10 000 iterations from each of 100 random initial conditions for a predetermined number of subpopulations (k) ranging from 2 to 7.

Indices of microsatellite diversity were quantified using ARLEQUIN, FreeNA (Chapius and Estoup 2007) and FSTAT version 2.9.3.2 (Goudet 1995). For each locus and population the total number of alleles (N_{all}), allelic richness (A_r), allele size range (A_{sr}), expected heterozygosity (H_e) and observed heterozygosity (H_o) were calculated. Microchecker (Van Oosterhout et al. 2004) was used to test all loci and populations for Hardy-Weinberg-equilibrium (HWE) and to determine whether deviations from HWE resulted from technical artefacts (null alleles or large allele dropout) or non-random breeding (e.g. Wahlund effect). As Microchecker indicated the presence of null alleles at all loci in most populations (Tables 4.2 and 4.3), FreeNA was used to estimate allele frequencies (corrected for the presence of null alleles) that could be used in subsequent analyses. Corrected allele frequencies were then analysed in DISPAN to estimate expected (H_s) and total heterozygosity (H_T). H_S and H_T were estimated across all populations and for each population pair.

The fixation indices F_{ST} (Wright 1943) and G''_{ST} (Gerlach et al. 2010), and Jost's (2008) unbiased estimator of population differentiation D_{est} were then used to investigate spatial variation in the genetic composition of *A. stutchburyi* populations. Global and population pairwise F_{ST} were calculated in FreeNA using corrected allele frequencies, while global and pairwise G''_{ST} was estimated as per Meirmans and Hedrick's (2011) equation 4:

$$G''_{ST} = [k^*(H_S-H_T)]/[(k^*H_T-H_S)^*(1-H_S)]$$

Table 4.3 *Austrovenus stutchburyi* genetic diversity at each sampled location and locus. The number of individuals genotyped for microsatellite loci (*N*) is provided as well as allelic richness (A_r), null allele frequency (NULL), observed (H_O) and expected (H_E) heterozygosity and Hardy-Weinberg equilibrium probability test *p*-values (*P*). Null allele frequencies were estimated in FreeNA using the EM algorithm. Heterozygosities and their significance were calculated in ARLEQUIN.

Population	*N*	Locus	A_r	NULL	H_o	H_e	*P*
		Cocdi 5	27.6	0.08	0.79	0.95	0.01
N1	42	Cocdi 13	17.8	0.28	0.38	0.94	<0.001
		Cocdi 15	24.1	0.13	0.67	0.94	<0.001
		Cocdi 5	25.8	0.11	0.71	0.94	<0.001
N2	42	Cocdi 13	16.6	0.22	0.48	0.91	<0.001
		Cocdi 15	19.8	0.19	0.57	0.94	<0.001
		Cocdi 5	32.5	0.06	0.85	0.97	0.001
N3	48	Cocdi 13	17.0	0.26	0.42	0.92	<0.001
		Cocdi 15	20.8	0.20	0.54	0.94	<0.001
		Cocdi 5	27.0	0.09	0.78	0.95	0.006
N4	45	Cocdi 13	16.1	0.27	0.38	0.9	<0.001
		Cocdi 15	19.0	0.18	0.56	0.92	<0.001
		Cocdi 5	30.1	0.09	0.78	0.96	0.003
N5	40	Cocdi 13	19.5	0.17	0.6	0.93	<0.001
		Cocdi 15	19.6	0.18	0.58	0.94	<0.001
		Cocdi 5	27.3	0.11	0.73	0.96	<0.001
N6	44	Cocdi 13	16.2	0.17	0.57	0.91	<0.001
		Cocdi 15	26.7	0.18	0.59	0.96	<0.001
		Cocdi 5	26.4	0.19	0.56	0.95	<0.001
S1	39	Cocdi 13	17.8	0.19	0.56	0.93	<0.001
		Cocdi 15	23.7	0.19	0.54	0.92	<0.001
		Cocdi 5	22.0	0.07	0.81	0.94	0.157
S2	37	Cocdi 13	16.0	0.29	0.35	0.91	<0.001
		Cocdi 15	24.0	0.13	0.68	0.95	<0.001

Global and pairwise bias-corrected D_{est} (Jost 2008) were calculated in DEMEtics version 0.8 (Gerlach et al. 2010), with significance calculated from 1000 bootstrap replicates.

Mantel tests were then implemented in ARLEQUIN at regional and national sampling scales. F_{ST}, G''_{ST} and D_{est} were regressed against geographic distance to assess the relationship between genetic and geographic distance and to test for a pattern of IBD. The significance of Mantel correlations were calculated using 1000 random bootstrap replicates. Global and inter-population estimates of the number of migrants (N_m) per generation under the assumptions of the island model of population structure were then calculated as per Meirmans and Hedrick (2011):

$$N_m = (1-G''_{ST})/(4*F_{ST})$$

F_{ST} values used for this calculation were corrected for the presence of null alleles in FreeNA and the G''_{ST} estimates used were those calculated above. Although many of the island model's assumptions are likely to be violated in *real* populations, for example equilibrium, nonspatial migration, equal population sizes and neutrality, N_m can still be used as a relative measure of population connectivity (Lowe and Allendorf 2010; Miermans and Hedrick 2011). Lastly, SAMOVA was implemented at the national sampling scale to further identify spatial population structure. As per the COI analysis, SAMOVA ran for 10 000 iterations from each of 100 random initial conditions for between two and seven populations.

4.3 Results

4.3.1 Genetic Diversity

One hundred and eight *A. stutchburyi* were sequenced for the mitochondrial COI gene. Of the 658 positions analysed, 63 were variable leading to the delineation of 58 haplotypes (Table 4.1). The most abundant haplotype (H1; Fig. 4.2), was recorded for 25 individual specimens (23% of the total sample) and occurred in all sampled populations. Another two haplotypes were recorded in 10 specimens from five populations, two haplotypes were recorded in three specimens, and four haplotypes were recorded twice, while the remaining 49 haplotypes were recorded only once. Haplotype diversity was high throughout all populations with at least seven haplotypes recorded at each location ($\overline{H}_e$ = 0.93; Table 4.1). In contrast to haplotype diversity, nucleotide diversity was low with most COI sequences differing by only a small number of base changes ($\overline{\pi}_1$ = 3.7, $\overline{\pi}_2$ = 0.006; Table 4.1).

The haplotype network generated in TCS revealed a 'star-like' topology in which the most common haplotype (H1) was central within the network and surrounded by an array of less common haplotypes linked to H1 by a maximum of eight mutational steps (Fig. 4.2a). Three haplotypes (H2-4) separated from H1 by between one and three mutational steps were also central within smaller 'star-like' topologies, while haplotype H5 was relatively abundant and located towards the exterior of a non-star-like region of the network (G5). Of the eight COI haplogroups that were defined from the network topology (G1-8; Fig. 4.2a), two groups (G1 and G7) consisted of single haplotypes, while haplogroup G2

