Population biology and genetics of paua, hapuku, and kingfish: sourcing fish for broodstock development

Peter J. Smith

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Abstract

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The genetic principles of broodstock selection, and the genetic population structure and phenotypic variation in marine fishes, were briefly reviewed prior to considering specific aspects of the population biology and genetics of kingfish *Seriola lalandi*, hapuku *Polyprion oxygeneios*, and black foot paua *Haliotis iris*. Ideally the founding broodstock would be based on a minimum of 50 wild caught individuals (25 males and 25 females, which are assumed to be unrelated) to secure a wide genetic base. When broodstock are chosen from widely separated locations there is a risk of outbreeding depression in the F2 and later generations. The level of genetic divergence among regional populations should be assessed, and consideration given to maintaining separate broodstock lines when selection programmes are based on broodstock derived from divergent wild populations.

Patterns of genetic variation in both mitochondrial and nuclear genes have been assessed in a wide range of marine fishes and invertebrates, including New Zealand species. For many marine fishes most of the genetic variation measured with selectively neutral markers has been found within individual populations and genetic population differentiation is evident only at ocean wide scales. The large population sizes, high fecundities, and high mobilities, all restrict genetic divergence. Invertebrates, particularly species with sessile adults, often show greater genetic divergence over similar spatial scales to fishes, and even genetic patchiness at small spatial scales, although some species with apparent weak dispersal potential show low regional genetic differentiation.

There are limitations in using selectively neutral molecular markers to estimate genetic divergence because these tools do not provide information on population differences in adaptive traits that are of importance in aquaculture, such as survival, growth rate, disease resistance, and stress adaptability. Nevertheless, species with high dispersal potential such as kingfish and hapuku are likely to show local adaptation only at broad spatial scales, determined by oceanic circulation patterns that might restrict dispersal of larval juvenile stages.

Kingfish are widely distributed in coastal waters from the Kermadec Islands to Foveaux Strait, with the majority of the commercial and recreational catch taken around the North Island. There is no evidence for genetic differentiation among samples from New South Wales and New Zealand based on selectively neutral DNA markers. Movements derived from tagging have revealed some long distant (trans-Tasman) returns although many adults may be resident following recruitment. The long pelagic juvenile phase also promotes dispersal and restricts genetic divergence. Meristic differences observed between the northeast and northwest coasts, when coupled with information on water currents indicate that there might be two groups of juvenile kingfish in New Zealand waters. However, the long distance movements and lack of genetic divergence with selectively neutral markers would suggest a low adaptive divergence within New Zealand waters and a low risk of outbreeding depression. A model to maximise genetic variability in the founding broodstock would be to chose equal numbers of kingfish from east and west Northland.

Hapuku or groper are widely distributed in temperate and subtropical waters in the southern Indian, Pacific, and Atlantic Oceans. Around New Zealand, hapuku occur from Northland to Stewart Island, on the Stewart/Snares Shelf and along the Chatham Rise and have a long pelagic juvenile phase (3−4 years) that promotes dispersal. Tagging studies have shown some long distant movement up to 1300 km, but no evidence for exchange of adults between the northeast North Island and central New Zealand. An early genetic study found no evidence for spatial differentiation among samples from central New Zealand with allozyme markers. In the closely related wreckfish *Polyprion americanus* there was no evidence for

genetic differentiation among samples from Australia and New Zealand with selectively neutral DNA markers. There are no regional data sets on life history and phenotypic traits.

A model to maximise genetic variability in the founding broodstock would be to chose equal numbers of hapuku from two widely separated regions such as east Northland and South Westland. An alternative and conservative model, based on adaptive divergence, would select broodstock from one region, to reduce the risk of outbreeding depression, and also select broodstock from the region where the stock were to be ongrown (assuming that this will be in sea cages). Data on adaptive traits in hapuku would be useful to determine the most appropriate broodstock model for hapuku. It should be noted that the late age at sexual maturity (10−13 years) coupled with large size make hapuku a difficult species for selective breeding unless age at maturation is reduced in captivity.

The black foot paua is widely distributed from Northland to Stewart Island generally in water <6 m deep. Paua have limited larval dispersal (potential dispersal range ~120 km) with the Chatham Island stocks beyond the limits of normal larval dispersal. A preliminary genetic study based on selectively neutral markers found significant differentiation among four regional samples from east Northland, Taranaki, Stewart Island, and the Chatham Islands. The Chatham Island sample was significantly different from the three mainland samples with a mtDNA marker. Mean shell length and growth rates vary among localities, but exhibit significant differences at small spatial scales (~100 m). Size at maturity is smaller in fast growing populations with 'stunted' populations in Taranaki and the Marlborough Sounds. The genetic basis, if any, for the differences in size at sexual maturity are unknown.

A conservative model, based on adaptive divergence, would select paua broodstock from three regions, Northland, Southland, and the Chatham Islands, to maximise genetic diversity in the founding broodstock. In order to reduce the risk of outbreeding depression, the three broodstock lines should be maintained and selected as three independent lines, and used to provide appropriately selected seed for ongrowing in different regions.

1. Population biology and genetics of marine fishes

1.1 Background

Three species, kingfish *Seriola lalandi*, hapuku *Polyprion oxygeneios*, and paua *Haliotis iris*, have been chosen for selective breeding programmes in the NIWA aquaculture programme. This report provides brief reviews of the genetic principles of broodstock selection and the genetic population structure and phenotypic variation in marine fishes, prior to considering specific aspects of the population biology and genetics of three key aquaculture species.

Genetic improvement programmes of aquaculture species are limited by the availability of genetic variation in the broodstock (Silverstein et al. 2006, Taniguchi 2003), consequently there is a need to consider the level of and spatial distribution of genetic diversity in wild populations prior to selecting broodstock (Borrell et al. 2007, Butts 2007, Gaffney et al. 2007). Patterns of genetic variation in both mitochondrial and nuclear genes have been assessed in many marine fishes and invertebrates, including New Zealand species. In general marine fishes have higher levels of genetic variation than anadromous species, which in turn are more variable than freshwater species (Gyllenstein 1985, Ward et al. 1994), a trend that probably results from larger evolutionary effective population sizes in marine fishes (Dewoody et al. 2000). However, most marine fishes show less spatial genetic differentiation than anadromous and freshwater species, due to the fewer barriers to dispersal and gene flow in the marine environment (Gyllensten 1985, Ward et al.1994). For many marine fishes most of the genetic variation measured with selectively neutral markers has been found within individual populations (Ball et al. 2000, Hauser et al. 1998, Thacker 2008); genetic population differentiation is evident only at ocean-wide scales, possibly because the large population sizes, high fecundities, and high mobilities restrict genetic divergence. Invertebrates, particularly species with sessile adults, often show greater genetic divergence over similar spatial scales to fishes, and even genetic patchiness at small (<5 km) spatial scales (Brown et al. 1992, Hancock 2000, Johnson et al. 1984), although some species with apparent weak dispersal potential show low regional genetic differentiation (Dias et al. 2006).

There are limitations in using selectively neutral molecular markers to estimate genetic divergence because these tools measure divergence at evolutionary and not ecological timescales, and do not provide direct information about population differences in adaptive traits that are of importance in aquaculture, such as survival, growth rate, disease resistance and stress adaptability (Houston et al. 2008, Silverstein et al. 2006, Waples 1998). Where there is significant genetic differentiation among populations with selectively neutral markers, then the information can be used to infer lack of gene flow. The converse, lack of genetic differentiation among sites, may not be due to current gene flow, and needs to be considered in parallel with phenotypic and life history data. New molecular techniques may allow the detection of adaptive variation by examining functional genes (Hemmer-Hansen et al. 2007, Schöffmann et al. 2007), as applied to the identification of populations in marine fishes (Jónsdóttir et al. 2008, Larsen 2007, Pogson et al. 2004). Genes under selection, or associated with markers under selection, are expected to show higher levels of population divergence than neutral markers, in particular for organisms with large population sizes, where selection will be a more powerful force than genetic drift (Endler 1986).

1.2 Genetic principles for broodstock selection

Genetic variation allows populations and species to persist through changing environments over evolutionary timescales. Levels of genetic variation and population differentiation in marine fishes and invertebrates are determined by the combined effects of mutation, random genetic drift, selection, and gene flow. Mutations are the ultimate source of genetic variation, but are rare (around 10^{-6} per gene per replication) and are not important over the time scale of aquaculture development projects.

The level of genetic variation present in aquaculture broodstock is a major factor in determining the fitness of seed, and genetic protocols for selection programmes are well documented (Bentsen et al. 2002, Fao 1981, 1993, Munro 1993, Myers 2001, Taniguchi 2003). Here the focus is on two genetic principles to be considered in the selection of the **founding** broodstock: inbreeding and outbreeding, and which are determined by the minimum numbers and the source of stock(s) used in the founding population(s).

Population genetic theory shows that a minimum effective population, N_e of 50 (N_e - the effective population size - is a measure of the number of individuals contributing to the next generation) with an equal sex ratio, is required to retain 99% of genetic variation in the founding population. Unequal sex ratios and variations in reproductive success reduce N_e which maybe considerably smaller than the number of broodstock N (Frankham 1995, Reed et al. 2000).

The broodstock N_e is the most important genetic parameter for broodstock management and selection because it is inversely related to genetic drift and inbreeding (Borrell et al. 2007). Small effective population sizes lead to loss of genetic variation in the founding broodstock, and restrict the response to selection, and lead to a risk of inbreeding in subsequent generations. (The rate of inbreeding per generation is $\Delta F = 1 / (2 N_e)$). Inbreeding and loss of genetic variation are cumulative processes increasing at each generation in small populations, and are most commonly observed in domesticated animals where they are expressed as lower survival or reproductive rates. Inbreeding depression is the reduced fitness in a stock as a result of breeding between related individuals (Lynch 1991). Inbreeding is a major issue for selective breeding in fish hatcheries (Bartley et al. 1992, Hedrick et al. 1995), with inbreeding depression observed as declines in survival and weight at age, and increases in fry mortalities (Kincaid 1995, Nakadate et al. 2003, Shikano et al. 2003, Su et al. 1996).

The effects of inbreeding differ among quantitative traits indicating that while inbreeding can decrease the performance of some traits the decrease is not uniform (Gallardo et al. 2004, Nakadate et al. 2003). Inbreeding has lead to a reduction in fitness in oysters *Crassostrea virginica*, scallops *Argopecten circularis* and *Pecten maximus*, and abalone (see references in Deng et al 2005; Park et 2006). In contrast, no inbreeding depression in larval survival was observed after two generations of inbreeding in the Pacific oyster *Crassostrea gigas* (Lanan 1980); and there was no difference in larval size in inbred and outbred lines of the American oyster *Crassostrea virginica* (Mallet et al. 1983). In the Pacific abalone *Haliotis discus hannai*, higher deformity rates of veliger larvae and lower survival rates in juveniles were reported in inbred populations but no changes in fertilisation rate and juvenile growth rates (Park et al. 2006), while in *Haliotis discus hannai*, inbreeding led to reduced larval growth and metamorphic success (Deng et al. 2005).

Simulation studies have shown that substantial responses to selection can be obtained in aquaculture mass selection programs with inbreeding rates as low as 1% per generation and little loss of genetic variation, though the use of a minimum of 50 pairs of breeders (Bentsen et al. 2002). Reducing the number of broodstock pairs increased the rate of inbreeding by as much as 6–8% per generation, and the accompanying loss of genetic variation reduced the response to selection by more than one third (Bentsen

et al. 2002). Optimal contribution models applied to salmon have been used to maximize genetic gain at a fixed rate of inbreeding, with the largest genetic gains obtained by sampling broodstock from at least 4 subpopulations (Holtsmark et al. 2006).

Outbreeding depression occurs when offspring from crosses between individuals from different populations have lower fitness than progeny from crosses between individuals in the same population, and is due to the breakdown of co-adapted gene complexes that have evolved in divergent populations (Goldberg et al. 2005, Lynch 1991, 2005). Outbreeding depression has been reported in salmonids (Gharrett et al. 1999, Gilk et al. 2004, Tymchuk et al. 2007) and guppies (Nakadate et al. 2003). Line crossing, as used by plant and animal breeders to gain hybrid vigor, results in an increase in productivity traits in the first generation, but can be followed by outbreeding depression in subsequent generations (Lynch 2005).

Breeding trials with largemouth bass *Micropterus salmoides* crossed from two geographically and genetically distinct populations suffered a reduction in fitness of approximately 14% relative to the parental stocks (Goldberg et al. 2005). Viral replication was more rapid in F2 fish than in F1 hybrids or wild-type parental fish, and was attributed to the disruption of co-adapted gene complexes in the immune systems of the F2 outbred fish. Increased susceptibility to infectious disease may be an important but overlooked mechanism through which outbreeding reduces the fitness of individuals and by which novel infectious diseases emerge in hatchery populations (Goldberg et al. 2005).

Theoretical and empirical studies show that both inbreeding depression and outbreeding depression can lead to the decline in fitness of populations, and both effects may take several generations to become apparent. Delay of the effects until the second generation makes inbreeding and outbreeding depression especially insidious for longer lived species (Lynch 2005). For some marine cultured species it could take more than a decade for outbreeding depression to be manifest, and subsequent reconstruction of a co-adapted genome would require several generations at reduced productivity, depending on the number of loci involved and their linkages (Lynch 2005, Takagawa et al. 2006). Thus, minimising the risks of inbreeding and outbreeding when establishing the founding broodstock is a better option than waiting two, or more, generations before attempting corrective actions.

In summary the founding broodstock should ideally be based on a minimum of 50 wild caught individuals (25 males and 25 females, which are assumed to be unrelated) to capture a wide genetic base. If broodstock are chosen from widely separated locations there is a risk of outbreeding depression in the F2 and later generations. The level of genetic divergence among regional populations should be assessed, and consideration given to maintaining separate broodstock lines when selection programmes are based on broodstock derived from divergent wild populations.

1.3 Molecular tools and measures of genetic variation in marine populations

A range of molecular tools are available for estimating genetic differentiation within and among natural populations. These tools are briefly outlined in Table 1. Two key measures are used to describe genetic variation within and between populations: heterozygosity and gene diversity. Observed heterozygosity (H_o) is the proportion of heterozygotes at a locus in a population sample. H_e, the expected heterozygosity under Hardy-Weinberg equilibrium, uses allele frequencies instead of genotype counts. As an estimate of the within-population genetic variability, heterozygosity is averaged across many loci: $H_e = 1/r \sum(1 - \sum p_{ij}^2)$, where p_{ij} if the frequency of allele i at locus j, and r the number of loci (Nei 1973, 1987). Marine fish tend to have higher heterozygosities and more alleles than anadromous species, which in turn are more variable than freshwater fishes measured with both allozyme (Gyllenstein 1985, Ward et al. 1994) and

microsatellite markers (Dewoody et al. 2000). This is due to the larger evolutionary effective population sizes in marine fishes (Dewoody et al. 2000).

The measure for between-population genetic differences, gene diversity F_{ST} (Wright 1951) is based on the Wahlund effect (a deficiency of heterozygotes relative to Hardy-Weinberg expectations in mixed samples taken from populations with different allele frequencies). Under the assumption of Hardy-Weinberg equilibrium in the local populations, Nei (1987) expanded F_{ST} to allow for multiple alleles (using H_e above): $G_{ST} = 1\text{-}H_S/H_T$, where H_T is the expected heterozygosity in the pooled population samples and H_S is the average of the expected heterozygosities in the local populations under study. F_{ST} and G_{ST}, and the analogue Φ_{ST} that takes into account haplotype and sequence divergence (Nei 1973, 1987), are relative measures ranging between 0 (identical allele frequencies in the populations) and 1 (different alleles fixed at each locus). A review of the allozyme literature showed that the mean G_{ST} for marine fish was 0.06, anadromous species 0.11, and freshwater fishes 0.22 (Ward et al. 1994).

Comparisons of hatchery produced stocks with wild stocks frequently demonstrate a lower diversity in hatchery seed, most probably due to the small number of founding broodstock; examples include Atlantic cod (Pampoulie et al. 2006), flatfishes (Porta et al. 2007, Sekino et al. 2002, Sekino et al. 2004), sea breams (Gonzalez et al. 2008), oysters (Dendanto et al. 2000), scallops (Li et al. 2007, Wang et al. 2007) and abalone (EvansBartlett et al. 2004, Li et al. 2004), including *Haliotis iris* (Smith et al. 1992).

