New Zealand Journal of Marine and Freshwater Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tnzm20

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Available online: 19 Feb 2010

To cite this article: Peter J. Smith (2009): Genetic principles for freshwater restoration in New Zealand, New Zealand Journal of Marine and Freshwater Research, 43:3, 749-762

To link to this article: http://dx.doi.org/10.1080/00288330909510039

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Genetic principles for freshwater restoration in New Zealand

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Abstract Habitats fragmentation and destruction can lead to loss of genetic diversity within and among populations. One goal of restoration projects is to minimise loss of genetic diversity and to preserve genetic structure. Restoration may involve the release of wild-caught or hatchery-produced individuals to re-establish or augment populations, and targets species with weak dispersal potential. Key criteria to consider in restoration projects are genetic divergence among the source populations and the number of individuals to transfer. Molecular tools provide quick and simple tools for assessing genetic diversity, but most markers are selectively neutral and should be supplemented with data on life-history traits. The effective size of the population, $N_e$, can be considerably smaller than the census $N$. Low numbers of founders will lead to a loss of genetic diversity, whereas subsequent breeding between closely related individuals will lead to inbreeding depression. Outbreeding depression can occur when offspring are produced from crosses between individuals from divergent populations, leading to the breakdown of co-adapted gene complexes. Both inbreeding and outbreeding depression can lead to declines in fitness, and these effects may take several generations to become evident.

Keywords effective population size; outbreeding and inbreeding depression; genetic restoration

INTRODUCTION
Restoration of biota, the augmentation or re-establishment of extirpated populations or communities, is becoming increasingly common as freshwater populations are reduced through habitat loss and degradation, species introductions, and overfishing (e.g., Clewell & Aronson 2007). Restoration typically involves the supplementation of populations with translocated stock or seed from wild populations or from hatcheries (Ryman 1991; Falk et al. 2006). Although the focus of restoration projects has been necessarily ecological (Anon. 2004a; Clewell & Aronson 2007; Katz et al. 2007), some genetic impacts are irreversible if novel genes or genotypes are inadvertently introduced from foreign source populations (McKay et al. 2005). The general genetic principles of restoration have been considered (McKay et al. 2005; Falk et al. 2006) and specifically reviewed for key freshwater species in Australia (Hughes 2007). Restoration genetics is a relatively new branch of conservation genetics (Tallmon et al. 2004; Hedrick 2005; McKay et al. 2005; Falk et al. 2006), which draws on genetics theory and its applications to small populations (Soule 1988; Nunney & Campell 1993). Genetic principles have been applied in plant restoration projects (McKay et al. 2005; Ramp et al. 2006; Takagawa et al. 2006) and widely considered in the restoration of commercial fish populations, notably salmonids (Ryman 1991; Utter 2004; Verspoor et al. 2007). Genetic guidelines have been developed for the restoration and enhancement of commercially important shellfish (Munro 1993; Benzie & Williams 1996; Gaffney 2006) and fish stocks (FAO 1993; ICES 1996). Increasingly, genetic data are being considered in restoration projects of other non-salmonid freshwater fishes and invertebrates (Vrijenhoek 1998; Sheller et al. 2006; Cook et al. 2007; Demand & Bjorklund 2007; Hughes 2007).

The success and timescale of re-colonisation will be driven by dispersal potentials of the organisms, which range from strong dispersers such as migratory fishes and winged insects to weak dispersers such as crustaceans (Malmqvist 2002; Hughes 2007).
Even sessile species such as mussels may be able to disperse over tens to hundreds of kilometres through the juvenile stages which are obligate parasites on fishes (e.g., Elderkin et al. 2007). Restoration projects that depend on natural re-colonisation (passive restoration) of organisms need to ensure that restoration sites and habitat connectivity networks are established at appropriate scales to promote re-colonisation. Restoration projects targeting weak dispersers need to consider translocation of key organisms. Successful reintroduction of populations to restored habitats will be dependent upon a wide range of ecological and demographic factors for which an understanding of genetic differentiation among potential source populations is desirable (Falk et al. 2006).