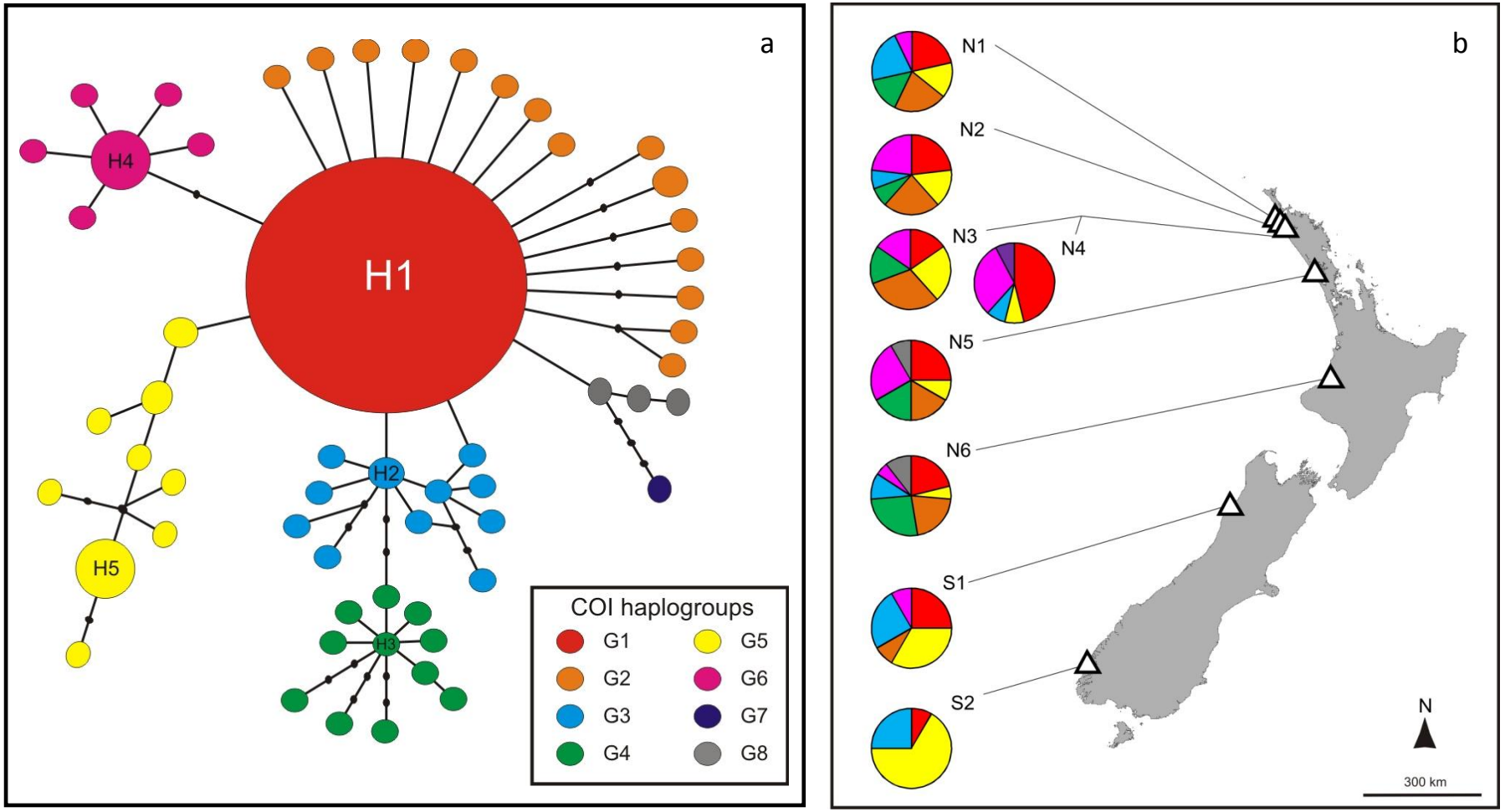

Figure 4.2 (a) Haplotype network of the 108 *Austrovenus stutchburyi* cytochrome c oxidase subunit I (COI) haplotypes. Each haplotype is represented by a circle with its size proportional to the number of individual specimens bearing the haplotype. Small black circles indicate undetected intermediate haplotypes. The most common haplotypes have been labelled (H1-5) and groups of closely related haplotypes assigned to haplogroups (G1-8) as indicated by circle colouration. (b) Sampling locations of *A. stutchburyi* populations and the frequencies of COI haplogroups in each population. Pie segment colours refer to COI haplogroups defined in the haplotype network, while segment size indicates the relative frequency of each haplogroup in each population. Labels refer to estuary locations detailed in Table 1.

consisted of 15 haplotypes one or two steps divergent from H1 and not linked to any other haplotypes.

The three dinucleotide repeat microsatellite loci were highly polymorphic with 49, 28 and 37 alleles recorded for Cocdi 5, Cocdi 13 and Cocdi 15 respectively (Table 4.2). Allelic richness (A_r) was high in all loci and for all populations (Tables 4.1 and 4.3). H_O was always less than H_E for all loci, with significant deviations from HWE detected in 23 of 24 of the locus-by-population comparisons (Table 4.3). Microchecker indicated that deviations from HWE were caused by an excess of homozygotes, most likely due to the presence of null alleles. Null allele frequencies estimated in FreeNA were consistent across populations (Table 4.3), but varied between loci (0.13-0.27; Table 4.2).

4.3.2 Local scale (within estuary) analyses

Analysis of mitochondrial COI sequences indicated genetic differences among *A. stutchburyi* collected from two locations within a single estuary (N3 and N4; Figures 4.1a and 4.2). A total of seventeen haplotypes were recorded of which only two were shared between the two locations. Five of the eight COI haplogroups delineated from the haplotype network were recorded at each sampling location. Three of these haplogroups were common to both inner (N3) and outer harbour (N4) locations (Fig. 4.2). However, the frequencies at which shared haplogroups were recorded varied between N3 and N4. Haplogroups G1 and G6 were more abundant at N4 (46% and 31% respectively) than at N3 (15% and 15%), while haplogroup G5 was recorded with greater frequency in specimens from N3 (23% vs. 8%).

In contrast to analyses of COI sequences, microsatellite allele frequencies did not indicate significant genetic divergence between N3 and N4. Within-estuary F_{ST} and G''_{ST} were low relative to global estimates calculated among populations at regional and national sampling scales (Table 4.4). D_{est} also suggested that divergence between N3 and N4 was small and not significant. Migration between N3 and N4, estimated according to Meirmans and Hedrick (2011), was an order of magnitude greater than migration estimated among populations at the regional scale and two orders greater than at the national scale (N_m; Table 4.4).

Table 4.4 Measures of global differentiation calculated at local, regional and national sampling scales. F_{ST} values were estimated in FreeNA using the EM algorithm (Chapius and Estoup 2007). G''_{ST} values were estimated according to Meirmans and Hedrick's (2011) equation 4. D_{est} values were estimated in DEMEtics, with significance calculated from 1000 bootstrap replicates. The significance of F_{ST} values could not be calculated as FreeNA does not perform bootstrapping for analyses with fewer than four loci.

	Measures of global differentiation		
	Local	**Regional**	**National**
F_{ST}	<0.0001	0.0008	0.0031
G''_{ST}	0.11	0.15	0.19
D_{EST}	0.065^{ns}	0.072^{ns}	0.124**
N_m	2521	279	64

4.3.3 Regional scale analyses

At the regional scale, analyses of COI sequence data indicated that genetic differences among estuaries were small. Differences observed among estuaries were in fact less than those observed between sites within an estuary (N3 and N4; Fig. 4.2). All eight of the COI haplogroups defined from the haplotype network were recorded in populations sampled at the regional sampling scale. Haplogroup frequencies indicated that *A. stutchburyi* at locations N1, N2 and N5 were genetically similar to those sampled from the outer harbour N3 population but differed from those sampled at the inner harbour N4. Haplogroups G1 (15-23%), G2 (15-31%), G4 (8-15), G5 (8-23%) and G6 (8-23%) were detected at similar frequencies in populations N1, N2, N3 and N5, while at population N4, haplogroups G1 (46%) and G6 (31%) were more abundant and haplogroups G2 and G4 absent (Fig. 4.2). In spite of these differences, estimates of Φ_{ST} were small among all population pairs (Table 4.5a) suggesting that the observed genetic differences were not significant. A Mantel test failed to detect a relationship between geographic and genetic (Φ_{ST}) distance (Table 4.6) indicating that gene flow among populations at this sampling scale is not limited by geographic separation.

Pairwise G''_{ST} and D_{est} estimates based on microsatellite allele frequencies were also non-significant, again suggesting limited genetic differentiation at this sampling scale (Tables 4.5b and 4.5c). However, G''_{ST} and D_{est} estimated between N5 (the most geographically isolated population at this sampling scale) and populations N2 and N4 were relatively large and for D_{est} only marginally non-

Table 4.5 Population pairwise comparisons of (a) Φ_{ST}, (b) D_{est}, (c) N_m (below diagonal) and G''_{ST} (above diagonal). Significance of Φ_{ST} and D_{est} (above diagonals) calculated using 1000 bootstrap replicates in ARLEQUIN and DEMEtics respectively. N_m and G''_{ST} values were calculated according to Meirmans and Hedrick (2011). Population site labels are detailed in Table 1 and locations displayed on Figure 1. Significant Φ_{ST} and D_{est} values are indicated in bold. **P*<0.05, ***P*<0.01, ****P*<0.001.

