Multi-species coastal marine connectivity: a literature review with recommendations for further research

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EXECUTIVE SUMMARY

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This report is a review of 58 studies which have described population genetic structure in 42 different taxa (plants, invertebrates and vertebrates) of New Zealand's coastal marine biota. Examination of the data from these studies (published and unpublished) reveals that 36 of them have focused on rocky intertidal and subtidal taxa, and that macro-invertebrates are particularly well represented (28 of 42 taxa). In terms of genetic population structure, 16 studies reported no structure, 9 reported an isolation by distance model, 12 reported divergence within and/or among populations, 1 reported an east-west difference, and 20 reported a north-south differentiation. Identification of coverage in terms of habitat types, taxa and spatial (geographic) extent reveals that significant knowledge gaps exist across New Zealand, that preclude assessment of patterns of population genetic structuring for the majority of coastal taxa. We also review the state of ongoing population genetics research and identify taxa which are presently the focus of research. Recommendations for further study to progress knowledge about the population genetic structuring of New Zealand's coastal marine biota are based on the need to try to fill some of the knowledge gaps that we have identified in our study. We suggest that four appropriate taxa for future study are: the pipi (*Paphies australis*), the tuatua (*Paphies subtriangulatum*), Sand flounder (*Rhombosolea plebeia*), and the yellow belly flounder (*Rhombosolea leporina*). Recommendations are made for a standardised collecting protocol from open sandy shores and estuaries, and for the subsequent development and application of microsatellite markers to quantify the population genetic structure and the coastal connectivity of these taxa.

1. INTRODUCTION

1.1 Overview

The overall objective of this report is to determine patterns of regional connectivity in a broad range of New Zealand (NZ) coastal marine organisms, and, from this, define the geographic units of genetic diversity for protection of marine species and the dispersal processes that maintain this diversity. Specifically this involves:

- a review of studies of marine connectivity and population genetics in NZ coastal organisms to determine the preliminary range of patterns observed,
- the identification of the principal gaps (taxonomic, geographic, and habitat) in our understanding, and
- recommendations for further research to address these gaps.

1.2 Scientific background

The conventional view that marine environments are demographically 'open' has been challenged by many genetics studies over recent times, which have shown subpopulation subdivision on a limited geographic scale (Jones et al. 1999, Hellberg et al. 2002). We now know that marine species can vary enormously in their population structure and this can be the result of many different environmental factors and, most importantly, the biological traits of different species. For example, most marine coastal species (macroalgae, invertebrates, and fish) are characterised by possessing a biphasic lifestyle. That is, the post-metamorphic adult stage is often sedentary and may be benthic for macroalgae and most invertebrates, and pelagic or benthic for fish. In contrast, the pre-metamorphic stage (spores in macroalgae, larvae in invertebrates and fish) is usually pelagic for a period of time. It is this pre-metamorphic stage that for many species is the dispersive stage, but dispersal is thought to be achieved much more by passive movement (e.g., wind and tidal dispersal) than by active dispersal (e.g., swimming) because the spores or larvae are usually small (mostly under 0.5 mm diameter) and because they are not generally well adapted for long periods of active locomotion, although they are able to actively move over small distances. The duration of the larval stage may be short (a few hours to a day or two – e.g., paua) in which gene flow (or genetic connectivity) is often assumed to be limited, but in most species the larval phase is moderate in duration (2–4 weeks – e.g., mussels) and in a few species it may be unusually long (12–18 months – e.g., rock lobsters). The adults of some marine organisms can also be highly dispersive (fish, floating algae), such that the dispersal of these adults and their subsequent mating will contribute to population connectivity. In the latter cases gene flow between populations is expected to be moderate to high. Thus, as a general rule, the duration of the larval or spore phase is thought to contribute to the extent of gene flow and connectivity among coastal taxa (e.g., Bell 2008a, 2008b). Earlier studies of genetic connectivity using biochemical markers such as allozymes (the protein products of the DNA) tended to confirm and reinforce this view, although exceptions were noted (e.g. Burton & Feldman 1982, Burton 1983, Scheltema & Williams 1983, Scheltema 1986, Allcock et al. 1997, Grant & da Silva-Tatley 1997). New types of molecular genetic markers have tended to question the universality of this thinking, and there is now evidence for many species in many different locations that larval duration alone cannot explain the observed population genetic structure.

As a generalisation, high levels of gene flow tend to result in low levels of population genetic structuring because extensive gene flow prevents the accumulation of genetic differences among populations (Ross et al. 2009, Kelly & Palumbi 2010, White et al. 2010). It is however important to note that this homogenising aspect of gene flow can be over-ridden by strong selective pressure differences among sites. Likewise, low levels of gene flow among

populations tend to result in greater levels of genetic differentiation among populations over multiple generations (the number of generations depends on a number of factors, including population size and life history characteristics and may range from 10s to 1000s). Thus, gene flow (connectivity) can be thought of as a homogenising force, and the absence of gene flow can be thought of as a factor promoting population genetic differentiation. Genetic connectivity and population genetic structuring are therefore usually strongly and negatively associated.

With the advent of new methodologies and a new generation of genetic markers to assess gene flow among populations (e.g., Hellberg et al. 2002, Palumbi 2004, Bay et al. 2006, Cowen et al. 2006, Levin 2006), there is increasing evidence that not all populations of coastal taxa are as "open" as was previously believed. Based on life-history characteristics and the speed of coastal currents it was generally believed that larvae and spores could and would move many kilometres from their natal sites and that, in effect, all new recruits were immigrants. This view of connectivity has changed in the last 30 years, and it is now increasingly being recognised that despite their life-history characteristics which promote dispersal, many populations are partially "closed": that is, they rely to a greater or lesser extent on self-recruitment. This has been particularly well documented for tropical reef fish (e.g., Jones et al. 1999, Swearer et al. 1999), and is increasingly being demonstrated for other groups too (e.g., Wright et al. 2000, Taylor & Hellberg 2003, Bell & Okamura 2005, Cowen et al. 2006, Johnson & Black 2006, Wood & Gardner 2007). The extent to which some/all populations rely on self-recruitment and whether this reliance is stable in time and space are questions of great importance, but which currently remained unanswered.

The quantification of gene flow among populations and/or stocks is particularly important in many areas of science. In marine biology, one of the great, largely unanswered, questions has been about connectivity – that is, the extent of gene flow among populations, the extent of self-recruitment within populations, and the identification of source and sink populations. These fundamental questions are important in many applied marine science areas, including fisheries management, marine protection and the establishment of a network of marine reserves, and biosecurity. Until we have a much better understanding of the magnitude of gene flow among populations it will be almost impossible to successfully manage all exploited species (because recruitment to a population is a key determinant of population size and stock determination), to prevent invasive species from spreading from one location to another (because gene flow from an invaded to a non-invaded site can result in the establishment of new populations of the invasive species), or to conserve New Zealand's biodiversity (because we don't know where recruits come from or how frequently they are exchanged among populations). The reason for this limited knowledge stems from the fact that the larvae and spores are very small (difficult to track), they experience high levels of mortality (often over 99.999%), and their dispersal is subject to coastal processes such as wave action, wind driven currents, upwelling and downwelling, and other turbulent and temporally infrequent oceanographic activities.

1.3 The New Zealand context

New Zealand is an archipelago of more than 700 islands, spanning from the subtropical north (Kermadec Islands, 29° S) to the subantarctic south (Campbell Island, 52° S) (Ross et al. 2009). The NZ marine estate – the Exclusive Economic Zone (EEZ), which extends 200 nautical miles from the territorial sea boundary (but may extend further in places under the UNCLOS extended continental shelf agreement) – is the fourth largest in the world and covers about 428 million hectares (480 000 km^2), which is about 18 times the area of the land, and the NZ coastline is estimated to be about 15 000 km long (Gardner et al. 2009, Walls 2009). Connectivity among coastal species is known to be strongly influenced by coastal (and to a lesser extent oceanic) currents (e.g., Ross et al. 2009, Kelly & Palumbi 2010, White et al. 2010). A number of the papers reviewed here provide background information on NZ's coastal currents: this information is summarised in Figure 1.

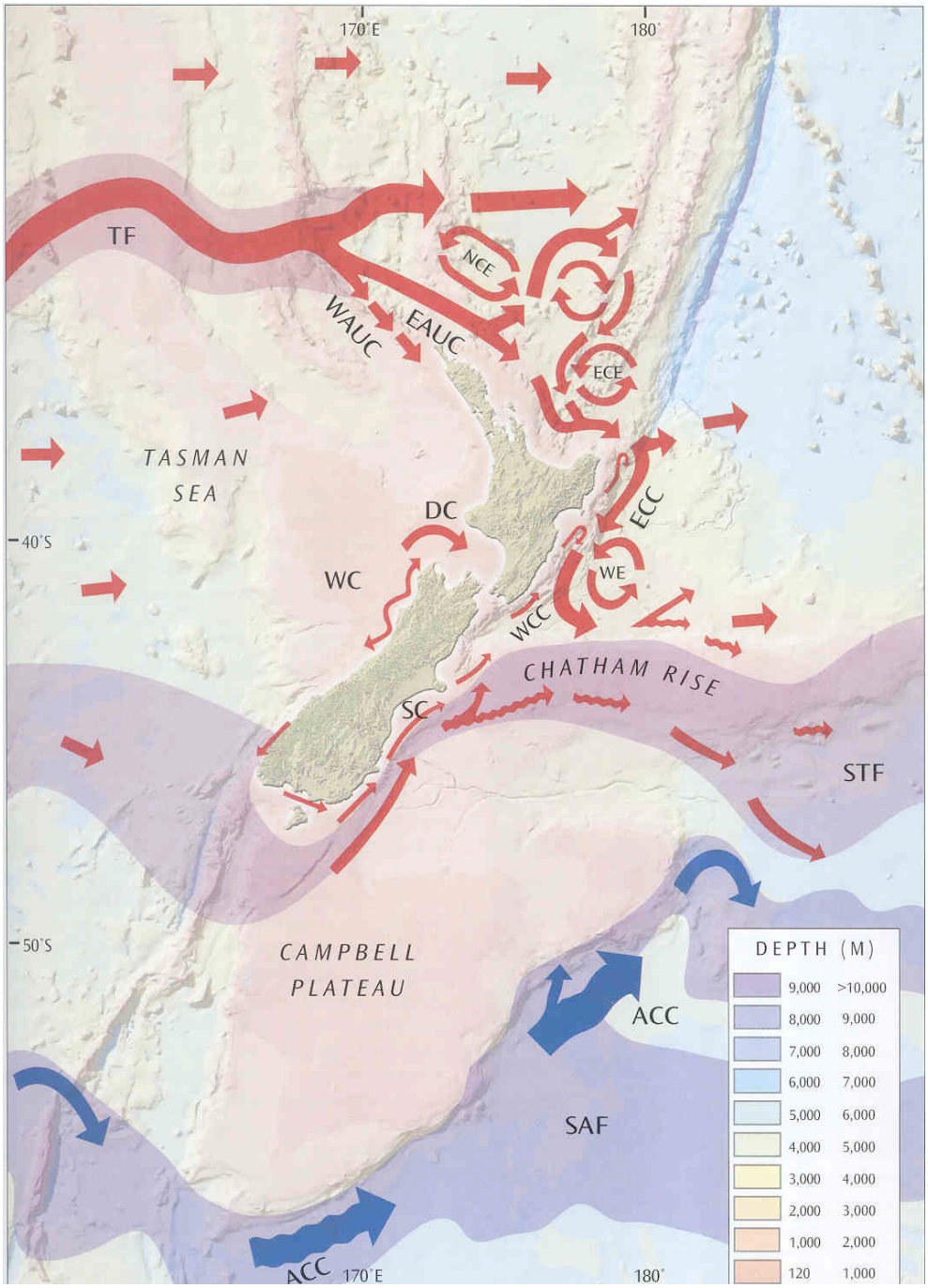

Figure 1: New Zealand's major coastal and offshore currents. ACC, Antarctic Circumpolar Current; DC, D'Urville Current; EAUC, East Auckland Current; ECC, East Cape Current; ECE, East Cape Eddy; NCE, North Cape Eddy; SAF, Subantarctic Front; SC Southland Current; STF, Subtropical Front; TF, Tasman Front; WAUC, West Auckland Current; WC, Westland Current; WCC, Wairarapa Coastal Current; WE, Wairarapa Eddy. Taken from Will & Gemmell (2008) and modified from Laing & Chiswell (2003).

New Zealand's marine environment is characterised by a mix of subtropical and warm temperate species in the far north, through to a range of cool temperate and subantarctic taxa in the far south. This diversity of marine estate is reflected in the NZ Department of Conservation's recognition of 14 different marine biogeographic provinces, of which 9 are associated with NZ mainland (North, South and Stewart islands) (Figure 2). New Zealand also has a large proportion of endemic species within all major groups across the macroalgae,

invertebrates and vertebrates. This biota is derived from a wide variety of different sources, and thus has a mixed and interesting evolutionary history. For its continued preservation a greater understanding is required of the patterns of genetic structure that exist among species of the NZ biota, where those different genetic groups (stocks) exist, how such stocks arose and are currently maintained, and the extent of connectivity (gene flow) among the different stocks.

Ultimately, no single study is likely to be able to identify and explain patterns of genetic structure and the processes that contribute to these. The reason is that different taxa have different histories that will reflect different patterns of genetic structure, and also have different life history characteristics (e.g., pelagic larval duration – the length of time the larval stage spends in the water column and which is thought to reflect the time available for dispersal) that help to maintain or erode the present structuring. Wallis & Trewick (2009) provided a comprehensive overview of NZ's geological history that is relevant in the context of processes contributing to and explaining population genetic structuring among coastal taxa. Their review of NZ's terrestrial, freshwater, and marine phylogeography highlights interesting and important patterns of population structuring at a continent-wide level, and helps to identify processes that have contributed to their establishment, as well as processes that help maintain or erode the current situation.