In New Zealand there are many local and national initiatives in freshwater restoration (see for example: www.nzfreshwater.org/home.html; www.lernz.co.nz/; www.sustain.canterbury.ac.nz/waterways/resources.shtml). Specific studies have considered restoration of fishes (Richardson & Jowett 2005; Jowett et al. 2009, this issue; Leathwick et al. 2009, this issue), invertebrates (Blakely et al. 2006; Winterbourn et al. 2007), and lake macrophytes (de Winton et al. 2000); whereas species recovery plans have been developed for the threatened non-diadromous mudfishes (Eldon 1993; Anon. 2003) and galaxiid fishes (Anon. 2004b). Here the genetic principles of restoration are considered with a focus on weak dispersers that are unlikely to establish populations in restored habitats over ecological timescales of a few generations. Although restoration genetics is intimately entwined with ecology and life-history traits, the key genetics questions can be distilled into two areas: selection of appropriate source populations and the numbers of individuals to transfer.

Genetic goals of restoration

Genetic goals of restoration projects are not always explicitly stated but share those of conservation genetics, to preserve evolutionary processes and the ecological viability of populations (Moritz 1994; Waples 1995; Falk et al. 2006), and specifically to maintain genetic resources without avoidable and irreversible loss of genetic diversity during the restoration process (Ryman 1991). However, the focus of conservation genetics is on rare and endangered species and differs subtly from restoration genetics, which may target species that are common outside the restoration area. One goal of conservation genetics is to restore or increase genetic diversity within small fragmented populations through translocation (Frankham et al. 2004; Allendorf & Luikart 2006), whereas a translocation goal of restoration projects is to avoid loss of genetic diversity in the restored population (Ryman 1991; Falk et al. 2006). In the absence of information on the ecological and evolutionary value of most genes or populations, the main focus of restoration projects has been aimed at maintaining genetic diversity within and between populations (Ryman 1991). Retention of genetic diversity has been an important component in the restoration and stocking programmes developed for freshwater Salmonidae (Utter 2004; Page et al. 2005) and Percidae (Wilson et al. 2007) in North America. To maintain genetic resources and minimise the risk of loss of genetic diversity, the source populations should be genetically close to those of the restored populations; and it is generally recognised that it is prudent to avoid the transfer of individuals among genetically discrete units (Moritz 1994). Consequently, a primary goal of freshwater restoration genetics is to define population genetic structure (Vrijenhoek 1998; Hurwood et al. 2003; Hogg et al. 2006; Hughes 2007). The second, main goal is to ensure that restored populations persist over time (Falk et al. 2006) and this goal requires that sufficient numbers of founders are established, and, if necessary, supplemented with additional introductions.

Genetic diversity and identification of source populations for restoration

There is a considerable literature that discusses and defines conservation units below the species level. Ryder (1986) first proposed the term Evolutionary Significant Unit (ESU) (Box 1), which has been widely applied and re-defined in conservation biology, although the differences lie more in the criteria used to define ESUs than in their fundamental essence (Fraser & Bernatchez 2001; De Guia & Saitoh 2007). Guia & Saitoh (2007) introduced the terms partial and full ESUs to recognise situations where partial ESUs were defined on the basis of one aspect (e.g., molecular based ESU), and full ESUs based on information derived from both neutral and adaptive genetic variation. Moritz (1994) used the term management unit (MU) (Box 1), which concerns current population structure, and is broadly equivalent to the stock concept used in fisheries management. The terms conservation unit (CU), and operational conservation unit have been used at a more pragmatic level to refer to either ESUs, MUs, or geographical units that managers...
consider important to conserve (Manel et al. 2003; Johnson & Belk 2007). Population is widely used in the ecological and evolutionary literature, and in a review of population definitions, Waples & Gaggiotti (2006) pointed out that although there is no consensus over a quantitative definition of a population, there are two main definitions that reflect ecological and evolutionary paradigms (Box 1).