(a)

	N1	N2	N3	N4	N5	N6	S1	S2
N1		-	-	-	-	-	-	***
N2	0.01		-	-	-	-	*	***
N3	-0.03	-0.02		-	-	-	-	***
N4	0.03	-0.02	0.01		-	*	-	***
N5	-0.02	-0.02	-0.04	-0.01		-	-	**
N6	0	0.03	-0.02	**0.07**	-0.02		-	***
S1	-0.01	**0.06**	0	0.05	0.03	0.05		*
S2	**0.13**	**0.28**	**0.16**	**0.25**	**0.20**	**0.19**	**0.08**	

(b)

	N1	N2	N3	N4	N5	N6	S1	S2
N1		-	-	-	-	-	-	**
N2	0.06		-	-	-	-	*	**
N3	0.06	0.03		-	-	-	-	*
N4	0.04	0.12	0.06		-	*	*	**
N5	0.06	0.14	0.01	0.11		*	*	**
N6	0.09	0.09	0.06	**0.15**	**0.16**		-	-
S1	0.11	**0.16**	0.10	**0.16**	**0.12**	0.16		*
S2	**0.22**	**0.19**	**0.16**	**0.24**	**0.19**	0.11	**0.15**	

(c)

	N1	N2	N3	N4	N5	N6	S1	S2
N1		0.15	0.13	0.12	0.19	0.19	0.20	0.23
N2	307		0.12	0.14	0.17	0.17	0.19	0.22
N3	2165	2212		0.11	0.13	0.16	0.17	0.20
N4	2207	65	2521		0.18	0.20	0.19	0.23
N5	329	44	2179	70		0.20	0.19	0.27
N6	134	64	144	33	44		0.19	0.22
S1	131	37	130	56	100	55		0.24
S2	21	18	34	19	24	62	40	

significant (p = 0.093 and 0.057 respectively). Estimates of global F_{ST} , G''_{ST} and D_{est} at the regional sampling scale were greater than those calculated at the local scale suggesting a greater degree of differentiation, although global D_{est} was again non-significant (Table 4.4). Estimates of migration among all populations at the regional scale were less than between sites within an estuary (local scale; Table 4.4) although pairwise estimates of N_m were highly variable and not related to the geographic separation of populations (Table 4.5c). As with the analysis of COI sequence data, Mantel tests again failed to detect any relationship between geographic and genetic (F_{ST}, G''_{ST} and D_{est}) distance (Table 4.6).

Table 4.6 Mantel correlations between geographic distance and measures of genetic dissimilarity as estimated from the analysis of cytochrome *c* oxidase I sequences (Φ_{ST}) and microsatellite allele frequencies (F_{ST}, G''_{ST} and D_{est}). Correlations and their significance were calculated in ARLEQUIN using 1000 permutations. Significant correlations are indicated in bold. **P<0.01, ***P<0.001.

Mantel correlations

	Regional scale	National scale
Φ_{ST}	0.26	**0.72*****
F_{ST}	0.16	**0.57****
G''_{ST}	0.58	**0.63****
D_{EST}	0.07	**0.61*****

4.3.4 Large scale analyses

At the national sampling scale, COI analyses indicated significant spatial variation in the genetic composition of *A. stutchburyi* populations. Variability in the frequency at which haplogroups were recorded indicated that population N6 was similar to populations sampled at the regional sampling scale (excluding N4), although more G4 haplotypes and fewer G6 haplotypes were recorded at N6 (Fig. 2). More substantial genetic differences were evident when comparing the two South Island populations to populations located in the North Island. G5 haplotypes were recorded in high frequencies at both S1 and S2 populations (31% and 62% respectively vs. 8-23%), while G2 haplotypes were not (8% and 0% vs. 15-31%). G3 haplotypes were also recorded at a higher frequency in S1 and S2 than at any other population except N1.

Pairwise Φ_{ST} estimates indicated that population S2, from Doubtful Sound, Fiordland, was significantly different from all other populations. Pairwise comparisons also revealed significant divergence between populations S1 and N2 and between N6 from N4. A Mantel test revealed a highly significant positive correlation between geographic and genetic distances (Φ_{ST}) among all sampled populations ($P < 0.001$; Fig. 4.3a). Distance between estuaries accounted for 72% of inter-population COI variability, a result indicative of genetic isolation-by-distance (IBD), implying that at this spatial scale dispersal among populations may be limited by geographic separation.

Table 4.7 *Austrovenus stutchburyi* population clusters as defined in SAMOVA using (a) COI sequence data and (b) microsatellite allele frequencies. Measures of differentiation and their significance were calculated for 10 000 iterations from each of 100 random initial conditions for a predetermined number of subpopulations (*k*) ranging from 2 to 7. Significant values are indicated in bold. **P*<0.05, ***P*<0.01, ****P*<0.001.

(a)

k	**2**	**3**	**4**	**5**	**6**	**7**
	N1	N1	N1	N1	N1	N1
	N2	N2	N2	N3		
	N3	N3	N3	N5	N3	N3
	N4	N4	N4		N5	
	N5	N5	N5	N4		N5
	N6	N6		N2	N4	
	S1		N6		N2	N4
		S1		N6		N2
	S2		S1		N6	
		S2		S1		N6
			S2		S1	
				S2		S1
					S2	
						S2
FSC	0.009	**0.002**	-0.012	-0.028	-0.035	-0.038
FST	0.164***	**0.107*** **	**0.077** **	**0.060** **	**0.055*** **	**0.052** **
FCT	0.155	**0.105***	**0.087** **	**0.086** **	**0.086** **	**0.087***

(b)

k	**2**	**3**	**4**	**5**	**6**	**7**
	N1	N1	N1	N1	N1	N1
	N2	N2	N2	N3		N2
	N3	N3	N3	N4	N4	
	N4	N4	N4	N5	N3	N3
	N5	N5	N6		N6	
	N6	N6		N2		N5
	S2		N5		N2	
		S1		N6		N4
	S1		S1		N5	
		S2		S1		N6
			S2		S1	
				S2		S1
					S2	
						S2
FSC	**0.014***	**0.004**	0.002	0.008	0	0.02
FST	**0.094*** **	**0.072*** **	**0.054*** **	**0.042*** **	**0.039** **	**0.035*** **
FCT	0.081	**0.068***	0.052*	0.035	0.039	0.015

SAMOVA obtained its best partitioning of genetic variance when populations were assigned to three groups (Table 4.7a). While F_{ST} was significant for all grouping options (k = 2 to 7), significantly differentiated population clusters were only produced when populations were split into three or four groups, with k = 3 producing the most significant genetic structure (F_{CT} = 0.068; F_{SC} = 0.004; Table 4.7a). At k = 3 the SAMOVA assigned all North Island populations to a single group while splitting S1 and S2 from populations N1-6 and each other.

Estimates of global F_{ST}, G''_{ST} and D_{est} generated from microsatellite allele frequencies were greater at the national sampling scale than at regional or local scales (Table 4.4). D_{est} was also highly significant (p = 0.001) indicating significant genetic differentiation among the populations sampled at this scale. Population pairwise estimates of D_{est} indicated that population S2 differed significantly from all populations except N6, that populations S1 and N6 both differed significantly from N4 and N5, and that population S1 differed significantly from N2. Mantel tests revealed significant positive correlations between geographic distance and three measures of genetic difference ($F_{ST,}$ G''_{ST} and D_{est}) among all sampled populations (Table 4.6; Figures 4.3b and 4.3c). Distance between estuaries accounted for between 57 and 63% of inter-population microsatellite variability, results indicative of genetic IBD.