As noted by Wallis & Trewick (2009), it is now widely established that marine species can show significant phylogeographic structure despite the apparent continuity of the marine realm. Phylogeographic splits can arise for a variety of different reasons, including both contemporary events (e.g., related to a species' ecology) and also historical events such as past geological and/or climatic changes. A number of authors have compiled lists of studies describing the population genetic or phylogeographic structure of NZ's coastal species, including Goldstien (2005), del Mundo (2009), Ross et al. (2009) and Wallis & Trewick (2009). The most recent and comprehensive review of the phylogeography of NZ's coastal benthos (Ross et al. 2009) looked at published information from 1980 to 2008. These authors identified 42 molecular studies, which provided data for 29 different coastal benthic invertebrates and one coastal benthic plant species (*Zostera muelleri*). The authors tried to reveal generalities and/or patterns that existed in this data set, and also tested for processes that might contribute to genetic structuring. Consistent with an ever-expanding body of work (e.g., Bradbury et al. 2008; Kelly & Palumbi 2010; White et al. 2010), Ross et al. (2009) showed in their assessment of 29 NZ studies that a statistically significant negative relationship exists between pelagic larval duration (PLD) and genetic differentiation. That is, taxa with longer PLDs exhibit less genetic differentiation (presumably because longer-lived larvae can travel greater distances and therefore "homogenise" population genetic structuring); this led the authors to suggest that PLD may be used as an index of dispersal potential. Importantly, among taxa with short (less than10 days) PLDs, Ross et al. (2009) observed greater variability in genetic differentiation than among taxa with long PLDs, suggesting that factors (e.g. variable reproductive success and predation) other than PLD alone contribute to genetic connectivity. The paper by Ross et al. (2009) and other international reviews of a similar nature (e.g., Bradbury et al. 2008, Kelly & Palumbi 2010, White et al. 2010) strongly suggest that there is no "one size fits all" answer to the question of which factors contribute to genetic connectivity – multiple factors are implicated and how these interact will create different scenarios for different taxa in different geographic settings.

The Ross et al. (2009) paper provides a good foundation for the present report, but does not include information on fishes, on macroalgae (seaweed), on environment and/or habitat type, or data after 2008 (published in the peer-reviewed literature, published in the form of theses or reports, or work that is unpublished because it is ongoing). We have used the information in the report by Ross et al. (2009), as well as information provided by Goldstien (2005), del Mundo (2009), and Wallis & Trewick (2009) as a starting point for the present report. We

have added to this information by including new information and by updating the existing information.

1.4 Objectives

This report has been produced under Ministry of fisheries contract ZBD2009-10, which has the following objectives:

Overall objective

1. Determine overall patterns of regional connectivity in a broad range of NZ coastal marine organisms to define the geographic units of genetic diversity and the dispersal processes that maintain this diversity.

Specific objective

1. Review previous studies of marine connectivity and population genetics in NZ coastal organisms to identify the preliminary range of patterns observed, and the principal gaps (including taxonomic, geographic and ecological information) in our understanding.

2. In a range of invertebrate and vertebrate marine organisms, determine geographic patterns of genetic variation using standardised sampling and molecular techniques.

3. Analyse data across past and present studies to reveal both common and unique patterns of genetic connectivity around the NZ coastline, and the locations of common barriers to dispersal.

2. OUTCOMES

The purpose of conducting the literature review is to permit identification of the principal gaps (including taxonomic, geographic, and habitat) in the coverage to date that hinders our ability to fully interpret and understand the extent of population genetic structuring and connectivity (gene flow) among coastal taxa. Based on the findings of the literature review will be a series of recommendations about:

- the most appropriate standardised sampling protocol,
- the most appropriate habitats in which to sample,
- the most appropriate suite of species to be sampled, and
- the most appropriate genetic markers to be employed for the subsequent lab-based assessment of connectivity.

Additional to the above, outcomes of the literature review include:

- a section which demonstrates the practical application of molecular genetics in identifying populations that are at risk (due to their lack of diversity) and how such populations should be managed or protected
- a comprehensive discussion of how the molecular tools (i.e., microsatellite markers) that are proposed to be used in the subsequent research will inform our understanding of genetic biodiversity in New Zealand
- an outline of what level of information can reasonably be expected from the use of molecular tools for the study of marine connectivity, and
- information that demonstrates how the study of the four proposed taxa (to be identified by the review process) at the 12 locations will contribute to our understanding of genetic biodiversity in New Zealand.

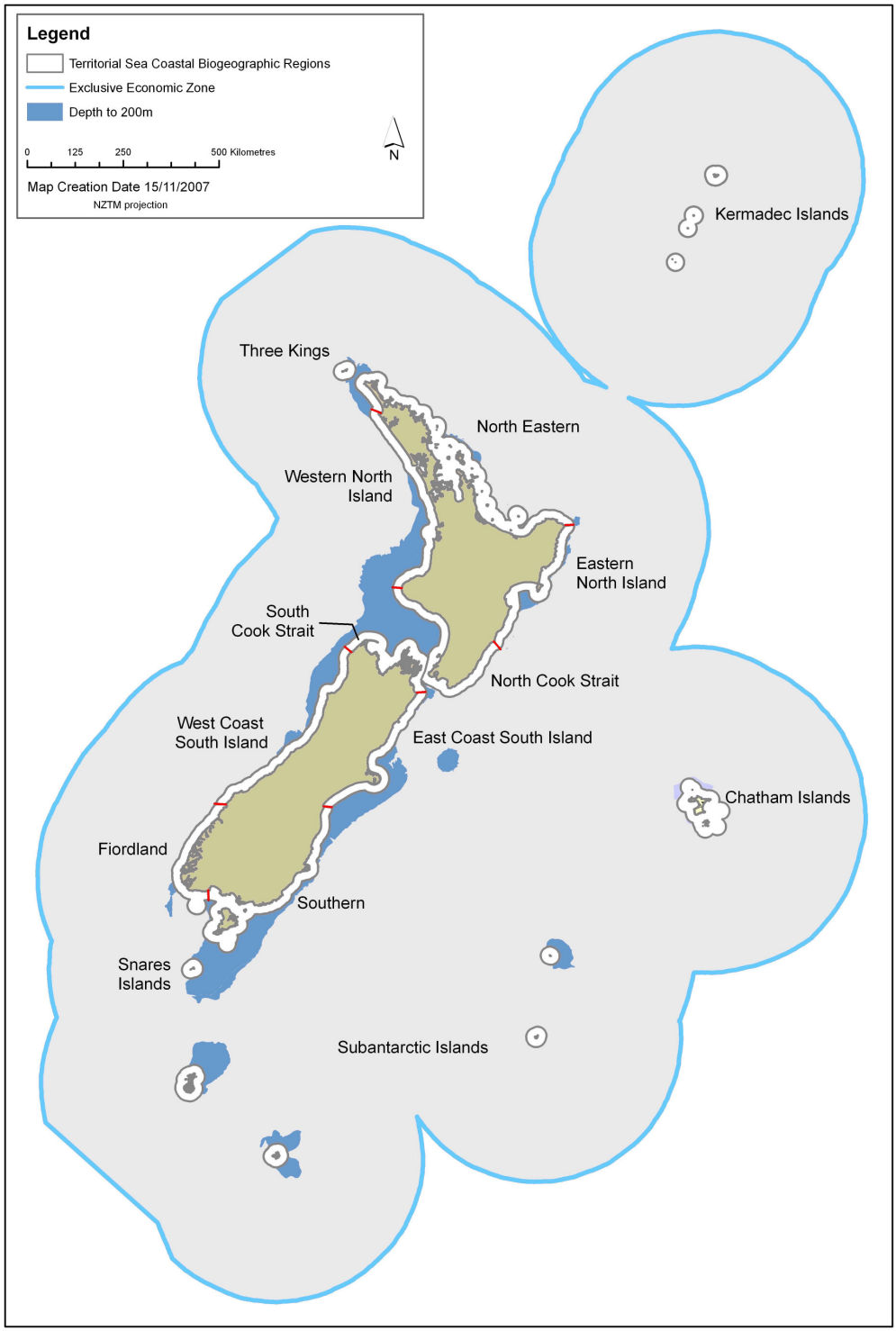

Figure 2: The 14 main biogeographic provinces recognised by the Department of Conservation. Nine of these 14 provinces are associated with the New Zealand mainland. Five are offshore island groups, and are not considered further in this report. Taken from Walls (2009).

3. METHODS

A number of different approaches have been employed to ensure that as much of the relevant information as possible has been obtained to conduct this review.

Searches of literature bases, such as ISI Web of Knowledge, Current Contents, and Aquatic Sciences and Fisheries Abstracts, have been conducted to ensure that full representation of all published work has been obtained. Articles have been sourced directly from the VUW library's e-journal subscription or via inter-library loans. Additionally, "grey literature" such as unpublished NZ Government reports and unpublished university theses (Masters, PhD) were sourced from the agencies or universities themselves.

Colleagues at NZ institutions have been contacted to see what unpublished information they hold and if they have plans to publish relevant material during the course of this tender. Where possible, copies of such data have been secured. However, under certain circumstances (e.g., unpublished student data yet to be used in a thesis or awaiting formal publication) this was not always possible.

All material collected was reviewed for relevance and a brief scientific summary of each article has been made. The reference and the article summary have been stored in a searchable database in EndNote. The EndNote library has also been used to catalogue communication and correspondence with other researchers to provide a record of where data have been successfully obtained and where not.

Having compiled the database of relevant studies the focus of the review has been on:

1. taxonomic coverage
2. geographic coverage
3. habitat coverage

3.1 Habitat types

For this study we have placed each taxon into one of seven different categories according to habitat type and tidal height. The purpose here is to help identify knowledge gaps in different environments that have not been extensively studied. More detailed information about these habitat types can be found in standard NZ reference books such as Leslie (1968), Morton & Miller (1968), Healy (1980), Kingsford & Battershill (1998), and Morton (2004), and also in text books such as Levinton (1999), Mann (2000) and Nybakken (2001).

3.1.1 Estuaries

This section includes data from studies that have looked at taxa in both intertidal and subtidal regions of estuaries (including all soft and hard substrates). These regions are typified by their salinity variation on a tidal basis (may range from 0 to 32 practical salinity units - PSU), in many cases on a 6-hourly basis when the tides are semi-diurnal.

3.1.2 Fiords

This section includes data from studies that have examined only NZ Fiordland populations, even if the taxon in question is more widely distributed throughout NZ (i.e., populations exist but were not sampled from outside Fiordland) and/or taxa which are only or mostly found in the fiords. May include studies of sites within the fiords that are characterised by little or no salinity variation (e.g., at the open coast end of the fiord) and also sites that are characterised by profound salinity variation (e.g., at the head of the fiord). In the context of a NZ-wide appraisal of coastal marine connectivity this section is of limited value because of its small spatial extent.

3.1.3 Open coast

This section includes a limited amount of data from studies for species which have wide spread distributions and do not conveniently fall into only one of the other categories above. Such species tend to be highly mobile and/or migratory, for example, Hector's dolphins and bottlenose dolphins.

3.1.4 Rocky intertidal

This section includes studies that are focused on taxa living exclusively or mostly in the intertidal region and are associated in large part (i.e., mostly as post-metamorphic stages) with open rocky shore environments. This association may be exclusively with reefs, but may also involve a wider association with habitat heterogeneity that includes, for example, life under cobbles or on boulders which are soft substrate patches such as sand. Environmental variables, such as salinity, are representative of open coast situations (32–34 PSU), rather than of estuaries (0-30 PSU). The distinction between rocky intertidal and rocky subtidal habitat may not be clear cut for some species, because their distributions may span both regions. In such a case, emphasis here is placed more on the region of the shore from which specimens were collected. For example, the endemic NZ greenshell mussel (*Perna canaliculus*) is found in the rocky intertidal zone but its distribution also extends into the shallow subtidal rocky region. Most studies of this species have collected from intertidal regions, hence we place *Perna canaliculus* in the rocky intertidal category.

3.1.5 Rocky subtidal

This section includes studies that are focused on taxa living exclusively or mostly in the subtidal region and are associated in large part (i.e., mostly as post-metamorphic stages) with open rocky shore reef environments. This association may be exclusively with reefs, but may also involve a wider association with habitat heterogeneity which includes, for example, soft substrate patches such as sand inter-digitated with the reef complex. Environmental variables such as salinity are representative of open coast situations (32–34 PSU), rather than of estuaries (0–30 PSU).

3.1.6 Soft substrate intertidal

This section includes data from studies which have focused on species that live on, or more usually in, soft substrate open coast intertidal habitats such as, but not limited to, sandy beaches on open coast wave-swept beaches. Environmental variables such as salinity are more representative of protected soft beaches (28–32 PSU) and/or open coast situations (32–34 PSU), rather than of estuaries (0–30 PSU).

3.1.7 Soft substrate subtidal

This section includes data from studies that have focused on species living on, or more usually in, soft substrate open coast subtidal habitats such as, but not limited to, sandy beaches on open coast wave-swept beaches. Environmental variables such as salinity are more representative of protected soft beaches (28–32 PSU) and/or open coast situations (32–34 PSU), rather than of estuaries (0–30 PSU).

Where possible and appropriate, we have broken down the database into different taxonomic categories (principally by phylum), reviewed sample site information to better understand geographic coverage, and categorised the habitat types of the taxa that have been studied. This three-way division of information has been used to identify knowledge gaps, from which recommendations for future study can be made.

3.2 Studies that are not included in this report

While information exists about genetic connectivity and/or genetic stock structure for the following, they are not included in this report.

1. Orange roughy, scampi, southern blue whiting, and southern bluefin tuna. These species are not coastal and therefore fall beyond the scope of the present work.
2. Different coastal taxa, such as Kermadec-endemic limpets and NZ subantarctic islands bull kelp, that are associated with offshore islands. Such studies do not reveal anything about patterns or processes of mainland genetic structuring and therefore fall beyond the scope of the present work.
3. Invasive species such as ascidians and amphipods. Such species are not native and therefore fall beyond the scope of the present work.

4. RESULTS

The details of the papers, reports, and theses addressing aspects of the population genetic structuring and genetic connectivity of NZ's coastal marine biota are summarised in Tables 1–3 and Figures 3–10.

Observed population genetic structuring is a function of sampling effort in terms of sample size, taxonomic coverage, spatial coverage, and habitat coverage. As outlined above, genetic structuring also reflects taxon-specific life history characteristics such as the pelagic larval duration (PLD), natural selection acting differentially on individuals at different temporal and spatial scales, the geological history of the taxon, and the molecular marker type that has been used (Anne 2006). For example, as pointed out by Ross et al. (2009), taxa with very specific habitat requirements, such as island-restricted or estuarine species, may only exist as discrete populations because of the patchy nature of the habitat and may therefore be separated by greater distances than continuously distributed taxa, which do not have such specific habitat requirements.

It is important to appreciate that not all of the studies listed in Tables 1–3 are equally informative. Ideally, a review of population genetic structure and genetic connectivity would focus on a number of independent studies, each of which was carried out specifically to address the topic, using high information-content markers such as microsatellites or DNA sequencing, and with a genuinely NZ-wide sampling regime of 30 or more populations. Most of the studies do not come close to meeting these requirements.