In practice, the identification of MUs, CUs, and evolutionary populations has been dominated by the application of molecular tools (Box 2), because of the relative ease with which genetic data can be collected. Restoration projects may not have sufficient time or resources to establish ESUs, and there is a risk that identification of MUs and CUs based on one type of data (particularly neutral genetic markers) may overlook genetic population differentiation. For example, a laboratory study of genetic variation in Drosophila showed no relationship between the levels of variation with molecular markers and life-history characters (Lynch et al. 1999). Adaptive divergence may occur rapidly through accumulation of genetic differences driven by local selection (Lynch 1996; Reznick et al. 1997; Bell 2001; Binks et al. 2007). Rapid changes in allozyme (Box 2) frequencies occurred in large mouth bass Micropterus salmoides exposed to warm water effluent from power stations (Smith et al. 1983), and rapid changes in allozyme frequencies and shell shape were reported for translocated populations of the marine intertidal snail Bembicium vittatum (Binks et al. 2007). New molecular techniques may allow the detection of adaptive variation by examining functional genes (Schöffmann et al. 2007), as applied in the identification of populations in marine fishes (Pogson & Mesa 2004; Larsen 2007). Genes under selection, or associated with markers under selection, are expected to show higher 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).

**Genetic diversity in freshwater fishes and invertebrates**

Freshwater species in general show greater population divergence (i.e., more MUs) than marine or terrestrial species, owing to the greater number of barriers to dispersal and migration (Gyllenstein 1985). The most widely used measure of genetic differentiation between populations is $G_{ST}$, or its analogue $\Phi_{ST}$ that takes into account haplotype and sequence divergence (Nei 1973, 1987). $G_{ST}$ is a relative measure ranging between 0 (identical

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**Box 1** Populations and conservation units below the species level.

**Evolutionary Significant Unit (ESU)**
- Populations possessing genetic attributes significant for present and future generations (Ryder 1986).
- Reciprocally monophyletic for mitochondrial (mt)DNA alleles and show significant divergence of allele frequencies at nuclear loci (Moritz 1994).
- A lineage demonstrating highly restricted gene flow from other such lineages within the higher organisational level of the species (Fraser & Bernatchez 2001).

**Management Unit (MU)**
- Populations with significant divergence of allele frequencies at nuclear or mitochondrial loci regardless of the phylogenetic distinctiveness of the alleles (Moritz 1994).

**Conservation Unit (CU) and Operational Conservation Unit (OCU)**
- A population or group of populations important to be conserved (Manel et al. 2003; Geist & Kuehn 2005a,b; Araguas et al. 2007).
- Continuous area limited by geographical boundaries, and inhabited by one or more populations sharing the same genetic pattern (Araguas et al. 2007).

**Evolutionary population**
- A group of individuals of the same species living in close enough proximity that any member of the group can potentially mate with any other member (Waples & Gaggiotti 2006).

**Ecological population**
- A group of individuals of the same species that co-occur in space and time and have an opportunity to interact with each other (Waples & Gaggiotti 2006).
allele frequencies in the populations) and 1 (different alleles fixed at each locus). Genetic differentiation, measured with allozyme markers, was inversely related to dispersal ability across 333 species of vertebrates and invertebrates from terrestrial, marine, and freshwater environments (Bohonak 1999). A review of the literature on allozymes (see Box 2) showed that the mean $G_{st}$ for marine fishes was 0.06, for anadromous species 0.11, and for freshwater fishes 0.22 (Ward et al. 1994). It should be noted that 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 & Avise 2000), owing to the larger evolutionary effective

**Box 2** Molecular tools for estimating genetic diversity in natural populations.

Molecular biology has provided a range of tools for measuring genetic variation in natural populations. Most markers have been assumed to be selectively neutral, and statistical tests are available to detect natural selection in patterns of genetic variation (Ford 2002).

**Polymerase chain reaction** (PCR)

PCR revolutionised molecular genetic studies, by enabling specific segments of DNA to be amplified to provide millions of copies, which can be visualised or further manipulated to analyse genetic variation. Amplification of DNA allows genetic analyses from non-lethally collected tissue samples.

**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), but can be underestimated because changes that do not result in a charge difference are not detected. The technique was widely used for 30 years in population-genetic studies, but has been largely replaced by tools that measure genetic variation directly at the DNA level.

**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 (CTA)$_n$. 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.

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 & Avise 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 $N_e$ (effective population size) of mtDNA to ¼ of that for nuclear DNA, increasing the potential for genetic drift among populations. There are several approaches to analysis, from fragmentation of the overall mtDNA genome with restriction enzymes (restriction fragment length polymorphisms = RFLPs) through to direct sequencing. Some regions of the mitochondrial region are non-coding and often highly variable (the control region) and used for population studies, while the less variable coding regions are used for phylogenetic studies.