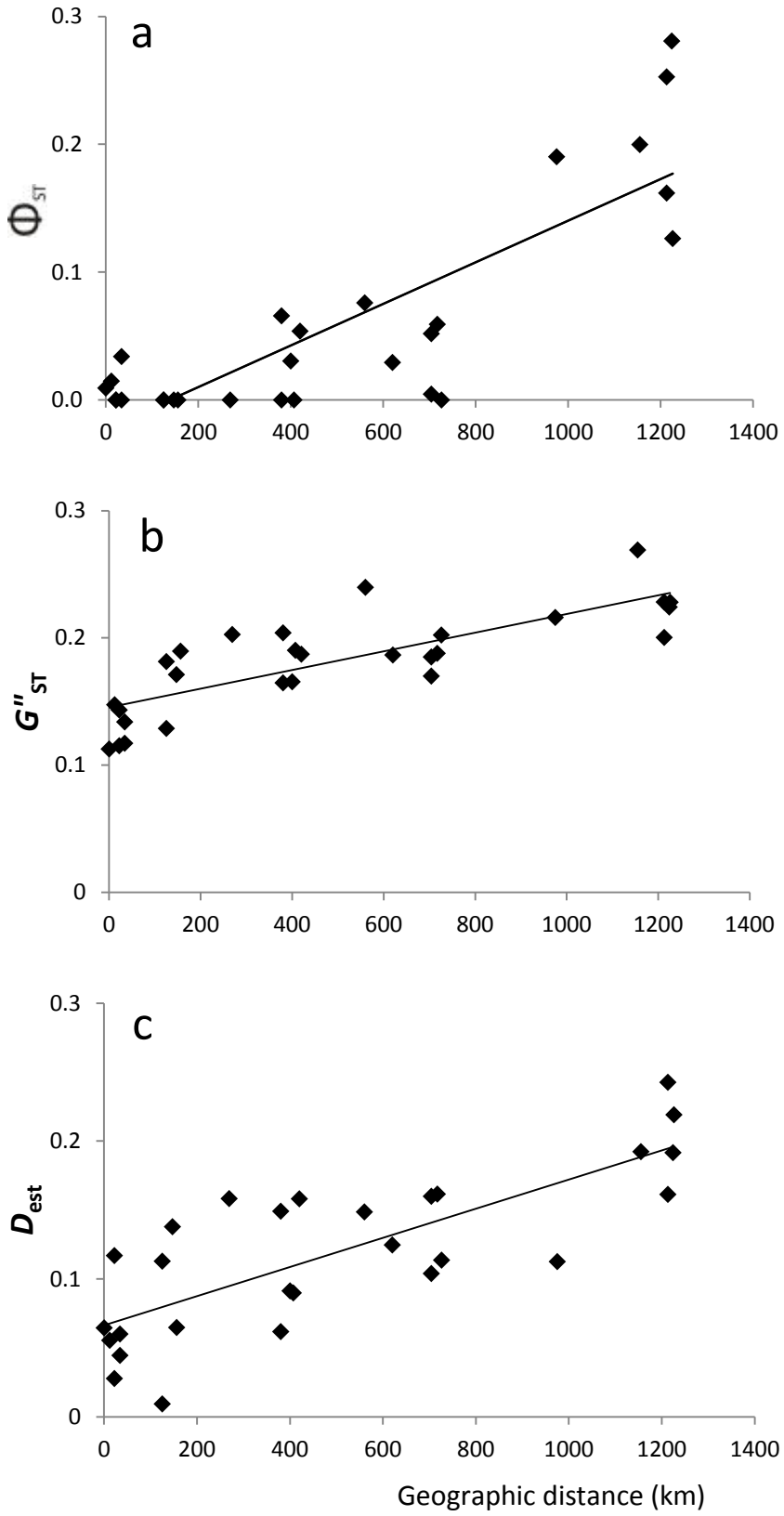

Figure 4.3 Relationships between geographic distance (shortest distance over water) and estimates of genetic dissimilarity (Φ_{ST}, G''_{ST} and D_{est}) for *Austrovenus stutchburyi*. Mantel relationships were significant ((a) F_{ST}: R^2=0.72, P<0.001; (b) G''_{ST}: R^2=0.63, P<0.01; (c) D_{est}: R^2=0.61, P<0.001)

Global N_m at the national sampling scale was an order of magnitude less than estimates of among populations migration at the regional scale. Pairwise estimates of N_m between the most geographically isolated populations N6, S1 and S2 and all other populations were low relative to most N_m estimates at regional and local sampling scales (Table 4.4). Although pairwise estimates of N_m at this scale were variable, estimates of migration over distances greater than 200 km were always low relative to the maximum values attained for population pairs located in closer proximity (< 200 km; Table 4.5c).

SAMOVA again obtained its best partitioning of genetic variance when populations were assigned to three groups (Table 4.7b). F_{ST} was significant for all grouping options (k = 2 to 7) and F_{CT} significant when populations were split into three or more groups. However, as with the SAMOVA of COI sequence data, k = 3 produced the most significant genetic structure (F_{CT} = 0.105; F_{SC} = 0.002; Table 4.7b), again grouping all North Island populations while splitting S1 and S2 from populations N1-6 and each other.

4.4 Discussion

Population genetic analyses using both mtDNA COI sequences and newly developed microsatellite loci, show that gene flow among *A. stutchburyi* populations is limited at large spatial scales. Genetic diversity was high for both mitochondrial and microsatellite markers, which is consistent with previous genetic analyses of *A. stutchburyi* (Lidgard 2001; Ross et al. 2011) and some other New Zealand benthic invertebrates (Waters and Roy 2004; Waters et al 2005; Veale 2007). The high levels of genetic diversity observed here may

partially explain the widespread distribution and high abundance of *A. stutchburyi* in estuarine ecosystems. Genetic diversity implies the capacity for local adaptation (Britten 1996; David 1998; Mitton 1998) and accordingly, *A. stutchburyi* can be found at high densities on intertidal flats in estuaries from New Zealand's subtropical north (34.5°S) to the subantarctic south (Auckland Islands; 50.9°S). Populations occur across steep environmental gradients, not just at a national scale but also within estuaries where water and habitat quality vary through time and space. Ross et al. (2011; Chapter 3) observed a north-south cline in COI haplotype frequency that was consistent with latitudinal environmental gradients. While local adaptation was suggested as a possible explanation for the genetic cline, having only analysed a single molecular marker, it was not possible to distinguish between natural selection and other processes such as limited gene flow.

Pelagic larval duration (PLD) was once thought to be a good predictor of dispersal ability and population connectivity (Bohonak 1999). However, recent meta-analyses have for many taxa demonstrated a decoupling of larval duration and connectivity (Bradbury et al. 2008; Shanks 2009; Ross et al. 2009; Weersing and Toonen 2009). For example, panmixia over 100s or 1000s of km has been reported for taxa either lacking or with limited capacity for larval dispersal, whereas fine spatial scale genetic subdivision has been reported for taxa with long-lived pelagic larvae (Ayre and Hughes 2000; Holmes et al. 2003; Miller and Ayre 2008). The results of the present study further suggest that factors other than PLD are important determinants of gene flow. In spite of a 2-3 week larval duration which might facilitate widespread dispersal, *A. stutchburyi* populations

were not genetically homogenous. Furthermore, the detection of a highly significant pattern of IBD suggests that dispersal among geographically distant populations is limited (Wright 1943).

Although it would have been desirable to incorporate a greater number of nuclear loci into these analyses, microsatellite development for *A. stutchburyi* has so far been only moderately successful, generating only the three loci used in this investigation (Appendix I). However, as similar patterns of connectivity were suggested by COI and microsatellite analyses (at regional and national scales) I have greater confidence in the findings of this study than would be warranted had analyses examined the microsatellite loci alone.

Divergence at the regional sampling scale was generally low, suggesting high connectivity among populations separated by between 12 and 156 km. However, divergence between the most remote estuary sampled at this scale (N5) and more northern populations (N2 and N4) was somewhat higher than other pairwise comparisons. This perhaps indicates that a 125 km stretch of open coast between adjacent estuaries may, given the local hydrodynamic conditions, be an impediment to regular gene flow. Sutton and Bowen (2011) deployed an array of current meters in coastal waters outside the Hokianga Harbour (N5) and described flows as typically weak and dominated by directional variability. Mean current speeds were calculated to be 2-4 cms^{-1} for inshore waters and 3-8 cms^{-1} offshore. If *A. stutchburyi* larvae are treated as passive particles and advected at a constant velocity of 8 cms^{-1} for three weeks (the upper estimate of PLD), dispersal estimates are approximately 145 km. However, if directional variability (noted as a dominant feature in this region), weaker inshore currents and larval

behaviour (currently unknown) were to be taken into account, estimates of dispersal could be considerably less. Accordingly, dispersal between N5 and the three northern estuaries would be expected to occur less frequently than among the northern estuaries themselves (separated by 12-34 km). The detection of IBD and variation in estimates of genetic differentiation and migration (N_m) suggest this is the case.