4.1 What patterns of coastal marine genetic structuring exist on mainland NZ?

4.1.1 No structure

Sixteen of 58 studies (28%) reported no evidence of population genetic structure (Table 1). This absence of structure has been reported across a broad range of taxa, from studies which have sampled at small and large spatial scales, and using a variety of different marker types. This result may be genuine in that the taxon under investigation may really not exhibit population genetic structuring for reasons such as a very long pelagic larval duration (which

permits extensive gene flow among populations and acts to prevent population genetic differentiation). Alternatively, this result may simply be a reflection of insufficient marker resolution. For example, Apte & Gardner (2001) observed no evidence of genetic structure among 35 populations of the greenshell mussel (*Perna canaliculus*) based on allozyme analysis. Subsequently, Apte and Gardner (2002) using mtDNA NADH IV SSCPs on a subset of 22 of the same populations, and Star et al. (2003) using RAPDs on a subset of 19 of the same populations, both reported pronounced north-south differentiation at about 42° S for this taxon. Apparent absence of structure may also reflect insufficient sampling resolution. As a general observation we caution against accepting evidence of no structure based on allozymes alone, even though allozymes may be excellent markers for identifying structure under certain conditions, such as when there is likely to be strong differentiation and/or effects of selection). We suggest that it is appropriate to accept evidence of no structure only when appropriate molecular markers (in particular highly polymorphic or variable markers such as microsatellites or RAPDs or mtDNA D-loop sequencing are employed, when the number sampled populations is large (over 20), and when the location of sampled populations encompasses the full extent of the known distribution of the taxon in question. In practice, these criteria are rarely met in any study.

4.1.2 Isolation by distance

Nine of 58 studies (16%) listed in Table 1 reported an isolation by distance (IBD) model or a latitudinal cline (which can be thought of as a special case of IBD). The IBD model was reported for a variety of different taxa (invertebrates and fish) from a variety of different habitat types, and using a variety of different marker types. The IBD model is described by a negative linear relationship between some measure of population genetic differentiation (e.g., F_{ST} expressed as [[1- F_{ST})/ F_{ST}]]) and geographic distance (usually in km) between pairs of populations. Thus, populations which are close together spatially have greater genetic similarity because it is easier for them to exchange larvae/genes, whereas populations that are further apart exhibit greatest genetic differentiation because it is harder for them to exchange larvae/genes. Given the latitudinal gradient that exists from the far north to the far south of NZ, and the fact that a significant number of species span most or all of this range, we would expect to observe the IBD model at reasonable frequency.

4.1.3 Divergence within and/or among populations

Twelve of 58 studies (21%) reported some form of unspecified population genetic differentiation within and/or among populations (Table 1). Seven reports were for studies conducted wholly or largely in the NZ fiords and presumably reflect the unusual hydrologic conditions of this habitat type. In the two studies of open coast taxa (Hector's and bottlenose dolphins) the analyses revealed distinct genetic clusters of individuals corresponding to known present-day dolphin distributions. The remaining three cases were reported from the rocky intertidal habitat. In several instances where divergence within and/or among populations has been described, numbers of sampled populations were low (less than 10). Therefore, one possible explanation for the presence of population genetic divergence and the absence of obvious structure to that divergence is that sampling effort was too low to permit detection of structure. Another possible explanation is that no cohesive structure exists, or at least could not be detected with the markers used. The detection of population genetic differentiation is important because it tells us something about the (limited) extent of genetic connectivity. The absence of clear structure to the differentiation is quite common among published reports, but does not aid our interpretation of the underlying causes for the differentiation.

4.1.4 East-west differentiation

Only one study (2%) reported east-west differentiation alone among populations (Table 1). This was for an allozyme assessment of snapper, which by its natural distribution is restricted to warmer waters in central and northern NZ (Smith et al. 1978). More recent analysis of snapper populations using molecular markers has indicated the existence of a north-south split (Bernal-Ramirez et al. 2003).

4.1.5 North-south differentiation

Twenty of 58 studies (33%) reported a form of north-south population genetic differentiation at the NZ-wide scale (Table 1). Such reports were across a range of habitat types (except the NZ fiords), and were most abundant for intertidal rather than subtidal regions. The reports included a macroalga, a higher plant, many invertebrates, and several fish species. Although the north-south pattern of differentiation may be the most commonly occurring of the five categories recognised here, there was considerable variability within this group in terms of the co-existence of other patterns of differentiation (e.g., east-west as well as north-south differentiation) and/or the geographic location of the north-south split. In other instances the spatial distribution of sampling effort simply did not permit the identification of the latitude at which the north-south exists. Several studies reported a north-south split variously in the range of about 37° S to 39° S, but where a reasonably accurate geographic location can be pinpointed most studies highlight the region at about 42° S as being important. A significant number of studies, principally of invertebrates from the rocky intertidal zone, have used a variety of different genetic markers to demonstrate that a relatively homogeneous northern grouping exists above 42° S. Thus, all sites in the North Island and sites at the top of the South Island (including all in Golden Bay, Tasman Bay, and the Marlborough Sounds) fall into the northern group. Sites in the South Island below 42° S on both coasts fall into the southern group. The papers may report that the northern group for any one taxon is reasonably homogeneous (indicative of high levels of gene flow among northern populations), whereas the southern group is more heterogeneous and may exhibit some degree of east-west differentiation. The exact geographic locations of the boundaries between the north and south groups are unclear, but evidence from a number of different taxa point to the region around Cape Campbell on the east coast and somewhere between Westhaven and Little Wanganui River on the west coast (Wei et al. in press). These boundaries match almost exactly with the biogeographic boundaries proposed by DoC (Figure 2) at the north end of the South Island.

4.2 Taxonomic coverage

A total of 42 different NZ coastal marine taxa have been examined for their population genetic structuring. Of these, one is a higher plant, one is a macroalga, 28 are invertebrates, and 12 are vertebrates (Table 2; Figures 3–10).

Within the invertebrates, the following higher order groupings are represented (number of times): Digenea (1), Brachiopoda (2), Cnidaria (3), Arthropoda (6), Echinodermata (6), Mollusca (9), and the Chordata (1).

Within the vertebrates, the following higher order groupings are represented (number of times): Elasmobranchii (1), Teleostei (9), and Mammalia (2).

As expected, the focus of most studies has been on macroinvertebrates, and to a lesser extent on vertebrates (specifically fish). Macroalgae and higher plants are both very poorly represented, although we note elsewhere (Table 4) that a number of studies involving

macroalgae are currently underway. Within the invertebrates, particular focus has been on major groups such as the crustaceans, the echinoderms, and the molluscs. This represents a variety of different factors, including the economic importance of certain taxa, their ecological importance and widespread distributions, their ease of collection, and perhaps also their customary interest and value. Major invertebrate groups such as the annelids (polychaetes), the sponges and the bryozoans are not represented at all, whereas other groups such as the brachiopods, the cnidarians, and the chordates are only poorly represented. Of the cartilaginous fishes, only one species is represented, and among the teleosts there is a clear focus on triplefins, with other groups such as the sparids being only poorly represented, and all other groups being absent. Despite the protected status of all marine mammals in New Zealand, only two different species (both are dolphins) are represented.

Overall, the taxonomic coverage is perhaps best summed up by saying that the bias in coverage reflects the taxonomic bias (personal interests) of individual researchers or groups of researchers. At present, no single study has taken a taxonomically diverse approach to addressing the question of the patterns of population genetic structuring that exist among NZ coastal marine taxa, or the process or processes that give rise to them. Generally, there is a relative dearth of information from plants (higher plants and macroalgae) and from fish species.

Table 1: Summary of 58 studies of New Zealand's coastal marine connectivity.

Habitat type	Species	Classification	Reference	Geographic coverage – (n) number of populations studied	Marker type(s)	Genetic structure
Estuarine	*Gracilaria chilensis*	Red alga - Gracialiares, Rhodophyta	Intasuwan et al. 1993	NZ wide (17)	Allozymes	Northern group down to ~38° S plus NZ wide group
Estuarine	*Maritrema novaezealandensis*	Trematode parasite – Microphallidae, Digenea	Keeney et al. 2008	Otago Harbour (3)	Microsatellites	No structure
Estuarine	*Paracorophium excavatum*	Amphipod – Arthropoda, Crustacea	Schnabel 1998; Schnabel et al. 2000	NZ east coast (4)	Allozymes	North-south differentiation
Estuarine	*Paracorophium excavatum*	Amphipod – Arthropoda, Crustacea	Stevens & Hogg 2004	NZ wide (21)	Allozymes	North-south differentiation at ~39° S
Estuarine	*Paracorophium lucasi*	Amphipod – Arthropoda, Crustacea	Schnabel 1998; Schnabel et al. 2000	Central NZ (11)	Allozymes	East-west & north-south differentiation
Estuarine	*Paracorophium lucasi*	Amphipod – Arthropoda, Crustacea	Stevens & Hogg 2004	NZ wide (18)	Allozymes	East-west & north-south differentiation
Estuarine	*Austrovenus stutchburyi*	Common cockle – Bivalvia, Mollusca	Lidgard 2001	NZ wide (10)	Allozymes	No structure
Estuarine	*Mustelus lenticulatus*	Rig – Triakidae, Elasmobranchii	Hendry 2004	NZ wide (8)	Allozymes and mtDNA RFLPs	No structure
Estuarine	*Grahamina nigripenne*	Estuarine triplefin - Tripterygiidae, Teleosti	Hickey et al. 2009	NZ wide (11)	mtDNA control region	Isolation by distance – and north-south differentiation at ~44° S
Fiord	*Antipathes fiordensis*	Black coral – Anthozoa, Cnidaria	Miller 1997	Fiordland and Stewart Island (28)	Allozymes	Divergence within and among fiords

Fiord	*Antipathes fiordensis*	Black coral – Anthozoa, Cnidaria	Miller 1998	Doubtful Sound, Fiordland (3)	Allozymes	Divergence within and among fiords
Fiord	*Errina novaezealandiae*	Red coral – Anthozoa, Cnidaria	Miller et al. 2004	Fiordland (9)	Allozymes	Divergence within and among fiords
Fiord	*Liothyrella neozelanica*	White brachiopod – Articulata, Brachiopoda	Ostrow 2004	Fiordland (6)	AFLPs	Differentiation within and among fiords
Fiord	*Terebratella sanguinea*	Red brachiopod – Articulata, Brachiopoda	Ostrow 2004	Fiordland & Stewart Island (23)	Allozymes and AFLPs	Some differentiation among fiords
Fiord	*Astrobrachion constrictum*	Snake star - Ophiuroidea, Echinodermata	Steele 1999	Fiordland (7)	Allozymes and mtDNA COI	No structure
Fiord	*Coscinasterias muricata*	Eleven-arm starfish – Asteroidea, Echinodermata	Perrin 2002; Perrin et al. 2004	Fiordland plus 2 South Island and 1 North Island sites (17)	mtDNA D-loop	Isolation by distance amongst northern fiords; restricted gene flow between southern fiords
Fiord	*Coscinasterias muricata*	Eleven-arm starfish – Asteroidea, Echinodermata	Skold et al. 2003	Fiordland plus 2 South Island and 1 North Island sites (16)	Allozymes	Differentiation among fiords
Fiord	*Evechinus chloroticus*	Kina – Echinoidea, Echinodermata	Perrin 2002	Fiordland plus 2 South Island and 2 North Island sites (20)	Microsatellites	Differentiation within and among fiords
Open coast	*Cephalorhynchus hectori*	Hector's dolphin – Mammalia, Chordata	Pichler et al. 1998	NZ wide (n=34 individuals)	mtDNA control region	North Island group, west coast of South Island, east coast of South Island
Open coast	*Tursiops truncates*	Bottlenose dolphin - Mammalia, Chordata	Tezanos-Pinto et al. 2009	NZ wide (3)	mtDNA control region	Three groups – Northland, Marlborough Sounds, Fiordland

Rocky intertidal	*Actinia tenebrosa*	Waratah anemone - Anthozoa, Cnidaria	Veale 2007	NZ wide (27)	Microsatellites	Isolation by distance
Rocky intertidal	*Cnemidocarpa nisiotis*	Sea tunicate – Ascidiacea, Chordata	del Mundo 2009	NZ wide (5)	mtDNA COI	No structure
Rocky intertidal	*Amphipholis squamata*	Common brittlestar - Ophiuroidea, Echinodermata	Sponer 2002; Sponer & Roy 2002	NZ wide (16)	mtDNA 16s, (nDNA ITS)	North-south differentiation
Rocky intertidal	*Ophiomyxa brevirima*	Brittle star - Ophiuroidea, Echinodermata	Garrett 1994	South and Stewart Islands (4)	Allozymes	Differentiation between populations
Rocky intertidal	*Patiriella regularis*	Cushion star – Asteroidea, Echinodermata	Waters & Roy 2004	NZ wide (19)	mtDNA control region	North-south differentiation at ~42° S
Rocky intertidal	*Patiriella regularis*	Cushion star – Asteroidea, Echinodermata	Ayers & Waters 2005	NZ wide (22)	mtDNA control region	North-south differentiation at ~42° S
Rocky intertidal	*Pinnotheres novaezelandiae*	Pea crab – Arthropoda, Crustacea	Stevens 1990	NZ North Island (5)	Allozymes	Differentiation within and among locations
Rocky intertidal	*Pinnotheres atrinicola*	Pea crab – Arthropoda, Crustacea	Stevens 1991	NZ North Island (7)	Allozymes	Latitudinal cline
Rocky intertidal	*Cellana flava*	Golden limpet – Gastropoda, Mollusca	Goldstien 2005; Goldstien et al. 2006	NZ wide (8)	mtDNA cytochrome b	North-south differentiation
Rocky intertidal	*Cellana ornate*	Ornate limpet - Gastropoda, Mollusca	Goldstien 2005; Goldstien et al. 2006	NZ wide (31)	mtDNA cytochrome b	North-south differentiation at ~42° S
Rocky intertidal	*Cellana radians*	Radiate limpet - Gastropoda, Mollusca	Goldstien 2005; Goldstien et al. 2006	NZ wide (31)	mtDNA cytochrome b	North-south differentiation at ~42° S
Rocky intertidal	*Haliotis iris*	Blackfoot paua – Gastropoda, Mollusca	Frusin 1982	NZ wide (3)	Allozymes	No structure within 2 mainland populations
Rocky intertidal	*Haliotis iris*	Blackfoot paua - Gastropoda, Mollusca	Smith & McVeagh 2006	NZ wide (4)	mtDNA COI and microsatellites	Differentiation between sites