1.4 Adaptive molecular variation

Many molecular polymorphisms have been applied on the premise that the markers are selectively neutral, and the patterns of spatial differentiation are due to genetic drift and restricted gene flow (Mcdonald 1994). Non-coding regions and degeneracy of the genetic code (where silent nucleotide substitutions do not result in a change in amino acid) support selective neutrality of many molecular markers. Adaptive divergence may occur rapidly through accumulation of genetic differences driven by local selection (Bell 2001, Binks 2007, Lynch 1996, Reznick et al. 1997), but would not be detected with selectively neutral genetic markers.

Some allozyme loci (Table 1) appear to be under selection or tightly linked to genes under selection. Changes in allele frequencies in perturbed populations (Mitton et al. 1975, Smith et al. 1983), and physiological differences between alleles are indicative that specific allozyme loci are under selection or tightly linked to genes under selection (Mitton 1997, Powers et al. 1991). Rapid changes in allozyme frequencies and shell shape were reported in translocated populations of the inter-tidal snail *Bembicium vittatum* (Binks 2007).

Species occupying steep thermal or salinity gradients often show significant genetic differentiation among regional populations. The killifish *Fundulus heteroclitus* occurs over 14^o latitude off the east coast of North America and northern and southern populations are nearly fixed for different alleles at the allozyme lactate dehydrogenase locus, *LDH-B**. The *LDH-B** alleles and genotypes differ in catalytic efficiencies, development rates, hatching times and swimming speeds; such functional differences at the whole organism level indicate that *LDH-B** is affected by selection (Powers et al. 1991, Schulte 2001). In Atlantic cod *Gadus morhua,* genotypes at two allozyme loci show different survival rates over the early juvenile stages (Mork et al. 1985). Haemoglobin genotypes are associated with growth rate (Imsland et al. 2007, Naevdal 1992) and feeding behaviour (Salvanes et al. 2000) in Atlantic cod, and correlated with physiological performance in turbot *Scophthalmus maximus* (Imsland et al. 1997).

Table 1: Molecular tools for estimating genetic diversity in natural populations.

***Allozymes** Allozyme loci are protein coding genes. Genetic variation is detected indirectly, through changes in the overall charge on the protein molecule, and migration through a gel (gel electrophoresis). The technique was widely used for 30 years in population-genetic studies, but has been replaced for the most part by tools that measure genetic variation directly at the DNA level.

The **polymerase chain reaction** (PCR) revolutionised molecular genetic studies, by enabling specific segments of DNA to be amplified and to provide millions of copies, which can be visualised or further manipulated to analyse genetic variation. Amplification of DNA allows genetic analyses from both small and historical tissue samples.

***Microsatellite DNA** Microsatellites are highly variable regions of DNA. They are characterised by short segments of DNA that contain a repeated sequence of 1−5 basepairs (bp), such as CTACTACTACTA. Microsatellites are widely dispersed along the chromosomes, with no known coding functions (unlike genes which code for specific proteins). The lack of coding constraints ensures that mutations accumulate more quickly than in coding regions of the DNA. These highly variable neutral markers have become a key tool for parentage analyses and pedigree tracing in aquaculture (Porta et al. 2006).

In fishes, allozyme loci typically have 2−3 alleles, occasionally up to 10 alleles per locus, with heterozygosities ranging from 0% to 18%; microsatellite loci have 5−30 alleles, sometimes more than 50, with heterozygosities >70% (Dewoody et al. 2000). The very high level of genetic diversity can present problems for analysis and biological interpretation of microsatellite data sets (Hedrick 1999).

***Mitochondrial DNA** Mitochondrial (mt)DNA is the small genome found within the mitochondria. In vertebrates and most invertebrates mitochondria are passed from mother to offspring in the egg. The haploid genome, with lack of recombination, reduces the N_e of mtDNA to ¼ of that for nuclear DNA. There are several approaches to analysis, from cutting the overall mtDNA genome into fragments with restriction enzymes (restriction fragment length polymorphisms = RFLPs) through to direct sequencing. Some regions of the mitochondrial region are highly variable (e.g., the control region) and used for population studies, while the less variable regions are used for phylogenetic studies.

***Microarrays** DNA microarray technologies have the ability to detect and measure thousands of distinct DNA sequences simultaneously, and are being developed as high throughput quantitative tools. Microarrays have recently been applied to marine fishes (Douglas 2006), specifically European flounder *Platichthys flesus* (Larsen 2007), chum salmon *Oncorhynchus keta* (Moriya et al. 2007, Moriya et al. 2004), and to *Crassostrea* oysters (Jenny et al. 2007).

Several other nuclear DNA tools are available, some such as the multilocus ***RAPDs***, Random Amplified Polymorphic DNA (Smith 2005) were quickly surpassed; others such as ***introns***, non coding regions of nDNA (Belshaw et al. 2006); ***SNPs***, Single Nucleotide Polymorphisms; ***MHC***, Major Histocompatiblity Complexes; and ***AFLPs*** Amplified Fragment Length Polymorphisms (Bensch et al. 2005), have not been widely applied in population studies of fishes, other than salmonidae (Dionne et al. 2007, Hansen et al. 2007, Langefores et al. 1998, Rogers et al. 2007, Vasemagi et al. 2005).

Allozyme loci in the Atlantic eel *Anguilla rostrata* show clinal variation and allele frequency differences between adults and elvers (Koehn et al. 1978, Williams et al. 1973). The stability of allozyme clines, the assumed single spawning ground, and the absence of mtDNA differentiation (Avise et al. 1986) suggest that the clines are maintained by natural selection (Koehn et al. 1978). Discordant patterns of genetic

differentiation with different molecular markers, provide evidence for natural selection acting on some loci, because genetic drift and gene flow would be expected to act on all loci (Mitton 1997). Such patterns of differentiation were first reported among allozyme loci in the eel pout *Zoarces viviparous* (Christiansen et al. 1988) and have been reported for allozyme (low G_{ST}) and nuclear DNA (high G_{ST}) markers in Atlantic cod (Pogson et al. 1995). However, a review of comparative studies concluded that the few examples of discordant variation were driven by exceptional loci (Allendorf et al. 2000), although more recent studies indicate that the level of divergence between markers is more common. Higher divergences have been measured with the pantophysin *Pan I* locus than with selectively neutral microsatellite DNA markers among populations of Atlantic cod *Gadus morhua* (Pogson et al. 2004) and the walleye pollock *Theragra chalcogramma* (Canino et al. 2005), and in cod the *Pan I* genotypes are linked to growth rate (Jónsdóttir et al. 2008). A fivefold higher genetic differentiation, measured by F_{ST}, was found at heat-shock cognate protein *Hsc70* (a gene involved in the response to thermal/osmotic stress and pollution), than at the microsatellite markers in the European flounder *Platichthys flesus* (Hemmer-Hansen et al. 2007). The strong temperature/salinity gradient occurring in the Baltic Sea could represent the selective force acting on *Hsc70* (Hemmer-Hansen et al. 2007).

It is noteable that the higher levels of genetic differentiation have been reported in fishes that occur over steep thermal and salinity gradients (e.g. flounder in the Baltic, killifish along the east coast of North America, and Atlantic cod in Norwegian fjords). Many of the larger commercial species occur in coastal and oceanic waters and exhibit low levels of genetic differentiation with selectively neutral molecular markers (Ball et al. 2000, Hauser et al. 1998, Nugroho et al. 2001, Sedberry et al. 1996, Smith et al. 2005).It is also likely that species with high dispersal potentials, such as kingfish and hapuku, will only show adaptation at broad spatial scales, determined by oceanic circulation patterns that restrict dispersal of larval and juvenile stages (see discussion below, section 7). For weak dispersers, such as paua, there is greater potential for local adaptive variation and consideration should be given to developing several regional broodstock lines in order to provide seed adapted for local on-growing conditions

1.5 Phenotypic variation in fish populations

Fish are phenotypically more variable than other vertebrates, and show large intra-specific differences in quantitative traits such as growth rate, size and age at maturity, fecundity, spawning times, meristics, and morphometrics (Allendorf et al. 1987, Brodziak et al. 2000). Often greater population differentiation is observed with phenotypic than selectively neutral molecular markers, and because gene flow acts equally on all genetic markers, higher phenotypic variation is either due to local selection and adaptation, or to phenotypic plasticity, non-genetic environmental variation. Evidence for adaptive phenotypic variation in fishes comes mostly from salmonids and freshwater and estuarine species amenable to hatchery rearing (Carvalho 1993, Taylor 1991), but developments in marine aquaculture and the elegant experimental studies of Conover and co-workers (Conover 1998, Conover et al. 2006) have provided insight into adaptive variation in marine fishes. Experimental transplant studies with freshwater guppies *Poecilia reticulata* have shown rapid divergence in life history traits after just 11 years and 18 generations (Reznick et al. 1990, Reznick et al. 1997).

Geographic differences in phenotypic variation have implications for broodstock selection (Butts 2007), but it is not easy to untangle the genetic and environmental components of phenotypic variation in wild fisheries, for example life history traits, have a genetic base and are known to change in response to fish density and fishing pressure (Jørgensen et al. 2007, Olsen et al. 2005). Hence “common garden” experiments (rearing fish under standardised laboratory conditions) are used to determine the genetic and environmental components of phenoytypic variation (Conover 1998, Conover et al. 2006, Schultz et al. 2002). Traits in some species exhibit a high genetic component, e.g. Atlantic silverside *Menidia menidia*

(Conover 1998), while in other species such as Atlantic cod *Gadus morhua*, there is a high environmental component to phenotypic variation (Godo et al. 1987).

The phenotypic variance in a quantitative character (V_P) is determined by the sum of genetic effects (V_G), environmental effects (V_E), genotype–environment interaction (V_{GxE}), and the covariance between G and E sources of variance (Cov(G,E)): $V_P = V_G + V_E + V_{GxE} + 2Cov(G,E)$. Heritability estimates from aquaculture species demonstrate a genetic component (V_G) to many traits in teleosts. The V_{GxE} interaction corresponds to the reaction norm measured across a range of conditions in fisheries (Heino et al. 2002, Olsen et al. 2005). The covariance term represents the non-random distribution of genotypes across environmental gradients (Conover et al. 1995, Conover 1998) and it is this population aspect that is considered here (see Fig. 1).

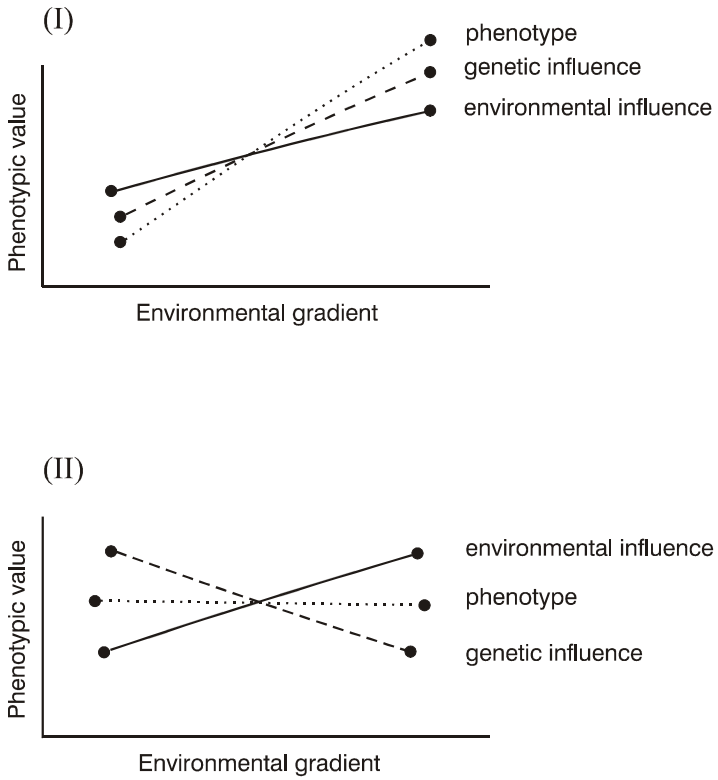

Figure 1. Genetic and environmental influences on phenotype across different environments. The covariance can either amplify or reduce the phenotypic change. (I) Positive covariance (or co-gradient variation) occurs with parallel genetic and environmental influences. (II) Negative covariance (or counter- gradient variation) occurs when genetic influences oppose environmental influences. Source: Conover (1998).

In several species growth rate is higher at a given temperature in high latitude populations (Jonassen et al. 2000), which may be an adaptation to shorter growing seasons in higher latitudes. A latitudinal compensation for growth rate is probably common in marine fishes (Conover et al. 1995, Conover et al. 2006); the length of the growing season declines with increasing latitude yet body size at the end of the first year is similar across the species range. This countergradient variation (Fig. 1) reduces phenotypic variation and obscures genetic variation across environments (Conover 1998).

Geographically separated populations of Atlantic cod *Gadus morhua* exhibit life history variation, with higher growth rates found in populations in warmer water (Brander 1995). The countergradient variation hypothesis predicts that northern populations should outperform fish from southern areas in growth at all temperatures, but experimental studies with juvenile cod from two regions in the northwest Atlantic and reared under identical conditions found no significant difference in growth between the two populations (Wijekoon et al. 2003). Cod from two Norwegian fisheries, with different growth and age at maturity traits, both grew faster and matured earlier when reared under identical and favorable conditions, indicating that the differences between the fisheries were not genetic (Godo and Moksness 1987). However significant genetic differences have been detected among Arctic and coastal Atlantic cod stocks off Norway, and among coastal stocks with a range of molecular markers (e.g. Dahle et al. 2006) and life history traits differ among broodstock groups collected from different stocks (Otterå et al. 2006).

Hatchery experiments with summer flounder *Paralichthys dentatus* (Malloy et al. 1994), winter flounder *Pseudopleuronectes americanus* (Butts 2007), Dover sole *Solea solea* (Exadactylos et al. 1999), Atlantic halibut *Hippoglossus hippoglossus* (Jonassen et al. 2000), and turbot *Scophthalmus maximus* (Imsland et al. 2000, 2001) have revealed regional genetic differences in growth rates, particularly in the juvenile and larval stages. For turbot and Dover sole no population differentiation had been detected with neutral molecular markers (Exadactylos et al. 1999, Imsland et al. 2000). The degree of phenotypic difference among populations can also differ with the trait, with larger differences observed in morphometric than physiological traits in the Japanese flounder *Paralichthys olivaceus* (Shikano et al. 2008).

1.6 Genetic and ecological stocks

The commercially important marine fishes have been subdivided into stocks or management units that exhibit varying degrees of isolation, and described as metapopulations - a series of local, connected populations (Smedbol et al. 2000) While there is no universal definition of a stock, most definitions include spatial and temporal isolation, and often reproductive isolation (Ihssen et al. 1981). Pullin (2000) used the term fish genetic resources to describe aquatic genetic resources that have distinctive properties, while (Moritz 1994) used the term “management unit” for demographically independent populations with distinct allele (= forms of a gene) frequencies. A wide range of approaches have been applied to the discrimination of marine fish stocks and include morphology and meristics, trace element chemistry, parasites, distribution and biology, tagging and marking, and genetics. With the exception of direct tagging, the connectivity of populations is inferred from biological patterns, and the methods estimate the consequences rather than determinants of dispersal (Sale et al. 2003).

In considering the different methods applied to stock discrimination of marine species it is useful to consider the mechanisms that lead to stock differentiation:

- genetic drift, or the random fluctuations in gene frequencies between generations;
- genetic selection, through a differential mortality on genotypes;
- environmental modification of traits due to local differences in the physical environment, such as temperature and salinity;
- environmental modification of traits due to local differences in the biotic environment, such as food availability;
- accumulation of trace elements or parasites due to local differences in the physical and biotic environments experienced by individuals.

These mechanisms fall into two broad categories: ecological and genetic. Ecological approaches to stock discrimination, based on differences in phenotypic characters, acquired markers, and life history traits, provide a measure of stock relationships, but may reflect differences in post-settlement habitat quality and

might not represent reproductively isolated or genetically differentiated populations. Much of the ecological information on stock structure is not relevant for assessing genetic diversity within and among populations. For example differences in parasite loads might reflect geographical distribution of the parasite and differences in trace elements reflect exposure to different water masses rather than local genetic adapations.

Genetic methods, based on different frequencies of inherited characters, provide an alternative approach to stock discrimination. A significant genetic difference at a neutral genetic marker is a sufficient but not necessary condition for separate stock management of wild fisheries. Genetic stocks have continuity over time; larvae and juveniles recruit back to their natal stock and remain discrete from other stocks over time, whereas ecological stocks may recruit from a common larval pool but undergo differentiation in the juvenile and adult feeding areas due to environmental differences.