At the national sampling scale, global and pairwise estimates of divergence ranged from low to high, indicating various degrees of connectivity (or lack thereof) among populations separated by between 269 and 1226 km. The detection of significant IBD indicates that gene flow is more frequent among populations in close proximity than among distant populations; an intuitive result when considering larvae with a finite pelagic duration. Analyses also suggest that connectivity between N6 in the central North Island and the northern estuaries is somewhat limited and that gene flow between North Island and South Island estuaries and between the two sampled South Island populations is very limited. Although distance between populations explains much of the observed divergence (57-72%), a number of other processes may be responsible for or contribute to the population structure recorded at this spatial scale. For example, long stretches of open coast separate adjacent estuaries in the South Island and lower North Island. If an inter-estuary of distance of 125 km (between N3/4 and N5) is an impediment to regular gene flow at the regional scale, it is feasible that inter-estuary distances of between 200 and 300 km constitute rarely traversed barriers to *A. stutchburyi* dispersal. Genetic subdivision across a similarly sized habitat gap has been documented for southeast Australian

intertidal invertebrates. Ayre et al. (2009) analysed population structure for a number of taxa across a 300 km stretch of coastline devoid of suitable habitat for obligate residents of exposed rocky reefs. Subdivision across this habitat gap was reported for exposed reef specialists, while taxa with more relaxed habitat requirements were able to use patches of suboptimal habitat to traverse the habitat gap over multiple generations. Like these Australian open coast specialists, *A. stutchburyi* has specific habitat requirements and is therefore unable to use non-estuarine habitats as stepping stones between estuaries.

SAMOVA identified a genetic boundary between North and South Island *A. stutchburyi* populations. A similar central New Zealand genetic division, in the vicinity of, but not aligned with, the strait between North and South Islands, has been previously reported for *A. stutchburyi* (Chapter 3; Ross et al. 2011) as well as other estuarine and open coast invertebrates (reviewed in chapter 2 and Ross et al. 2009). Although the cause of this shared genetic boundary is uncertain, hydrodynamic and historic mechanisms have been suggested as possible explanations (Chapter 2; Ross et al. 2009). As this genetic division has been reported in taxa for which IBD was not, it is possible that a yet to be identified dispersal barrier rather than IBD is responsible for the divergence of northern and southern *A. stutchburyi*. However, the sharing of alleles between islands and a fairly linear relationship between geographic and genetic distance provide support for the hypothesis of contemporary IBD over present or historic hydrodynamic dispersal barriers. Ross et al. (2011) also suggested local adaptation across a steep environmental gradient as a possible driver of both the central New Zealand divergence and a north-south cline in *A. stutchburyi* COI

haplotype frequencies. The detection of IBD and genetic subdivision in unlinked molecular markers (COI and microsatellites) in the present study suggest that limited gene flow or genetic drift are more likely explanation than natural selection (Schmidt et al. 2008).

SAMOVA also split S2 from S1 and from N1-6, with pairwise comparisons indicating that S2, from Doubtful Sound in Fiordland, was significantly different from all other sampled locations. Given the geographic separation of S2 from other populations, a high degree of divergence is expected under a model of IBD. However, highly divergent fiord populations have been reported both in New Zealand (Mladenov et al. 1997; Miller 1997; Miller et al. 2004; Perrin et al. 2004) and elsewhere (Cunningham et al. 2009). The semi-closed estuarine circulation characteristic of fiords has been suggested as a strong impediment to larval exchange between fiord and coastal waters (Lamare 1998; Wing et al. 2003). While the observed relationships between geographic and genetic distance (COI and microsatellite) in the present study were on the whole fairly linear, COI pairwise comparisons between S2 and other populations suggest a higher level of divergence than would be expected under IBD alone. The inclusion of COI sequences from two previously sampled South Island locations (Chapter 3; SW2 and SW4) increases the strength of the IBD relationship. With this additional data the relationship remains linear with the possible exception of S2 comparisons which again appear slightly elevated. I therefore suggest that fiord associated hydrodynamic barriers decrease connectivity between fiord and open coast populations and in concert with IBD are responsible the high observed divergence of S2. Analysis of additional South Island populations, particularly

those intermediate to S1 and S2 and from other fiords would help clarify the nature of this genetic relationship and the mechanisms responsible.

It was only at the within-estuary (local) sampling scale that COI and microsatellite analyses indicated contrasting patterns of population structure. Microsatellite analyses showed low divergence and substantial migration between N3 and N4, while COI analyses revealed differences in haplogroup frequency between inner and outer harbour sampling locations. The genetic composition of the outer estuary N3 population was similar to that of other North Island populations while the composition of N4 was not. Given the close proximity of the sampled locations and the high estimate of inter-population migration derived from microsatellite allele frequencies, physical barriers to within-estuary larval exchange are an unlikely explanation for the observed differences. An alternative explanation is adaptation to local environmental conditions.

Natural selection has previously been suggested as a driver of genetic structure in estuaries (Perrin 2002; Koehn et al. 1980), where the mixing of fresh and coastal waters generates steep gradients in physical, chemical and biological parameters (Telesh and Khlebovich 2010). In the present study, sampled populations were, with the exception of N4, located in lower estuarine regions, in relatively sandy areas of high *A. stutchburyi* biomass. In contrast, N4 specimens were collected from the upper Hokianga Harbour in an area of low *A. stutchburyi* biomass in what probably constitutes marginal habitat for this species. At N4, *A. stutchburyi* were restricted to a narrow strip of uppermost intertidal as the area below this strip was too muddy to be habitable. It is

possible that selection either for or against specific haplotypes in these contrasting environments accounts for within-estuary differences in haplotype frequency. However, as upper harbour populations were not sampled in other estuaries it is uncertain whether the within-estuary differences recorded in the Hokianga Harbour occur elsewhere.

A similar partitioning of genetic variation was reported for fiord populations of the New Zealand sea urchin *Evichinus chloroticus* (Perrin 2002). Perrin (2002) genotyped *E. chloroticus* populations throughout Fiordland, and found populations clustered into two groups corresponding to inner and outer fiord environments. It was suggested that local adaptation to the steep environmental gradients that are a dominant feature of fiords in this region (Brewin et al. 2011; Wing and Leichter 2011) has maintained habitat specific genetic differences despite high inter- and intra-fiord larval exchange. Further investigation of habitat driven genetic differentiation in *A. stutchburyi* is warranted, as the occurrence of within-estuary differences will have implications for the implementation and interpretation of inter-population genetic comparisons.

4.4.1 Conclusions and implications

Estimates of migration and differentiation derived from COI and microsatellite data suggest that for *A. stutchburyi,* gene flow is high within estuaries and at regional scales where estuaries occur in close proximity. In contrast, the detection of population subdivision and IBD at the national sampling scale, particularly in areas where estuaries are sparse, suggests that gene flow is infrequent over large distances. Analyses suggest that for *A. stutchburyi,* inter-estuary distances of 125 km or more may constitute barriers to

inter-estuary larval exchange. Sampling of additional populations may resolve some uncertainty about whether distance alone accounts for the observed genetic structure or whether other processes (dispersal barriers) interact with distance to determine rates of gene flow.

Previous genetic analyses of open coast taxa on New Zealand's west coast have not detected IBD and concluded that these taxa possess widely dispersing larvae (Mladenov et al. 1997; Waters and Roy 2003; Waters and Roy 2004). An alternative explanation is that multi-generational stepping-stone migration across patches of closely spaced habitat is masking the genetic evidence for shorter single generation dispersal. If this were the case, a lack of genetic structure could easily be misinterpreted as an indication of high dispersal capacity. This could result in an overestimation of connectivity and ultimately the implementation of inappropriate fisheries or conservation management strategies. Estimates of gene flow in patchily distributed taxa might therefore provide a more accurate representation of dispersal capacity for taxa occurring in more common habitats. However, a better understanding of the role of life history traits and larval behaviour in dispersal is required if cross-species inferences are to be made with any confidence.