Rocky intertidal	*Haliotis iris*	Blackfoot paua - Gastropoda, Mollusca	Will & Gemmell 2008	NZ wide (25)	mtDNA COI and ATP8-ATP6; microsatellites	North-south differentiation at ~42° S
Rocky intertidal	*Nerita atramentosa*	Black nerite – Gastropoda, Mollusca	Waters et al. 2005	Northern NZ (10)	mtDNA COI	No structure
Rocky intertidal	*Perna canaliculus*	Greenshell mussel – Bivalvia, Mollusca	Smith 1988	NZ wide (6)	Allozymes	North-south differentiation
Rocky intertidal	*Perna canaliculus*	Greenshell mussel – Bivalvia, Mollusca	Gardner et al. 1996	NZ wide (10)	Allozymes	Isolation by distance
Rocky intertidal	*Perna canaliculus*	Greenshell mussel – Bivalvia, Mollusca	Apte & Gardner 2001	NZ wide (35)	Allozymes	No structure
Rocky intertidal	*Perna canaliculus*	Greenshell mussel – Bivalvia, Mollusca	Apte & Gardner 2002	NZ wide (22)	mtDNA NADH IV	North-south differentiation at ~42° S
Rocky intertidal	*Perna canaliculus*	Greenshell mussel – Bivalvia, Mollusca	Star et al. 2003	NZ wide (19)	RAPDs	North-south differentiation at ~42° S
Rocky intertidal	*Perna canaliculus*	Greenshell mussel – Bivalvia, Mollusca	Apte et al. 2003	NZ wide (36)	Allozymes, mtDNA and RAPDs	North-south differentiation at ~42° S
Rocky intertidal	*Sypharochiton pelliserpentis*	Snakeskin chiton – Polyplacophora, Mollusca	Veale 2007	NZ wide (28)	mtDNA COI	North-south differentiation
Rocky intertidal	*Bellapiscis lesleyae*	Mottled twister - Tripterygiidae, Teleosti	Hickey et al. 2009	NZ wide (5)	mtDNA control region	Isolation by distance
Rocky intertidal	*Bellapiscis medius*	Twister - Tripterygiidae, Teleosti	Hickey et al. 2009	NZ wide (8)	mtDNA control region	Isolation by distance
Rocky subtidal	*Jasus edwardsii*	Red rock lobster – Arthropoda, Crustacea	Smith et al. 1980	NZ wide (3)	Allozymes	No structure
Rocky subtidal	*Jasus edwardsii*	Red rock lobster – Arthropoda, Crustacea	Ovenden et al. 1992	NZ east coast (2)	mtDNA RFLPs	No structure
Rocky subtidal	*Sagmariasus (Jasus) verreauxi*	Packhorse lobster - Arthropoda, Crustacea	Brasher et al. 1992	NZ northeast coast (2)	mtDNA RFLPs	No structure

Rocky subtidal	*Coscinasterias muricata*	Eleven-arm starfish – Asteroidea, Echinodermata	Waters & Roy 2003	NZ wide (4)	mtDNA COI (and nDNA ITS2)	No structure
Rocky subtidal	*Evechinus chloroticus*	Kina – Echinoidea, Echinodermata	Mladenov et al. 1997	NZ wide (6)	Allozymes	No structure (except Doubtful Sound distinct from all other populations)
Rocky subtidal	*Forsterygion lapillum*	Common triplefin – Tripterygiidae, Teleosti	Hickey et al. 2009	NZ wide (12)	mtDNA control region	Isolation by distance
Rocky subtidal	*Forsterygion varium*	Variable triplefin - Tripterygiidae, Teleosti	Hickey et al. 2009	NZ wide (8)	mtDNA control region	No structure
Rocky subtidal	*Grahamina capito*	Spotted robust triplefin - Tripterygiidae, Teleosti	Hickey et al. 2009	NZ wide (15)	mtDNA control region	Isolation by distance
Rocky subtidal	*Grahamina gymnota*	Tasmanian robust triplefin - Tripterygiidae, Teleosti	Hickey et al. 2009	NZ wide (9)	mtDNA control region	No structure
Rocky subtidal	*Ruanoho whero*	Spectacled triplefin - Tripterygiidae, Teleosti	Hickey et al. 2009	NZ wide (5)	mtDNA control region	No structure
Rocky subtidal	*Pagrus auratus*	Snapper – Sparidae, Teleosti	Smith et al. 1978	Northern and central NZ (12)	Allozymes	East-west differentiation
Rocky subtidal	*Pagrus auratus*	Snapper – Sparidae, Teleosti	Bernal-Ramirez et al. 2003	Northern and central NZ (6)	Microsatellites and mtDNA hypervariable region	North-South differentiation at ~39° S on east coast and ~37° S on west coast of North Island
Soft substrate intertidal	*Zostera muelleri*	Seagrass – Magnoliophyta, Plantae	Jones 2004; Jones et al. 2008	NZ wide (8)	RAPDs	North Island and South Island
Soft substrate subtidal	*Paphies subtriangulata*	Tuatua – Bivalvia, Mollusca	Smith et al. 1989	NZ wide (13)	Allozymes	North-south differentiation; (Chatham Islands differentiation)

Table 2: Summary of 58 studies (42 species) of New Zealand coastal marine connectivity according to taxonomic coverage.

Taxonomic group	Classification – higher grouping	Classification – lower grouping	Common name	Species	Reference(s)
Macroalga	Rhodophyta	Gracialiares	Red alga	*Gracilaria chilensis*	Intasuwan et al. 1993
Higher plant	Plantae	Magnoliophyta	Seagrass	*Zostera muelleri*	Jones 2004 Jones et al. 2008
Invertebrate	Digenea	Microphallidae	Trematode parasite	*Maritrema novaezealandensis*	Keeney et al. 2008
Invertebrate	Brachiopoda	Articulata	White brachiopod	*Liothyrella neozelanica*	Ostrow 2004
Invertebrate	Brachiopoda	Articulata	Red brachiopod	*Terebratella sanguinea*	Ostrow 2004
Invertebrate	Cnidaria	Anthozoa	Black coral	*Antipathes fiordensis*	Miller 1997 Miller 1998
Invertebrate	Cnidaria	Anthozoa	Red coral	*Errina novaezealandiae*	Miller et al. 2004
Invertebrate	Cnidaria	Anthozoa	Waratah anemone	*Actinia tenebrosa*	Veale 2007
Invertebrate	Arthropoda	Crustacea	Amphipod	*Paracorophium excavatum*	Schnabel 1998 Schnabel et al. 2000 Stevens & Hogg 2004
Invertebrate	Arthropoda	Crustacea	Amphipod	*Paracorophium lucasi*	Schnabel 1998 Schnabel et al. 2000 Stevens & Hogg 2004
Invertebrate	Arthropoda	Crustacea	Red rock lobster	*Jasus edwardsii*	Smith et al. 1980 Ovenden et al. 1992
Invertebrate	Arthropoda	Crustacea	Packhorse lobster	*Sagmariasus (Jasus) verreauxi*	Brasher et al. 1992
Invertebrate	Arthropoda	Crustacea	Pea crab	*Pinnotheres novaezelandiae*	Stevens 1990
Invertebrate	Arthropoda	Crustacea	Pea crab	*Pinnotheres atrinicola*	Stevens 1991
Invertebrate	Echinodermata	Asteroidea	Eleven-arm starfish	*Coscinasterias muricata*	Perrin 2002 Skold et al. 2003 Waters & Roy 2003 Perrin et al. 2004
Invertebrate	Echinodermata	Asteroidea	Cushion star	*Patiriella regularis*	Waters & Roy 2004 Ayers & Waters 2005
Invertebrate	Echinodermata	Echinoidea	Kina	*Evechinus chloroticus*	Mladenov et al. 1997 Perrin 2002
Invertebrate	Echinodermata	Ophiuroidea	Common brittlestar	*Amphipholis squamata*	Sponer 2002 Sponer & Roy 2002
Invertebrate	Echinodermata	Ophiuroidea	Snake star	*Astrobrachion constrictum*	Steele 1999

Invertebrate	Echinodermata	Ophiuroidea	Brittle star	*Ophiomyxa brevirima*	Garrett 1994
Invertebrate	Mollusca	Polyplacophora	Snakeskin chiton	*Sypharochiton pelliserpentis*	Veale 2007
Invertebrate	Mollusca	Gastropoda	Golden limpet	*Cellana flava*	Goldstien 2005 Goldstien et al. 2006
Invertebrate	Mollusca	Gastropoda	Ornate limpet	*Cellana ornate*	Goldstien 2005 Goldstien et al. 2006
Invertebrate	Mollusca	Gastropoda	Radiate limpet	*Cellana radians*	Goldstien 2005 Goldstien et al. 2006
Invertebrate	Mollusca	Gastropoda	Blackfoot paua	*Haliotis iris*	Frusin 1982 Smith & McVeagh 2006 Will & Gemmell 2008
Invertebrate	Mollusca	Gastropoda	Black nerite	*Nerita atramentosa*	Waters et al. 2005
Invertebrate	Mollusca	Bivalvia	Greenshell mussel	*Perna canaliculus*	Smith 1988 Gardner et al. 1996 Apte & Gardner 2001 Apte & Gardner 2002 Apte et al. 2003 Star et al. 2003
Invertebrate	Mollusca	Bivalvia	Tuatua	*Paphies subtriangulata*	Smith et al. 1989
Invertebrate	Mollusca	Bivalvia	Common cockle	*Austrovenus stutchburyi*	Lidgard 2001
Invertebrate	Chordata	Ascidiacea	Sea tunicate	*Cnemidocarpa nisiotis*	del Mundo 2009
Vertebrate - fish	Elasmobranchii	Triakidae	Rig	*Mustelus lenticulatus*	Hendry 2004
Vertebrate - fish	Teleosti	Tripterygiidae	Mottled twister	*Bellapiscis lesleyae*	Hickey et al. 2009
Vertebrate - fish	Teleosti	Tripterygiidae	Twister	*Bellapiscis medius*	Hickey et al. 2009
Vertebrate - fish	Teleosti	Tripterygiidae	Common triplefin	*Forsterygion lapillum*	Hickey et al. 2009
Vertebrate - fish	Teleosti	Tripterygiidae	Variable triplefin	*Forsterygion varium*	Hickey et al. 2009
Vertebrate - fish	Teleosti	Tripterygiidae	Spotted robust triplefin	*Grahamina capito*	Hickey et al. 2009
Vertebrate - fish	Teleosti	Tripterygiidae	Estuarine triplefin	*Grahamina nigripenne*	Hickey et al. 2009
Vertebrate - fish	Teleosti	Tripterygiidae	Tasmanian robust triplefin	*Grahamina gymnota*	Hickey et al. 2009
Vertebrate - fish	Teleosti	Tripterygiidae	Spectacled triplefin	*Ruanoho whero*	Hickey et al. 2009
Vertebrate - fish	Teleosti	Sparidae	Snapper	*Pagrus auratus*	Smith et al. 1978 Bernal-Ramirez et al. 2003
Vertebrate - mammal	Chordata	Mammalia	Hector’s dolphin	*Cephalorhynchus hectori*	Pichler et al. 1998
Vertebrate - mammal	Chordata	Mammalia	Bottlenose dolphin	*Tursiops truncates*	Tezanos-Pinto et al. 2009

4.3 Geographic coverage

To provide some sort of context for the question of geographic coverage of studies described in Table 1, the Department of Conservation's map of NZ marine biogeographic regions (Figure 2) is useful. DoC recognises 14 different marine biogeographic regions, of which 9 are associated with the NZ mainland (North, South and Stewart islands). For the purposes of the present report, the offshore island regions (Kermadec, Three Kings, Chathams, Snares, Subantarctic) are not informative and are not considered further.

As illustrated in Figures 3–10, all of the nine mainland biogeographic regions have been sampled, but inevitably there are some hot spots for sampling effort, and also some areas that are under-represented. Sampling hot spots arise for a number of different reasons, including:

- ease of access and proximity to major urban centres
- proximity to research institutions (particularly important for student projects)
- comparisons of ports/harbours with areas of reduced or absent shipping pressure
- areas of particular oceanographic and/or conservation interest such as the fiords
- testing of hypotheses about geographic splits in regions such as Cook Strait
- sampling from habitat types that are infrequently encountered or poorly represented – e.g., mangroves in the north of NZ, estuaries generally

In terms of reduced sampling effort, and therefore poor geographic coverage, certain areas stand out as being under-represented. These include:

- the eastern North Island region
- the Bay of Plenty region
- much of the west coast of the South Island (not including the fiords)
- the southern part of the South island including Stewart Island
- the western coast of the North Island

4.4 Habitat coverage

For classification purposes we recognise seven different habitat types (Table 3; Figures 3–10). Habitat coverage in terms of sampling effort per habitat type is impossible to calculate for NZ because we do not have information about the areal extent of each habitat type along the country's coastline.

Table 3. Summary of results by habitat type, number of studies, and number of different species.

Habitat type	Number of studies	Number of populations[1]	Number of different species
Estuarine	9	103	7
Fiord	9	129	7
Open coast	2	4	2
Rocky intertidal	24	386	16
Rocky subtidal	12	84	11
Soft substrate intertidal	1	8	1
Soft substrate subtidal	1	13	1
TOTALS	**58**	**727**	**42[2]**

[1] This is the sum of the number of populations given in Table 1. It is not the number of *different* populations studied (because some studies used the same populations in different studies employing different markers – e.g. work of Gardner and colleagues for *Perna canaliculus*). It is provided as an approximate indication of sampling effort within each habitat type.