1.7 Genetic differentiation, dispersal potential, and ocean currents

Genetic differentiation is inversely related to dispersal ability, as estimated across 333 species of vertebrates and invertebrates from terrestrial, marine, and freshwater environments (Bohonak 1999). Dispersal in the marine environment is constrained by the length of the pelagic larval and juvenile stages, by behavioural mechanisms, and by physical barriers such as gyres and ocean fronts. An inverse relationship has been reported between genetic differentiation and dispersal potential in small shore-fishes (Waples 1987) and reef-fishes (Doherty et al. 1995), and appears to apply to some of the commercially important marine fishes around New Zealand (Smith et al. 2005). Species with high dispersal potential (pelagic juvenile stages > 1 year), such as kingfish, black oreo *Allocyttus niger*, smooth oreo *Pseudocyttus maculatus*, and hoki *Macruronus novaezelandiae*, show little genetic differentiation over wide sea areas (Milton et al. 1987, Nugroho et al. 2001, Smith et al. 2002, Smith et al. 1996, Ward et al. 1998). In contrast snapper (Bernal-Ramirez et al. 2003, Smith et al. 1978) and orange roughy (Elliott et al. 1992, Smith et al. 1997, Smith et al. 1996, Smolenski et al. 1993), with limited dispersal potential (pelagic eggs and larval stages <1 month, demersal juveniles) show genetic differentiation when sampled over similar ranges to the high dispersers.

Patterns of intra-specific genetic differentiation may vary according to the spatial scale of the sampling programme (Planes et al. 2002). Isolation by distance, in which geographically close populations are genetically more similar than distant populations, has been reported for some marine fishes and invertebrates (Couceiro et al. 2007, Laurent et al. 2007, Pogson et al. 2001). Sardine *Sardina pilchardus* from the North Sea to the Mediterranean Sea show a weak but significant structure (F_{ST} = 0.057), produced by a change in the frequency of the common allele at one allozyme locus over the greatest distances (Laurent et al. 2007). In the netted dog whelk *Nassarius reticulatus* in Europe, an analysis of the COI mtDNA marker revealed a weak and non significant population structure (overall Φ_{ST} = 0.00013), but pairwise Φ_{ST} values revealed a slight but significant increase in genetic isolation with distance, indicating that populations were not panmictic. By assuming a stepping stone model of gene flow, the level of genetic differentiation among populations of *N. reticulatus* was consistent with a larval dispersal of ~70 km per generation (Couceiro et al. 2007). Simulations of stepping stone models of dispersal for coastal fishes and invertebrates showed that isolation by distance is most obvious in populations separated by mean larval dispersal distances of 25-150 km (Palumbi 2003).

In the Atlantic cod *Gadus morhua* correlations between gene flow and geographic distance have been detected at both small and large spatial scales and imply that dispersal distances and effective population sizes are smaller than predicted. In this respect the recent age of the populations (which have diverged

since the last ice ages in the North Atlantic Ocean) rather than extensive gene flow, may be responsible for the weak population structure (Pogson et al. 2001).

The application of isolation by distance models may offer limited insight if the sampling regime is inadequate. For example, in the Atlantic redfish *Sebastes mentella* an apparent isolation by distance across the Atlantic, resulted from three genetically differentiated groups: western, in the Gulf of St Lawrence and Newfoundland; Panoceanic, from the Grand Banks to the Faroes; and eastern, off Norway and in the Barents Sea (Roques et al. 2002). Genetic homogeneity observed over 6000 km within the Panoceanic group may result from larval gene flow via the cyclonic circulation of the central North Atlantic (Roques et al. 2002).

The pattern of genetic homogeneity over wide areas within the same current system, but significant genetic differentiation over small spatial scales at hydrological barriers is common in marine fishes. Species of redfish *Sebastes* and lingcod *Ophiodon elongates* in the northeast Pacific Ocean show genetic homogeneity along the open coast, but genetically differentiated populations in Puget Sound (Buonaccorsi et al. 2002, Jagielo et al. 1996, Seeb 1998, Seeb et al. 1988). Off the same coast, *Sebastes helvomaculatus* shows a significant genetic break between populations separated by the divergence of the northward flowing Alaskan current and southward flowing California current (Rocha-Olivares et al. 1999).

Genetic differentiation has been reported in several coastal fishes at a biogeographic boundary between the Gulf of Mexcio and Atlantic coast of North America (Avise 2000, Gold et al. 2001, Gold et al. 1997, Gold et al. 1998, Gold et al. 1999), but not in large pelagic species (Buonaccorsi et al. 2001, Heist et al. 1999) or sharks (Heist et al. 1999). In the red drum *Sciaenops ocellatus* there is a shallow isolation by distance over 3000 km within the Gulf of Mexico, but genetic differentiation between populations in the Gulf and the western Atlantic with a genetic neighbourhood of 500−600 km, within the Gulf of Mexico (Gold et al. 2001, Gold et al. 1999).

The major oceanic features around New Zealand that might isolate stocks and lead to genetic differentiation are shown in Figure 2. Off the east coast of the North Island the southward flowing East Auckland Current (EAC) moves subtropical water down the east coast of the North Island (Fig. 2). The EAC diverges near East Cape with some water flowing north and east, and the remainder flowing southwards as the East Cape Current ECC, until it reaches the Chatham Rise and is deflected eastwards. The Wairarapa Coastal Current (WCC) transports cooler water northwards along the south-east coast of the North Island (Fig. 2). The WCC is a mix of waters from the Southland current, flowing northwards along the east coast South Island, and the D'Urville Current, flowing eastwards through Cook Strait (Chiswell 2000). The WCC acts as a barrier for some coastal fishes, for example there is a repeatable genetic break in snapper populations that corresponds with the northern boundary of the WCC between Hawke Bay and East Cape (Bernal-Ramirez et al. 2003).

Most of the west coast is influenced by the northward drift of the Tasman Current, and is hydrologically more uniform than the east coast (Roberts et al. 1978). The West Auckland Current (WAC) influences Ninety Mile Beach off the northwest coast of the North Island (Roberts et al. 1978).

Several independent genetic studies of New Zealand coastal species have indicated that genetic structure is associated with coastal currents and genetic differentiation occurs where there are physical barriers that could isolate stocks. In the snapper *Pagrus auratus* significant genetic differentiation was found between samples from the north-east and west coasts of the North Island, areas contained within the Tasman Current and the EAC respectively (Fig. 2). Genetically isolated populations were found in Tasman Bay and Hawke Bay (Bernal-Ramirez et al. 2003, Smith et al. 1978), and associated with ocean currents, the D'Urville Current isolating Tasman Bay from the west coast, and the Wairarapa Coast Current isolating Hawke Bay and the East Coast (Fig. 1). Genetic homogeneity occurred over wide areas, e.g the northeast

coast of the North Island, rather than simple geographic distance (Bernal-Ramirez et al. 2003). The spatial differentiation was temporally stable and detected with allozyme markers in the 1970s (Smith et al. 1978) and microsatellite markers in the late 1990s (Bernal-Ramirez et al. 2003).

An early allozyme study of genetic variation in the tuatua *Paphies subtriangulata* indicated three genetic groups: central, northern and Chatham Islands (Smith et al. 1989). A similar allozyme study of the greenshell mussel *Perna canaliculus* showed genetic divergence among northern and southern populations (Smith 1988). DNA analyses revealed genetic differentiation among mussels from the North Island/Cook Strait and lower South Island, particularly South Island west coast and Stewart Island populations (Apte et al. 2002, Apte et al. 2003, Star et al. 2003). A similar genetic sub division was reported off the east coast at around 42^{o}S in the seastar *Patiriella regularis*, and appears to coincide with hydrographic features (Ayers et al. 2005, Waters et al. 2004).

A genetic discontinuity among North and South Island populations of three intertidal limpets, *Cellana ornata*, *C. radians* and *C. flava* was associated with water masses in the Cook Strait region (Goldstein et al. 2006). The small spatial scale discrepancies in the barriers to gene flow in the invertebrate studies may in part be due to lack of parallel sampling sites (Goldstein et al. 2006, Waters et al. 2004), or reflect different dispersal patterns of the organisms.

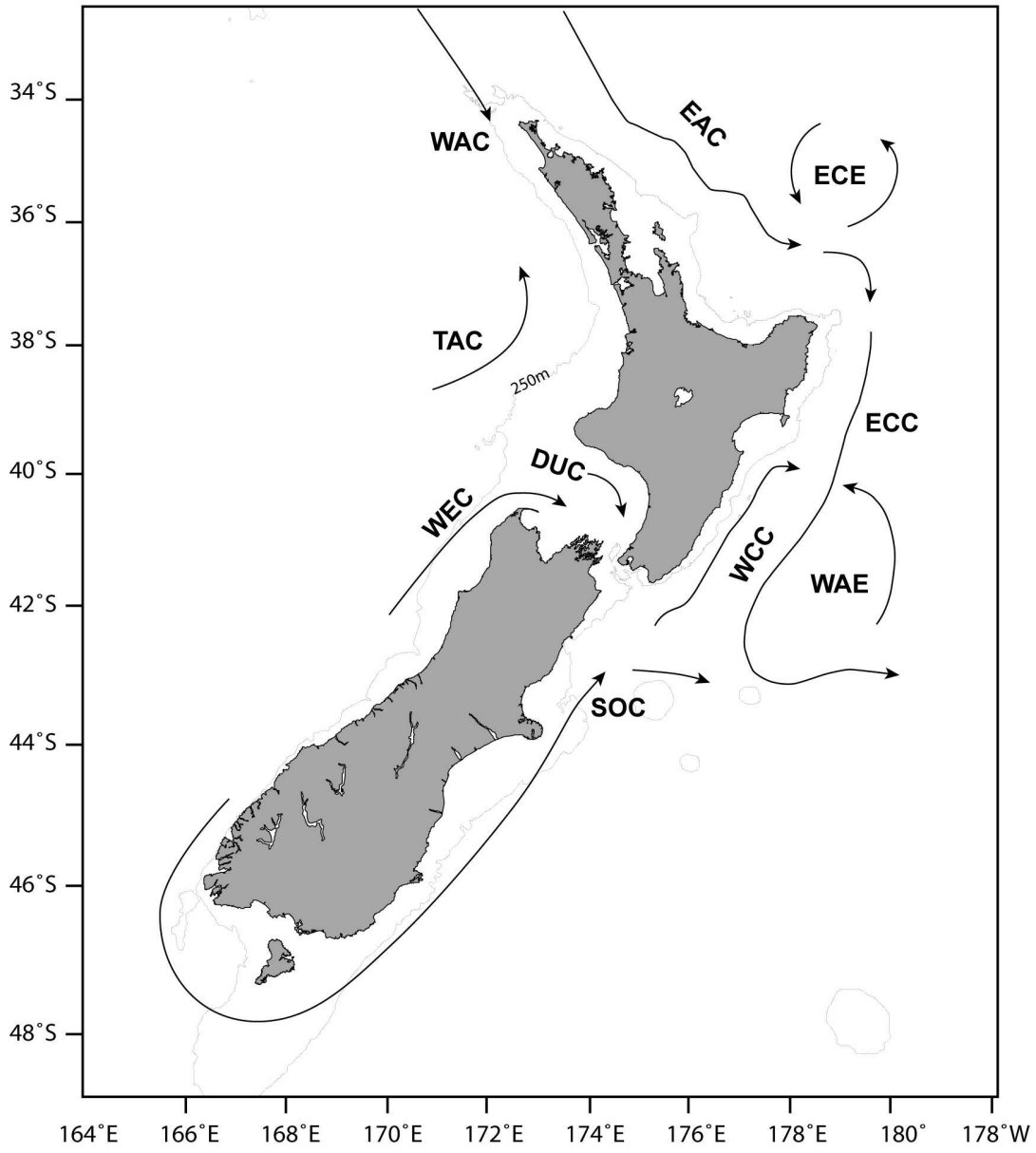

Figure 2: Major current systems around New Zealand. Currents: DUC = D'Urville Current; EAC = East Auckland Current; ECC = East Cape Current; SOC = Southland Current; TAC = Tasman Current; WAC West Auckland Current; WEC = Westland Current; WCC = Wairarapa Coastal Current. Eddies: ECC = East Cape Eddy; WAE = Wairarapa Eddy.

2. Population biology and genetics of key aquaculture species

2.1 Kingfish (*Seriola lalandi*)

2.1.1 Kingfish distribution and biology

Kingfish have a circumtropical distribution and are found in temperate waters in the Indo-Pacific, in the eastern Pacific, and the eastern Atlantic. Three subspecies have been recognised: *Seriola lalandi lalandi* in the Southern Hemisphere, *S. lalandi aureovittata* around Asia, and *S. lalandi dorsalis* off the west coast of America (http://www.fishbase.org/search.php).There is significant genetic heterogeneity (G_{ST} = 0.046, P <0.001) among samples from Japan and Australasia, with area-specific microsatellite alleles, and fixed differences in mtDNA haplotypes (Nugroho et al. 2001).

In New Zealand waters, kingfish are widely distributed from the Kermadec Islands (29° S) to Foveaux Strait (46° S) in depths down to 200 m (Sullivan et al. 2005). The majority of the commercial and recreational catch is taken around the North Island, off the east coast in Fishstocks KIN 1 and KIN 2 (Figure 3), and the west coast KIN 8 (Sullivan et al. 2005). The key literature on *S. lalandi* in New Zealand waters has been reviewed by NIWA staff (Mckenzie et al. 2000, Walsh et al. 2003) and the stock structure investigated using meristic, parasite, and life history characters (Smith et al. 2004). Adult kingfish are large (> 1m) predators and are most common in open coastal waters around rocky outcrops and reefs.

Spawning kingfish have not been observed in New Zealand waters, but gonad studies indicate that individuals have the capacity for multiple spawning between October and January (Portenaar et al. 2001). Kingfish juveniles appear to be pelagic, based on coloration and analogy with congeners, e.g., the amberjack *Seriola quinqueradiata* (Sakakura et al. 1997). Juveniles of *S. quinqueradiata* utilise drifting algae as a nursery ecosystem and drift northwards in the Kuroshio current from the spawning area (34° N) to populate adult stocks in Sendai Bay, around 38°N (Safran et al. 1990). Juveniles of *S. lalandi* have been reported around floating objects off northeast New Zealand (Holdsworth 1995, Kingsford 1992), and large numbers (>100) of juvenile kingfish have been observed under fish aggregation devices placed outside a marine reserve at the Poor Knights. However the juvenile distribution around New Zealand is unknown. Kingfish larvae produced off the west coast are likely to drift northwards as pelagic juveniles, while those produced off the east coast are likely to drift southwards (Fig. 2) along the northeast and east coast, before recruitment to reefs.

2.1.2 Kingfish genetics

Use of three microsatellite loci and composite haplotypes of the mtDNA control region showed high genetic diversity, but no significant differentiation among samples of *S. lalandi* taken along the east coast of Australia (30−35° S) and from New Zealand (Nugroho et al. 2001). The same markers showed highly significant levels of divergence among the Australia/New Zealand populations and Japan (Nugroho et al. 2001). Finding a lack of significant genetic differentiation in kingfish samples across the Tasman Sea is typical of genetic studies of pelagic species such as tunas, marlin, and swordfish which show little or no genetic differentiation within, but differentiation between ocean basins (Hauser et al. 1998). The lack of genetic differentiation across the Tasman Sea is compatible with the tagging results.

There have been few population genetic studies of other species of *Seriola*. Two subpopulations or stocks of greater amberjack *Seriola dumerili*, were postulated in different water masses in the northern Gulf of Mexico and along the U.S. Atlantic coast, based on mtDNA haplotype frequencies (Gold et al. 1998).