Low levels of inter-population connectivity imply vulnerability to natural or anthropogenic disturbances, as recovery from significant disturbances will potentially depend on external recruitment (Thrush et al. 1996, 2005). The results of this study suggest that for *A. stutchburyi*, long distance dispersal is rare and that geographically isolated populations experience reduced connectivity relative to populations occurring in close proximity. Consequently, patchily

distributed taxa, or those restricted to rare habitats may need to be managed at relatively small spatial scales and with some caution to ensure the persistence of isolated populations and their functionality as stepping stones for multi-generational long distance dispersal.

Chapter 5

General conclusions

5.1 Summary

The dynamics of marine communities are to a large extent determined by larval dispersal (Hastings and Harrison 1994; Botsford et al. 2001). However, due to difficulties associated with the direct measurement of the dispersal process, information about the mechanisms that control larval transport and therefore drive variation in the supply of recruits has been elusive (Levin 2006). Consequently, indirect methods, such as population genetics, have been used to estimate larval dispersal and connectivity. In this thesis, I review previous studies of population genetic structure for New Zealand's coastal benthos and conduct genetic analyses for the estuarine clam *Austrovenus stutchburyi.* By identifying shared and species specific patterns of population genetic structure, my aim was to enhance our understanding of connectivity and dispersal among populations of New Zealand's coastal benthos, as well as identify physical and biological processes that might influence population genetic structure.

In Chapter 2 of this thesis, I present a quantitative review of studies examining geographic variation in the genetic structure of New Zealand's coastal benthos. The results of these studies are to a large extent dependent on the molecular markers used and the geographic distribution of sampled populations. The reported population structures can generally be placed into one or more of

the following four classes: (a) no genetic structure; (b) divergence between northern and southern populations; (c) divergence between eastern and western populations; (d) small-scale population structure. Divergence between northern and southern populations was the most frequently reported population genetic structure in the reviewed literature. In most cases the boundary between subpopulations was located in the vicinity of Cook Strait, central New Zealand. However, the processes or mechanisms generating this genetic boundary remain unclear. Present and historical hydrodynamic barriers to dispersal have been suggested as possible explanations as have vicariance and natural selection.

In examining the relationship between pelagic larval duration (PLD) and reported population genetic structure, I detected a significant negative correlation between PLD and estimates of genetic differentiation. This correlation suggests that larval duration may be a useful proxy for dispersal potential. However, it is also apparent that, among taxa with short PLDs, there is considerable variability in genetic differentiation relative to taxa with longer PLDs. This variability implies that PLD on its own does not determine rates of gene flow or genetic structure. When PLD is short, other factors (biological or physical) may take on additional importance in determining the distances over which larvae disperse.

As further studies examine the population genetic structure of New Zealand's coastal benthos, new or common patterns may become apparent. Recent studies have, for the most part sampled populations over larger geographic areas and at greater densities than earlier research. This increased spatial resolution combined with a larger sample size and the examination of

taxa from a wider range of habitats will allow more complex meta-analyses to be conducted, improving our ability to detect patterns and make generalisations about the influence of larval characteristics, species distribution and dispersal barriers on gene flow and connectivity (as demonstrated by Toonen et al. 2011).

In Chapter 3 of this thesis I examined the population genetic structure of the New Zealand endemic estuarine clam *A. stutchburyi.* Analyses of mitochondrial cytochrome *c* oxidase subunit I (COI) sequences revealed a pattern of genetic isolation-by-distance (IBD) indicating that for this species, dispersal is limited and gene flow is most likely between populations in close proximity. Six divergent subpopulations were identified with genetic boundaries detected through central New Zealand, about the East and North Capes and in the south of the South Island. A greater number of subpopulations were detected here relative to much of New Zealand's open coast benthos, although similar boundaries have been reported for other estuarine taxa. That the population structure of *A. stutchburyi* is more similar to that of estuarine taxa without pelagic larvae than to coastal taxa with putatively similar dispersal potential suggests that distribution and habitat requirements, as well as larval characteristics, may determine patterns of gene flow. The examination of additional estuarine taxa will be required to determine whether the high level of subdivision reported here is common among estuarine taxa with pelagic larvae or is unique to *A. stutchburyi*.

Although the detection of IBD indicates that limited contemporary dispersal may account for the additional genetic structure observed in *A. stutchburyi* and other estuarine taxa, partial congruence between genetic and

biogeographic boundaries suggests that historical events and natural selection may also contribute to the observed genetic boundaries. The observed congruence also suggests that in the absence of genetic data, biogeographic classifications could be a useful tool for defining population management units.

In Chapter 4 of this thesis I present the results of a multi-scale genetic analysis of *A. stutchburyi* populations on New Zealand's west coast. Analyses of COI and nuclear microsatellite loci showed populations to be well connected within estuaries and at the regional sampling scale (≤ 156 km) although not at a national scale (≤ 1226 km). Distance among populations explained much of the observed population structure as did distance between estuaries. High rates of inter-population gene flow were estimated between estuaries separated by up to 22 km of open coast, while an inter-estuary distance of 125 km appeared to limit regular gene flow. In regions where inter-estuary distances were large, estimates of gene flow were much less suggesting 200-300 km stretches of open coast represent rarely traversed barriers to dispersal among *A. stutchburyi* populations. However, as several of these long stretches of open coast coincide with putative hydrodynamic barriers to gene flow there is some uncertainty about the roles of these barriers versus inter-estuary distance in generating the observed divergences. Additional sampling of *A. stutchburyi* populations in the vicinity of Cook Strait and Fiordland could provide some clarification in this regard.

Within-estuary genetic differences were also revealed by COI analyses and may result from local adaptation to steep within-estuary environmental gradients. This phenomenon warrants further investigation as the occurrence of

within-estuary genetic differences will have implications for the implementation and interpretation inter-estuary genetic comparisons.

5.2 Conclusions and future research

Prior to commencing my PhD research in 2006, genetic analyses of New Zealand's coastal benthos had for the most part not identified genetic subdivision at local or regional scales for taxa with pelagic larvae or produced any evidence for dispersal limitation (Chapter 2). While it was generally believed that the dispersal capacity of larvae with pelagic durations of days to weeks would be insufficient to connect populations over 100's to 1000's of km (Bohonak 1999), genetic data were not available to support this hypothesis. Although genetic subdivision had been reported through central New Zealand this was thought to reflect local oceanographic features rather than limited dispersal of propagules with finite PLDs (Apte and Gardner 2002).

In contrast to much of the earlier research (Chapter 2), my analyses identified subdivision among *A. stutchburyi* populations at local, regional and national scales and provide evidence of limited dispersal. This thesis, therefore, adds to the growing body of literature challenging the paradigm that a pelagic larval phase guarantees widespread dispersal and high inter-population connectivity (Weersing and Toonen 2009). Data presented in Chapters 3 and 4 of this thesis indicate that migration is most likely between populations in close proximity and that previously identified dispersal barriers and long stretches of open coast may act as barriers to inter-estuary larval transport.

As low levels of connectivity imply vulnerability to disturbance (Thrush et al. 2005), the estimates of connectivity presented here suggest that *A. stutchburyi* may be vulnerable to regional scale disturbances on coastlines where estuaries are common and to more localised perturbations where estuaries are scarce. Consequently, resource managers will need to consider the geographical distribution of estuaries when determining the spatial scale at which estuarine taxa are managed and the level of protection they require.