[2] The total here is 42 (not 44 as per column total) because two species occur in both fiord and rocky subtidal habitat groupings

Most studies listed in Table 3 are of rocky reef species, either from intertidal (Figure 7) or shallow subtidal regions (Figure 8). This reflects the widespread availability of this habitat type, its ease of accessibility for sample collecting, and the personal bias or preference of many scientists for hard over soft substrates. Similarly, the NZ fiords (despite their small spatial extent and relative isolation), are reasonably well represented in terms of numbers of different taxa and numbers of populations studied (Figure 5). Generally, estuaries are poorly represented with only nine studies of seven different taxa from 103 populations (Figure 4). Estuaries are important habitats for a number of different reasons, including their widespread distribution around the coasts of New Zealand, their high levels of biological productivity, their role as nursery areas for many different species (in particular fish), and the increasing degradation that they face from ongoing human activities such as land reclamation, increasing sedimentation, and discharges of various forms of waste products which may contribute to the formation of unique environments. Open coastal habitats (reflective of more pelagic species that are highly mobile than of any easily defined ecological habitat type) are poorly represented with only two examples (both dolphin species) (Figure 6), and both intertidal and subtidal soft substrate habitats are also very poorly represented with a total of only two examples (Figures 9 and 10). We note that there are no recorded studies from the mangrove habitat that occurs in northern NZ. This is an important ecotype of high conservation value and one that is regarded as being iconic of the northern part of the country. However, in a wider context, studies of genetic connectivity of taxa from mangrove systems cannot tell us much about the patterns of genetic structuring across mainland NZ.

5. ONGOING STUDIES

Before making recommendations about which taxa and which habitats should be sampled for future research, it is appropriate to give consideration to studies which are currently ongoing, so that duplication of effort is avoided. Below (Table 4) is a list of research that we know to be underway and for which we expect to see some form of published results (thesis, report, peer-reviewed papers) in the near future. With the exception of ongoing studies at Victoria University we do not have access to these data sets or their interpretation.

Knowledge about these studies is important because we make the assumptions that (1) there may be no need for new work on these species in the future, unless the marker type listed in the table below is not microsatellites in which case further work may be required to ensure data/information compatibility; (2) the spatial coverage of the study listed in the table below is sufficiently comprehensive (NZ wide) to be informative in the present case; and (3) some samples appropriate to our proposed research have already been obtained and may be made available for additional analyses with microsatellite markers if such work has not already been planned.

Table 4: Studies which are presently ongoing and which are expected to contribute at a future date to a greater understanding of the patterns of population genetic structuring of New Zealand's coastal marine biota.

Taxon	Common name	Marker type	Habitat type	Contact person	Institution
Macroalgae					
Carpophyllum maschalocarpum	Macroalga	mtDNA	Rocky subtidal	Dr G Zuccarello	Victoria University of Wellington
Carpophyllum maschalocarpum	Macroalga	Microsatellites	Rocky subtidal	Dr G Zuccarello	Victoria University of Wellington

Lessonia variagata	Macroalga	mtDNA	Rocky subtidal	Dr G Zuccarello	Victoria University of Wellington
Lessonia variagata	Macroalga	Microsatellites	Rocky subtidal	Dr G Zuccarello	Victoria University of Wellington
Macrocystis pyrifera	Macroalga	mtDNA	Rocky subtidal	Dr G Zuccarello	Victoria University of Wellington
Macrocystis pyrifera	Macroalga	Microsatellites	Rocky subtidal	Dr G Zuccarello	Victoria University of Wellington
Invertebrates					
Amphibola crenata	New Zealand mud snail	Microsatellites	Estuarine	Dr Ann Wood	Victoria University of Wellington
Austrolittorina antipodum	Winkle	Microsatellites	Rocky intertidal	Pelayo Salinas, PhD student	Victoria University of Wellington
Austrolittorina cincta	Winkle	Microsatellites	Rocky intertidal	Pelayo Salinas, PhD student	Victoria University of Wellington
Austrovenus stutchburyi	NZ cockle or little neck clam	Microsatellites	Estuarine	Phil Ross, PhD student	University of Waikato
Cominella spp.	Whelk – speckled, banded	Mitochondrial DNA sequencing	Estuarine or rocky intertidal	Dr Hamish Spencer	Otago University
Cominella spp.	Whelk – speckled, banded	Nuclear DNA sequencing	Estuarine or rocky intertidal	Dr Hamish Spencer	Otago University
Diloma nigerrima	Bluish top shell	Microsatellites	Rocky intertidal	Dr Kirsten Donald	Otago University
Diloma subrostrata	Mudflat top shell	Microsatellites	Estuaries and mudflats	Dr Kirsten Donald	Otago University
Diloma sp	Top shell – species unspecified	Unknown	Rocky intertidal	Dr Shane Lavery	University of Auckland
Haliotis iris	Blackfoot paua	Microsatellites	Rocky intertidal	Prof Neil Gemmell	Otago University
Hemigrapsus edwardsi	Common rock crab	mtDNA	Rocky intertidal	Dr Shane Lavery	University of Auckland
Hemigrapsus crenulatus	Hairy handed crab	mtDNA	Rocky intertidal	Dr Shane Lavery	University of Auckland
Jasus edwardsii	Red rock lobster	Microsatellites	Rocky subtidal	Dr James Bell	Victoria University of Wellington
Mytilus spp.	Blue mussels	Microsatellites	Rocky intertidal	Kristen Westfall, PhD student	Victoria University of Wellington
Onchidella nigricans	Pulmonate slug	Microsatellites	Rocky intertidal	Dr Ann Wood	Victoria University of Wellington
Ophiomyxa brevirima	Brittle star	Microsatellites	Rocky intertidal	Dr Graham Wallis	Otago University
Perna canaliculus	Greenshell mussel	Microsatellites	Rocky intertidal	Dr Jonathan Gardner	Victoria University of Wellington

Siphonaria australis	Limpet	Microsatellites	Rocky intertidal	Dr Ann Wood	Victoria University of Wellington
Turbo smaragdus	Cat's eye	Unknown	Rocky intertidal	Dr Shane Lavery	University of Auckland
Fish					
Arripis trutta	Kahawai	mtDNA	Estuaries and open coasts	Dr Peter Richie	Victoria University of Wellington
Galeorhinus galeus	school shark	mtDNA	Estuaries and open coasts	Dr Peter Richie	Victoria University of Wellington
Notolabrus celidotus	Spotty (Wrasse)	mtDNA	Rocky subtidal	Dr Peter Richie	Victoria University of Wellington

NOTE 1 - Because much of the research listed in this table has been funded by other agencies and/or at other institutions we cannot provide these data sets to MFish as part of this (or any other) tender. Information and interpretation from these studies will await publication of peer-reviewed papers, theses, or reports.
NOTE 2 – In a progress report from Auckland UniServices to the Department of Conservation (Lavery et al. 2007) mention is made of collections and in some cases ongoing work on a number of different species. Included in the table above, we have listed only those species for which there is NZ wide sampling coverage and for which published results have not been reported.

6. RECOMMENDATIONS

The purpose of conducting the literature review has been to identify the principal taxonomic, geographic, and ecological knowledge gaps which hinder our ability to fully interpret and understand the extent of population genetic structuring and connectivity (gene flow) among NZ's coastal taxa.

In making any recommendations for future research we note that information return (in terms of number of different taxa to be studied, number of populations to be collected from, number of individuals to be collected per population, and habitat type to be sampled from) has to be balanced against funding available to support the research. At present, under the terms of this contract from MFish to Victoria University of Wellington, the agreement is for a minimum of 4 taxa to be studied, at a minimum of 12 sites (populations), and with a minimum of 20 individuals per taxon per site to be sampled. Subsequently, 6 microsatellite markers will be developed for each taxon and applied to all individuals (as technical aspects permit) of each population.

Based on the findings of the literature review the following recommendations are made.

6.1 The most appropriate habitats in which to sample

The analysis-by-habitat-type of published research indicates that open sandy coastlines and sandy embayments and also estuaries are under-represented (e.g., compare sample coverage of Figures 7 and 8 with Figures 9 and 10). As a consequence, we recommend that future sampling effort be focused away from rocky reef environments and towards open soft substrate shores and towards estuaries. The inclusion of new sampling effort in these habitat types will provide greater information return about the existence of specific (e.g., small spatial scale) and widespread (e.g., NZ-wide) patterns of genetic structuring among NZ's coastal marine benthos.

Based on existing contract details we will sample from six North Island and six South Island locations for each individual taxon. Depending on habitat specificity the same site may be sampled for two or more of the four taxa that are to be assayed. In terms of geographic coverage (the actual location of each sampling site) we recommend that where possible the same sites as have been previously sampled by other studies conducted in the same habitats be sampled again. This will provide as much of a standard approach to sampling location as it is possible to achieve across multiple studies. Additionally, we recommend that sites at the north end of the South Island be specifically targeted to allow for testing of the location of the frequently reported north-south split (Section 4.1).

6.2 The most appropriate suite of species to be sampled

Based on the identification of sampling effort that is required in sandy and estuarine environments, and also the funding that is available to support this research (4 taxa from a minimum of 12 different sites, but not necessarily the same 12 sites for all species), we recommend that the following taxa be sampled and studied for new research into coastal marine genetic connectivity (Table 5).

Pipi – *Paphies australis*
Tuatua – *Paphies subtriangulatum*
Sand flounder – *Rhombosolea plebeia*
Yellow belly flounder – *Rhombosolea leporina*

These four species are recommended for new research because they are all NZ-wide in their distributions, they are abundant at sites along open sandy shores, sandy embayments and/or estuaries, all four of them are in the Quota Management System, all are of customary importance, and all of them can be collected relatively easily in terms of sampling effort and travel/logistical constraints.

Table 5: Species identified as possible candidates for new study in future research, based in large part on recommendations about which habitat types need to be sampled.

Group	Common name	Binomial	Habitat type	Species in QMS?	Comments
Mollusca, Bivalvia	Pipi	*Paphies australis*	Estuaries	Yes	No previous studies; NZ wide; endemic
Mollusca, Bivalvia	Tuatua (surf clam)	*Paphies subtriangulatum*	Open sandy coast	Yes	One previous study of allozymes – north-south differentiation; endemic
Arthropoda, Decapoda	Paddle crab	*Ovalipes catharus*	Sandy bays	Yes	No previous studies; NZ wide; endemic
Teleosti, Mugilidae	Yellow-eyed mullet	*Aldrichetta forsteri*	Estuaries, harbours, sheltered bays	Yes	No previous studies; NZ wide distribution.
Cnidaria, Anthozoa	Small brown sea anemone	*Anthopleura aureoradiata*	Estuaries, protected sand flats. Often on cockle shells	No	No previous studies; NZ wide
Elasmobranchi,	Rig (shark)	*Mustelus*	Estuaries	Yes	One previous study

Triakidae		*lenticulatus*			of allozymes and mtDNA – no structure; endemic
Teleosti, Pleuronectidae	Common sole	*Peltorhamphus novaezeelandiae*	Estuaries, harbours and bays	No	No previous studies; NZ-wide; endemic
Teleosti, Pleuronectidae	Dab, Sand flounder	*Rhombosolea plebeia*	Shallow tidal sandy or mud flats, harbours and estuaries	Yes	No previous studies; NZ wide; endemic
Teleosti, Pleuronectidae	Yellow belly flounder	*Rhombosolea leporina*	Shallow tidal sandy or mud flats, harbours and estuaries	Yes	No previous studies; NZ wide; endemic
Teleosti, Sparidae	Snapper	*Pagrus auratus*	Rocky subtidal	Yes	Two previous studies (allozymes; mtDNA and microsatellites); snapper are not NZ-wide - they are mostly found in northern and central NZ
Teleosti, Pinguipedidae	Blue cod	*Parapercis colias*	Rocky subtidal	Yes	No previous studies; blue cod are not NZ-wide - they are mostly found in southern and central NZ; endemic
Teleosti, Latridae	Blue moki	*Latridopsis ciliaris*	Rocky subtidal; over sand or mud bottom	Yes	No previous studies; NZ-wide distribution. Fertilisation is external; larval duration is 8–12 months; one major spawning ground off Gisborne
Teleosti, Labridae	Banded wrasse	*Notolabrus fucicola*	Rocky subtidal	No	No previous studies; NZ-wide – easy to catch. Sex-change which is often found among wrasses does not always occur in banded wrasses

6.3 The most appropriate standardised sampling protocol

6.3.1 Fish

Fish species will be caught by line, net, or in cage traps, most individuals will have a small piece of fin tissue non-destructively sampled and then will be released back into the sea, but a

small number will be preserved and taken back to the laboratory for further analysis and documentation analysis. Genetic differentiation among marine populations is often observed to be low and it can become difficult to discriminate true signal from the genetic "noise" associated with limited sampling. Ward & Elliot (2001) have shown that sample sizes should be at least 50 individuals from each location, and preferably 100 or more to obtain reasonable statistical resolution. We will attempt to achieve this, but we note that MFish funding may not permit this to occur.

6.3.2 Bivalves

Pipis and tuatuas will be collected from the low intertidal and/or shallow subtidal region by digging. Whole animals will be collected and small tissue samples will be removed and preserved in 100% ethanol.

All samples will be individually labelled and all collecting sites will be located using handheld GPS. Photographs of collecting sites – general site pictures plus specific details of exact locations – will be made. In all cases, estimates of time spent collecting (i.e., duration of net deployment, time spent digging, time spent hand collecting) will be recorded to provide an estimate of CPUE for each species at each site.

6.4 The most appropriate genetic markers to be employed

For the present study we recommend the use of microsatellite markers (refer to Appendices I–III for further information about genetic markers and their application). These are highly polymorphic co-dominant markers that are ideally suited for the present application. Given their information content, their increasing use (we now have a better understanding of their limitations and also their utility), and the decreasing cost and increasing speed of their development, microsatellites are now regarded as the markers of choice for population genetic studies.

Next-generation sequencing techniques have simplified the process of microsatellite marker development. Firstly, genomic DNA is randomly fragmented and analysed using a 1/8 run on a Roche 454 massively parallel sequencing platform (based at Otago University). About 18,000 reads with an average length of 250 base pairs are typically obtained. The DNA sequence information will be mined for microsatellite repeat motifs using bioinformatic tools (e.g., MsatCommander). Specific PCR primers will be designed, synthesised and tested for polymorphism using the DGGE unit in a subsample of individuals. A final panel of six loci will be chosen for each species.