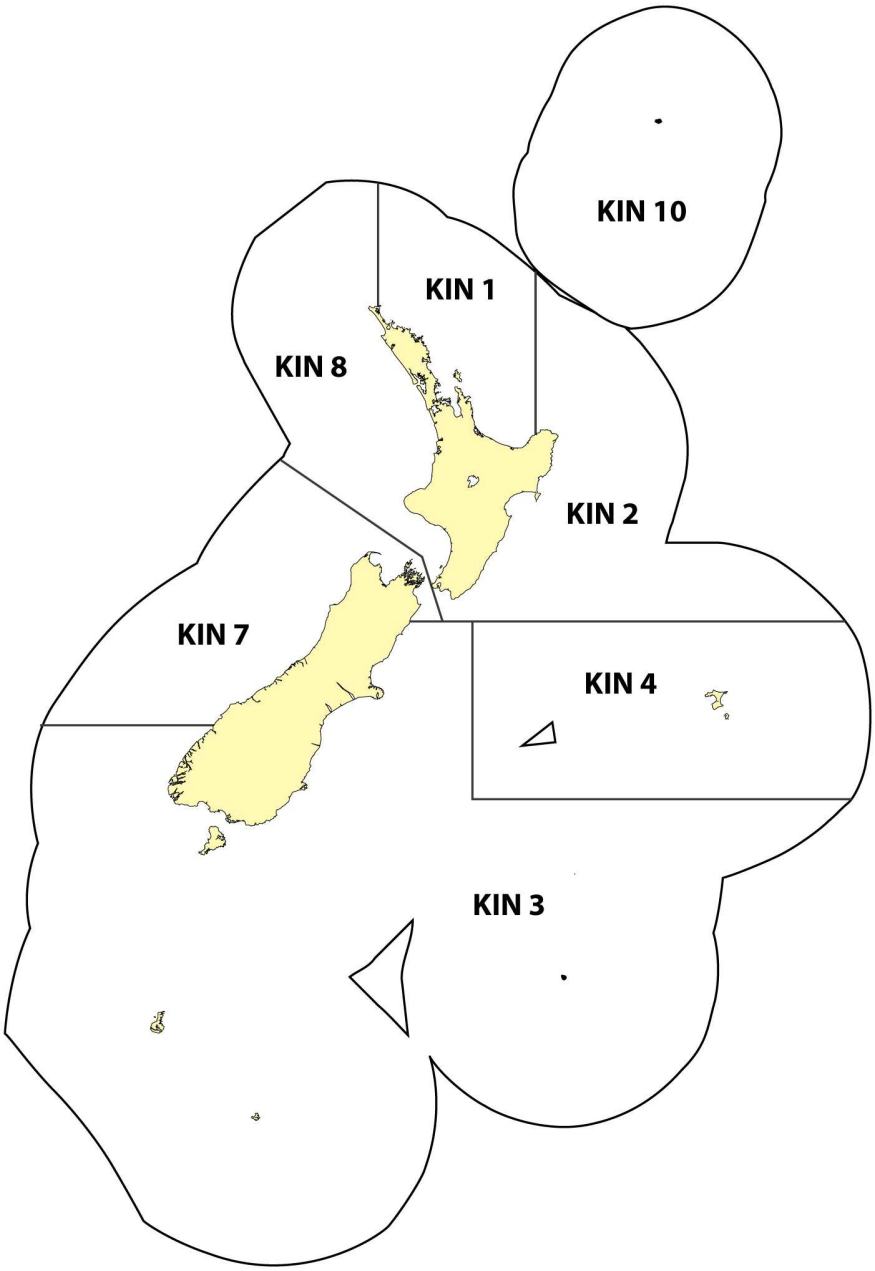

Figure 3: Kingfish fishery management areas around New Zealand.

2.1.3 Kingfish life history

In northern New Zealand waters, kingfish mature at a larger size than in New South Wales (Gillanders et al. 2001, Portenaar et al. 2001); and it is possible that these regional differences are due to warmer water producing faster growth rates and earlier maturity in New South Wales (Portenaar et al. 2001).

Around New Zealand, differences in the mean length at which 50% (L_{50}) of kingfish are mature were compared between east Northland and the west coast using data collected in the spawning months of October to January (Portenaar et al. 2001). Differences in L_{50} were evident between males and females (Table 2), but the male and female L_{50} estimates for the east Northland and the west coast samples were within the respective 95% confidence bounds, leading to the conclusion that there was no evidence for area specific differences in length-at-maturity (Smith et al. 2004). The preliminary data on length-at-maturity suggests either that the processes influencing maturation are similar on the northeast and west coasts, or that kingfish on both coasts belong to the same stock. The Bream Bay captive F1 kingfish spawned for the first time between February and April 2008, at five years of age and size ranges 73−87 cm (J. Symonds NIWA, pers. comm.).

Table 2. Kingfish mean length at 50% maturity by area and sex, data taken from Smith et al. 2004.

Area	N Males	50% maturity cm	95% c.i.
East Northland	146	83.97	80.64 ~ 87.30
West coast North Island	50	83.85	76.86 ~ 90.84
	N Females	**50% maturity cm**	**95% c.i.**
East Northland	170	97.98	93.99 ~ 101.98
West coast North Island	65	102.77	93.06 ~ 112.48

2.1.4 Kingfish meristics

Meristic characters have a genetic basis (Christiansen et al. 1988, Purdom et al. 1969) but their phenotypic expression is influenced by environmental factors, such as water temperature, salinity, oxygen, pH, and food availability, and particularly water temperature experienced during early development in the egg and larval stages (Fahy 1972, Lindsey 1988, Taning 1946), after which the characters are fixed (Lindsey 1988). Thus, population differences in meristic characters measured in adult fishes can result from environmental, genetic, or a combination of environmental and genetic influences.

Fin rays (dorsal I, dorsal II, anal, pectoral, and pelvic) and gill rakers (on the first left arch) were counted in adult kingfish from northeast coast 44 fish (KIN 1), west coast 87 fish (KIN 8), and Hawke Bay 63 fish (KIN 2). Only two characters showed significant between-area differences: dorsal II fin ray counts between the northeast and west coast samples, and anal fin ray counts between northeast and west coast samples (Smith et al. 2004). The limited meristic data on kingfish reject the single stock hypothesis, and indicate that kingfish on the west and northeast coasts are derived from separate spawning populations, exposed to different environmental conditions during the early larval stages. The corresponding lack of

meristic differences among the northeast and Hawke Bay samples could be interpreted as fish sharing a common larval history or discrete ecological stocks subject to similar environmental conditions.

Average December water temperatures on the west coast are one degree warmer than those on the northeast coast, which in turn are on average around one degree warmer than those in Hawke Bay (NIWA data). While laboratory experiments have shown the meristic counts are correlated with water temperature (Fahy 1972, Lindsey 1988, Taning 1946), it is unknown if a temperature difference of one degree Celsius would be sufficient to generate the observed differences in dorsal II and anal fin ray counts (between the west and northeast coasts). For both meristic characters the counts are higher in the northeast (warmer water) than west coast samples. In general, vertebral number in marine fishes is negatively correlated with water temperature in the larval rearing period, but dorsal fin ray counts are positively correlated in some species (Lindsey 1988). In a review of meristic variation in fishes Lindsay (1988) concluded that in different species the same meristic series may have different response patterns, and different meristic series in the same species may have different responses.

2.1.5 Kingfish tagging

New Zealand gamefish tagging programmes have included kingfish (Hartill et al. 1999, Saul et al. 1992). A total of 9277 kingfish have been tagged and 869 recaptured (Hartill et al. 2001). Of the 869 returns, 86% occurred within KIN 1 where the fish were released. A large number of kingfish have been tagged in the eastern Bay of Plenty, where 94% of returns have been from the same area. Long distance returns of kingfish tagged in the Bay of Plenty (KIN 1) show movement to Hawke Bay, the northeast coast of the North Island, the west coast of the North Island, and Australia (Saul et al. 1992). The New Zealand tagging data do not support a substantial north-south movement (Holdsworth et al. 1998), as might have been predicted from the seasonal pattern of catches, rather there may be an inshore-offshore seasonal movement of adult fish, associated with water turbidity, with kingfish actively seeking clear water for visual feeding and only entering inshore waters and reefs over spring-summer (J.Holdsworth & K.Michael, NIWA, pers. comm.).

The New Zealand tagging results, with a high percentage of recaptures in the release area and a few exceptional long distance returns, are broadly similar to results obtained in large-scale tagging programmes in New South Wales with 1376 recaptures out of 17190 releases (Gillanders et al. 2001). Most tagged kingfish were recaptured within 50 km of the release site, but there were some large-scale movements (>500 km) within coastal waters off New South Wales, and a few long distant movements (>2400 km) in both directions across the Tasman Sea (Gillanders et al. 2001). The results from the New South Wales tagging programme lead to the conclusion that the kingfish population off New South Wales is well mixed and it is unlikely that more than one stock exists (Gillanders et al. 2001). Adult kingfish, once recruited may be vulnerable to localised depletion and in this respect kingfish in New Zealand might be managed as a series of local ecological stocks (Mckenzie et al. 2000) as opposed to one genetic stock.

2.1.6 Kingfish summary

- Biology: pelagic juvenile phase that promotes dispersal and restricts genetic divergence.
- Movement: some long distant (trans-Tasman) tag returns, although many adults may be resident following recruitment.
- Meristics: differentiation between samples from the northeast and northwest coast of the North Island.

- Genetics: no evidence for genetic differentiation among samples from New South Wales and New Zealand with selectively neutral DNA markers.

The meristic differences observed between the northeast and northwest coasts, when coupled with information on water currents indicate that there might be two groups of juvenile kingfish in New Zealand waters, but given the mobility of the adults there are not likely to be genetic differences. In the absence of genetic data on adaptive traits, then a model to maximise genetic variability in the founding broodstock would be to chose equal numbers of fish from east and west Northland. The long distance movement from tag returns and lack of genetic divergence with selectively neutral markers would suggest a low adaptive divergence within New Zealand waters and a low risk of outbreeding depression.

2.2 Hapuku (*Polyprion oxygeneios*)

2.2.1 Hapaku distribution and biology

The hapuku or groper is a large demersal fish found in temperate and subtropical waters in the southern Indian, Pacific, and Atlantic Oceans. Hapuku support fisheries around New Zealand, the Juan Fernandez Archipelago off Chile, and are taken as by-catch in fisheries off southeast Australia and the Tristan da Cunha Group of the South Atlantic (Barreiros et al. 2004, Kukharev 1998, Sullivan et al. 2005). (See http://www.fishbase.org/Summary/speciesSummary.php?ID=350&genusname=Polyprion&speciesname=oxygeneios). Around New Zealand, hapuku occur from Northland to Stewart Island, on the Stewart/Snares Shelf and along the Chatham Rise, but are not reported from the Campbell Plateau or Challenger Plateau. A similar species, the bass or wreckfish *Polyprion americanus*, is caught in the New Zealand fishery and the catch statistics for *P. oxygeneios* and *P. americanus* are combined and the two species managed as eight fishstocks (Sullivan et al. 2005), see Fig. 4. Some change to these fishstock boundaries have been suggested, in particular the boundaries which subdivide the largest fishery in Cook Strait (Paul 2002a). The early biological and meristic data have been reviewed for the Cook Strait population (Johnston 1983). A more recent review of the stock structure of both *P. oxygeneios* and *P. americanus* lead to the conclusion that there was insufficient data to describe the stock structures in New Zealand waters (Paul 2002a). There are no new data that would change that position for *P. oxygeneios*.

Adult hapuku are most abundant between 100−300 m, and generally occur over rough ground; juveniles are pelagic for the first 3-4 years (Francis et al. 1999, Roberts 1996), and are found in surface waters where they are associated with floating weed and debris (Roberts 1996). In European populations of *P. americanus* the size at settlement is 55−65 cm (Machias et al. 2003). Paul examined the size range of hapuku reported in New Zealand trawl catches and noted that small immature fish (~50 cm) occurred on the Stewart/Snares shelf, and east coast South Island, with larger immature fish (55−70 cm) on the Chatham Rise (Paul 2002b, 2002c). However, the size structure was estimated from fish caught during trawl surveys, which tend to avoid rough ground, and it is possible that larger fish which might favour rough ground were not sampled (Paul 2002c). Size at 50% maturity for hapuku in Cook strait was 80−85 cm for males and 85−90 cm for females (Paul 2002c), but running ripe fish are seldom caught and the spawning grounds are not known (Sullivan et al. 2005). Occasionally ripe hapuku have been caught in Cook Strait, where pre-spawning fish are caught in June and spent fish in October (Johnston 1983). Off the south east cast of the South Island developing fish are caught in May−June and spent fish in October (Graham 1956).

In New Zealand waters, hapuku are long-lived (up to 60 years) with both sexes maturing at about 10−13 years (Francis et al. 1999). Faster growth rates have been reported in hapuku from the Juan Fernandez Islands with maximum ages of only 12 years, but this maybe an underestimation due to errors in otolith

reading (Francis et al. 1999). Late onset of sexual maturity will delay the potential for selective breeding in hapuku, unless sexually maturity can be advanced by aquaculture, as for example with other cultured species in which hatchery fish mature earlier than wild fish, e.g., black bream *Acanthopagrus butcheri* (Doupe 2005). The F1 hapuku (3.75 years, average weight 4.9 kg, length 66 cm) held at Bream Bay had not spawned as at March 2008 (J. Symonds NIWA, pers. comm.).

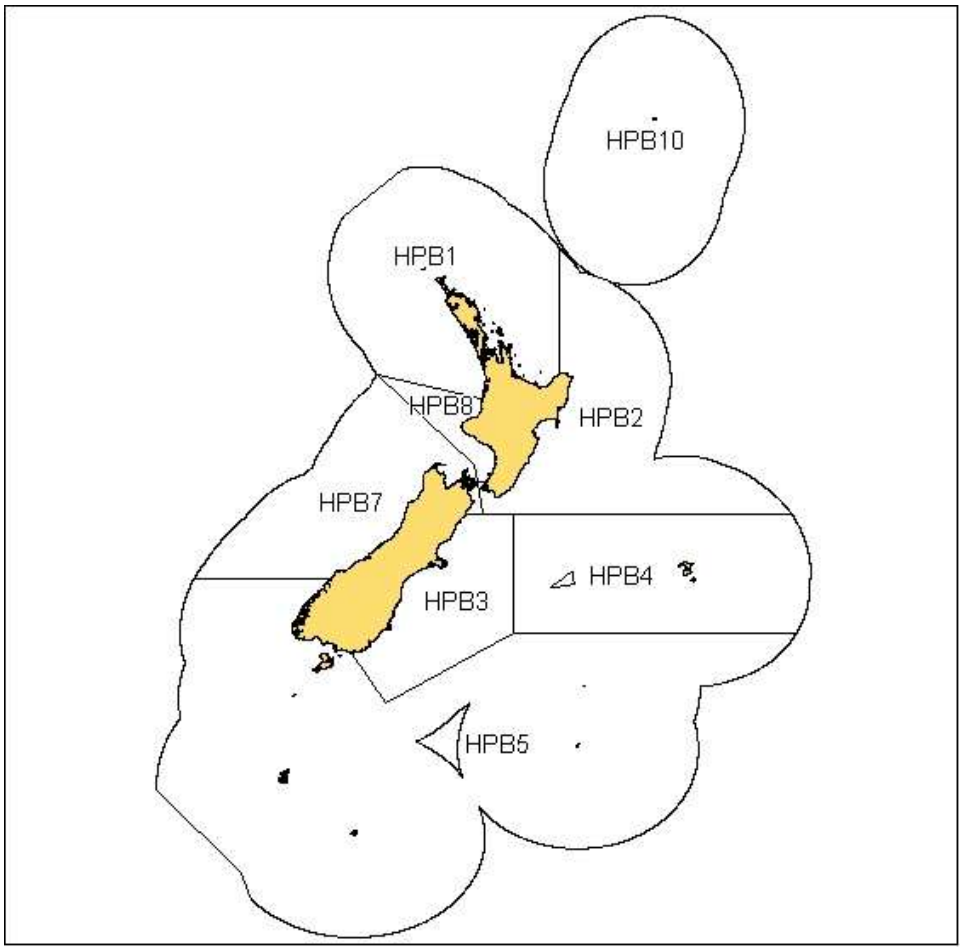

Figure 4: ***Polyprion oxygeneios*** **and** ***Polyprion americanus*** **fishery management areas around New Zealand.**

2.2.2 Hapuku tagging

Hapuku have been tagged off the southeast South Island (SESI), in Cook Strait (CS), and off the Poor Knights Islands (PK) in separate programmes aimed at determining movements. A total of 1623 hapuku have been tagged with an overall recapture rate of 16.3% and the results reviewed by Beentjes & Francis (1999). Some hapuku were recaptured at the tag release sites (13% in SESI, 39% in CS, and 40% off PK), often after long periods at liberty, maximum 10.2 years (Beentjes et al. 1999). The greatest distance travelled was 1389 km, by two fish tagged off the SESI and recaptured off Ninety Mile Beach and Tauranga. The median distance travelled increased with hapuku length at recapture, but some smaller immature hapuku travelled several hundred kilometres. Hapuku tagged in Cook Strait appeared to travel

shorter distances than those tagged off the SESI, but this may have been an artefact of the lower hapuku fishing effort outside the Cook Strait-Kaikoura region; several Cook Strait hapuku moved to Kaikoura, and one to Oamaru. Hapuku tagged around the Poor Knights Islands showed limited movements, with 80% recaptured within 10 km of the release site, and no movement to Cook Strait or the South Island. The tagging results were consistent with a single stock in the southeast South Island and Cook Strait region, and possibly a separate stock in northern New Zealand (Beentjes et al. 1999).