Studies of New Zealand's open coast taxa typically suggest higher levels of genetic connectivity than reported for *A. stutchburyi* and other estuarine taxa (Chapters 2, 3 and 4). However, it is uncertain whether these higher estimates of gene flow imply a greater dispersal capacity. An alternative hypothesis is that estuarine and open coast taxa have comparable dispersal potentials (when PLD and larval behaviour are similar) and that differences in habitat availability determine realised dispersal and therefore population genetic structure (Chapter 4; Pelc et al. 2009). Because open coast habitats (e.g. rocky reef and soft sediment) generally occur at high frequencies and close proximity along New Zealand coastlines, dispersal over small distances would be expected, even for taxa with limited PLDs. When this process is repeated over multiple generations, genetic material may be transported over large distances in small increments (i.e. stepping stone-dispersal; Kimura and Weiss 1964), potentially resulting in genetic homogeneity at large scales. In contrast, for coastlines where estuaries are separated by large stretches of open coast, inter-estuary gene flow may be infrequent and populations divergent. In this way, taxa with similar dispersal capacities but different habitat requirements may exhibit entirely different

population genetic structures (e.g. Pelc et al 2009). The estimation of dispersal potential from genetic data should therefore be performed with some caution, particularly where life history and habitat distribution data are not available. Where stepping-stone dispersal does occur, genetic analyses may overestimate the distances that larvae are able to disperse. Such misinterpretation will have implications for our ability to predict a population's response to disturbance and may influence the way in which species are managed. Genetic analyses of patchily distributed taxa may provide more accurate estimates of dispersal potential, especially where the spatial arrangement of habitat patches can be used to test connectivity across a range of inter-population distances. While such analyses may be useful in estimating dispersal for other taxa inhabiting more common habitats, a better understanding of the influence of life history and larval behaviour in dispersal is needed before inter-specific inferences can be made with any confidence.

It is worth noting that relatively low rates of gene flow, possibly as low as a single migrant exchanged per generation (Spieth 1974), are enough to maintain genetic homogeneity among populations. It is therefore likely that the estimates of connectivity made here for *A. stutchburyi* represent the upper limits of this species' dispersal potential. Rates of inter-population migration sufficient for population maintenance and replenishment (i.e. ecologically meaningful connectivity) probably occur over smaller spatial scales (Cowen et al. 2007). However, determining the spatial extent of ecologically meaningful larval connectivity has proven difficult (Levin 2006). The main exception is for taxa that disperse over very short distances (Olson and McPherson 1987). Further analyses

of patchily distributed taxa may prove useful in making such estimates for more widely dispersing larvae.

In the absence of reliable estimates of larval dispersal and ecologically meaningful connectivity, resource managers must make decisions based on the best estimates currently available. This thesis demonstrates for New Zealand's coastal benthos that genetic connectivity among populations of estuarine taxa is generally low relative to coastal species. The data presented here also suggest that migration among patchily distributed habitats, such as estuaries, will be dependent on habitat distribution. Consequently, patchily distributed taxa may experience low rates of ecologically meaningful connectivity, requiring relatively cautious management at small spatial scales to ensure the persistence of intact biological communities in the face of persistent anthropogenic and natural disturbances.

Significant gaps in our knowledge of larval dispersal and connectivity may be filled through (a) determination of larval origins and dispersal pathways through direct and indirect methods (e.g. population genetics, elemental fingerprinting), and (b) a better understanding of the biological and hydrodynamic process involved in the transport of larvae (Cowen et al. 2007). I propose that the following avenues of research should be pursued in the near future: (1) analysis of additional *A. stutchburyi* populations in the vicinity Cook Strait and Fiordland to provide clarification of the role of local dispersal barriers versus inter-estuary distance in generating the divergences reported in Chapter 4 of this thesis; (2) the population genetic structure of other estuarine taxa should be examined to determine whether the high level of subdivision reported here is

common among estuarine taxa with pelagic larvae or is unique to *A. stutchburyi*; (3) the population genetic structure of patchily distributed non-estuarine taxa should be examined to investigate whether high levels of population structure are a function of estuary endemism or patchy distribution regardless of habitat restrictions; (4) the population genetic structure of previously examined but poorly sampled taxa (e.g. *Pinnotheres novaezelandiae*, Stevens 1990) should be re-examined to provide a more complete picture of their population structure; (5) the population genetic structure of additional taxa with a range of life histories and habitat requirements should be examined; (6) multi-species population genetic analyses such as the analysis conducted by Toonen et al. (2011) should be used to identify concordant patterns of connectivity among New Zealand's coastal benthos.

In the longer term, our requirements for fundamental knowledge regarding the role of biological and hydrodynamic processes in determining rates of population connectivity may be met through the following areas of research. (1) Investigations of the larval behaviour and life history traits of taxa utilised in dispersal research. This knowledge can assist in interpretation of indirect dispersal estimates, be used to generate testable hypotheses and will help determine where cross-species inferences regarding realised dispersal are appropriate. (2) The development of hydrodynamic models that incorporate realistic larval behaviour and mortality terms (Cowen et al. 2007, White et al. 2010). The extent to which physical models will mimic reality is dependent on the quality of data with which they are generated. Knowledge of life history and behavioural data will be critical as will fine-scale oceanographic measurements

(e.g. Selkoe et al. 2010). Such models will facilitate the testing of hypotheses regarding interactions between biological and physical processes. (3) Rapid advances in sequencing technology will soon enable very large numbers of genetic markers, randomly distributed throughout a target genome to be sampled simultaneously (e.g. whole-genome sequencing and SNP assays). The move towards large genome-wide data sets will not only enhance our ability to identify biologically meaningful population structure, but will allow for the identification of DNA regions that are under natural selection (or linked to such regions) greatly improving our understanding of physical vs. realised (incorporates survivorship and reproductive success) larval dispersal (Pineda et al. 2007). (4) Ultimately, direct measurement of larval transport may be necessary to resolve the relationship between physical dispersal and population genetic structure. While direct measurement will undoubtedly prove difficult, the methods required to make these measurements already exist. For example, large spatial scale plankton surveys (e.g. Shanks and Brink 2005) using genetic methods for rapid larvae identification and quantification (e.g. Henzler et al. 2010). Such techniques may be best applied to taxa occurring in geographically isolated populations, for example *A. stutchburyi*, as the presence of multiple larval sources may complicate analyses. The multi-disciplinary approach suggested here will further our understanding of the processes determining dispersal and population connectivity, providing opportunities to answer questions regarding the origins and fate of dispersing larvae.

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Appendix I

Isolation and characterization of microsatellite markers for the New Zealand endemic estuarine clam *Austrovenus stutchburyi.*

The following work was conducted in collaboration with Craig H. Newton (ATG genetics Inc, Malsapina University, Nanaimo, Canada) and Richard J. Wilkins (University of Waikato, Hamilton, New Zealand). Funding for microsatellite development was provided by Carolyn J. Lundquist (National Institute of Water and Atmospheric Research Ltd, Hamilton, New Zealand) and Ian Hogg (University of Waikato, Hamilton, New Zealand).

The clam *Austrovenus stutchburyi* is an intertidal suspension feeding bivalve, endemic to New Zealand and restricted to estuarine habitats. *A. stutchburyi* is found throughout New Zealand with populations spanning more than 15° of latitude and consequently, a wide range of temperatures, salinities and sediment types. The genetic structure of *A. stutchburyi* populations has previously been characterised using the mitochondrial gene cytochrome *c* oxidase subunit I (COI; Chapter 3; Ross et al. 2011). Although significant population subdivision was detected, the use of a single non-recombining molecular marker (COI) has made it difficult to determine the processes responsible for the observed population structure. The analysis of additional autosomal molecular markers was suggested as a means of gaining a better understanding of the causes of the observed population structure and obtaining more reliable estimates of the spatial and

temporal scales of connectivity and subdivision (Chapter 3; Ross et al., 2011). In this study, microsatellite markers were developed for *A. stutchburyi* for the purpose of investigating the population genetic structure of this estuarine taxon and determining the mechanisms responsible for any observed population structure.

A. stutchburyi genomic DNA was digested with restriction enzyme *Hae*III and fragments ligated to double-stranded adaptors M28 (5'-CTCTTGCTTGAATTCGGACTA) and M29 (5'-pTAGTCCGAATTCAAGCAAGAGCACA) with T4 DNA ligase in the presence of Psh A1. Adaptor-modified *A. stutchburyi* DNA was then hybridized separately with 5' biotin-labelled di- and tetranucleotide simple sequence repeat (SSR) motif mixtures; $(GA)_{17}$, $(CATA)_{20}$ $(GATA)_{20}$ and enriched for a single round using streptavidin-conjugated paramagnetic beads (Dynabeads M-270, Invitrogen).

Biotin-selected genomic fragments were then amplified using primer M28, digested with *Eco*RI and ligated into the dephosphorylated *Eco*RI sites of plasmid vector pGEM3z+ (Promega) for propagation in *Escherichia coli* (strain SURE, Stratagene). Random bacterial colonies were picked into 0.1 ml YT/10% glycerol and amplified with the M13 universal primers (M13forward 5'-GTTGTAAAACGACGGCCAGT & M13reverse 5'-CAGGAAACAGCTATGACC). Positive microsatellite clones were identified by dot blot hybridization with appropriate mixes of biotin labelled SSR oligonucleotides.