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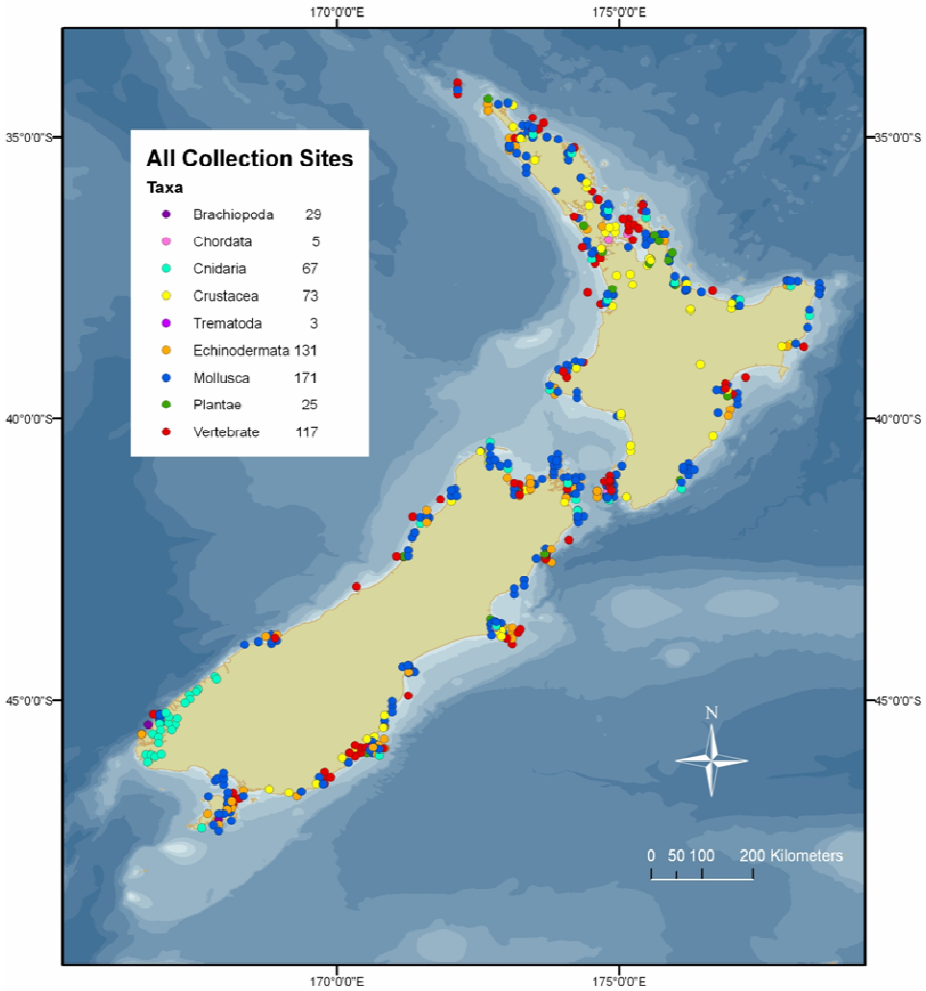

Figure 3: Map of New Zealand showing all genetic study collection sites regardless of habitat type from studies listed in Table 1, with site-specific information about taxonomic coverage.

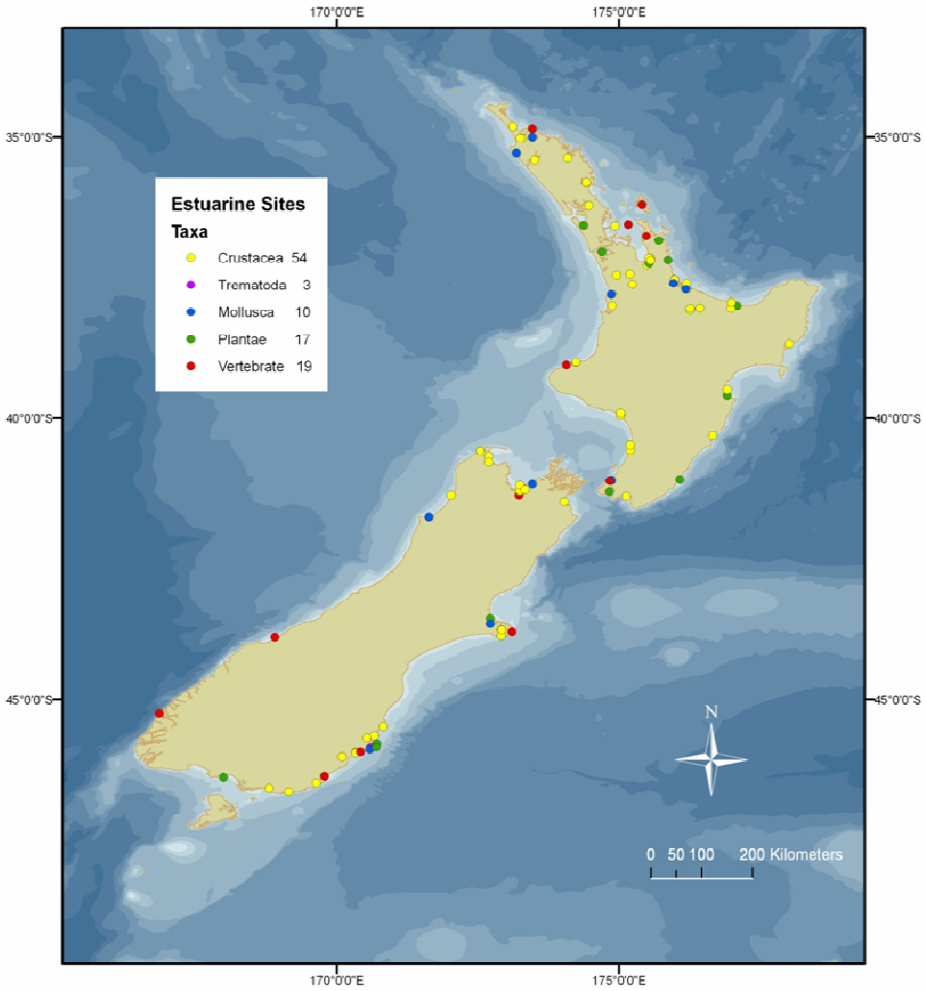

Figure 4: Map of New Zealand showing all estuarine genetic study collection sites from studies listed in Table 1, with site-specific information about taxonomic coverage.

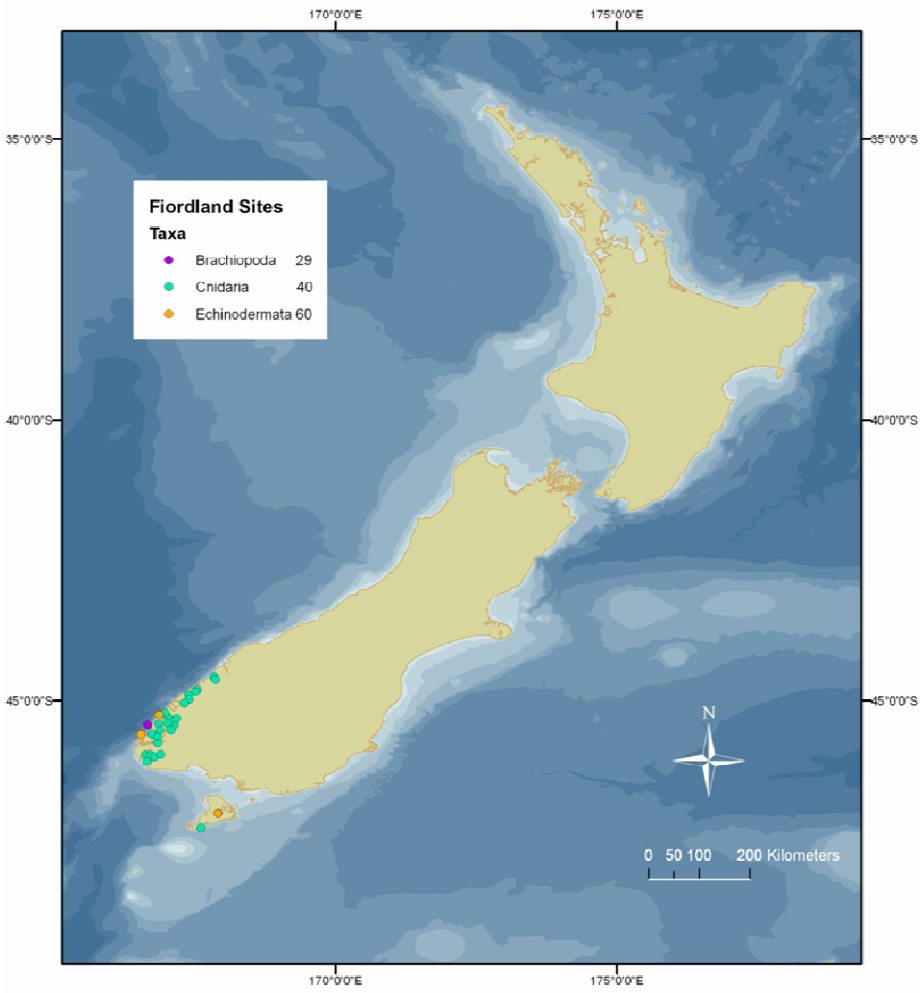

Figure 5: Map of New Zealand showing all Fiordland genetic study collection sites from studies listed in Table 1, with site-specific information about taxonomic coverage.

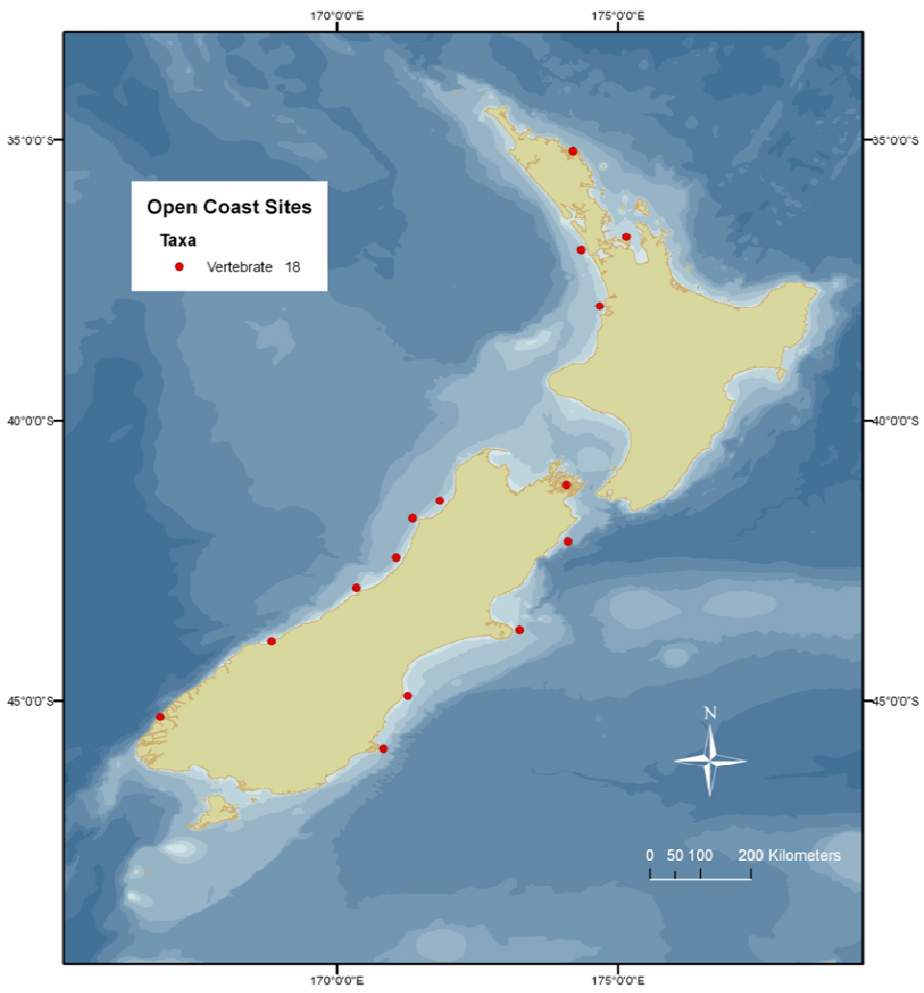

Figure 6: Map of New Zealand showing all open coast genetic study collection sites from studies listed in Table 1, with site-specific information about taxonomic coverage.

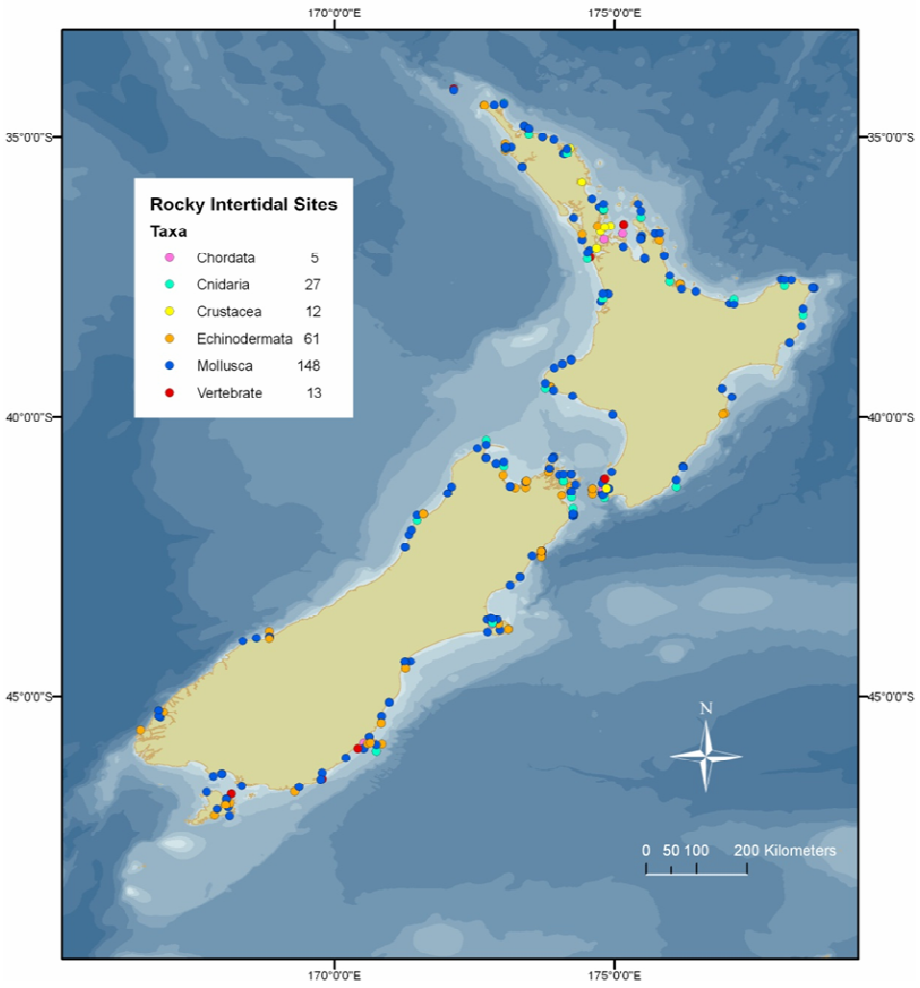

Figure 7: Map of New Zealand showing all rocky intertidal genetic study collection sites from studies listed in Table 1, with site-specific information about taxonomic coverage.