2.2.3 Polyprion genetics

An early genetic study based on two allozyme loci showed significant heterogeneity within and between areas for hapuku samples taken in central New Zealand (Smith et al. 1985). At one allozyme locus, *GPI*, there was a significant difference between samples collected at the same time period in Cook Strait and the central east coast and between Cook Strait and west coast (New Plymouth). Overall, genetic differentiation within Cook Strait was as great as that observed between the central east (Tolaga Bay to Kaikoura) and west coasts, with no evidence for spatial differentiation in central New Zealand (Smith et al. 1985).

Several bottom-living oceanic species that utilise offshore reefs and seamounts, such as armorhead *P. wheeleri*, alfonsino *Beryx splendens*, and wreckfish *Polyprion americanus*; show little genetic differentiation within ocean basins (Ball et al. 2000, Hoarau et al. 2000, Humphreys et al. 1989, Martin et al. 1992, Sedberry et al. 1996), and all have extensive dispersal through pelagic juvenile stages that last for months to years. In the wreckfish, microsatellite allele frequencies were similar in the eastern and western North Atlantic and Mediterranean (Ball et al. 2000). Overall, three genetically distinct stocks were recognised: the North Atlantic and Mediterranean, the South Atlantic (one sample from Brazil), and the South Pacific (one sample each from Australia and New Zealand) (Ball et al. 2000). There were no significant differences observed between Australia and New Zealand, but significant differences between Brazil and Australia/New Zealand, and the results interpreted as indicating a genetically homogeneous population in the South Pacific Ocean but with a lack of gene flow among populations in the South Atlantic and South Pacific Oceans (Ball et al. 2000). The large genetic divergence among the southern populations lead the authors to suggest that *Polyprion moene* (originally described from New Zealand) may be a valid species and further revision of *Polyprion* is required (Ball et al. 2000). An earlier DNA study based on RFLPs of one region of mtDNA (the ND1 gene) in *P.americanus* showed fixed differences between samples from the North and South Atlantic Ocean, suggesting little gene flow across the tropics (Sedberry et al. 1996). The lack of genetic differences among samples from Brazil and Australia/New Zealand lead to the erroneous conclusion that gene flow occurred between these latter areas through long distance dispersal of pelagic juveniles (Sedberry et al. 1996).

2.2.4 Hapuku summary

- Biology: long pelagic juvenile phase (3−4 years) that promotes dispersal. It should be noted that the late age at sexual maturity (10−13 years) coupled with large size make hapuku a difficult species for selective breeding unless age at maturation is reduced in captivity.

- Movement: some long distant (1300+ km) tag returns within New Zealand (east coast South island to Ninety Mile Beach and Tauranga); but no evidence for exchange of adults between northeast coast North Island and central New Zealand.

- Genetics: no evidence for spatial genetic differentiation among samples from central New Zealand with selectively neutral DNA markers. In the closely related wreckfish *Polyprion americanus* there was no evidence for genetic differentiation among samples from Australia and New Zealand with selectively neutral DNA markers.

- Life history and phenotypic traits: no regional data.

In the absence of a genetic data set, especially for adaptive traits, then a model to maximise genetic variability in the founding broodstock would be to chose equal numbers of fish from two widely separated regions such as east Northland and south Westland. An alternative and conservative model, based on adaptive divergence, would select broodstock from one region, to reduce the risk of outbreeding depression, and also select broodstock from the region in which the stock were to be ongrown (assuming that this will be in sea cages). Data on adaptive traits in hapuku would be useful to determine the appropriate broodstock model for hapuku.

2.3 Paua (*Haliotis iris*)

2.3.1 Paua distribution and biology

The black foot paua *Haliotis iris* is widely distributed around the coastline of New Zealand generally in water <6 m deep. Most of the commercial catch is taken from the Wairarapa coast southwards, with the major fisheries off the South Island, Stewart Island, and the Chatham Islands. Under the Quota Management System *H. iris* (PAU) has been subdivided into eight wide-scale fishstocks (Figure 5) , but the boundaries may not represent discrete stocks (Sullivan et al. 2005). The southern QMA PAU 5 was divided into three QMAs: Fiordland (PAU 5A), Stewart Island (PAU 5B), and Catlins/Otago (PAU 5D) in 1995. Subsequently the subareas PAU 5A, PAU 5B, and PAU 5D were further divided in 1997 into statistical areas for catch reporting purposes (Sullivan et al. 2005). There is no biological basis to the subdivisions. Two other species occur in New Zealand waters: the yellowfoot paua *Haliotis australis* and the white foot paua *H. virginea*. Globally there is one genus in the abalone family Haliotidae, with more than 100 species recognised, mostly in cool temperate waters in the Southern Hemisphere and North Pacific Ocean.

The larval period for *H. iris* is about 7−9 days at temperatures between 13−15^{o}C (Tong et al. 1992); while at 10^{o}C the larval period is extended to around 14 days. Assuming average water flows of 5−10 cm s^{-1} then larval dispersal is potentially 40−80 km, with a maximum of ~120 km. The Chatham Islands, at 860 km east of the South Island, are clearly beyond the limits of typical larval dispersal of *H. iris*.

2.3.2 Haliotis genetics

Genetic studies of abalone populations in Asia, Australia, North America, and South Africa have lead to different conclusions about population structure, ranging from genetic homogeneity promoted by larval dispersal (Withler et al. 2003) to localised recruitment (Hancock 2000). In abalone, as in many other species, microsatellite DNA markers have revealed more genetic variation than traditional genetic markers such as allozymes (Sekino et al. 2001), and microsatellites have become the nuclear DNA marker of choice for population and breeding studies, e.g., *Haliotis rubra* (Evans et al. 2000), *H. asinina* (Selvamani et al. 2000), *H. rufescens* (Kirby et al. 1998), and *H. midae* (Bester et al. 2004). Mitochondrial (mt) DNA

markers have also shown genetic differentiation among some abalone populations (Conod et al. 2002, EvansSweijd et al. 2004, Jiang et al. 1995), and the complete mitochondrial DNA of the blacklip abalone *H. rubra* has been published (Maynard et al. 2005).

Patterns of genetic differentiation revealed with selectively neutral molecular markers correspond with oceanographic barriers in several abalone species: genetic differentiation occurs among populations of *H. rubra* separated by Bass Strait (Conod et al. 2002), among populations of *H. midae* separated by Cape Agulhas (Conod et al. 2002, EvansSweijd et al. 2004, Klinbunga et al. 2003), and among populations of *H. asinina* and *H. ovina* in the Andaman Sea and Gulf of Thailand (Klinbunga et al. 2003, Tang et al. 2005). Seasonally variable currents can also influence patterns of genetic differentiation. Off the California coast, the black abalone *H. cracherodii* has a relatively short summer spawning season that corresponds with the period of limited current movement and exhibits genetic differentiation among populations (Hamm et al. 2000). In contrast *H. rufescens*, which spawns throughout the year and experiences greater variance in oceanographic conditions promoting larval dispersal, exhibits little genetic differentiation (Hamm et al. 2000).

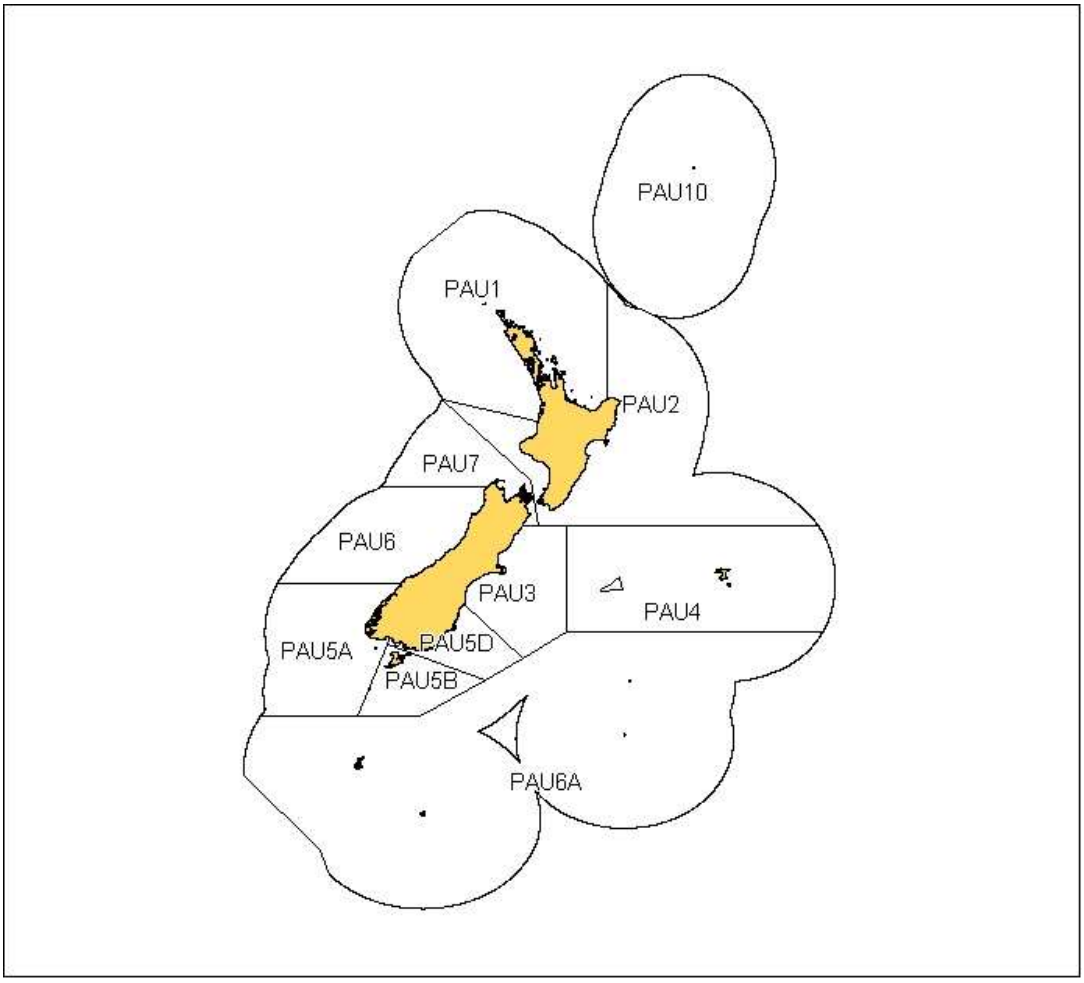

Figure 5: *Haliotis iris* management areas around New Zealand.

In the absence of oceanographic barriers, larval dispersal among large populations of *H. kamtschatkana* along the coast of British Columbia (Withler et al. 2003) appears to have been sufficient to prevent local

genetic differentiation. Less than 1% of the total microsatellite DNA variation was found among samples collected along the coast of British Columbia and off southeast Alaska. An alternative interpretation is that large population sizes coupled with recent isolation, following dispersal from glacial refuges during the last ice age (which finished approximately 12000 years ago), may have provided insufficient time for differentiation to develop at selectively neutral markers (Withler et al. 2003).

Genetic studies of three Australian species of abalone (*H. rubra, H. roei and H. laevigata*) have revealed low levels of genetic differentiation over wide areas (Brown et al. 1992, Brown 1991, Conod et al. 2002, Hancock 2000). An allozyme study of *H. rubra* found a relationship between genetic distance and geographic distance around southern Australia, fitting an isolation by distance model, with an average neighbourhood size of ~500 kms (Brown 1991). Studies, based on microsatellite and RAPD markers, indicated genetic differentiation among populations of *H. rubra* <100 km apart (Huang et al. 2000), although small sample sizes (n= 10) may have contributed to this result. A recent microsatellite study reported significant variation among sites separated by <1 km (K. Miller, University of Tasmania, unpublished data). Based on the early genetic results it was concluded that the Australian abalone species were subdivided into a series of local populations linked by larval dispersal (Shepherd et al. 1993). However, Conod et al. (2002) found little genetic differentiation among four regional samples around Tasmania, favouring a single large panmictic population, rather than isolation by distance, although there was significant genetic differentiation among samples separated by Bass Strait.

In the Western Australian abalone *H. roei* genetic differentiation fitted a simple isolation by distance model, in the absence of marked oceanographic boundaries (Hancock 2000). However heterogeneity was detected among some sites <20 km apart (Hancock 2000). Local heterogeneity has also been reported in *H. laevigata* (Brown et al. 1992) which occurs across the same geographic range as *H. rubra*, but has more patchy distribution, related to specialised habitat requirements (Shepherd 1973). Local genetic differentiation has been reported in several marine invertebrates and is unexpected in species with wide but continuous distributions. One explanation for local differentiation has been sweepstake events in recruitment that lead to genetic drift among small populations, and create a “chaotic genetic patchiness” or fine scale spatial and temporal heterogeneity (Hedgecock 1994, Johnson et al. 1984, Larson et al. 1999).

Data on composite mtDNA haplotypes in the South African *H. midae* revealed two major population groupings with almost 40% of the total genetic variance attributable to differences between Atlantic and Indian Ocean populations separated by Cape Agulhas (EvansSweijd et al. 2004). In the tropical abalone *H. ovina* low nucleotide divergence (0−1.7%) was found between geographically isolated samples, but significant heterogeneity among samples from the Gulf of Thailand and the Andaman Sea. Likewise *H. asinina* also showed low nucleotide divergence (0−0.47%) but significant population divergence between samples from the Philippines and Thailand (Klinbunga et al. 2003).

2.3.3 Genetic studies on *Haliotis iris*

In a preliminary assessment of genetic population structure, weak structure was observed with one mtDNA marker, in which a Chatham Island sample was significantly different from the three “mainland” samples (Smith et al. 2006). Six microsatellite (ms) DNA loci revealed high levels of genetic variation and highly significant differences in allele distributions among four area samples (F_{ST} = 0.048, P = 0.001), from east Northland (East Auckland current, Fig. 2), Stewart Island (Southland current), the central west coast (Taranaki – West Auckland/Tasman current), and the Chatham Islands (beyond limits of larval dispersal from the mainland). Overall levels of genetic differentiation indicated that 4−5% of the genetic diversity was due to differences among populations. It was noted that the preliminary differences need to be measured in additional samples to test for spatial differentiation at macro- and micro- spatial scales

(Smith et al. 2006), and to determine if an isolation by distance model is appropriate for mainland New Zealand or if there are there are regional stocks contained within water masses. Initial analyses of the msDNA markers applied to the Chatham Island and Tory Channel 2008 broodstock established at Mahanga Bay found significant differentiation at 9/11 loci, with region specific alleles at some loci (P. Smith & J. Symonds, NIWA, unpublished data).

A population genetic study of *H. iris* has been undertaken at Canterbury University. Preliminary results indicate that a Chatham Island sample was the most divergent, and there was limited genetic differentiation among regional mainland stocks, with a north-south split around Cook Strait; details are not available as of June 2008.

2.3.4 Paua morphometrics and life history

Morphometric variation has been reported in populations of *H. iris*, but variation among localities, while significant, is not large, with the greatest variation found among localities ~200 m apart (Mcshane et al. 1994). Likewise mean shell length and growth rates vary among localities, but also exhibit significant differences at small spatial scales (~100m) which correspond with exposure (Mcshane et al. 1995). Size at maturity is smaller in fast growing populations (Mcshane et al. 1995) accounting for the observation of 'stunted' populations in Taranaki and the Marlborough Sounds. The genetic basis, if any, for the differences in size at sexual maturity are unknown.

2.3.5 Paua summary

- Biology: wide distribution from Northland to Stewart Island, with limited larval dispersal (potential dispersal range ~120 km). Chatham Island stocks beyond the limits of normal larval dispersal.
- Genetics: spatial genetic differentiation among four regional samples from east Northland, Taranaki, Stewart Island, and the Chatham Islands, with selectively neutral microsatellite DNA markers. Chatham Island sample most differentiated with mtDNA marker (COI).
- Life history and phenotypic traits: mean shell length and growth rates vary among localities, but exhibit significant differences at small spatial scales (~100m). Size at maturity is smaller in fast growing populations with 'stunted' populations in Taranaki and the Marlborough Sounds. The genetic basis, if any, for the differences in size at sexual maturity are unknown.