Twenty two of the sequenced clones were positive for the presence of dinucleotide repeats. From these, 17 primer pairs (Table 1) were designed either manually or using the program Primer 3 version 4.1 (Rozen & Skaletsky 2000)

and tested on *A. stutchburyi* from populations across the entire distribution of the species. PCR reactions were conducted in 10 µl volumes consisting of 1 µl unquantified DNA, 1x platinum Taq buffer, 1.5 mM $MgCl_2$, 1 µl reverse primer, 200 µM dNTPs and 0.5 U Platinum Taq DNA polymerase. The forward primer was labelled with ^{33}P using polynucleotide kinase and present in the reaction at a concentration of 0.1 µM. PCR amplification consisted of an initial denaturation (95°C, 2 min.), followed by 30 cycles of annealing (50-62°C, 20 s), elongation (72°C, 20 s) and denaturation (95°C, 20 s), and a final elongation period (72°C, 5 min.). Annealing temperatures were set according to primer specific melting temperatures (Table 1). PCR products and ^{33}P-labelled 10 bp reference ladder were denatured and analysed on standard denaturing 6%w/v polyachrylamide sequencing gels (30 x 40 cm). Dried gels were exposed to Kodak Biomax XAR film overnight. Alleles were assigned by visual inspection using the 10-nt ladder as a reference.

Of the 17 primer pairs tested, only Cocdi 5, 13 and 15, were found to consistently produce PCR products that could be screened for polymorphisms. Forty *A. stutchburyi* from the Kaipara Harbour were then genotyped at these three loci. PCR amplifications were conducted in 20 µl reactions containing 0.2 µl Bioline MyTaq HS DNA polymerase, 4 µl 5x MyTaq reaction buffer, 4 pmol of each primer and 0.4 µl of unquantified template DNA. Forward primers were 5' end-labelled with the fluorescent dye 6-FAM to allow amplified DNA fragments to be visualised and their length quantified. PCR reactions consisted of an initial denaturing step (94°C, 3 min), followed by 30 thermocycles of denaturation (94°C, 30 s), annealing (52°, 57° or 61°C, 30 s; Table 2) and extension (72°C, 90s),

followed by a final extension period (72°C, 30 min). Amplified products were processed on an Applied Biosystems 3130 Genetic Analyser with LIZ600 size standard. Fragment size was calculated using Peak Scanner v1.0 (Applied Biosystems).

Indices of microsatellite diversity were quantified using ARLEQUIN (Excoffier et al. 2005), FreeNA (Chapius and Estoup 2007) and FSTAT version 2.9.3.2 (Goudet 1995). These included the total number of alleles (N_{all}), allelic richness (A_r), allele size range (A_{sr}), expected heterozygosity (H_e) and observed heterozygosity (H_o). Microchecker (Van Ooosterhout et al. 2004) was used to test all loci and populations for Hardy-Weinberg-equilibrium (HWE) and to determine whether deviations from HWE resulted from technical artefacts (null alleles or large allele dropout) or non-random breeding (e.g. Wahlund effect).

The three dinucleotide repeat microsatellite loci were highly polymorphic with 31, 20 and 20 alleles recorded for Cocdi 5, Cocdi 13 and Cocdi 15 respectively (Table 2). Allelic richness (A_r) was high in all loci. H_O was less than H_E and significant deviations from HWE detected for all loci. Microchecker indicated that deviations from HWE were caused by an excess of homozygotes, most likely due to the presence of null alleles. Null allele frequencies estimated in FreeNA varied between loci (0.09-0.18; Table 2).

Table A1.1 Primer sequences and annealing temperatures (T_a) for microsatellite primer pairs tested for *Austrovenus stutchburyi.*

Locus	primer sequence 5'-3'	T_a (°C)
Cocdi1	CocdiF1 CCATTTGACCACGGATATCAA	64
	CocdiR1 GATAATCCAGACGTCAACAGAA	61
Cocdi2	CocdiF2 CTAAGCATCACAGGAGATGCA	63
	CocdiR2 CGTTGTTCATGAAAGAAAGACA	62
Cocdi3	CocdiF3 AGTAGGTGACACGTGATGCT	60
	CocdiR3 AAGTGTGAAGGACAAACTGCT	60
Cocdi4	CocdiF4 AAAGTGTCCTTATCAGCGGG	63
	CocdiR4 CTCTGAATAAATTGCATTATGAATG	61
Cocdi5	CocdiF5 CCCTAACCATGTTAAATAGGCA	61
	CocdiR5 CCACTGTCAGCTTGAAACATTC	64
Cocdi6	CocdiF6 AACTTCGAAATATATTGAAGGTC	57
	CocdiR6 CCTTCAGGGAACTAAAACC	58
Cocdi7	CocdiF7 ACCTATTCCACAGGAGTATGG	60
	CocdiR7 CTAGATTCCAAACTGGTCATG	59
Cocdi8	CocdiF8 CCAACTCCACAGTGACTTAAG	59
	CocdiR8 GAATTCAAGATTTGTTAGAGAG	54
Cocdi8.1	CocdiR8.1 GAATACATTTGATTTATTGACTCC	57
	CocdiF8.1 ACTGAAACTCTCTCTCACTGTAAC	57
Cocdi10	CocdiF10 AAGGATGCAATGTGTCATCC	62
	CocdiR10 TTTCACGTCAAAAGCATGCC	66
Cocdi13	CocdiF13 GTTTGTGGAAACCATAGATGCA	64
	CocdiR13 CAGTTAACGTCGTTGTTTATCA	59
Cocdi15	CocdiF15 CCAAGCATTTTAGAGAGAGA	56
	CocdiR15 AAGTTAATTGTCCTCAATGTGA	58
Cocdi15.1	CocdiF15.1 TGAGAGAGAGAGGGTGTG	56
	CocdiR15.1 CCACCAATCATATATAACCC	56
Cocdi16	CocdiF16 TCTGGAATGCGATCATGCCA	70
	CocdiR16 GTCTTATATGCTTTAAGATATTTTC	53
Cocdi18	CocdiF18 ACTTGCTAACCACTCAAACC	53
	CocdiR18 CTACTTGAGAAGTGATCACC	50
Cocdi20	CocdiF20 CCGGAAGGTTAAGGAATTTC	51
	CocdiR20 CAAATTCTTAAACTTCGTCATTTC	50
Cocdi23	CocdiF23 TGGTGTAAGGGAGGTAAGTC	53
	CocdiR23 CACCATTTTGCCGAGCATC	55

Table A1.2 Characteristics and diversity indices of three microsatellite loci for *Austrovenus stutchburyi* specimens from Kaipara Harbour. Primer sequences are provided as well as the repeat motif as first identified (Repeat), annealing temperature (T_a), number of alleles recorded across all populations (N_{ALL}), observed (H_O) and expected (H_E) heterozygosity, Hardy-Weinberg equilibrium probability test *p*-values (*P*) and the null allele frequency for each locus (NULL). Heterozygosities and their significance were calculated in ARLEQUIN with exact tests using a Markov chain (100 000 steps; 1 000 000 chain length).

Locus	Primer sequences (5'-3')	Repeat	T_a(°C)	N_{ALL}	Allele size range (bp)	A_r	H_o	H_e	*P*	NULL
COCDI 5	F: CCCTAACCATGTTAAATAGGCA R: CCACTGTCAGCTTGAAACATTC	$(CT)_6(CT)_9$	61°C	31	180-242	30.1	0.78	0.96	0.003	0.09
COCDI 13	F: GTTTGTGGAAACCATAGATGCA R: CCACTGTCAGCTTGAAACATTC	$(AG)_{15}(GA)_{10}$	57°C	20	214-264	19.5	0.6	0.93	<0.001	0.17
COCDI 15	F: CCAAGCATTTTAGAGAGAGA R: AAGTTAATTGTCCTCAATGTGA	$(AG)_{15}(GA)_{10}$	52°C	20	143-193	19.6	0.58	0.94	<0.001	0.18

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