**Nuclear-encoded ribosomal RNA genes**

Ribosomal RNA genes are among the most abundant elements of DNA in the cell and exist as repeat units separated by the internal transcribed spacers (ITS). The conserved 18S and 28S ribosomal sequences have been used in phylogenetic studies, whereas the less conserved ITS regions have been used to study closely related species.

Several other nuclear DNA tools are available, such as the multilocus RAPDs, random amplified polymorphic DNA (Kliber & Eckert 2005) were quickly surpassed; others such as introns, non-coding regions of nDNA (Belshaw & Bensasson 2006); SNPs, single nucleotide polymorphisms; MHC, major histocompatibility complexes; and AFLPs amplified fragment length polymorphisms (Bensch & Akesson 2005), have not been widely applied in population studies of freshwater organisms to date, other than Salmonidae (Langefores et al. 1998; Vasemagi et al. 2005; Dionne et al. 2007; Hansen et al. 2007; Rogers et al. 2007).
population sizes in marine fishes (Dewody & Avise 2000).

Comparisons of genetic diversity in Australian freshwater species have shown winged insects were the most extensive dispersers across catchment boundaries whereas non-diadromous fish and insects were good dispersers within catchments, although fish dispersal was limited to species in lowland streams (Hughes 2007). It was concluded that with the exception of fish in lowland streams and insects, natural re-colonisation of restored sites was only likely to occur from within the same stream (Hughes 2007).

There are limitations in using selectively neutral molecular markers to estimate genetic divergences, because these tools measure divergence at evolutionary and not ecological timescales. Where there is significant genetic differentiation among populations/sites, then differentiation can be used to infer lack of gene flow. The converse, lack of genetic differentiation among sites, may not be owing to current gene flow, and needs to be considered in parallel with ecological and life-history data. Lack of genetic differentiation among populations of winged insects in neighbouring catchments might reflect pre-fragmentation connectivity rather than present day gene-flow via adult flight (Monaghan et al. 2001; Finn & Adler 2006; Smith et al. 2006a). Extensive de-forestation over the past 200 years may have created barriers to dispersal and extirpated populations in lowland catchments, where dispersal among populations may have been less constrained by geography than among populations in the hill country (Hughes 2007).

**Minimum numbers for active genetic restoration**

Genetic variation allows populations and species to persist through changing environments over evolutionary timescales, and is determined by the combined effects of mutation, random genetic drift, selection, and gene flow (Box 3). Mutations are the ultimate source of genetic variation, but are rare and not important over the timescale of restoration projects. Restored populations based on small numbers of founders with low genetic variation may risk extinction long after population size has recovered, because genetic variation is only restored through accumulation from mutations over numerous generations or through gene-flow (Lynch et al. 1999). Population genetic theory shows that a minimum effective population size, \( N_e \) (Box 3) of 50, with an equal sex ratio, is required to retain 99% of genetic variation.

**Box 3 Genetic processes in natural populations.**

**Genetic drift and \( N_e \) (effective population size)**
The genetic composition of a population can change over generations owing to chance events, which are most relevant in small populations. Alleles (genetic variants) may become fixed or lost from small populations regardless of their adaptive value. The effective size of the population, \( N_e \) (Wright 1951)—a measure of the number of individuals contributing to the next generation—can be considerably smaller than the census population \( (N) \) owing to variations in population size between generations, reducing the effective population size to the harmonic mean over generations (Wright 1940). Unequal sex ratios and random variations in reproductive success further reduce \( N_e \).

**Selection**
Selection is the differential reproductive success of genotypes. Under natural selection, the more fit individuals leave more offspring than less fit individuals, leading to adaptive change in populations. Selection may act at one or a few loci whereas genetic drift and gene flow act on all loci. Specific genotypes can have different selective values under different environmental conditions leading to balanced polymorphisms. Evidence for natural selection in fish populations is limited to a few well researched cases for qualitative (Mitton 1997) and quantitative markers (Reznick et al. 1997), and can be rapid (Smith et al. 1983; Stockwell & Weeks 1999; Bell 2001).