A conservative model, based on adaptive divergence, would select broodstock from three regions, Northland, Southland, and the Chatham Islands, to maximise genetic diversity in the founding broodstock. In order to reduce the risk of outbreeding depression, the three lines of broodstock should be maintained and selected as three independent lines, and used to provide appropriate selected seed for ongrowing in different regions.

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References

Allendorf, F.; Ryman, N.; Utter, F. (1987). Genetic and fisheries management: past, present and future. *In Population Genetics and fisheries management Eds Ryman, N and Utter, F.M. University of Washington Press, Seattle.*: 1-20.

Allendorf, F.; Seeb, L. (2000). Concordance of genetic divergence among sockeye salmon populations at allozyme, nuclear DNA, and mitochondrial DNA markers. *Evolution 54*: 640-651.

Apte, S.; Gardner, J.P. (2002). Population genetic subdivision in the New Zealand greenshell mussel (*Perna canaliculus*) inferred from single-strand conformation polymorphism analysis of mitochondrial DNA. *Molecular Ecology 11*: 1617-1628.

Apte, S.; Star, B.; Gardner, J.P. (2003). A comparison of genetic diversity between cultured and wild populations, and a test for genetic introgression in the New Zealand greenshell mussel *Perna canaliculus* (Gmelin 1791). *Aquaculture 219*: 193-220.

Avise, J.; Helfman, G.; Saunders, N.; Hales, L. (1986). Mitochondrial DNA differentiation in North Atlantic eels: population genetic consequences of an unusual life history pattern. *Proceedings of the National Academy of Sciences USA 83*: 4350-4354.

Avise, J.C. (2000). Phylogeography: the history and formation of species. *Cambridge Massachusetts, Harvard University Press.*: 477.

Ayers, K.L.; Waters, J.M. (2005). Marine biogeographic disjunction in central New Zealand. *Marine Biology 147*: 1045-1052.

Ball, A.O.; Sedberry, G.R.; Zatcoff, M.S.; Chapman, R.W.; Carlin, J.L. (2000). Population structure of the wreckfish *Polyprion americanus* determined with microsatellite genetic markers. *Marine Biology 137*: 1077-1090.

Barreiros, J.; Machado, L.; Hostim-Silva, M.; Sazima, I.; Heemstra, P. (2004). First record of *Polyprion oxygeneios* (Perciformes: Polyprionidae) for the southwest Atlantic and a northernmost range extension. *Journal of Fish Biology 64*: 1439-1441.

Bartley, D.M.; Bagley, M.; Gall, G.; Bentley, B. (1992). Use of linkage disequilibrium data to estimate effective population size of hatchery and natural fish populations. *Conservation Biology 6*: 365-375.

Beentjes, M.; Francis, M. (1999). Movement of hapuku (*Polyprion oxygeneios*) determined from tagging studies. *New Zealand Journal of Marine & Freshwater Research 33*: 1-12.

Bell, M. (2001). Lateral plate evolution in the threespine stickleback: getting nowhere fast. *Genetica 112*: 445-461.

Belshaw, R.; Bensasson, D. (2006). The rise and falls of introns. *Heredity 96*: 208-213.

Bensch, S.; Akesson, M. (2005). Ten years of AFLP in ecology and evolution: why so few animals? *Molecular Ecology 14*: 2899-2914.

Bentsen, H.; Olesen, I. (2002). Designing aquaculture mass selection programs to avoid high inbreeding rates. *Aquaculture 204*: 349-359.

Bernal-Ramirez, J.H.; Adcock, G.H.; Hauser, L.; Carvalho, G.R.; Smith, P.J. (2003). Temporal stability of genetic population structure in the New Zealand snapper, *Pagrus auratus*, and relationship to coastal currents. *Marine Biology 142*: 567-574.

Bester, A.E.; Slabbert, R.; D'amato, M.E. (2004). Isolation and characterization of microsatellite markers in the South African abalone (*Haliotis midae*). *Molecular Ecology Notes 4*: 618-619.

Binks, R.K., Wj; Johnson, Ms. (2007). Rapid evolutionary responses in a translocated population of intertidal snail (Bembicium vittatum) utilise variation from different source populations. *Conservation Genetics 8*: 1421-1429.

Bohonak, A. (1999). Dispersal, gene flow and population structure. *Quarterly Review of Biology 74*: 21-45.

Borrell, Y.; Carleos, C.; Asturiano, J.; Bernardo, D.V., E; Corral, N; Sanchez, Ja; Blanco, G. (2007). Use of microsatellites and a combinatorial optimization approach in the acquisition of gilthead seabream (*Sparus aurata* L.) broodstocks for hatcheries. *Aquaculture 269*: 200-210.

Brander, K. (1995). The effect of temperature on growth of Atlantic cod (*Gadus morhua* L.). *Journal of Marine Science 52*: 1-10.

Brodziak, J.; Mikus, R. (2000). Variation in life history parameters of Dover sole, *Microstomus pacificus*, off the coasts of Washington, Oregon, and northern California. *Fishery Bulletin 98*: 661-673.

Brown, L.; Murray, N. (1992). Population genetics, gene flow, and stock structure in *Haliotis rubra* and *Haliotis laevigata*. *Abalone of the World: biology, fisheries, and culture Ed SA Shepherd, MJ Tenger, SA Guzman*: 24-33.

Brown, L.D. (1991). Genetic variation and population structure in the blacklip abalone, Haliotis rubra. *Marine & Freshwater Research 42*: 77-90.

Buonaccorsi, V.; Starkey, E.; Graves, J. (2001). Mitochondrial and nuclear DNA analysis of population subdivision among young-of-the-year Spanish mackerel (*Scomberomorus maculatus*) from the western Atlantic and Gulf of Mexico. *Marine Biology 138*: 37-45.

Buonaccorsi, V.P.; Kimbrell, C.A.; Lynn, E.A.; Vetter, R.D. (2002). Population structure of copper rockfish (*Sebastes caurinus*) reflects postglacial colonisation and contemporary patterns of larval dispersal. *Canadian Journal of Fisheries & Aquatic Sciences 59*: 1374-1384.

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

Canino, M.F.; O'reilly, P.T.; Hauser, L.; Bentzen, P. (2005). Genetic differentiation in walleye pollock (*Theragra chalcogramma*) in response to selection at the pantophysin (PanI) locus. *Canadian Journal of Fisheries & Aquatic Sciences 62*: 2519-2529.

Carvalho, G.R. (1993). Evolutionary aspects of fish distribution: genetic variability and adaptation. *Journal of Fish Biology 43 (Suppl A)*: 53-73.

Chiswell, S.M. (2000). The Wairarapa Coastal Current. *New Zealand Journal of Marine & Freshwater Research 34*: 303-315.

Christiansen, F.B.; Nielsen, V.H.; Simonsen, V. (1988). Genetics of *Zoarces* populations XV. Genetic and morphological variation in Mariager Fjord. *Hereditas 109*: 99-12.

Conod, N.; Bartlett, J.P.; Evans, B.S.; Elliott, N.D. (2002). Comparison of mitochondrial and nuclear DNA analyses of population structure in the blacklip abalone *Haliotis rubra* Leach. *Marine & Freshwater Research 53*: 711-718.

Conover, D.; Schultz, E. (1995). Phenotypic similarity and the evolutionary significance of countergradient variation. *Trends in Ecology and Evolution 10*: 248-252.

Conover, D.O. (1998). Local adaptation in marine fishes: evidence and implications for stock enhancement. *Bulletin of Marine Science 62*: 477-493.

Conover, D.O.; Clarke, L.M.; Munch, S.B.; Wagner, G.N. (2006). Spatial and temporal scales of adaptive divergence in marine fishes and the implications for conservation. *Journal of Fish Biology 69*: 21-47.

Couceiro, L.; Barreiro, R.; Ruiz, J.; Sotka, E. (2007). Genetic isolation by distance among populations of the netted dog whelk *Nassarius reticulatus* (L.) along the European Atlantic coastline. *Journal of Heredity*.

Dendanto, D.; Brown, B.; Davis, C.; Kornfieid, I. (2000). Analysis of genetic diversity in a commercially important line of oysters selected for fast growth. *Journal of Shellfish Research 19*: 613-614.

Deng, Y.; Liu, X.; Zhang, G.; Guo, X. (2005). Inbreeding depression and maternal effects on early preformance of Pacific abalone. *North American Journal of Aquaculture 67*: 231-236.

Dewoody, J.A.; Avise, J.C. (2000). Microsatellite variation in marine, freshwater and anandromous fishes compared with other animals. *Journal of Fish Biology 56*: 461-473.

Dias, G.M.; Duarte, L.F.L.; Solferini, V.N. (2006). Low genetic differentiation between isolated populations of the colonial
ascidian *Symplegma rubra* Monniot, C. 1972. *Marine Biology 148*: 807-815.

Dionne, M.; Dodson, J.J.; Bernatchez, L.; Miller, K.M.; Caron, F. (2007). Clinal variation in MHC diversity with temperature: Evidence for the role of host-pathogen interaction on local adaptation in Atlantic salmon. *Evolution 61*: 2154-2164.

Doherty, P.J.; Planes, S.; Mather, P. (1995). Gene flow and larval duration in seven species of fish from the Great Barrier reef. *Ecology 76*: 2373-2391.

Douglas, S. (2006). Microarray studies of gene expression in fish. *OMICS A Journal of Integrative Biology 4*: 474-489.

Doupe, R.G.L., Alan J. (2005). Genetic covariation in production traits of sub-adult black bream *Acanthopagrus butcheri* after grow-out. *Aquaculture 36*: 1128-1132.

Elliott, N.; Ward, R. (1992). Enzyme variation in orange roughy (*Hoplostethus atlanticus*) samples from southern Australian and New Zealand waters. *Australian Journal of Marine and Freshwater Research 45*: 51-67.

Endler, J. (1986). Natural selection in the wild. *Princeton University Press, Princeton, New Jersey*.

Evans, B.; Bartlett, J.; Sweijd, N.; Cook, P.; Elliott, N. (2004). Loss of genetic variation at microsatellite loci in hatchery produced abalone in Australia (*Haliotis rubra*) and South Africa (*Haliotis midae*). *Aquaculture 233*: 109-127.

Evans, B.; Sweijd, N.; Bowie, R.; Cook, P.; Elliott, N. (2004). Population genetic structure of the perlemoen *Haliotis midae* in South Africa: evidence of range expansion and founder events. *Marine Ecology Progress Series 270*: 163-172.

Evans, B.; White, R.; Elliott, N. (2000). Characterization of microsatellite loci in the Australian blacklip abalone (*Haliotis rubra*, Leach). *Molecular Ecology 9*: 1171-1193.

Exadactylos, A.; Geffen, A.; Thorpe, J. (1999). Growth and genetic variation in hatchery reared larval and juvenile Dover sole *Solea solea* (L.). *Aquaculture 176*: 209-226.

Fahy, W.R.E. (1972). Influence of temperature change on number of vertebrae and caudal fin rays in *Fundulus majalis* (Walbaum). *Journal du Conseil International pour l'Exploration de la Mer 34*: 217-231.

Fao. (1981). Conservation of the genetic resources of fish: problems and recommendations. *FAO Fisheries Technical Paper 217*: 43 pp.

Fao. (1993). Report of the expert consultation on utilization and conservation of aquatic genetic resources. *FAO Fisheries Report 491*: 59 pp.

Francis, M.; Mulligan, K.; Davies, N.; Beentjes, M. (1999). Age and growth estimates for New Zealand hapuku, *Polyprion oxygeneios*. *Fishery Bulletin 97*: 227-242.

Frankham, R. (1995). Effective population size/adult population size ratios in wildlife. *Genetical Research 66*: 95-107.

Gaffney, P.M.; Rupnow, J.; Domeier, M.L. (2007). Genetic similarity of disjunct populations of the giant sea bass *Stereolepis gigas*. *Journal of Fish Biology 70*: 111-124.

Gallardo, J.; García, X.; Lhorente, J.; Neira, R. (2004). Inbreeding and inbreeding depression of female reproductive traits in two populations of Coho salmon selected using BLUP predictors of breeding values. *Aquaculture 234*: 111-122.

Gharrett, A.; Smoker, W.; Reisenbichler, R.; Taylor, S. (1999). Outbreeding depression in hybrids between odd- and even-broodyear pink salmon. *Aquaculture 173*: 117-129.

Gilk, S.E.; Wang, I.A.; Hoover, C.L.; Smoker, W.W.; Taylor, S.G.; Gray, A.K.; Gharrett, A.J. (2004). Outbreeding depression in hybrids between spatially separated pink salmon, *Oncorhynchus gorbuscha*, populations: marine survival, homing ability, and variability in family size. *Environmental Biology of Fishes 69*: 287-297.

Gillanders, B.M.; Ferrel, D.J.; Andrew, N.L. (2001). Estimates of movement and life-history parameters of yellow tail kingfish (*Seriola lalandi*): how useful are data from a cooperative tagging programme? *Marine & Freshwater Research 52*: 179-192.

Godo, O.; Moksness, E. (1987). Growth and maturation of Norwegian coastal cod and northeast Arctic cod under different conditions. *Fisheries Research 5*: 235-242.

Gold, J.; Burridge, C.; Turner, T. (2001). A modified stepping-stone model of population structure in red drum, *Sciaenops ocellatus* (Sciaenidae), from the northern Gulf of Mexico. *Genetica 111*: 305-317.

Gold, J.R.; Kristmundsdottir, A.Y.; Richardson, L.R. (1997). Mitochondrial DNA variation in king mackerel (*Scomberomorrus cavalla*) from the western Atlantic Ocean and Gulf of Mexico. *Marine Biology 129*: 221-232.

Gold, J.R.; Richardson, L.R. (1998). Population structure in greater amberjack, *Seriola dumerili*, from the Gulf of Mexico and the western Atlantic Ocean. *Fishery Bulletin 96*: 767-778.

Gold, J.R.; Richardson, L.R.; Turner, T.F. (1999). Temporal stability and spatial divergence of mitochondrial DNA haplotype frequencies in red drum (*Sciaenops ocellatus*) from coastal regions of the western Atlantic Ocean and Gulf of Mexico. *Marine Biology 133*: 593-602.

Goldberg, T.; Grant, E.; Inendino, K.; Kassler, T.; Claussen, J.; Phillip, D. (2005). Increased infectious disease susceptibility resulting from outbreeding depression. *Conservation Biology 19*: 455-462.

Goldstein, S.J.; Schiel, D.R.; Gemmell, N.J. (2006). Comparative phylogeography of coastal limpets across a marine disjunction in New Zealand. *Molecular Ecology 15*: 3259-3268.

Gonzalez, E.; Nagasawa, K.; Umino, T. (2008). Stock enhancement program for black sea bream (*Acanthopagrus schlegelii*) in Hiroshima Bay: Monitoring the genetic effects. *Aquaculture 276*: 36-43.

Graham, D. (1956). A treasury of New Zealand fishes. *Reed, Wellington, New Zealand*: 424 p.

Gyllenstein, U. (1985). The genetic structure of fish: differences in the intraspecific distribution of biochemical genetic variation between marine, anadromous, and freshwater species. *Journal of Fish Biology 26*: 691-699.

Hamm, D.E.; Burton, R.S. (2000). Population genetics of black abalone, *Haliotis cracherodii*, along the central California coast. *Journal of Experimental Marine Biology and Ecology 254*: 235-247.

Hancock, B. (2000). Genetic subdivision of Roe's abalone, *Haliotis roei* Grey (Mollusca: Gastropoda), in south-western Australia. *Marine & Freshwater Research 51*: 679-687.

Hansen, M.M.; Jensen, L.F.; Bekkevold, D.; Mensberg, K.-L.D.; Skaala, O.; Jensen, L.F. (2007). Gene flow, effective population size and selection at major histocompatibility complex genes: Brown trout in the Hardanger Fjord, Norway. *Molecular Ecology 16*: 1413-1425.

Hartill, B.; Davies, N.M. (1999). New Zealand billfish and gamefish tagging 1998-99. *NIWA Technical Report 79*: 20 pp.

Hartill, B.; Davies, N.M. (2001). New Zealand billfish and gamefish tagging 1999-2000. *NIWA Technical Report 106*: 10 pp.