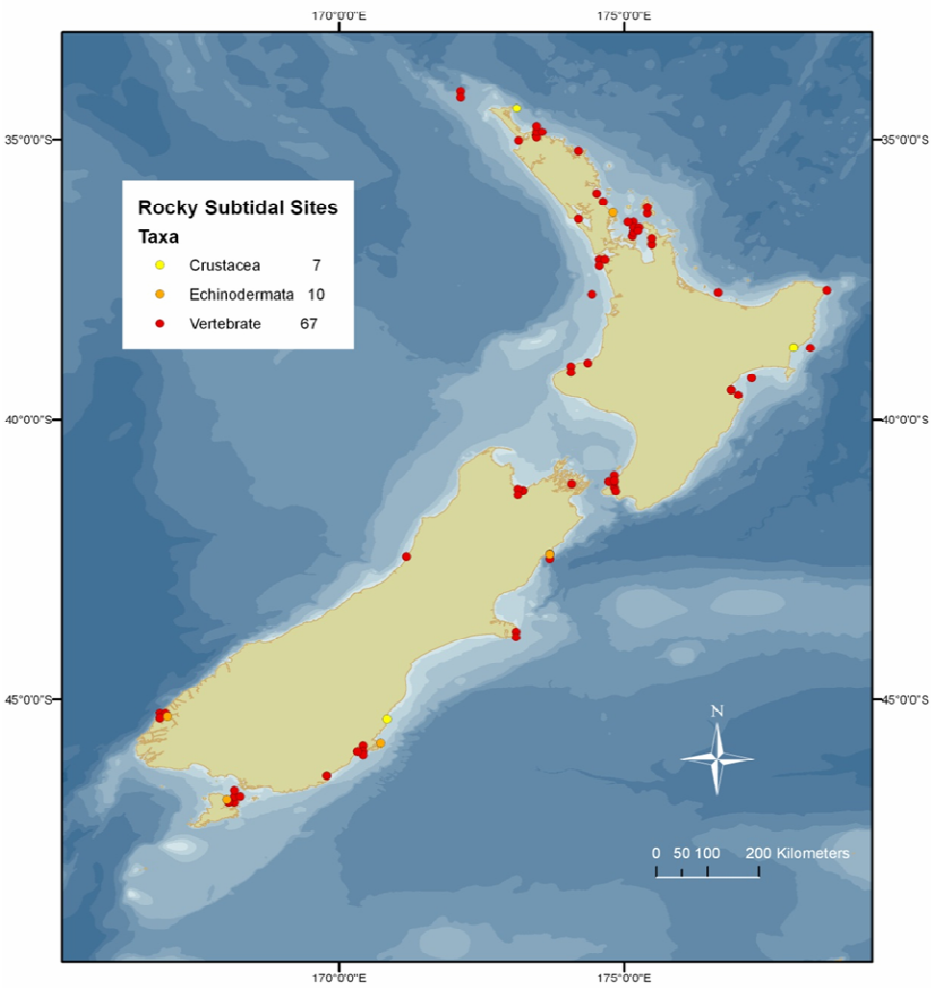

Figure 8: Map of New Zealand showing all rocky subtidal genetic study collection sites from studies listed in Table 1, with site-specific information about taxonomic coverage.

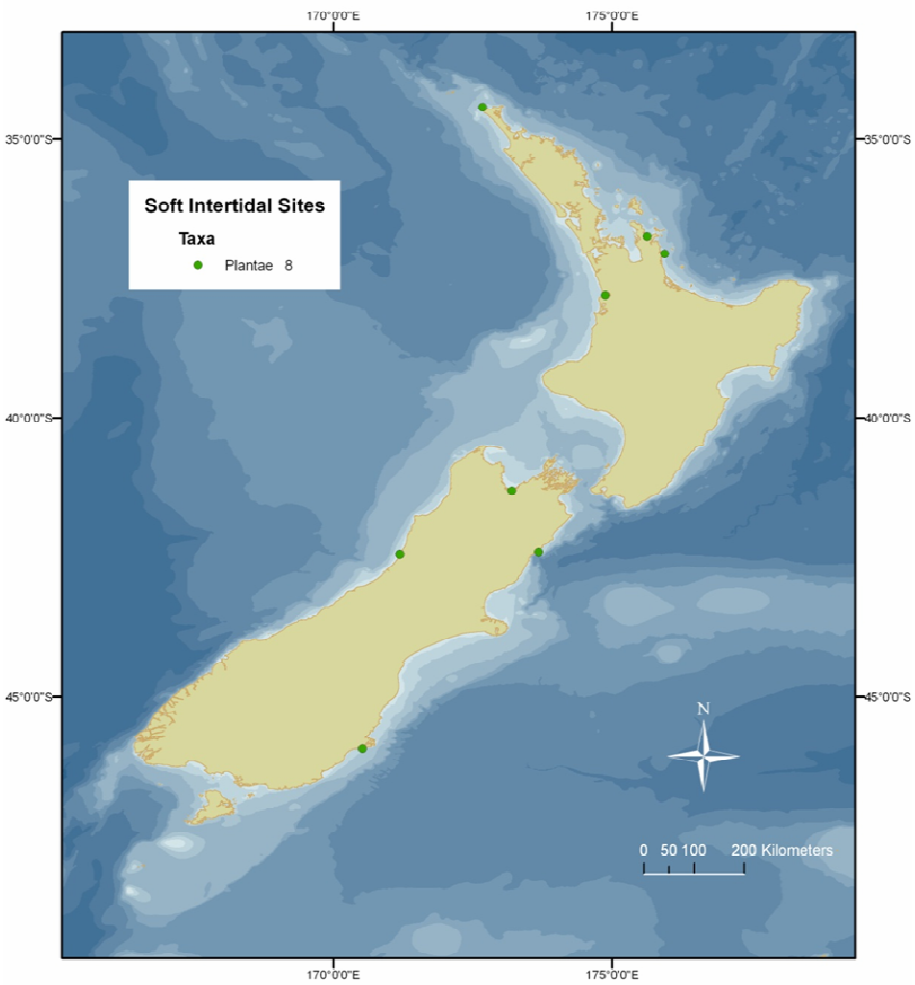

Figure 9: Map of New Zealand showing all soft substrate intertidal genetic study collection sites from studies listed in Table 1, with site-specific information about taxonomic coverage.

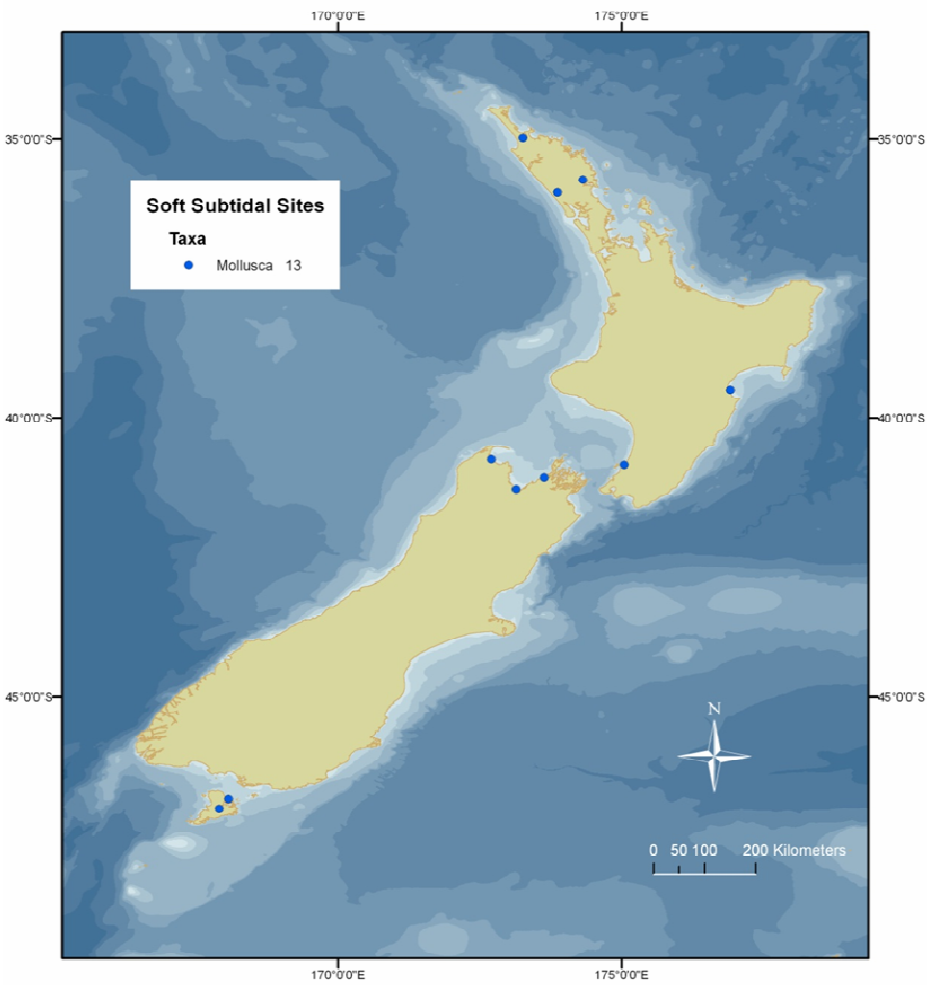

Figure 10: Map of New Zealand showing all soft substrate subtidal genetic study collection sites from studies listed in Table 1, with site-specific information about taxonomic coverage.

Appendix I: Genetic markers – horses for courses

To aid in the understanding of the review of studies carried out of population genetic structuring of NZ coastal marine species, and also to help understand the recommendations for use of certain marker types, below we provide a brief background and set of definitions for most of the commonly used marker types. The important point to appreciate is that different marker types provide different levels of information content because of their level of variability, their mutation rates, their mode of inheritance (maternal only versus bi-parental), and their cost of development, testing, and application. Published reviews of marker types and their appropriate applications were provided by Hadrys et al. (1992), Chambers & MacAvoy (2000), Hellberg et al. (2002), Jeffreys (2005), Anne (2006), Selkoe & Toonen (2006), and Adbelkrim et al. (2009) among others.

Proteins – Proteins are organic compounds made of a linear series of amino acids, some of which are charged, that fold to make a globular form. The sequence of the amino acids is determined by the gene sequence and therefore variability between gene sequences can result in changes in protein characteristics.

Allozymes – Allozymes are different forms of an enzyme coded by different alleles at a locus. Enzymes are proteins and are therefore made of amino acid chains, which are charged. Enzymes have a net electric charge, depending on the combination of amino acids comprising the protein. Any mutation or change in the DNA encoding an amino acid comprising the protein may change the confirmation (shape) of the protein along with its charge. These changes in charge and shape effect the migration of the protein in an electric field and therefore genetic variation can be detected using gel electrophoresis. The advantages of this technique include: its simplicity as it does not require DNA extraction (however, optimisation can be time consuming for some species); the marker is co-dominant (inherited from both parents, and expressed equally) which enables differentiation between heterozygotes and homozygotes, and it is highly reproducible. Disadvantages include generally low levels of genetic variation that can be detected, requires large amounts of tissue, only about 30% of the variation in the DNA code results in amino acid changes (and hence protein changes) meaning a large proportion of the variation cannot be detected. Finally, this marker is usually considered to be neutral, but some allozymes have been shown to be under selective pressure (patterns of structure may therefore not represent gene flow *per se*, but may be the result of selection). This technique is no longer widely used and has been replaced with molecular (DNA-based) markers. Allozymes have been used in the past in many population genetics studies, including measurements of population structuring and population divergence, and for measuring outcrossing rates. For further information see May (1992).

Mitochondrial DNA – Mitochondrial DNA (mtDNA) is the DNA that is located within the mitochondria (the structures within cells that convert food to energy). mtDNA is a double stranded circular molecule which is about 16 500 base pairs in length in most animals. mtDNA is uniparentally (maternally) inherited in most organisms (with the exception of mussels and a few other species).

Chloroplast DNA – Chloroplast DNA (ctDNA) is the DNA that is located within the chloroplasts of plants (the structures in plant cells that convert sunlight to energy). ctDNA is usually 2–3 times larger than mtDNA, and is maternally inherited. ctDNA is a double stranded circular molecule that generally encodes genes that are involved in the photosynthetic pathway.

Nuclear DNA – Nuclear DNA (nDNA) is the DNA contained within the nucleus of eukaryotic organisms. nDNA is passed sexually via both parents, rather than matrilineally via one parent. nDNA has a lower mutation rate than either ctDNA or mtDNA.

RFLPs – Restriction Fragment Length Polymorphisms are differences in homologous DNA molecules arising from the different locations of restriction sites (DNA with a specific sequence which can be cut by bacterial enzymes) in the genome of all organisms. This method involves the digestion (cutting) of DNA with one or more restriction enzymes: the resulting fragments (that are off different lengths) are separated according to their length using agarose gel electrophoresis. They are visualised by direct staining of the gel with ethidium bromide or by transferring them to a membrane (Southern blotting). Hybridisation of a DNA marker probe to the membrane then identifies the length of the fragments that are complimentary to the probes. An RFLP occurs when the length of a detected fragment varies between individuals. Each fragment length is considered to be an allele. The advantages of this marker type include its relatively high level of polymorphism, common occurrence and wide distribution across the genome, and its high reproducibility. The disadvantages are that they technically challenging and expensive to produce, and they also require large amounts of DNA (because this is not a PCR technique). RFLPs are a very versatile marker that can be used for a range of different applications including phylogenetic studies and studies examining differences between populations.

RAPDs – Randomly Amplified Polymorphic DNA markers (pronounced as "rapids") are DNA fragments that are amplified by PCR using a single short (usually around 10 bp) synthetic primer. These primers are usually able to amplify fragments from 1 to10 genomic sites simultaneously. Amplified fragments are usually within the 500–5000 bp size range and can be separated by agarose gel electrophoresis. The gel is stained with ethidium bromide and polymorphisms are detected as the presence or absence of bands of particular sizes. These polymorphisms are generally the result of variation in the primer annealing sites. The advantages include no requirement for prior knowledge of the DNA sequence, it is a quick and simple technique, the RAPDs are widely distributed across the genome, and it is a reasonably cheap system. The main disadvantage of this technique is the low levels of reproducibility (although many researchers dispute this problem) due to the sensitivity of the technique to PCR conditions. In addition, RAPDs are dominant markers – that is, the researcher does not know what the fragment is or from which parent it has come. RAPDs have been used for many purposes, ranging from studies at the individual level (e.g., genetic identity) to studies involving closely related species. For further information see Hadrys et al. (1992).