**Gene flow, migration, and dispersal**
Gene flow is the movement of genes and their establishment in neighbouring populations and counteracts the effects of drift and selection by introducing new alleles into populations. In two populations of size \( N \), which exchange a proportion \( m \) through immigrants at each generation, substantial differentiation (at neutral loci) will not occur when \( Nm > 1 \) (Slatkin 1987). Because \( N \) is large in many wild populations, a small absolute number of immigrants at each generation will prevent populations from diverging owing to drift. Migration is the long-distance movement of large numbers of individuals, in the same general direction at the same time, and includes the seasonal movement between feeding and breeding areas, whereas dispersal is the random movement of individuals between localities (Endler 1977; Bilton 2001)). For salmon, migration includes the return of adults to their natal site (= homing). Mark-recapture studies provide an indication of dispersal and migration, but not a measure of reproductive success and gene flow.
variation per generation. Experimental studies with the housefly Musca domestica have shown that $N_e > 50$ is necessary for populations to retain fitness and escape extinction, even in the short term (Reed & Bryant 2000). A minimum $N_e$ of 500 has been suggested for the long-term preservation of genetic diversity (Franklin 1980; FAO 1981; Soule 1988) and $N_e$ of 5000 to retain evolutionary potential in natural populations (Lande 1995). Unequal sex ratios and variations in reproductive success reduce $N_e$ and can result in $N_e$ several orders of magnitude smaller than census size (Frankham 1995a). Estimates of $N_e / N$ average 0.1 in 102 species (Frankham 1995a), and in insects range from <0.0001 to 1 (Frankham 1995a) and in fishes from 0.01 to 1 (Frankham 1995a; Miller & Kapuscinski 1997; Hedrick et al. 2000).

Small effective population size will result in a loss of rare alleles in the founding population, and lead to a risk of inbreeding in subsequent generations (Lynch 2005). The rate of inbreeding is given by: $\Delta F = 1/(2N_e)$, where $\Delta F$ is the rate of inbreeding per generation and $N_e$ is the effective population size. Inbreeding is cumulative because it increases from one generation to the next. Inbreeding depression is the reduced fitness in a population as a result of breeding between related individuals (Lynch 2005). Inbreeding depression is difficult to demonstrate in wild populations and is more commonly observed in domesticated animals and laboratory populations as lower survival or reproductive rates (Visscher et al. 2001). Although inbreeding is unlikely in many wild populations, it could occur when populations have been severely reduced in size and are supplemented with hatchery or translocated stock derived from a few parents (Bartley et al. 1992; Hedrick et al. 1995). Inbreeding in small populations ($N < 1000$) may be more important than chance variations in survival and reproduction, and has contributed to the decline and extinction of several rare mammals and birds (Frankham 1995b). Yet, some vertebrate populations have survived despite severe reductions in population size ($N < 100$) e.g., elephant seals (Mirounga angustirostrus) (Bonnell & Selander 1974), European bison (Bison bonasus) (Lacy 1997), and park cattle (Bos taurus) (Visscher et al. 2001).

One scenario to avoid inbreeding would be to use outbreeding enhancement by transferring stock from different populations to establish the restored population. This form of line crossing is used by plant and animal breeders to gain hybrid vigor, but the increase in productivity traits obtained in the first generation can be followed by outbreeding depression in subsequent generations (Lynch 2005). Outbreeding depression appears when offspring from crosses between individuals from different populations have lower fitness than progeny from crosses between individuals in the same population, and a result of the breakdown of coadapted gene complexes that have evolved in divergent populations (Lynch 2005). Outbreeding depression might also occur through the swamping of locally adapted genes when large numbers of individuals are introduced into small populations, as has occurred in some salmon populations (Hindar et al. 1991; Ryman 1991; Bartley et al. 1992; Ayllon et al. 2006). Swamping displaces the adaptive gene complexes through the introduction of genes that are adapted to the hatchery environment or to some other locality.

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. Thus prevention may be a better restoration option than waiting several generations before attempting corrective actions (Lynch 2005; Takagawa et al. 2006).