Hauser, L.; Ward, R.D. (1998). Population identification in pelagic fish: the limits of molecular markers. *In Carvalho G (ed) Advances in Molecular Ecology. IOS, Amsterdam*: pp 191-224.

Hedgecock, D. (1994). Temporal and spatial genetic structure of marine animal populations in the California Current. *Reports of California Cooperative Oceans Fisheries Investigations 35*: 73-81.

Hedrick, P.W. (1999). Perspective: highly variable loci and their interpretation in evolution and conservation. *Evolution 53*: 311-318.

Hedrick, P.W.; Hedgecock, D.; Hamelburg, S. (1995). Effective population size in winter run Chinook salmon. *Conservation Biology 9*: 615-624.

Heino, M.; Dieckmann, M.U.; Godo, O. (2002). Reaction norm analysis of fisheries induced adapative change and the case of the Northeast Arctic cod. *ICES CM2002/Y:14*.

Heist, E.; Gold, J. (1999). Microsatellite DNA variation in sandbar sharks *(Carcharhinus plumbeus*) from the Gulf of Mexico and mid-Atlantic bight. *Copeia 1999*: 182-186.

Hemmer-Hansen, J.; Nielsen, E.; Frydenberg, J.; Loeschcke, V. (2007). Adaptive divergence in a high gene flow environment: *Hsc70* variation in the European flounder (*Platichthys flesus* L.). *Heredity 99*: 592-600.

Hoarau, G.; Borsa, P. (2000). Extensive gene flow within sibling species in the deep sea fish Beryx splendens. *Life Sciences 323*: 315-325.

Holdsworth, J. (1995). Drifting buoy offers clues on juvenile kingfish. *Seafood New Zealand 3(3)*.

Holdsworth, J.; Saul, P. (1998). New Zealand billfish and gamefish tagging, 1995-96. *NIWA Technical Report 16*: 10 pp.

Holtsmark, M.; Sonesson, A.; Gjerde, B.; Klemetsdal, G. (2006). Number of contributing subpopulations and mating design in the base population when establishing a selective breeding program in fish. *Aquaculture 258*: 241-249.

Houston, R.; Haley, C.; Hamilton, A.; Guy, D.; Tinch, A.; Taggart, J.; Mcandrew, B.; Bishop, S. (2008). Major quantitative trait loci affect resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmo salar*). *Genetics 178 (2)*.

Huang, B.; Peakall, R.; Hanna, P. (2000). Analysis of genetic structure of blacklip abalone (*Haliotis rubra*) populations using RAPD, minisatellite and microsatellite markers. *Marine Biology 136*: 207-216.

Humphreys, R.; Winans, G.; Tagami, D. (1989). Synonymy and life history of the North Pacific pelagic armorhead *Pseudopentaceros wheeleri* Hardy (Pisces: Pentacerotida). *Copeia 1989*: 142-153.

Ihssen, P.; Booke, H.; Casselman, J.; Mcglade, J.; Payne, N.; Utter, F. (1981). Stock identification: materials and methods. *Canadian Journal of Fisheries & Aquatic Sciences 38*: 1838-1855.

Imsland, A.; Brix, O.; Naevdal, G.; Samuelsn, E. (1997). Hemoglobin genotypes in turbot (*Scophthalmus maximus* Rafinesque) their oxygen affinity propertires and relation with growth. *Comparative Biochemistry and Physiology 116A*: 157-165.

Imsland, A.; Foss, A.; Naevdal, G.; Johansen, T.; Stefansson, S.; Jonassen, T. (2007). New haemoglobin genotypes in Atlantic cod, *Gadus morhua*: Possible relation with growth. *Comparative Biochemistry and Physiology 147 A*: 955-960.

Jagielo, T.; Leclair, L.; Vorderstrasse, B. (1996). Genetic variation and population structure of lingcod. *Transactions of the Amercian Fisheries Society 125*: 372-386.

Jenny, M.; Chapman, R.; Mancia, A.; Chen, Y.; Mckillen, D.; Trent, H.; Lang, P.; Escoubas, J.; Bachere, E.; Boulo, V.; Liu, Z.; Gross, P.; Cunningham, C.; Cupit, P.; Tanguy, A.; Guo, X.; Moraga, D.; Boutet, I.; Huvet, A.; De Guise, S.; Almeida, J.; Warr, G. (2007). A cDNA Microarray for *Crassostrea virginica* and *C-gigas*. *Marine Biotechnology 9*: 577-591.

Jiang, L.; Wu, W.; Huang, P. (1995). The mitochondrial DNA of Taiwan abalone *Haliotis diversicolor* Reeve, 1846 (Gastropoda: Archaeogastropoda: Haliotidae). *Molecular Marine Biology and Biotechnology 4*: 353-364.

Johnson, M.; Black, R. (1984). Pattern beneath the chaos: The effect of recruitment on genetic patchiness in an intertidal limpet. *Evolution 38*: 1371-1383.

Johnston, A. (1983). The southern Cook Strait groper fishery. *New Zealand Ministry of Agriculture and Fisheries Technical Report 159*: 33 p.

Jonassen, T.; Imsland, A.; Fitzgerald, R.; Stefansson, M.; Bonga, S.; Ham, E.V.; Naevdal, G.; Stefansson, S. (2000). Interpopluation variation in growth and growth efficiency of juvenile Atlantic halibut (*Hippoglossus hippoglossus* L.) related to latitude. *Journal of Fish Biology 56*: 279-294.

Jónsdóttir, I.; Marteinsdóttir, G.; Pampoulie, C. (2008). Relation of growth and condition with the Pan I locus in Atlantic cod (*Gadus morhua* L.) around Iceland. *Marine Biology 154*: 867-874.

Jørgensen, C.; Enberg, K.; Dunlop, E.; Arlinghaus, R.; Boukal, D.; Brander, K.; Ernande, B.; Gårdmark, A.; Fjohnston; Matsumura, S.; Pardoe, H.; Raab, K.; Silva, A.; Vainikka, A.; Dieckmann, U.; Heino, M.; Rijnsdorp, A. (2007). Managing evolving fish stocks. *Science 318*: 1247-1248.

Kincaid, H. (1995). An evaluation of inbreeding and effective population size in salmonid broodstock in federal and state hatcheries. *Proceedings of the American Fisheries Society Symposium 15*: 193-204.

Kingsford, M. (1992). Drift algae and small fish in coastal waters of northeastern New Zealand. *Marine Ecology Progress Series 80*: 41-45.

Kirby, V.; Villa, R.; Powers, D. (1998). Identification of microsatellites in the California red abalone, *Haliotis rufescens*. *Journal of Shellfish Research 17*: 801-804.

Klinbunga, S.; Pripue, P.; Khamnamtong, N.; Puanglarp, N.; Tassanakajon, A.; Jarayabhand, P.; Hirono, I.; Aoki, T.; Menasveta, P. (2003). Genetic diversity and molecular markers of the tropical abalone (*Haliotis asinina*) in Thailand. *Marine Biotechnology 5*: 505-517.

Koehn, R.; Williams, G. (1978). Genetic differentiation without isolation in the American eel *Anguilla rostrata* 11. Temporal stability of geographic variation. *Evolution 32*: 624-637.

Kukharev, N.P., as; Timokhin, Ig; Ivanin, Na. (1998). The history and the present state of deepwater fisheries for the Indian Ocean fish. *Trudy Yuzhnogo Proceedings of the Southern Scientific Research Institure Marine Fisheries and Oceanography 44*: 148-159.

Lanan, J. (1980). Broodstock management of Crassostrea gigas, IV. Inbreeding and larval survival. *Aquaculture 21*: 353-356.

Langefores, A.; Von Schantz, T.; Widegren, B. (1998). Allelic variation of Mhc class 11 in Atlantic salmon; a population genetic analysis. *Heredity 80*: 568-575.

Larsen, P.N., Ee; Williams, Td; Hemmer-Hansen, J; Chipman, Jk; Kruhoffer, M; Gronkjaer, P; George, Sg; Dyrskjot, L; Loeschcke, V. (2007). Adaptive differences in gene expression in European flounder (*Platichthys flesus*). *Molecular Ecology 16*: 4674-4683.

Larson, R.J.; Julian, R.M. (1999). Spatial and temporal genetic patchiness in marine populations and their implications for fisheries management. *Reports of California Cooperative Oceans Fisheries Investigations 40*: 94-99.

Laurent, V.; Caneco, B.; Magoulas, A.; Planes, S. (2007). Isolation by distance and selection effects on genetic structure of sardines *Sardina pilchardus* Walbaum. *Journal of Fish Biology 71*: 1-17.

Li, Q.; Park, C.; Endo, T.; Kijima, A. (2004). Loss of genetic variation at microsatellite loci in hatchery strains of the Pacific Abalone (*Haliotis discus hannai*). *Aquaculture 235*: 207-222.

Li, Q.; Xu, K.; Yu, R. (2007). Genetic variation in Chinese hatchery populations of the Japanese scallop (*Patinopecten yessoensis*) inferred from microsatellite data. *Aquaculture 269*: 211-219.

Lindsey, C.C. (1988). Factors controlling meristic variation. *Fish physiology XIB(Eds Hoar, WS and Randal DJ)*: 179-274.

Lynch, M. (1991). The genetic interpretation of inbreeding depression and outbreeding depression. *Evolution 45*: 622-629.

Lynch, M. (1996). A quantitative genetic perspective on conservation issues. *In Conservation Genetics: case histories from nature. Ed JC Avise and J Hamrick Chapman and Hall, New York*: 471-501.

Lynch, M. (2005). Inbreeding depression and outbreeding depression. *NOAA Technical Memo NMFS Genetic Effects of Straying of Non-Native Hatchery Fish into Natural Populations NWFSC-30*.

Machias, A.; Somarakis, S.; Papadroulakis, N.; Spedicato, M.; Suquet, M.; Lembo, G.; Divanach, P. (2003). Settlement of the wreckfish (*Polyprion americanus*). *Marine Biology 142*: 45-52.

Mallet, A.; Haley, L. (1983). Effects of inbreeding on larval and spat performance in the American oyster. *Aquaculture 33*: 229-235.

Malloy, K.; T.E., T.T. (1994). Effects of ration limitation and low temperature on growth, biochemical condition, and survival of juvenile summer flounder from two Atlantic coastal nurseries. *Transactions of the American Fisheries Society 123*: 182-193.

Martin, A.; Humphreys, R.; Palumbi, S. (1992). Population genetic structure of the armourhead *Pseudopentaceros wheeleri* in the North Pacific Ocean: application of the polymerase chain reaction to fisheries problems. *Canadian Journal of Fisheries & Aquatic Sciences 49*: 2386-2391.

Maynard, B.; Kerr, L.; Mckiernan, J.; Jansen, E.; Hanna, P. (2005). Mitochondrial DNA sequence and gene organization in Australian blacklip abalone *Haliotis rubra* (Leach). *Marine Biotechnology 7*: 645-658.

Mcdonald, J. (1994). Detecting natural selection by comparing geographic variation in protein and DNA polymorphisms. *In Golding, B. (ed) Non-neutral-evolution: theories and molecular data Chapman and Hall, New York*: 88-100.

Mckenzie, J.; Walsh, C.; Mcgregor, G.; Poortenaar, C.; Hartill, B.; Smith, M. (2000). Information available for the assesment of New Zealand kingfish stocks. *NIWA Fisheries Assessment Report 2000*: 24 pp.

Mcshane, P.; Naylor, J. (1995). Small-scale spatial variation in growth, size at maturity, and yield- and egg-per-recruit relations in the New Zealand abalone *Haliotis iris. New Zealand Journal of Marine & Freshwater Research 29*: 603-612.

Mcshane, P.; Schiel, D.; Mercer, S.; Murray, T. (1994). Morphometric variation in *Haliotis iris* (Mollusca: Gastropoda): Analysis of 61 populations. *New Zealand Journal of Marine & Freshwater Research 28*: 357-364.

Milton, D.; Shaklee, J. (1987). Biochemical genetics and population structure of blue grenadier, *Macruronus novaezelandiae* (Hector) (Pisces: Merluccidae), from Australian waters. *Australian Journal of Marine and Freshwater Research 38*: 727-742.

Mitton, J. (1997). Selection in natural populations. *Oxford Univeristy Press, Oxford.*

Mitton, J.; Koehn, R. (1975). Genetic organistaion and adaptive response of allozymes to ecological variables in *Fundulus heteroclitus. Genetics 79*: 97-111.

Moritz, C. (1994). Applications of mitochondrial DNA analysis in conservation: a critical review. *Molecular Ecology 3*: 401-411.

Moriya, S.; Sato, S.; Azumaya, T.; Suzuki, O.; Urawa, S.; Urano, A.; Abe, S. (2007). Genetic stock identification of chum salmon in the Bering Sea and North Pacific Ocean using mitochondrial DNA microarray. *Marine Biotechnology 9*: 179-191.

Moriya, S.; Urawa, S.; Suzuki, O.; Urano, A.; Abe, S. (2004). DNA microarray for rapid detection of mitochondrial DNA haplotypes of chum salmon. *Marine Biotechnology 6*: 430-434.

Mork, J.; Sundnes, G. (1985). 0-Group cod (*Gadus morhua*) in captivity: differential survival of certain genotypes. *Helgolander Meerescuntersuchungen 39*: 63-70.

Munro, P. (1993). Genetic aspects of conservation and cultivation of giant clams. *ICLARM Conference Proceedings 39*: 47 pp.

Myers, J.H., Po; Hudson, G; Iwamoto, Rn. (2001). Genetics and broodstock management of coho salmon. *Aquaculture 197*: 43-62.

Naevdal, G.F., A.; Otterlei, E.; Thorkildsen, S. (1992). Growth rate related to genotype of 0-group cod at three environmental temperatures. *Sarsia 77*: 71-33.

Nakadate, M.; Shikano, T.; Taniguchi, N. (2003). Inbreeding depression and heterosis in various quantitative traits of the guppy, Poecilia reticulata. *Aquaculture 220(1-4)*: 219-226.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings National Academy of Sciences 70*: 3321-3323.

Nei, M. (1987). Molecular evolutionary genetics. *New York, Columbia University Press*: 512 pp.

Nugroho, N.; Ferrell, D.J.; Smith, P.J.; Taniguchi, N. (2001). Genetic divergence of kingfish from Japan, Australia and New Zealand inferred by microsatellite DNA and mtDNA control region markers. *Fisheries Science 67*: 843-850.

Olsen, E.; Lilly, G.; Heino, M.; Morgan, M.; Brattey, J.; Dieckmann, U. (2005). Assessing changes in age and size at maturation in collapsing populations of Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries & Aquatic Sciences 62*: 811-823.

Otterå, H.; Agnalt, A.; Jørstad, K. (2006). Differences in spawning time in captive Atlantic cod from four regions of Norway, spawned under identical conditions. *ICES Journal of Marine Science 63*: 216-223.

Palumbi, S. (2003). Population genetics, demographic connectivity, and the design of marine reserves. *Ecological Applications 13*: 146-158.

Pampoulie, C.; Jörundsdóttir, T.; Steinarsson, A.; Pétursdóttir, G.; Stefánsson, M.; Daníelsdóttir, A. (2006). Genetic comparison of experimental farmed strains and wild Icelandic populations of Atlantic cod (*Gadus morhua* L.). *Aquaculture 261*: 556-564.

Park, C.; Li, Q.; Kobayashi, T.; Kijima, A. (2006). Inbreeding depression traits in Pacific abalone *Haliotis discus hannai* by factorial mating experiments. *Fisheries Science 72*: 774-780.

Paul, L. (2002a). Can existing data describe the stock structure of the two New Zealand groper species, hapuku (*Polyprion oxygeneios*) and bass (*P. americanus*)? *New Zealand Fisheries Assessment Report 14*: 15 pp.

Paul, L. (2002b). A description of the New Zealand fisheries for the two groper species, hapuku (*Polyprion oxygeneios*) and bass (*P. americanus*). *New Zealand Fisheries Assessment Report 2002/13*: 17 pp.

Paul, L. (2002c). Size structure of hapuku (*Polyprion oxygeneios*) and bass (*P. americanus*) populations in New Zealand. *New Zealand Fisheries Assessment Report 2002/16*: 17 pp.

Planes, S.; Fauvelot, C. (2002). Isolation by distance and vicariance drive genetic structure of a coral reef fish in the Pacific Ocean. *Evolution 56*: 378-399.