AFLPs - Amplified Fragment Length Polymorphisms are DNA fragments (80–500 bp) obtained from the digestion of DNA with restriction enzymes, followed by ligation of oligonucleotide adapters (a pair of primers) to the digestion products and selective amplification by the PCR. Thus, AFLPs involve both RFLPs and PCR. The AFLP banding profiles are the result of variations in the restriction sites or in the regions between the restriction sites, and the resulting fragments can be separated by agarose gel electrophoresis. Advantages include no need for prior genetic sequence information, high genome abundance and coverage, and because they involve two primers AFLPs tend to be highly reproducible. Disadvantages to this technique include the need for very high quality, pure DNA and only moderate levels of polymorphism. Additionally, AFLPs are dominant markers with the inherent uncertainty about band origin. For more information see Vos et al. (1995).

SSCPs – Single-Strand Conformation Polymorphisms are DNA fragments of approximately 200–800 bp that are amplified by PCR using specific synthetic primers (20–25 bp in length). Gel electrophoresis of single-stranded DNA is then used to detect nucleotide sequence variation among the amplified fragments. The method is based on the fact that the mobility of single-stranded DNA depends on the secondary structure (conformation) of the molecule, which is changed significantly when sequence mutations occur. Thus, SSCP provides a method to detect nucleotide variation among DNA samples without having to perform sequencing reactions. Advantages include the co-dominance of alleles and the low quantities of template DNA required as this is a PCR-based technique. The disadvantage is the need for prior sequence data to design suitable primers; there is also the problem that some mutations may not be detected (as

they might not change conformation), and there are sometimes difficulties in obtaining reproducible results. For more information see Hayashi (1992).

DGGE – Denaturing Gradient Gel Electrophoresis is a method for separating DNA fragments according to their mobilities under increasingly denaturing conditions. DGGE works by running a small sample of DNA in an electrophoresis gel that contains a denaturing agent (such as urea). As a result of the melting (dissociation of the double-stranded DNA) that is caused by the denaturing agent, the DNA spreads through the gel and the resulting patterns can be analysed to detect differences in DNA or identify mutations. This method has been commonly used to examine microbial diversity. For more information see Muyzer (1999) and Nakatsu (2007).

DNA sequencing – PCR-based DNA sequencing involves determination of the nucleotide sequence of a DNA fragment amplified by the PCR, using primers specific for a particular genomic site. The main advantage of this method enables all possible sequence differences within the amplified fragment to be resolved between individuals, and therefore sequencing provides the best measurement of genetic variation. As this technique has now been used for a number of years, universal primer pairs to target specific coding and non-coding DNA sequences in a wide range of species are available for the chloroplast, mitochondrial, and ribosomal genomes. Other advantages include the need for only a small amount of template DNA as this is a PCR technique, and the high level of reproducibility. Disadvantages may include low genome coverage and low levels of variation below the species level. Historically, this resulted primarily from our ability to sequence only small regions of DNA (to about 1000 bp). More recently (see SNPs) sequencing technologies have advanced considerably to sequence much longer DNA regions.

Minisatellites - Minisatellites are chromosomal DNA regions containing tandem repeat units of 10–50 bases, flanked by conserved DNA regions. Minisatellite analysis involves digestion of genomic DNA with restriction enzymes. Variation in the number of repeat units is thought to be the result of unequal crossing over or gene conversion. The main advantage of this marker is the high mutation rate of minisatellites and therefore their high level of polymorphism, generally resulting in unique multilocus profiles for different individuals within a population. This technique is often used for DNA fingerprinting, which is a method for uniquely identifying individuals. In a similar way to RFLPs, the disadvantages arise from the inability to identify individual alleles and loci, so similar sized fragment bands may be non-homologous. The genome coverage of these markers has also been questioned. This technique has particular use in forensic applications. For more information see Jeffreys (2005).

Microsatellites – Microsatellites, also known as Simple Sequence Length Polymorphisms (SSLP), Simple Sequence Repeats (SSR), and Sequence Tagged Microsatellites (STMS), are tandem repeating units of DNA, similar to minisatellites, but their repeat motifs are much shorter (1–6 bp). By determining nucleotide sequences in the flanking regions of the microsatellite, specific primers (generally 20–25 bp) can be designed to amplify the microsatellite by PCR. Microsatellites are widely used for population genetics studies because of their high level of polymorphism; it is a PCR-based technique, the alleles are co-dominant, reproducibility is high and the process can be automated. Finally, microsatellites are widely considered not to be under selective pressure. The disadvantages of this technique is the time and expense required to develop the primers for the microsatellite flanking regions, although recent advances in sequencing technology have reduced the time and expense considerably. For more information see Chambers & MacAvoy (2000) and Selkoe & Toonen (2006).

SNPs – Single Nucleotide Polymorphisms (pronunced as “snips”) are DNA sequence variations occurring when a single base or nucleotide (A, T, C, or G) in the genome differs at the same DNA position between members of the same species. Advances in sequencing technology and our ability to sequence much longer DNA sequences have led to increased interest in these

markers. These markers have been used in disease research and breeding programmes, and are now becoming employed in population genetic studies. Advantages to the use of SNPs includes their high frequency throughout the genomes of most organisms, their genome-wide coverage, their high level of information return, their reproducibility, and their increasing ease of development as new technologies become more efficient and cheaper to use. Their disadvantages include the fact that they are bi-allelic markers and therefore may be of limited use for certain types of studies (e.g., relatedness), there may be some ascertainment bias in their development (their identification depends on the individual samples used to generate them), as with any new technology there is limited information about their frequencies in and distributions among the genomes of many species, and initial costs for development are high (although dropping rapidly). For more information see Syvänen (2001) and Morin et al. (2004).

Appendix II: How microsatellite markers will inform our understanding of genetic biodiversity in New Zealand

Microsatellite markers have become one of the favoured tools of the molecular ecologist given their high levels of polymorphism, their species-specificity, their co-dominant nature, and wide genome coverage (Selkoe & Toonen 2006). In addition, these markers are widely believed to be selectively neutral, but this may not necessarily be the case (Kashi & Soller 1999). These features of microsatellites make them the preferred markers for many population genetics studies including: estimating the level of genetic diversity within populations (including the identification of founder events or bottlenecked populations), measuring connectivity, stock assessments, parental analysis, forensics, the identification of barriers, and determining source-sink dynamics.

The level of information we can reasonably expect from the present study, and the application of this information, depends on a number of factors, particularly the number of microsatellite markers we are able to develop and utilise, the biology and ecology of the species that are chosen, and the physical environment (Selkoe & Toonen 2006). The number of microsatellite loci and their level of polymorphisms will influence the resolution level at which we are able to distinguish between populations, although for this study we anticipate using six loci, with high levels of polymorphism, which is consistent with many previous studies (see previous sections). The aspects of species biology and ecology that are important in determining the level of information we can expect to obtain, and the scale at which the information will be useful, are particularly those features that influence the amount of exchange between populations.

The most important biological factor driving population connectivity is life-history strategy (Colson & Hughes 2004). Marine invertebrates display a spectrum of reproductive strategies that are a major driving force influencing gene flow and consequently the degree of genetic differentiation and connectivity between populations (Denny et al. 2004). Most marine animal species produce free-swimming pelagic larvae that can potentially disperse widely and link adult populations across broad spatial scales (Thorson 1950), although this depends on the amount of time that a larva spends in the water column, which may range from minutes to years, depending on the species. Other species lack pelagic larvae (direct development) and are expected to show lower levels of connectivity, although this will also be influenced by the amount of adult migration and rafting. Although larval duration is a primary driver of the differences in the level of connectivity between populations, other factors, including fecundity, habitat availability/distribution, population size and longevity, are also important. We expect the spatial scale at which we will be able to detect population structuring to be strongly influenced by larval development mode and larval duration. For species with long-lived larvae (weeks) it may be possible to detect population level differences only at scales of 100 km, while for those species with direct development, we might expect to detect differences at scales of 1 km or less (Bell 2008a).

Importantly, larval dispersal potential is not only influenced by species characteristics, but also by local and large-scale hydrodynamics (Swearer et al. 1999, Hellberg et al. 2002), such that the potential gene flow of a species may never be fully realised and may be highly variable among locations (Palumbi 2001, 2003, Palumbi et al. 2003, Almany et al. 2007). Furthermore, the effects of local oceanography on the connectivity between populations of marine invertebrates with larval dispersal should not be underestimated because temporal hydrographic changes can create significant variability in connectivity patterns to particular shores at a range of temporal and spatial scales (Barber et al. 2002); hence, it may possible for genetic disparity to occur at local (less than 1 to 10 km) as well as distant (100s to 1000s km) scales, even for organisms with long-lived (weeks) larvae. Our work is likely to be able to examine how large-scale (10s km) water hydrodynamics influence connectivity.

This study will provide information on the current levels of genetic diversity contained with multiple populations of four species around New Zealand. Since the microsatellite markers that will be developed are species-specific, it will not be possible to directly compare levels of genetic diversity between species. However, the genetic diversity information will enable us to examine how the genetic diversity is distributed within the species around New Zealand. This will enable us to determine if populations have gone through recent bottlenecks (rapid reductions in population size) (Colson & Hughes 2004) and possibly examine patterns of population expansion and contraction as a result of geological-scale changes in environmental conditions (Colson & Hughes 2006). We will also be able to make estimates of the effective population size for our study species and identify any populations that are inbred (Bell & Okamura 2005, Hoararu et al. 2005).

The genetic data that we collect will be used to estimate genetic connectivity between the sampled populations, which can be compared between species. This information will enable us to determine contemporary patterns of gene flow between these populations, which will enable us to identify barriers to gene flow (e.g., hydrographic barriers) and identify prominent patterns of exchange. It is important to note that estimates of gene flow reflect patterns of larval exchange over multiple generations, which contrasts with demographic connectivity which refers to more recent exchange events over the most recent generations. There are relatively few studies that have compared multiple species from the same sites to examine consistencies in genetic connectivity patterns, or for species with similar life-history characteristics (but see Bell 2008b). Our data are likely to include some species with similar habitat requirements and therefore we should be able to examine consistencies in patterns (and also compare our data with previous studies). In addition, since the species we will use are unlikely to be commercial species, our genetic patterns are unlikely to have been influenced by previous harvesting (Allendorf et al. 2008, Hauser et al. 2002), and therefore represent true patterns of connectivity.

We will also be able to use our data to estimate dispersal distances based on estimates of genetic connectivity (Kinlan & Gaines 2003, Kinlan et al. 2005). By identifying barriers and patterns of gene flow, and depending on the scale of our sampling sites, it may also be possible to examine source-sink population dynamics (Bell 2008a). For example, some population-pair comparisons may have higher levels of gene flow than others, thereby identifying potential source and sink populations. Finally, the information we collect will act as a baseline, by which any future temporal variation in genetic diversity can be measured (e.g., Riccioni et al. 2010), which may become increasingly important when considering the future anthropogenic effects on the marine environment.

Appendix III: The practical application of molecular genetics in identifying populations that are at risk (due to their lack of diversity) and how such populations should be managed or protected.

As described by Allendorf & Luikart (2007) and references therein, molecular genetics have been widely used in the past for a number of purposes relevant to identifying populations that might be at risk, including the following.

Identifying populations with levels of low genetic diversity (spatial variation in genetic diversity). Since genetic diversity is the basis of adaptation to change, reduced genetic diversity may reduce the chances of dealing with future environmental changes.

Identifying temporal changes in the levels of genetic diversity. As well as examining the spatial distribution of genetic diversity in populations using molecular tools, it is also possible to examine temporal changes in genetic diversity. For example, there is increasing evidence in the literature that harvesting has altered the genetic structure of marine populations, particularly by decreasing the levels of genetic diversity.

Evidence for demographic effects. We can use molecular genetics techniques for identifying populations that have experienced bottlenecks (rapid reductions in size) or have resulted from founder events (a low number of individuals found a new population).

Evidence of inbreeding. We can use molecular tools to determine if populations show evidence of inbreeding (i.e., an increased level of homozygotes compared to that expected based on allele frequencies).

Identification of isolated populations. Population genetics are routinely used to measure connectivity, and provide an indication of how well populations are connected. Isolated populations tend to be at greater risk from stochastic events, although they can also act as reservoirs of genetic diversity, and be protected from disease outbreaks.

Cryptic speciation. Molecular tools are routinely applied in taxonomy and there are many examples where cryptic species have been identified. This can result in the identification of one very abundant species and one very rare species.

Forensics. The application of molecular tools to forensic science has been very successful. Although not directly relevant to the identification of populations with low genetic diversity, it is relevant to the identification of protected or endangered species whose genetic diversity is already likely to have been reduced.

Identifying populations that might be at risk using molecular techniques is relatively straightforward; however, demonstrating that populations are actually at risk is much more difficult. Even though a population has low genetic diversity it does not necessarily mean it is at risk. For example, earlier allozyme work, and subsequent mtDNA work, showed that the northern elephant seal, which was heavily exploited and whose population numbers were reduced to fewer than 100 individuals, has no polymorphisms among 21 proteins encoded by 24 loci, and very little mtDNA variability (less than 1% sequence difference) compared with southern elephant seals, whose population size stayed higher than 1000 individuals (Hoelzel et al. 1993). The population size of the northern elephant now stands at over 100 000 individuals, but there appears to be no negative impact of this lack of genetic variation caused by hunting to near extinction. For any species where a decline in genetic diversity is identified, the key management action is to prevent further decline, since increasing genetic diversity at the species level will take millions of generations.

Marine fish and invertebrates generally have much larger census (Nc) and effective (Ne) population sizes than terrestrial vertebrates (Waples 1998). However, genetic diversity can be lost because Ne is often much smaller than the census size in many marine species. For example, Ne in New Zealand snapper (*Pagrus auratus)* was estimated to be 100 based on monitoring loss of heterozygosity and temporal changes in allele frequency (Hauser et al. 2002). This study, along with others, supports the conclusion that the Ne:Nc ratio might be extremely small in a variety of marine species. This suggests that even very large exploited marine fish populations might be in danger of losing genetic variation (Allendorf et al. 2008).