Although $N_e$ can be used to guide the minimum number of founders to establish a restored population, consideration has to be given to persistence over many generations. Population size is a major determinant of extinction risk and populations with $N_e < 100$ and $N < 1000$ are highly vulnerable to extinction (Lynch et al. 1995), but there is controversy as to how large populations need to be to ensure persistence. The minimum viable population (MVP) describes the smallest size at which a population or species can exist without facing extinction from natural disasters or demographic and genetic stochasticity (Reed et al. 2003). The size of MVPs are highly specific and dependent on the environmental and life-history characteristics of the species. MVPs have been simulated with population viability analyses (PVA), which provide a quantitative means for predicting the probability of extinction, by using population-specific life-history information to forecast future population sizes, and take into account the combined impacts of stochastic (demographic, environmental, and genetic) and deterministic factors such as habitat loss and over-exploitation (Reed et al. 2003). The mean and median estimates of MVPs in 102 vertebrate (mostly terrestrial) species, with a 99% probability of persistence over 40 generations, were 7316 and 5816 adults, respectively (Reed et al. 2003), leading the authors to conclude that the lack of long-term studies for many endangered species has led to widespread underestimations of extinction risk.
Census numbers are not known for most of the non-diadromous fishes in New Zealand, but the new species of *Galaxias* fishes recently described from South Island, New Zealand have restricted distributions and presumed small population sizes (McDowall & Waters 2002, 2003) and are recognised as threatened or data poor (Anon. 2004b). The longjaw galaxid *Galaxias cobitis* is restricted to the Kakanui and Waitaki catchments in the eastern South Island (McDowall & Waters 2002) with an estimated adult population of \( N < 250 \) individuals in the Kauer River (Kakanui catchment), and is recognised as Nationally Critical (Anon. 2004b).

**ESUs and MUs in New Zealand freshwater species**

The key freshwater taxa likely to be considered in restoration projects in New Zealand are the non-diadromous fishes (*Galaxias, Neochanna, and Gobiomorphus*), molluscs (*Eychiridella*), crustaceans (*Paranephrops and Paracalliope*), insects, in particular the EPT taxa, *Ephemeroptera* (mayflies), *Plecoptera* (stoneflies), and *Trichoptera* (caddisflies), and lake macrophytes. An initially unexpected but recurring finding in molecular studies has been the number of potentially cryptic species in fishes (Allibone et al. 1996; Gleeson et al. 1999; Ling et al. 2001; Waters & Wallis 2001; Smith et al. 2005), crustaceans (Hogg et al. 2006; Apte et al. 2007), and insects (Hogg et al. 2002), many of which have restricted distributions and small population sizes, making them vulnerable to land use changes and introduced predators. Ecological aspects of restoration of insects and fishes are discussed in other papers in this issue (Jowett et al. 2009; Leathwick et al. 2009) and in other reports for fishes (Anon. 2003, 2004b; Richardson & Jowett 2005). The requirements for captive breeding of mudfish *Neochanna* have been considered by Dunn & O’Brien (2005).

Extensive habitat loss and degradation along with wetland drainage and de-forestation over the past 200 years may have created barriers to dispersal and extirpated populations, particularly in lowland catchments where populations were less constrained by landscape than in the hill country. Species of mudfish *Neochanna* are threatened (Anon. 2003) as are many of the non-diadromous *Galaxias* (Anon. 2004b). Although many EPT taxa are not considered threatened, the recently isolated populations may be vulnerable to further de-forestation activities that reduce connectivity pathways and population sizes, because small effective populations are likely to retain lower levels of genetic diversity (Lynch et al. 1995; Allendorf & Luikart 2006). Present day populations of winged insects (EPT) may have been isolated over ecological as opposed to evolutionary timescales, and have had insufficient time to reach an equilibrium between gene flow (that restricts divergence) and drift (that enhances divergence) among populations. Even comparatively small environmental changes such as the placement of road culverts could act as partial barriers to upstream flight patterns of insects (Blakely et al. 2006), and consequently restrict adult dispersal and gene-flow.