Pogson, G.; Mesa, K. (2004). Positive Darwinian Selection at the Pantophysin (Pan I) Locus in Marine Gadid Fishes. *Molecular Biology and Evolution 21*: 65-75.

Pogson, G.; Mesa, K.; Boutilier, R. (1995). Genetic population structure and gene flow in the Atlantic cod *Gadus morhua*: a comparison of allozyme and nuclear RFLP loci. *Genetics 139*: 375-385.

Pogson, G.; Taggart, C.; Mesa, K.; Boutilier, R. (2001). Ioslation by distance in the Atlantic cod, *Gadus morhua*, at large and small geographic scales. *Evolution 55*: 131-146.

Porta, J.; Porta, J.; Canavate, P.; Martinez-Rodriguez, G.; Alvarez, M. (2007). Substantial loss of genetic variation in a single generation of Senegalese sole (*Solea senegalensis*) culture: implications in the domestication process. *Journal of Fish Biology 71B*: 223-234.

Porta, J.; Porta, J.M.; Martinez-Rodriguez, G.; Alvarez, M.D.C. (2006). Development of a microsatellite multiplex PCR for Senegalese sole (*Solea senegalensis*) and its application to broodstock management. *Aquaculture 256*: 159-166.

Portenaar, C.W.; Hooker, S.H.; Sharp, N. (2001). Assessment of yellowtail kingfish (*Seriola lalandi lalandi*) reproductive physiology as a basis for aquaculture development. *Aquaculture 201*: 276-286.

Powers, D.; Lauerman, T.; Crawford, D.; Smith, M.; Gonzalez-Villasenor, I.; Dimichele, L. (1991). The evolutionary significance of genetic variation at enzyme synthesising loci in the teleost *Fundulus heteroclitus*. *Journal of Fish Biology 39A*: 169-184.

Pullin, R.S.V. (2000). Management of Aquatic Biodiversity and Genetic Resources. *Reviews in Fisheries Science 8*: 379-393.

Purdom, C.E.; Wyatt, T. (1969). Racial differences in Irish Sea and North Sea plaice (*Pleuronectes platessa*). *Nature 222*: 780-781.

Reed, D.; Bryant, E. (2000). Experimental tests of minimum viable population size. *Animal Conservation 3*: 7-14.

Reznick, D.; Bryga, H.; Endler, J. (1990). Experimentally induced life history evolution in a natural population. *Nature 346*: 357-359.

Reznick, D.A.; Shaw, F.; Rodd, F.H.; Shaw, R.G. (1997). Evaluation of the rate of evolution in natural population of guppies (*Poecilia reticulata*). *Science 275*: 1934-1937.

Roberts, C. (1996). Hapuku and bass: the mystery of the missing juveniles. *Seafood New Zealand 4 (1)*: 17-21.

Roberts, P.E.; Paul, L.J. (1978). Seasonal hydrological changes in continental shelf waters off the west coast, North Island, New Zealand and comments on fish distributions. *New Zealand Journal of Marine & Freshwater Research 12*: 323-339.

Rocha-Olivares, A.; Veter, R. (1999). Effects of ocenaographic circulation on the gene flow, genetic strcuture, and phylogeography of the rosethorn rockfish (*Sebastes helvomaculatus*). *Canadian Journal of Fisheries & Aquatic Sciences 56*: 803-813.

Rogers, S.; Isabel, N.; Bernatchez, L. (2007). Linkage Maps of the dwarf and Normal Lake Whitefish (*Coregonus clupeaformis*) Species Complex and Their Hybrids Reveal the Genetic Architecture of Population Divergence. *Genetics 175*.

Roques, S.; Sevigny, J.M.; Bernatchez, L. (2002). Genetic structure of deepwater redfish, *Sebastes mentella*, populations across the North Atlantic. *Marine Biology 140*: 297-307.

Safran, P.; Omori, M. (1990). Some ecological observations on fishes associated with drifting seaweed off Tohoku coast Japan. *Marine Biology 105*: 395-402.

Sakakura, Y.; Tsukamoto, K. (1997). Age composition in the schools of juvenile yellow tail *Seriola quinqueradiata* associated with drifting seaweeds in the east China sea. *Fisheries Science 63*: 37-41.

Sale, P.F.; Kritzer, J.P. (2003). Determining the extent and spatial scale of population connectivity: decapods and coral reef fishes compared. *Fisheries Research 65*: 153-172.

Salvanes, A.G.; Hart, P.J.B. (2000). Is individual variation in competitive performance of reared juvenile cod influenced by haemoglobin genotype? *Sarsia 85*: 265-274.

Saul, P.; Holdsworth, J. (1992). Cooperative gamefish tagging in New Zealand waters 1975-90. *New Zealand Fisheries Technical Report 33*: 24 pp.

Schöffmann, J.; SušNik, S.; Snoj, A. (2007). Phylogenetic origin of *Salmo trutta* L 1758 from Sicily, based on mitochondrial and nuclear DNA analyses. *Hydrobiologia 575*: 51-55.

Schulte, P. (2001). Environmental adaptations as windows on molecular evolution. *Comparative Biochemistry and Physiology 128*: 597-611.

Schultz, E.; Lankford, T.; Conover, D. (2002). The covariance of routine and compensatory juvenile growth rates over a seasonality gradient in a coastal fish. *Oecologia 133*: 501-509.

Sedberry, G.R.; Carlin, J.L.; Chapman, R.W.; Eleby, B. (1996). Population structure in the pan-oceanic wreckfish *Polyprion americanus* (Teleostei: Polyprionidae), as indicated by mtDNA variation. *Journal of Fish Biology 49*: 318-329.

Seeb, L.W. (1998). Gene flow and introgression within and among rockfishes, Sebastes auriculatus, S. caurinus, and S. maliger. *Journal of Heredity 89*: 393-403.

Seeb, L.W.; Gunderson, D.R. (1988). Genetic variation and population structure of Pacific Ocean perch (*Sebastes alutus*). *Canadian Journal of Fisheries & Aquatic Sciences 45*: 78-88.

Sekino, M.; Hara, M. (2001). Microsatellite DNA loci in Pacific abalone *Haliotis discus discus* (Mollusca, Gastropoda, Haliotidae). *Molecular Ecology Notes 1*: 1-10.

Sekino, M.; Hara, M.; Taniguchi, N. (2002). Loss of microsatellite and mitochondrial DNA variation in hatchery strains of Japanese flounder *Paralichthys olivaceus*. *Aquaculture 213*: 101-122.

Sekino, M.; Sugaya, T.; Hara, M.; Taniguchi, N. (2004). Relatedness inferred from microsatellite genotypes as a tool for broodstock management of Japanese flounder *Paralichthys olivaceus*. *Aquaculture 233*: 163-172.

Selvamani, J.; Degnan, M.; Paetkau, D.; Degnan, M. (2000). Highly polymorphic microsatellite loci in the Heron Reef population of the tropical abalone *Haliotis asinina*. *Molecular Ecology 9*: 1184-1186.

Shepherd, S.A. (1973). Studies on southern Australian abalone (genus *Haliotis*). 1. Ecology of five sympatric species. *Australian Journal of Marine and Freshwater Research 24*: 217-257.

Shepherd, S.A.; Brown, L.D. (1993). What is an abalone stock: implications for the role of refugia in conservation. *Canadian Journal of Fisheries & Aquatic Sciences 50*: 2001-2009.

Shikano, T.; Shimada, Y.; Suzuki, H. (2008). Comparison of genetic diversity at microsatellite loci and quantitative traits in hatchery populations of Japanese flounder *Paralichthys olivaceus*. *Journal of Fish Biology 72*: 386-399.

Shikano, T.; Taniguchi, N. (2003). DNA markers for estimation of inbreeding depression and heterosis in the guppy *Poecilia reticulata*. *Aquaculture Research 34*: 905-911.

Silverstein, J.; Weber, G.; Rexroad, C.; Vallejo, R. (2006). Genetics and genomics - Integration of molecular genetics into a breeding program for rainbow trout. *Israeli Journal of Aquaculture 58 (4)*: 231-237.

Smedbol, R.; Woblewski, J. (2000). Metapopulation theory and northern cod population structure: interdependency of subpopulations in recovery of a groundfish population. *Canadian Stock Assessment 2000/087*: 29p.

Smith, M.H.; Smith, M.W.; Scott, S.L.; Liu, E.H.; Jones, J.C. (1983). Rapid evolution in a post-thermal environment. *Copeia 1983*: 193-197.

Smith, P. (1988). Biochemical-genetic variation in the green-lipped mussel *Perna canaliculus* around New Zealand and possible implications for mussel farming. *New Zealand Journal of Marine & Freshwater Research 22*: 85-90.

Smith, P.; Benson, P.; Mcveagh, S. (1997). A comparison of three genetic methods for stock discrimination of orange roughy, *Hoplostethus atlanticus*: allozymes, PCR amplified mitochondrial DNA and random amplified polymorphic DNA. *Fishery Bulletin 94*: 800-811.

Smith, P.; Francis, R.; Paul, L. (1978). Genetic variation and population structure in the New Zealand snapper *Chrysophrys auratus*. *New Zealand Journal of Marine & Freshwater Research 12*: 343-350.

Smith, P.; Johnston, A. (1985). Glucosephosphate isomerase and a glycerophosphate dehydrogenase electromorph frequencies in groper *Polyprion oxygeneios* from central New Zealand. *New Zealand Journal of Marine & Freshwater Research 19*: 173-177.

Smith, P.; Macarthur, G.; Michael, K. (1989). Regional variation in electromorph frequencies in the tuatua, *Paphies subtriangulata*, around New Zealand. *New Zealand Journal of Marine & Freshwater Research 23*: 27-33.

Smith, P.; Mcmiilan, P.; Bull, B.; Mcveagh, S. (2002). Genetic and meristic variation in black and smooth oreos in the New Zealand EEZ. *New Zealand Journal of Marine & Freshwater Research 36*: 737-750.

Smith, P.J. (2005). Random Amplified Polymorphic DNA (RAPD). *Stock Identification Methods. Ed. by Cadrin, S.X., Friedland, K.D., and Waldman, J. Academic Press Database book*: 371-387.

Smith, P.J.; Conroy, A.M. (1992). Loss of genetic variation in hatchery-produced abalone, *Haliotis iris*. *New Zealand Journal of Marine & Freshwater Research 26*: 81-85.

Smith, P.J.; Diggles, B.; Mckenzie, J.; Kim, S.; Maolagáin, C.Ó.; Notman, P.; Griggs, L.H. (2004). Stock structure of kingfish. *New Zealand Ministry of Fisheries Final Research Report, Project KIN2002/01 Sept 2004*.

Smith, P.J.; Mcveagh, S.M. (2006). Genetic population structure of blackfoot paua. *Ministry of Fisheries Final Research Report*: 17 pp.

Smith, P.J.; Mcveagh, S.M.; Ede, A. (1996). Genetically isolated stocks of orange roughy (*Hoplostethus atlanticus*) but not hoki (*Macruronus novaezelandiae*) in the Tasman Sea and Southwest Pacific Ocean around New Zealand. *Marine Biology 125*: 783-793.

Smith, P.J.; Taniguchi, N.; Chow, S. (2005). Genetic differentiation and dispersal potential in marine fishes. *Marine Biotechnology 6S*: 343-350.

Smolenski, A.J.; Ovenden, J.R.; White, R.W.G. (1993). Evidence of stock separation in southern hemisphere orange roughy (*Hoplostethus atlanticus*), Trachichthyidae) from restriction-enzyme analysis of mitochondrial DNA. *Marine Biology 116*: 219-230.

Star, B.; Apte, S.; Gardner, J.P. (2003). Genetic structuring among populations of the greenshell mussel *Perna canaliculus* revealed by analysis of randomly amplified polymorphic DNA. *Marine Ecology Progress Series 249*: 171-182.

Su, G.; Liljedhal, L.; Gall, G. (1996). Effect of inbreeding on growth and reproductive traits in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture 142*: 139-148.

Sullivan, K.J.; Mace, P.M.; Smith, N.W.M.; Griffiths, M.H.; Todd, P.R.; Livingston, M.E.; Harley, S.J.; Key, J.M.; Connell, A.M. (2005). Assessment plenary, May 2005: stock assessments and yield estimates. *Unpublished report held in NIWA library, Wellington May 2005*: 792 pp.

Takagawa, S.; Washitani, I.; Uesugi, R.; Tsumura, Y. (2006). Influence of inbreeding depression on a lake population of *Nymphoides peltata* after restoration from the soil seed bank. *Conservation Genetics 7*: 705-716.

Tang, S.; Tassanakajon, A.; Klinbunga, S.; Jarayabhand, P.; Menasveta, P. (2005). Population structure of tropical abalone (*Haliotis asinina*) in coastal waters of Thailand determined using microsatellite markers. *Marine Biotechnology 6*: 604-611.

Taniguchi, N. (2003). Genetic factors in broodstock management for seed production. *Reviews in Fish Biology and Fisheries 13*: 177-185.

Taning, A.V. (1946). Stage of determination of vertebrae in teleostean fishes. *Nature 157*: 594-595.

Taylor, E. (1991). A review of local adaptation in Salmonidae with particular reference to Pacific and Atlantic salmon. *Aquaculture 98*: 185-207.

Thacker, C.T., Ar; Roje, Dm; Shaw, Ey. (2008). New expansions in old clades: population genetics and phylogeny of *Gnatholepis* species (Teleostei : Gobioidei) in the Pacific. *Marine Biology 153*: 375-385.

Tong, L.J.; Moss, G.A.; Redfearn, P.; Illingworth, J. (1992). A manual of techniques for culturing paua, *Haliotis iris*, through to the early juvenile stage. *New Zealand Fisheries Technical Report 31*: 21 pp.

Tymchuk, W.; Sundstrom, L.; Devlin, R. (2007). Growth and survival trade-offs and outbreeding depression in rainbow trout (*Oncorhynchus mykiss*). *Evolution 61*: 1225-1237.

Vasemagi, A.; Gross, R.; Paaver, T.; Koljonen, M.L.; Saisa, M.; Nilsson, J. (2005). Analysis of gene associated tandem repeat markers in Atlantic salmon (*Salmo salar* L.) populations: implications for restoration and conservation in the Baltic Sea. *Conservation Genetics 6*: 385-397.

Walsh, C.; Mckenzie, J.; Mcgrego, G.; Poortenaar, C.; B, H.; Smith, M. (2003). Information available for the management of New Zealand kingfish (*Seriola lalandi lalandi*) stocks. *New Zealand Fisheries Assessment Report 25*: 57 pp.

Wang, L.; Zhang, H.; Song, L.; Guo, X. (2007). Loss of allele diversity in introduced populations of the hermaphroditic bay scallop *Argopecten irradians*. *Aquaculture 271*: 252-259.

Waples, R.S. (1987). A multispecies approach to the analysis of gene flow in marine shore fishes. *Evolution 41*: 385-400.

Waples, R.S. (1998). Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *Journal of Heredity 89*: 438-450.

Ward, R.D.; Elliott, N.G.; Grewe, P.; Last, P.R.; Lowry, P.S.; Innes, B.H.; Yearsley, G.K. (1998). Allozyme and mitochondrial DNA variation in three species of oreos (Teleostei: Oreosomatidae) from Australasian waters. *New Zealand Journal of Marine & Freshwater Research 32*: 233-245.

Ward, R.D.; Woodwark, M.; Skibinski, D.O.F. (1994). A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. *Journal of Fish Biology 44*: 213-232.

Waters, J.; Roy, M. (2004). Phylogeography of a high-dispersal New Zealand sea-star: does upwelling block gene-flow? *Molecular Ecology 13*: 2797-2806.

Wijekoon, M.; Puvanendran, V.; Brown, J. (2003). Interpopulation difference in growth of juvenile Atlantic cod (*Gadus morhua*) - implication for broodstock development. *Aquaculture Canada 2002, Proceedings of the 19th Annual meeting of the Aquaculture association of Canada*: 21-23.

Williams, G.; Koehn, R.; Mitton, J. (1973). Genetic differentiation without isolation in the American eel *Anguilla rostrata*. *Evolution 27*: 192-204.

Withler, R.E.; Campbell, A.; Shaorong, L.; Brouwer, D.; Supernault, K.J.; Miller, K.M. (2003). Implications of high levels of genetic diversity and weak population structure for the rebuilding of northern abalone in British Columbia, Canada. *Journal of Shellfish Research 22*: 839-847.

Wright, S. (1951). The genetical structure of populations. *Annals of Eugenics 15*: 323-354.