**Genetic diversity in New Zealand freshwater fishes**

New Zealand was long regarded as having a depauperate fish fauna (Ling et al. 2001) but molecular studies over the past decade have revealed a species flock, the *G. vulgaris* complex, in the South Island (Allibone et al. 1996; Waters & Wallis 2001), cryptic species of mudfish *Neochanna* (Gleeson et al. 1999; Ling et al. 2001), and high sequence divergence in the upland bully *Gobiomorphus breviceps* that might be indicative of a cryptic species (Smith et al. 2005). A common finding in molecular population studies has been catchment-specific mtDNA haplotypes with little evidence of dispersal among adjacent coastal catchments (Waters et al. 2001). Historical hydrographic patterns have been a key driver in determining the genetic structure of freshwater fish populations with examples of river capture (Waters et al. 2001; Waters et al. 2006). A main north-south discontinuity was found in the Canterbury galaxid *G. vulgaris* and in the dwarf galaxiid *G. divergens* in the northern South Island, with “Canterbury” and “Marlborough” lineages, separated by the Kaikoura ranges (Waters & Wallis 2000; Burridge et al. 2006). The upland bully *Go. breviceps* for the most part shows catchment-specific haplotypes (Smith et al. 2005; Burridge et al. 2006), but shared haplotypes among two neighbouring catchments in the northern South Island (Burridge et al. 2006), and among two neighbouring catchments in the lower North Island (Smith et al. 2005).

The dwarf or dune lakes inanga *G. gracilis* have been considered lake-locked populations of *G. maculatus*, with separate conservation units in each lake (Ling et al. 2001), and are recognised as in serious decline (Anon. 2004b). Significant genetic differentiation was found among lake-locked populations of koaro *G. brevipinnis* (King et al. 2003). In the threatened mudfish *Neochanna diversus*, three population groupings have been identified among
North Island wetland populations in northern and southern Northland and in Waikato (Ling et al. 2001). In the nationally endangered Canterbury (South Island) mudfish *N. burrowsi*, a study of the mtDNA control region found low diversity, but significant genetic subdivision among northern and southern populations in eastern Canterbury, with most wild populations representing distinct management units (Davey et al. 2003).

**Genetic diversity in New Zealand freshwater invertebrates**

Catchment specific haplotypes were found in freshwater crayfish, koura, *Paranephrops zealandicus* in the South Island and *P. planifrons* in the North Island and northern South Island (Apte et al. 2007), and specifically for *P. planifrons* among neighbouring catchments in the central west North Island (Smith & Smith 2009, this issue). An unexpected finding was three lineages of koura with a cryptic west coast South Island group in addition to *P. planifrons* and *P. zealandicus* (Apte et al. 2007). High sequence divergences among samples of the amphipod *Paracalliope fluviatilis* from the North and South Islands, and among eastern and western catchments in the North Island, suggested a complex consisting of at least four, and perhaps as many as six, geographically isolated and morphologically conservative cryptic species (Hogg et al. 2006).

In contrast, a molecular population study of the mussel *Echyridella menziesi* found weak isolation by distance with the most common mtDNA haplotype shared among southern catchments, and by inference the mussels are strong dispersers with gene-flow among catchments via fish hosts (Fenwick 2006). Shell morphology has shown significant differences among six lake populations in the central North Island (Roper & Hickey 1994), but morphological variation may represent phenotypic rather than genotypic variation.

Among the winged insects, some species appear to be strong dispersers. Three species of damselflies (Odonata: *Xanthocnemis, Austrolestes*, and *Ischnura*) in which the adults are relatively long-lived (and two are found on offshore islands) show low population differentiation leading to the conclusion that levels of gene flow within the North and South islands have been sufficient to maintain homogeneous population structures (Nolan et al. 2007). In contrast, the mayfly *Acanthophlebia cruentata* and the caddisfly *Orthopsyche fimbriata*, restricted to native forest streams in the North Island, exhibit genetically differentiated populations among catchments at the 70–100 km scale (Smith & Collier 2001; Smith et al. 2006a,b). A hierarchical analysis of *O. fimbriata* populations from 14 streams in eight neighbouring catchments in the central west North Island found high haplotype diversity with c. 56% of genetic diversity distributed among catchments (Smith & Smith 2009). Haplotypes were shared across catchments and only the most northern and southern catchments, separated by straight line distances <100 km, did not share haplotypes (Smith & Smith 2009). In the mayfly *Coloburiscus humeralis*, low divergence and weak differentiation found with allozyme markers led to the conclusion that there is wide dispersal (Hogg et al. 2002). However, low allozyme diversity might provide a weak test of population differentiation; low population differentiation found with allozyme markers in *A. cruentata* (Smith & Collier 2001) contrasts with high mtDNA haplotype diversity, with 58% of variation distributed among catchments and catchment-specific haplotypes at the 70–100 km scale (Smith et al. 2006b).

In the megalopteran *Archicauliodes diversus* genetic differentiation was found among Northland and central North Island populations, whereas fixed genetic differences at some allozyme loci among North and South Island populations may be indicative of cryptic species (Hogg et al. 2002).

**Guidelines for genetic restoration**

Guidelines for genetic restoration have been developed for plant populations (McKay et al. 2005; Falk et al. 2006) and for commercially important fish and shellfish (FAO 1981, 1993; Munro 1993; Benzie & Williams 1996; Gaffney 2006). Most guidelines are species- and/or site-specific and emphasise that genetics cannot be considered in isolation from ecology, but should be an integral component of the restoration process (Geist & Kuehn 2005a). Genetic principles will not apply equally to all species and all restoration sites, therefore specific projects will need to be developed for each New Zealand location (Richardson & Jowett 2005; Jowett et al. 2009; Leathwick et al. 2009). Many translocations have failed to establish sustainable populations (Sheller et al. 2006), and in one example, translocation of the Australian freshwater shrimp *Paratya australiensis* accidentally mixed two ESUs in different sub-catchments leading to the expiration of resident genotypes within seven generations (Hughes et al. 2003). Less than half of the early (pre 1987) translocation efforts with birds and mammals were successful (Wolf et al. 1996), whereas 50–70% of...
translocations of the endangered gila topminnow, *Poeciliopsis occidentalis*, in the southwest United States failed within the first 5 years (Sheller et al. 2006). Key factors that led to successful (= persistent) translocations of *Po. occidentalis* included the season in which the fish were translocated, the habitat type of the translocation site, and the genetic origin of the source population, leading the authors to emphasise that life-history attributes need to be considered for each translocated species (Sheller et al. 2006).

Rare and threatened species are more likely to require hatchery supplementation rather than translocation because sampling wild populations may further stress their status and provide insufficient numbers to establish viable populations. For an introduction of 50 individuals and assuming an initial survival of 30–50%, owing to mortalities during the collection, transport and release stages, 100–170 (50/0.5, 50/0.3) individuals would need to be collected (Brown & Briggs 1991; Falk et al. 2006). Removing 100+ individuals even from non-threatened populations may lead to local depletion, inadvertently creating a restoration problem in the donor population. Estimates of fish densities in New Zealand rivers are mostly for diadromous species and are typically low, <1 individual m⁻², range 0.008–3.0 individuals m⁻² (Jowett et al. 1999; Chadderton & Allibone 2000; Rowe et al. 2002; West et al. 2005); although inanga *G. maculatus* may form dense schools with 500–1000 individuals in single pools (Jowett et al. 1998). Densities of diadromous *Gobiomorphus* are typically <1 individuals m⁻² (Jowett & Richardson 1996; Jowett et al. 1996), whereas koura *Paranephrops* are 1–4 individuals m⁻² (Rabeni et al. 1997; Olsson et al. 2006). Using several source populations from neighbouring streams in the same catchment (20 individuals × 5–8 streams) may reduce local depletion while providing a representative sample of catchment genetic diversity without risking outbreeding depression. Given high initial mortalities, the translocations may need to be repeated over several years.

Eldon (1993) recommended translocation of juveniles as opposed to adults of the nationally endangered eastern South Island Canterbury mudfish *N. burrowsius* owing to ease of capture and handling. The key ecological criteria that contributed to the successful liberation of *N. burrowsius* into ponds and their short-term persistence over a few years have been identified (Eldon 1993), and the Waianiwaniwa River, on the Canterbury Plains, has been identified as the most important refuge free of larger predatory fish and reduced competition from other fish species (Harding et al. 2007). DNA studies have identified significant genetic structure that should be considered in future restoration projects (Davey et al. 2003).

Ultimately, restoration success will be dependent upon the application of ecological and genetic principles and will be measured by the persistence of restored populations over timescales beyond the working life span of most scientists.

ACKNOWLEDGMENTS

I thank Steph Parkyn, Brian Smith, and John Quinn (NIWA), and two anonymous referees for constructive comments on a draft manuscript. The project was supported by the Foundation for Research, Science and Technology programme on Restoration of Aquatic Ecosystems, CO1X0305.

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